Utility of chloride and adenosine deaminase measurement in cerebrospinal fluid for the early presumptive diagnosis of tuberculous meningitis

by

Hendré Swanepoel
Student number: SWNHEN002

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Supervisor: Dr Justyna Wojno
MBBCh, MMed, FC Path SA
Division of Medical Microbiology
Department of Pathology
Faculty of Health Sciences
University of Cape Town

Co–supervisor: Dr Fierdoz Omar
MBChB, MMed, FC Path SA
Division of Chemical Pathology
Department of Pathology
Faculty of Health Sciences
University of Cape Town

DECLARATION

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ABSTRACT

Background

Chloride and adenosine deaminase measurements in cerebrospinal fluid are still sporadically requested as part of tuberculous meningitis work–up. In the literature, evidence is contradictory and opinion is divided on their utility in clinical practice. The accuracy of both for the early presumptive diagnosis of tuberculous meningitis was investigated in patients in a region with high prevalence of tuberculosis and HIV infection in order to inform a decision on whether to continue offering these tests to clinicians.

Methods

A retrospective descriptive study of diagnostic accuracy was conducted at the National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa. Data were collected on all cerebrospinal fluid specimens submitted for tuberculosis culture between 1 January 2012 and 31 December 2014. Chloride and adenosine deaminase concentrations were compared with automated liquid culture for *Mycobacterium tuberculosis* as the reference standard.

Findings

There were 2531 cerebrospinal fluid specimens submitted for tuberculosis culture during the study period; exclusion of duplicates yielded 2081 specimens. Chloride was requested on 711 (34·2%) specimens; 44 (6·2%) were tuberculosis culture–positive. Adenosine deaminase was requested on 152 (7·3%) specimens; 20 (13·2%) were culture–positive. Chloride sensitivity (<120 mmol/L) for the detection of tuberculous meningitis was 93·2% (95% confidence interval 81·3–98·6), with specificity 62·4% (58·6–66·1), positive predictive value 14% (10·3–18·6), negative predictive value 99·3% (97·9–99·9), positive likelihood ratio 2·48 (2·18–2·81), and negative likelihood ratio 0·109 (0·037–0·326). Adenosine deaminase sensitivity (>6 U/L) was 70% (45·7–88·1), specificity 89·4% (82·8–94·1), positive predictive value 50% (30·6–69·4), negative predictive value 95·2% (89·8–98·2), positive likelihood ratio 6·6 (3·72–11·7), and negative likelihood ratio 0·336 (0·171–0·657).

Interpretation

In this patient population chloride and adenosine deaminase showed at best only modest performance as markers of tuberculous meningitis. However, very good negative predictive values could serve to identify patients highly unlikely to have the disease. [308 words]

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LIST OF ABBREVIATIONS

ADA	Adenosine deaminase
ADH	Antidiuretic hormone
AFB	Acid–fast bacilli
AIDS	Acquired Immune Deficiency Syndrome
ART	Anti–retroviral therapy
AUC	Area–under–the–curve
BCG	Bacillus Calmette–Guérin
CI	Confidence interval
CM	Cryptococcal meningitis
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DOR	Diagnostic odds ratio
ELISA	Enzyme–linked immunosorbent assay
ELISPOT	Enzyme–linked immunospot assay
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
INH	Isoniazid
ISO	International Standards Organization
IQR	Interquartile range
ISE	Ion–selective electrode
LIS	Laboratory information system
LJ	Löwenstein–Jensen
LP	Lumbar puncture

LPA	Line probe assay
LR–	Negative likelihood ratio
LR+	Positive likelihood ratio
MDR	Multi–drug resistant
MGIT	Mycobacterial growth indicator tube
MMed	Master of Medicine
MOTT	Mycobacteria other than TB
MTB	*Mycobacterium tuberculosis*
NAAT	Nucleic acid amplification test
NHLS	National Health Laboratory Service
NPV	Negative predictive value
PCR	Polymerase chain reaction
PPV	Positive predictive value
RCT	Randomised controlled trial
RIF	Rifampicin
ROC	Receiver–operator–characteristic
SCID	Severe Combined Immunodeficiency Disease
SIADH	Syndrome of Inappropriate Antidiuretic Hormone Secretion
STARD	Standards for the reporting of diagnostic accuracy studies
TB	Tuberculosis
TBM	Tuberculous meningitis
TTP	Time–to–positivity
UCT	University of Cape Town
UNAIDS	Joint United Nations Programme on HIV and AIDS
WHO	World Health Organization
ZN	Ziehl–Neelsen

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Goals of the literature review

The goals of this literature review were to obtain information on the following topics:

1 The epidemiology of tuberculosis, HIV infection, and tuberculous meningitis (TBM) in South Africa.
2 The history and available evidence on the measurement of chloride in the cerebrospinal fluid and its utility in the diagnosis of TBM.
3 The history and available evidence on the measurement of adenosine deaminase in the cerebrospinal fluid and its utility in the diagnosis of TBM.
4 The current clinical and laboratory approaches to TBM diagnosis.
5 The evolving role of commercial molecular platforms.

Search strategy

The PubMed digital archive was searched up to 17 December 2015 for papers on the above topics published in any language. For cerebrospinal fluid chloride (as one of the index tests in the research study) the search strategy used combinations of key terms as follows: "tuberculous meningitis" OR "TB meningitis" AND "cerebrospinal fluid chloride" OR "CSF chloride". The search identified 25 papers. For cerebrospinal fluid adenosine deaminase (as the other index test in the study) the search strategy used the following combinations of key terms: "tuberculous meningitis" OR "TB meningitis" AND "cerebrospinal fluid adenosine deaminase" OR "CSF adenosine deaminase" OR "cerebrospinal fluid ADA" OR "CSF ADA". This search identified 112 papers. For the topics on epidemiology, clinical and laboratory approaches, and molecular platforms, searches were performed using selected key terms. Further papers of interest on all topics were identified via manual searching of the reference lists of primary papers identified through the database search. Papers were retrieved via the Health Sciences Library of the University of Cape Town.

Quality criteria

The abstracts of all papers initially identified were reviewed to critically assess the subject matter for content, results presented, and applicability to the topics of this research study. Suitable papers were then retrieved and read in full by the author. Quality criteria applied during appraisal included consideration of the study methodology and whether it was adequate for the research question, statistical significance of results, number of papers published on the subject, number of study participants, choice and recruitment of participants, sample size, control of confounding variables, method of data analysis, and

ethics considerations. The main aim was to identify studies reporting on the utility of one or both of CSF chloride and CSF ADA measurement in the context of TBM diagnosis. Preference was given to work performed in Southern Africa. There were no time limits applied as to when the work was published. A similar procedure was followed in the appraisal of papers on the topics of epidemiology of TBM, clinical and laboratory approaches to diagnosis, and molecular platforms.

Summary of the literature

Epidemiology of tuberculosis, HIV, and tuberculous meningitis in South Africa

South Africa has one of the worst tuberculosis (TB) epidemics in the world.[1] Data published by the World Health Organization (WHO) in 2015[2] indicate that there were a total of 318193 cases of TB notified during 2014. This was inclusive of new cases and relapses. There were 272078 cases of pulmonary TB notified, both bacteriologically confirmed or clinically diagnosed; there were 34088 cases of extrapulmonary TB (including TBM) notified. The incidence rate of both TB and TB/HIV co–infection was 834 per 100000 of the population (which was estimated at 54 million in 2014). The TB/HIV co–infection incidence rate was 509 per 100000 of the population. There were 218231 cases tested for multi–drug resistant TB (MDR–TB), 18734 cases were laboratory–confirmed MDR–TB, and 11538 patients were started on MDR–TB treatment. During 2014 there were 295136 (93%) TB patients with known HIV status notified. There were 1148477 HIV–positive people screened for TB; 179756 HIV–positive patients were notified with TB. With respect to laboratory coverage as of 2014, Xpert MTB/RIF testing was offered at 207 sites and second–line drug susceptibility testing was available in the country. South Africa's national TB control programme budget stood at US$ 248 million in 2015, of which 84% was funded domestically, 8% was funded internationally, and 8% was unfunded.

South Africa also carries one of the heaviest burdens of HIV/AIDS globally.[1] Estimates published in 2013 by the Joint United Nations Programme on HIV and AIDS (UNAIDS)[3] showed that 6·3 million people were living with HIV, inclusive of 5·9 million adults aged 15 years and older, and 360000 children aged 0 to 14 years. There were 2·4 million orphans (aged 0 to 17 years) due to AIDS. The prevalence rate among adults aged 15 to 49 was 19·0%. Women aged 15 and older living with HIV numbered 3·5 million and there were 200000 deaths due to AIDS. Trend analyses from 1990 up to 2013 show that the HIV prevalence rate for ages 15 to 49 were unchanged since 2005, while the number of people living with HIV had increased slightly year–on–year since 2005 (prior years showed a steeper yearly increase). The HIV incidence rate (ages 15 to 49), the number of new

infections (all ages), and the annual number of AIDS deaths had all been decreasing steadily since the 2000–2005 period.

Tuberculous meningitis develops as a result of infection with *Mycobacterium tuberculosis*. It is one of the most severe extrapulmonary complications of TB and causes significant morbidity and mortality. It frequently presents in childhood, although people of all ages can develop the disease. A number of studies published in recent years have examined the epidemiology of TBM in the Western Cape province of South Africa, which is the setting for the proposed research study.[4–7]

Jarvis and colleagues[4] published findings on 4961 suspected cases of adult meningitis occurring in a setting of high TB and HIV prevalence. Patients undergoing diagnostic lumbar puncture at a public sector secondary–level referral hospital over a three–year period were studied. They recorded 5578 procedures performed on 4549 patients which represented 4961 clinical episodes. Of 820 microbiological diagnoses, cryptococcal meningitis (CM) accounted for 514 (63%) of cases, TBM for 227 (28%) of cases, and bacterial meningitis for 68 (8%) of cases. They highlighted the changing face of meningitis epidemiology, due to chronic meningitis caused by CM and TBM having assumed much greater prominence in recent years as a result of the HIV/AIDS pandemic.

Marais and co–workers[5] performed a retrospective review of CSF findings and clinical records over a period of six months in a similar setting in Cape Town, South Africa. They applied published case definitions of definite, probable, and possible TBM to their study population. TBM was diagnosed in 120 patients out of 211 enrolled (57%). In their paper, they identified TBM as the most common cause of meningitis when considering both microbiologically confirmed cases and cases diagnosed on clinical grounds. They found TBM accounted for 44% and CM accounted for 45% of all microbiologically confirmed cases of meningitis. They postulated that greater access to antiretroviral therapy (ART) would most likely account for the difference in their figures, compared to those of Jarvis and colleagues, resulting in smaller numbers of HIV–infected patients developing the severe levels of immunosuppression associated with CM.

Van Well and colleagues[6] reported on a retrospective cohort study performed in the Western Cape province of South Africa which examined twenty years of experience with paediatric TBM. It took place at a large university hospital and the study period ranged from January 1985 to April 2005. Their cohort consisted of 554 patients. They found that 82% of positive TBM diagnoses were in children younger than five years of age; mean age was 37 months,

other similar studies yielded mean ages ranging from 23 to 49 months. Presentation frequently was subacute, with non–specific early symptoms. Clinical clues identified to support early diagnosis included recent poor weight gain, vomiting, low–grade fever, and recent TB contact.

In summary, South Africa has a large number of people living with HIV and TB, including people co–infected. Cases of chronic meningitis due to TBM and CM have assumed greater prominence and these account for more presentations to health care facilities at present than do cases of acute bacterial meningitis in adults. In the paediatric population, disease often occurs in children younger than five. Presentation is frequently subacute, with variable and non–specific symptomatology. Morbidity and mortality remain unacceptably high.

Cerebrospinal fluid chloride

Chloride is the most abundant anion in plasma and interstitial fluid, including CSF. As such, it represents about 70% of the body's total anion content. A number of authors writing on the subject of chloride in CSF credit Mestrezat with first observing and describing a decrease in CSF chloride in meningitis,[8–10] and also noting that this decrease seemed to be most pronounced in cases of TBM. After a spate of these early reports, there followed a period in which very little was written on the subject. Present day texts still advocate for its measurement in suspected cases of TBM,[11] crediting a low CSF chloride with being virtually diagnostic of this disease.[12]

Linder and Carmichael[8] studied chloride and other ions in serum and CSF in four cases of meningitis, which comprised two cases of TBM, one case of meningococcal meningitis, and one case of streptococcal meningitis. They made two general observations. One was that a decrease in CSF chloride was dependent on a similar decrease in serum chloride, and that the relative concentrations between the two compartments remained the same. The other was that an associated increase in the bicarbonate concentrations of both fluid compartments took place. This was true for all four their cases. There was no attempt to differentiate between the different cases based on relative ion concentrations. Their study was limited by a very small number of participants. Fowweather[9] reported on a series of three cases of meningitis in children and concluded that a decrease in CSF chloride was a constant finding among cases. It was also stated that this reduction seemed to occur to a greater degree in cases of TBM than in other forms of meningitis. Study limitations included very few participants and no clear descriptions of reference standards. Gierson and Owens[10] published results on CSF findings of 1788 patients at the time of admission to hospital. Their observations were that chloride was normal in cases of poliomyelitis, viral meningitis, and encephalitis. Chloride

was moderately decreased in cases of pyogenic meningitis. In cases of tuberculous and fungal meningitis more pronounced decreases of chloride were found. A large number of cases were studied, but their conclusions were based only on simple comparison of chloride levels across different diagnostic groups, no diagnostic accuracy measures were calculated.

Ramkissoon and Coovadia[13] published a paper in 1988 describing a study on chloride and bromide levels in a series of 148 children with TBM, bacterial, or viral meningitis. They compared CSF chloride levels, blood chloride levels, and blood/CSF chloride ratios between the groups. The conclusion was that none of these measures were useful in differentiating between TBM, bacterial meningitis, and viral meningitis. This was based on comparison across groups, no measures of sensitivity, specificity, or predictive values were determined. In a letter to the editor in the Journal of the American Medical Association (JAMA) in 1984, Schoen[14] wrote that chloride measurement in CSF in cases of TBM was of little if any value. He quoted noted paediatric clinician Dr Edith Lincoln's work which showed that the decrease in CSF chloride reflected low total–body chloride secondary to protracted vomiting. This clinician's work was conducted in a first–world setting (the United States of America), thus limiting transferability of the results to settings in developing countries with higher disease prevalence.

Two other mechanisms may explain decreased CSF chloride in cases of chronic meningitis, such as TBM. One is that of dilution of whole body chloride mediated by the syndrome of inappropriate ADH secretion (SIADH).[15] In this syndrome there is a pathological increase in ADH secretion from the posterior pituitary gland which leads to increased renal reabsorption of water in the face of normal to increased total body water. The other is that of the electrolyte exclusion effect, also called the Donnan effect.[16] When protein molecules carrying a net charge is present on one side of a semi–permeable membrane, but not the other, and cannot diffuse across that membrane, the distribution of ions which can diffuse is altered. In TBM, large negatively charged protein molecules are present in the CSF. Small negatively charged chloride ions will preferentially diffuse across the blood–brain barrier until the amounts of negative charges on both sides of the barrier are equal. The extent to which each of these mechanisms contribute in any given case is not known though, and evidence on these phenomena is lacking.

To summarise, a small number of early papers reported on the observation that CSF chloride decreased in cases of meningitis, and that more pronounced decreases were seen in chronic meningitides such as TBM and fungal meningitis. Consensus among the more recent papers[13,14] seemed to be that there is little, if any, incremental diagnostic utility in measuring

CSF chloride in cases of meningitis. Measures of diagnostic accuracy were not reported on in any of the papers. No clear evidence supporting the current reference interval (120–130 mmol/L) could be found.

Cerebrospinal fluid adenosine deaminase

The enzyme adenosine deaminase (ADA) has a key role in purine metabolism, where it irreversibly hydrolyses adenosine or deoxyadenosine to inosine or deoxyinosine and ammonia.[17,18] Almost all mammalian cells contain the enzyme and it has a key function in maintenance of immune competence. Severe Combined Immunodeficiency Disease (SCID) is associated with a lack of ADA. AIDS, anaemia, lymphoma, and leukaemia are other conditions associated with altered levels of the enzyme. Developing T–cells, especially those associated with the stomach and the intestine, and also the fetal–maternal interface, have been shown to have high levels of ADA. Enzyme function is important in modulating the effects of adenosine in various systems. Adenosine has anticonvulsant and antihypoxic properties and it can modulate blood flow, platelet aggregation, lipolysis, glycogenolysis, and neurotransmission.[17]

Human ADA exists as one of three isoenzymes: ADA_1, ADA_{1+CP}, and ADA_2.[19] Ungerer and colleagues[19] studied the distribution of these after developing an electrophoretic technique to separate the three isoenzymes. Patterns were studied in cell and tissue homogenates and also in serum, both from healthy people and from patients with increased ADA levels due to hepatitis, infectious mononucleosis, tuberculosis, pneumonia, rheumatoid arthritis, or acute lymphoblastic leukaemia. Lymphocytes and monocytes were found to have the highest ADA activity overall. Lymphocytes had 84% ADA_1 activity and 16% ADA_{1+CP} activity, while monocytes had 82% ADA_1 activity and 18% ADA_2 activity. Differential distribution of these isoenzymes in CSF and neuronal tissue were not reported on.

There are many papers in the literature reporting on the measurement of ADA in CSF in the diagnosis of TBM. A PubMed search yielded more than 100 studies on the subject. Initial appraisal of these suggested the presence of a trend, in the sense that studies looking at smaller numbers of patients in settings of lower prevalence of TB reported ADA as having significant diagnostic utility;[20–22] while studies looking at larger numbers of patients in settings of high TB and TB/HIV prevalence reported ADA as having limited diagnostic utility and being unable to discriminate between patients with TBM and bacterial meningitis.[23,24]

Ribera and colleagues[20] studied ADA in 205 patients and 40 normal controls. Patients were grouped according to disease, which included TBM, viral meningitis, purulent meningitis, neoplasms, stroke, and other miscellaneous conditions. They found mean enzyme levels higher in patients with TBM, compared to the other groups. Sensitivity for diagnosis was 1 and specificity was 0·99. They concluded the test to be simple and reliable for early TBM diagnosis, as well as for follow–up of patients. Study limitations included a small number of patients, no clear description of the reference standard, and incomplete data analysis. In another paper, Agarwal and co–workers[21] reported on ADA measurement in 56 patients presenting to hospital with clinical features of meningitis. In their cohort, 32 patients were shown to have TBM and of these, 28 had CSF ADA at or above their cut–off of 10 U/L. They calculated sensitivity of 87·5%, specificity of 83·33%, positive predictive value of 87·5%, and negative predictive value of 83·33%. They found ADA to be simple, inexpensive, rapid, and specific for diagnosing TBM. Their study was limited by a small sample size and diagnoses of TBM made via clinical criteria only, also, no figures for prevalence or predictive values were given. Parra–Ruiz and colleagues[22] reviewed ADA utility in Spain, a region with low TB prevalence. They did a retrospective study of the accuracy of ADA in 190 patients, which were classified as certain TBM, probable TBM, or not TBM, based on clinical and laboratory criteria. Receiver–operating–characteristic (ROC) curve analysis yielded 11·5 IU/L as optimal cut–off in their setting. At this level, they calculated sensitivity of 91% and specificity of 77·7% for ADA accuracy in diagnosing TBM. They derived a predictive algorithm based on ADA and other CSF parameters and concluded that it offered improved utility in diagnosing TBM, compared to ADA alone, in their population. Diagnostic accuracy measures were incompletely calculated (no prevalence figures were given and little was said about predictive values) and TBM diagnosis was based on clinical criteria only.

Studies reporting on limited diagnostic accuracy are many. Two are highlighted here, one of which investigated TBM in HIV–positive patients,[23] and the second of which is a recent, large systematic review with meta–analysis on the subject.[24] Corral and co–workers[23] studied ADA levels in 417 patients with HIV infection who presented with neurological symptoms. They found no association between high ADA and HIV infection, HIV–associated neurological disorders, or progressive multifocal leuko–encephalopathy. ROC analysis among patients with meningitis in their cohort yielded an optimal ADA cut–off of 8·5 IU/L for TBM diagnosis, with a sensitivity of 57% and a specificity of 87%. False–positive results were due to neurological CMV disease and cryptococcal, lymphomatous and candida meningitis. Their conclusion was that ADA had limited utility for diagnosing TBM in HIV–positive patients. Tuon and colleagues[24] performed a systematic review with meta–analysis

on ADA in TBM. They included 13 studies in their final meta–analysis, out of 522 studies on the topic initially identified through their literature search. There were 380 patients with TBM among the 13 studies. They assessed ADA over a range of values. ADA of 1–4 U/L were helpful in excluding TBM (sensitivity > 93%, specificity < 80%). ADA from 4–8 U/L could neither confirm nor exclude TBM. ADA more than 8 U/L improved diagnosis of TBM (sensitivity < 59%, specificity > 96%). They found that none of these cut–off ranges could distinguish between TBM and bacterial meningitis, although they wrote that ranges of ADA values may be helpful in improving diagnosis once bacterial meningitis had been ruled out. Different assay methodologies and heterogeneous data reporting were highlighted as barriers to standardisation of the test as routine.

In summary, more papers have been published on CSF ADA than on CSF chloride in TBM. The selection of papers summarised above show that the settings in which ADA was studied vary quite significantly. Findings ranged along a spectrum, from some studies reporting very favourable findings, to other studies reporting limited to virtually no diagnostic utility for the marker. The meta–analysis by Tuon and colleagues[24] summarises much of the other work that had been done, and shows that ADA has limited accuracy, but that it may have value when applied in certain clinical situations.

Clinical and laboratory approaches to tuberculous meningitis diagnosis

Diagnosis of TBM remains challenging, even for experienced clinicians. Published guidelines[25] and consensus case definitions[26] aim to assist with the diagnostic process by recommending approaches and providing the rationale behind these approaches. Guidelines recommend starting with a thorough history and complete physical examination, and then progress to special investigations which would include various laboratory tests done on peripheral blood and CSF specimens, as well as imaging which would include visualisation of the CNS and chest, and other anatomical sites which are suspected to be involved.[25,26] Although TBM is generally accepted to present differently to the acute encephalo–meningitides, it is nevertheless prudent to take cognisance of guidelines for the acute CNS infections.[27] Recommendations by local experts developed for the local setting should also be taken into account.[28]

A number of recent papers highlight some important issues with respect to TBM diagnostics.[29–32] Thwaites and Hien[29] pointed out the non–specificity of clinical features, insensitivity of conventional bacteriology, and incompleteness of assessment of new diagnostic technology. Ho and colleagues[30] reviewed a number of novel diagnostic techniques such as interferon–gamma release assays and various biomarkers (MTB–specific

antigen and antibody, cytokines, gene expression profiles, and metabolomics), but emphasised that further evaluation would be required to establish their diagnostic utility. Brancusi and co–workers[31] also highlighted the absence of sensitive diagnostic tests for TBM. Additionally, they noted that new insights into the immunopathology of the disease, pathogen lineage (genotypes), and host genetics may influence susceptibility to TBM, and this may have implications for diagnosis. Garg[32] emphasised that bacteriological confirmation of disease is not always possible, and that serological tests do not have sufficient sensitivity and specificity.

Various groups have studied clinical and laboratory features in different patient populations and in a variety of settings with the aim of creating prediction rules and identifying features which would facilitate diagnosis.[33–40] One such study[33] identified five features as being predictive of TBM: age, length of history, white blood cell count, total CSF white cell count, and CSF neutrophil proportion. The same group of authors, in a different study,[34] found volume of CSF, duration of symptoms, and changes in CSF neutrophil count, lactate, and glucose all to be independently associated with confirmation of presence of TBM. Bhigjee and colleagues[35] studied diagnostic yield by polymerase chain reaction (PCR) in patients with suspected TBM in South Africa. They found that targeting the TB genome at multiple sites with conventional PCR did not improve diagnostic yield. They also found real–time PCR to be more sensitive. Their final conclusion was that none of the molecular techniques studied were sensitive enough to confidently exclude a diagnosis of TBM on laboratory grounds. In a study from Egypt, Youssef and co–workers[36] attempted to identify features which would enable early diagnosis. They found six parameters to be predictive in their model: duration of history > five days, headache, CSF white cell count of < 1000 cells/mm^3, clear CSF, lymphocyte proportion > 30%, and protein content of > 100 mg/dL (> 1 g/L).

Duration of symptoms ≥ five days, age over 30 years, CSF white cell count ≤ 1000 x 10^3 cells/mL (≤ 1000 cells/mm^3), and CSF lymphocytosis ≥ 70% were identified as predictive factors in a paper by Moghtaderi and colleagues.[37] Another group[38] found prodrome ≥ seven days, optic atrophy on fundal examination, focal neurological deficit, abnormal movements, and CSF neutrophil proportion < 50% to be useful predictors. A group from China[39] found independent factors for TBM diagnosis to be CSF protein > 1 g/L, CSF glucose < 2·2 mmol/L, CSF white cell count of 10–500 cells/μL (10–500 cells/mm^3), and neutrophil proportion > 50%. Pasco,[40] in a study done in the Philippines, investigated new–onset seizures, focal neurological deficit, pulmonary TB on chest X–ray, CSF pleocytosis with lymphocyte predominance, decreased CSF glucose, and increased CSF protein, as possibly being associated with TBM. At result analysis he found only the combination of CSF

pleocytosis with decreased CSF glucose and increased CSF protein as being associated with a diagnosis of TBM.

TBM has been studied also in patients with HIV infection.[41–44] One study,[41] which examined 96 HIV positive patients, found that neurological features of TBM are not changed by HIV infection but that survival rates are decreased. Another study[42] concluded that meningitis risk is increased in HIV–positive patients but that infection alters neither clinical manifestations nor outcome of TBM. This cohort was smaller; 35 patients with HIV infection were included, thus providing less robust evidence than the first study discussed. Yet another group[43] reported that the absence of CSF pleocytosis occurred more frequently in HIV–positive patients, that multidrug–resistant TB infection was more prevalent, and that mortality in hospital was higher in their HIV–positive cohort. A study from India[44] reported similar clinical features between HIV–positive and HIV–negative patients, but noted that cognitive dysfunction occurred more commonly among HIV–positive patients. They also examined radiological features and found meningeal enhancement to be minimal to absent, together with absence of communicating hydrocephalus on computed tomography (CT) scan in HIV–positive patients.

Adjunctive tests for TB diagnosis have been reviewed in the literature.[45,46] These tests include serology, Interferon–Gamma Release Assays (IGRAs; including ELISPOT), PCR, ELISA, lipoarabinomannan, and the string test. Some of these (ELISPOT, PCR, ELISA) have been evaluated in the setting of TBM. While all were shown to be useful to a certain extent, hard data on diagnostic accuracy measures are lacking.[45,46] Van Kampen and colleagues[47] did a study on automated liquid culture systems, utilising existing published data to assess system performance in both high and low prevalence settings. They found that TB cross–contamination rates increased exponentially as the prevalence rate of TB increased in the population of interest. They concluded that automated systems play a valuable role in TB diagnostics, but that awareness of increased risks of cross–contamination should be maintained in settings of high disease burden.

To conclude, published guidelines[25,28] and the uniform case definition criteria[26] were developed through consensus processes in order to empower clinicians to diagnose TBM. As can be gathered from the paragraphs above, numerous groups have done studies among different populations investigating all manner of variables (clinical, laboratory, and radiological) in order to try and improve diagnostic algorithms. It should be kept in mind that study findings cannot always be extrapolated to different populations and settings, and that

measures of diagnostic accuracy are sensitive to the context of the study, the prevalence of disease, and other factors.

Molecular diagnosis of tuberculous meningitis

Rapid progress in nucleic acid amplification test (NAAT) technology has shifted the paradigm in TB diagnostics. Initial evaluation and subsequent implementation of assays (first in research settings, followed by commercialisation) involved pulmonary specimens in the clinical setting of pulmonary TB. Following this, investigators started looking at applying the methods to test extra–pulmonary specimens, including CSF. A number of papers have investigated the accuracy of NAATs in diagnosis of TBM.[48–52]

Thwaites and colleagues[48] compared conventional bacteriology (Ziehl–Neelsen (ZN) stain and culture) with a NAAT (Gen–Probe amplified *Mycobacterium tuberculosis* direct test) in 341 CSF specimens. They reported a sensitivity of 64% for ZN staining, a sensitivity of 59% for the NAAT, and a sensitivity of 83% for the two tests combined, when done on repeated CSF specimens. They concluded that ZN staining was at least as good as the NAAT for rapid MTB diagnosis, much faster, and less expensive. Pai and co–workers[49] published a systematic review and meta–analysis on the diagnostic accuracy of NAATs in TBM. They found 14 papers which reported on commercial NAATs, and calculated summary estimates as follows: sensitivity 0·56 (95% confidence interval 0·46–0·66), specificity 0·98 (0·97–0·99), positive likelihood ratio 35·1 (19·0–64·6), negative likelihood ratio 0·44 (0·33–0·60), and diagnostic odds ratio 96·4 (42·8–217·3). Their conclusion stated that commercial NAATs would have a potential role in confirming TBM, but that they would not be useful as rule–out tests given their overall low sensitivity. In two other papers appraised,[50,51] the authors of one[50] found Xpert MTB/RIF to be a good rule–in test for TBM diagnosis. This applied to patients with HIV infection in a setting of high TB prevalence, and performing the assay on a centrifuged CSF pellet. In the other paper,[51] looking at NAATs (GenoType MTBDR*plus* and Xpert MTB/RIF) in childhood TBM, the authors found that commercial NAATs improved diagnostic accuracy incrementally, thus confirming disease rapidly microbiologically. However, they also found that these tests cannot serve to rule out TBM with any certainty.

A recent WHO publication examined the performance of Xpert MTB/RIF in the detection of MTB in CSF.[52] Combined sensitivity across 16 studies was 79·5% (62·0–90·2) while combined specificity was 98·6% (95·8–99·6). Although WHO in this document strongly recommends Xpert MTB/RIF in preference to conventional microscopy and culture as the

initial diagnostic test in patients with presumed TBM, given the urgency of the diagnosis, it does admit that current evidence for this recommendation is of poor quality.

To summarise, NAATs hold great promise for the future of TBM diagnostics, especially as the technology keeps on evolving. These tests have been shown to have incremental utility in the process of investigating for TBM. However, as authors have pointed out, due to the limitations of the assay, it is impossible to reliably rule out the presence of TBM in a patient, given a negative NAAT result, even if done on repeated specimens. The WHO document[52] shows that the number of patients assessed in studies of diagnostic accuracy of Xpert MTB/RIF on CSF specimens is still small; there is a need for more work in this area.

Identification of needs for further research

As a general note, studies of diagnostic accuracy has been the object of interest for some time, as authors have noted that these need to be better planned and conducted,[53] and that there should be adherence to certain principles.[54] The STARD initiative is a consensus effort to provide clear guidance to study authors in accomplishing these objectives.[55–57]

Further research needs, across the domains described in this literature review, are as follows:

1 Epidemiology: More research is needed in South Africa to accurately describe the epidemiology of TBM in our patient population.
2 CSF chloride and ADA: There is a need for diagnostic accuracy studies done locally to assess test accuracy against a reference standard in order to inform future decisions to continue offering the tests to clinicians.
3 Clinical and laboratory approaches to diagnosis: More work is needed in our setting to establish whether current guidelines are being followed and whether this has resulted in improved patient care and outcomes.
4 Commercial molecular platforms: Studies are needed to assess whether deployment of instruments has proceeded as planned and whether the instruments are being used optimally (especially looking at testing of extra–pulmonary specimens). There is also a need for more studies to examine the diagnostic utility of NAATs in our setting.

Aim and objectives of this research study

Aim

To retrospectively determine the diagnostic accuracy of CSF chloride and CSF ADA measurement compared to automated liquid TB culture as the reference standard, in the

assessment of patients with suspected TBM, in order to inform a decision of whether to continue offering these tests to clinicians.

Objectives

1 To determine the total number of specimens processed for TB culture in the laboratory and to identify among these all CSF specimens submitted for TB culture.

2 To determine the number of CSF specimens which had either chloride, or ADA, or both measured, and to correlate these results with those of TB culture.

3 To outline the distribution of HIV positivity, $CD4^+$ T–cell counts, and viral loads in the patient population studied.

4 To calculate sensitivities, specificities, predictive values, likelihood ratios, diagnostic odds ratios, and diagnostic efficiencies of the index tests.

5 To evaluate the index tests against the reference standard to determine whether they offer any diagnostic utility, in order to decide whether the results support continued offering of the tests to clinicians. [5272 words]

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CHAPTER 2: PUBLICATION–READY MANUSCRIPT

This manuscript was prepared according to the author guidelines as set out in the Information for Authors supplement of The Lancet Infectious Diseases (Appendix D).

Title

Utility of chloride and adenosine deaminase measurement in cerebrospinal fluid for the early presumptive diagnosis of tuberculous meningitis

Author names and preferred degrees

Hendré Swanepoel MBChB,[1,2,3] Justyna Wojno FC Path SA,[1,3] Fierdoz Omar FC Path SA[2,3]

Author affiliations

1 Division of Medical Microbiology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.

2 Division of Chemical Pathology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.

3 NHLS Clinical Pathology Laboratory, C17 & C18, Groote Schuur Hospital, Anzio Road, Observatory, Cape Town, South Africa.

Corresponding author

Hendré Swanepoel MBChB
NHLS Clinical Pathology Laboratory
C–floor, C17 & C18, Groote Schuur Hospital
Anzio Road, Observatory, 7925
Cape Town, South Africa
Office phone: +27 (21) 404 5303
Facsimile: +27 (21) 404 4105
Mobile: +27 (72) 319 1014
Email: hendreswanepoel@yahoo.com

Keywords

Sensitivity, specificity, predictive values, TB meningitis, chloride, adenosine deaminase

Abstract

Background

Chloride and adenosine deaminase measurements in cerebrospinal fluid are still sporadically requested as part of tuberculous meningitis work–up. In the literature, evidence is contradictory and opinion is divided on their utility in clinical practice. The accuracy of both for the early presumptive diagnosis of tuberculous meningitis was investigated in patients in a region with high prevalence of tuberculosis and HIV infection in order to inform a decision on whether to continue offering these tests to clinicians.

Methods

A retrospective descriptive study of diagnostic accuracy was conducted at the National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa. Data were collected on all cerebrospinal fluid specimens submitted for tuberculosis culture between 1 January 2012 and 31 December 2014. Chloride and adenosine deaminase concentrations were compared with automated liquid culture for *Mycobacterium tuberculosis* as the reference standard.

Findings

There were 2531 cerebrospinal fluid specimens submitted for tuberculosis culture during the study period; exclusion of duplicates yielded 2081 specimens. Chloride was requested on 711 (34·2%) specimens; 44 (6·2%) were tuberculosis culture–positive. Adenosine deaminase was requested on 152 (7·3%) specimens; 20 (13·2%) were culture–positive. Chloride sensitivity (<120 mmol/L) for the detection of tuberculous meningitis was 93·2% (95% confidence interval 81·3–98·6), with specificity 62·4% (58·6–66·1), positive predictive value 14% (10·3–18·6), negative predictive value 99·3% (97·9–99·9), positive likelihood ratio 2·48 (2·18–2·81), and negative likelihood ratio 0·109 (0·037–0·326). Adenosine deaminase sensitivity (>6 U/L) was 70% (45·7–88·1), specificity 89·4% (82·8–94·1), positive predictive value 50% (30·6–69·4), negative predictive value 95·2% (89·8–98·2), positive likelihood ratio 6·6 (3·72–11·7), and negative likelihood ratio 0·336 (0·171–0·657).

Interpretation

In this patient population chloride and adenosine deaminase showed at best only modest performance as markers of tuberculous meningitis. However, very good negative predictive values could serve to identify patients highly unlikely to have the disease.

Funding

None. [310 words]

Introduction

The diagnosis of tuberculous meningitis (TBM) requires the consideration of risk factors, clinical assessment, laboratory tests, and imaging modalities.[1,2] Rapid decision–making is important as this is a serious infection with severe long–term sequelae, requiring prompt initiation of appropriate therapy and referral for definitive management. At present, the interaction of various risk factors (such as tuberculosis contact, HIV, alcoholism, diabetes mellitus, malignancy, recent steroid use, socio–economic status, infant/young child, and parental education level) in the pathogenesis of this disease is incompletely understood. Furthermore, clinical assessment is frequently unhelpful, laboratory tests yield variable results, and imaging is subject to inter–operator variability. All of these hamper the diagnostic process. Despite this, confirming the presence of disease and delineating drug susceptibility patterns have become more important in the current setting of high tuberculosis (TB) prevalence and HIV/TB co–infection, emerging multi–drug resistant pathogens, and treatment access and adherence issues.[3,4]

Current internationally–accepted practice[1] with respect to laboratory testing for TBM includes obtaining a cerebrospinal fluid (CSF) specimen after due consideration for the presence of raised intracranial pressure. The usual analysis includes measurement of protein and glucose concentrations. Cell counts are performed either using manual cell counting chambers or automated technology. Improvements in automated haematology instrumentation and flow cytometry technology have led to a preference for these new platforms. Specific microbiological techniques for detection of *Mycobacterium tuberculosis* (MTB) in CSF include direct light microscopy of clinical specimens with Ziehl–Neelsen (ZN) staining, culture methods including automated liquid culture (mycobacterial growth indicator tube – MGIT) and manual culture on Löwenstein–Jensen (LJ) medium, and molecular platforms such as the Xpert MTB/RIF system (Cepheid, Sunnyvale, CA, USA).

Advances in molecular diagnostic techniques have spearheaded renewed efforts to address the threats posed by TB and HIV. Xpert MTB/RIF, the most rapid detection system, was found to have a pooled sensitivity of 79·5% (95% confidence interval (CI) 62·0–90·2) across 16 studies which examined 709 CSF specimens,[5] and thus cannot be utilised to reliably rule out the presence of TBM with a negative result.[1] New integrated approaches aimed at improving the diagnosis of TB meningitis have appeared in the literature.[6] These emphasise rational application of diagnostic criteria and advise consensus case definitions to be used to aid in comparison of studies, and to improve scientific communication and patient care.[6] The introduction of Xpert MTB/RIF, the evolution of our understanding of TBM, and changes in clinical and laboratory practice have brought into question the validity of continued

measurement of chloride and ADA in CSF, which were initially used as proxies for the presence of TBM. Clinicians still sporadically request these two tests and while they have been measured in many centres historically, there is contradictory evidence in the literature regarding their diagnostic utility.

Mestrezat was the first author to proclaim that very low CSF chloride levels were pathognomonic of TBM.[7] Subsequent papers on the topic are divided.[7–10] One series of 1788 cases of suspected meningitis found that chloride was markedly decreased in TBM, based on comparison of CSF levels between patients.[7] Another series of three cases made a similar observation; that chloride was decreased to a greater degree in TBM than in other forms of meningitis.[8] A study performed in South Africa among 148 children with meningitis concluded that TBM could not be distinguished from bacterial or viral meningitis, based on comparison of CSF chloride level, blood chloride level, and blood/CSF chloride ratio.[9] Similarly, another author pointed out that CSF glucose and protein were helpful in assessing the course of TBM, but that CSF chloride did not provide incremental utility.[10] It emerges that very few papers have been published on this topic, the studies investigated small numbers of patients, no measures of diagnostic accuracy were calculated, descriptions of methodology were inadequate or absent, study populations were inaccurately described, and incorrect statistical methods were applied to data sets.[7–10]

ADA gained prominence as a marker of TBM quite long after chloride. This enzyme catalyses the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine.[11,12] Enzyme activity is increased in activated T–lymphocytes, as in the case of TB.[11,12] While the exact mechanism accounting for increased activity in body fluids (including CSF) remains to be elucidated,[11] it has nevertheless been the subject of many studies of diagnostic utility in TBM. Papers on the topic examined variable numbers of patients in various different settings.[13–15] Not all populations were equally well described, and neither were all study procedures and ADA assay methods equally well outlined. For example, two small series found ADA to be a simple, fast, and accurate method for diagnosing TBM.[13,14] One paper based this on a sensitivity of 87·5% and a specificity of 83·3% in a cohort of 32 patients (classified as TBM based on limited clinical criteria, imaging, and CSF cell count and chemistry – neither CSF culture nor PCR were done).[13] The other paper defined a reference standard, but then applied this inconsistently (TB culture – but performed on fluids other than CSF in some cases, with cases then classified as TBM based on this and compatible symptoms).[14] A large systematic review and meta–analysis published in 2010 gave a balanced overview of the results of 13 different papers on ADA in TBM, concluding that ADA cannot distinguish between TBM and bacterial meningitis.[15] However, the authors also

stated that ranges of ADA levels might be useful in improving diagnosis of TBM after bacterial meningitis had been ruled out.[15]

This research study sought to address the question of whether to continue offering CSF chloride and ADA measurement in clinical laboratory practice by way of retrospective determination of diagnostic accuracy using TB culture as the reference standard.

Methods

Patients

A retrospective data analysis of laboratory tests on CSF specimens obtained from patients seen at 22 different health care facilities in the greater Cape Town Metro region and the Western Cape Province of South Africa was performed (Figure 1). All specimens taken and submitted to the laboratory between 1 January 2012 and 31 December 2014 were identified and screened for inclusion. To qualify for inclusion, each specimen had to have TB culture results as well as chloride and/or ADA results. Consecutive specimens meeting these criteria were identified and the corresponding patient data were captured into data collection spreadsheets using Microsoft Office Excel. Male and female patients of all ages (including children) and from all ethnic groups were included. Clinical data available were limited to information written on the laboratory requisition forms and thus at the discretion of requesting clinicians.

The Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town formally approved the study protocol (HREC REF: 497/2015). A waiver of informed consent was granted due to the retrospective nature of the study, utilisation of existing, de–identified laboratory data, no immediate risk of harm, and foreseeable difficulty in locating such a large number of patients.

Procedures

Laboratory data were extracted from the laboratory information system (LIS) via an algorithm which searched the laboratory database for the term “TB culture”. Management of data included tabulation by year (2012/2013/2014), identification of specimens which had ADA or chloride or both measured, and separation of results by age for the group which had chloride measured. The number of ADA results was insufficient to analyse by age for this group. ZN stain results were recorded. Results of CSF Xpert MTB/RIF tests, HIV tests, $CD4^+$ T–cell counts, and viral loads, when these were performed and available on the LIS, were documented. Patients who had multiple CSF specimens submitted for culture were identified and duplicates were excluded from analysis. In these instances the first specimen

received for the relevant patient was included for analysis. Specimens were processed at the clinical pathology laboratory located at Groote Schuur Hospital in Cape Town, South Africa. This is a routine diagnostic laboratory accredited to ISO 15189:2007 standards. Trained medical technologists perform all tests according to standard operating procedures. Chemistry tests were usually completed on the same day whereas TB culture results were dependent on time–to–positivity (TTP) which spanned days to weeks.

CSF chloride was measured on the Roche Modular (P–module) automated chemistry analyser (Roche, Basel, Switzerland) by indirect ion–specific electrode (ISE). The coefficient of variation (CV) for this method in this laboratory is 1·8% at a chloride level of 72·8 mmol/L and 1·6% at a chloride level of 107·3 mmol/L. CSF ADA was measured on the Cobas Integra 400 Plus instrument (Roche, Basel, Switzerland) using the Diazyme ADA Assay (Diazyme Laboratories, Poway, CA, USA). This is a spectrophotometric method based on the enzymatic deamination of adenosine to inosine. The CV for this assay is 2% at an ADA level of 10 U/L and 5% at an ADA level of 30 U/L.

Direct ZN stains were made from centrifuged CSF specimens and examined via light microscopy by experienced technologists. Standard BD BACTEC MGIT 960 culture bottles were inoculated and incubated as per manufacturer's instructions (Beckton Dickinson Microbiology Systems, Cockeysville, MD, USA). All cultures were incubated for a maximum of 42 days (six weeks) unless these flagged positive within this time. Positive cultures were removed from the instrument, aliquots of culture fluid were aspirated from bottles, slides of ZN stains were made of these, and the slides were examined by light microscopy under oil immersion for the presence of acid–fast bacilli (AFB). GenoType MTBDR*plus*96 line probe assays (Hain LifeSciences, Hehren, Germany) were used for organism identification and detection of the presence of common mutations conferring resistance to rifampicin (RIF) and isoniazid (INH).

Xpert MTB/RIF assays were performed according to the manufacturer's instructions. $CD4^+$ T–cell counts were performed using the CD45–assisted pan–leucogating method as described by Glencross and colleagues,[16] on Beckman Coulter Cytomics FC 500 MPL flow cytometers (Beckman Coulter, Brea, CA, USA). HIV viral load quantifications were performed with the Abbott RealTime HIV–1 assay (Abbott, Des Plaines, IL, USA).

Data analysis

Positive culture for MTB from CSF served as the reference standard. For analysis of chloride and ADA as index tests, specimens were separated into two groups based on which of the

two analytes were measured. Analysis of combined diagnostic accuracy was not possible, as only a very small number of specimens had both chloride and ADA measured. The chloride group was further stratified into two smaller groups based on age. Data analysis was performed with Stata Version 12 (StataCorp, College Station, TX, USA). Sensitivity, specificity, predictive values, likelihood ratios, diagnostic odds ratios, and diagnostic efficiency were calculated for chloride and ADA; 95% confidence intervals (CIs) were determined for all parameters. These measures were calculated for the chloride and ADA groups inclusive of all patients identified according to the inclusion criteria, and were also separately calculated for HIV–positive and HIV–negative subsets of patients. Receiver–operator–characteristic (ROC) curve analysis was performed for each test to delineate the sensitivities and specificities at various cut–off levels. Area–under–the–curve (AUC) values were determined, together with their respective 95% CIs. Simple descriptive statistics were used to describe the study population. Non–normally distributed data were summarised by median and interquartile ranges (IQR). In Stata "roctab" was used for ROC curve analysis, "diagti" was used to calculate measures of diagnostic accuracy with CIs, and "univar" was used to determine medians and IQRs.

Results

The laboratory processed 60758 specimens for TB culture during the three–year study period. Among these there were 2531 (4·2%) CSF specimens; exclusion of duplicates (two or more specimens submitted for the same patient within a couple of days) yielded 2081 (3·4%) CSF specimens. Of these, 182 (8·7%) cultured TB. Exclusion of duplicate cultures (more than one culture on the same patient specimen) yielded 140 (6·7%) TB culture–positive CSF specimens. In 2012, 693 specimens were processed with 51 (7·4%) culture–positive results, while 716 specimens were processed in 2013 with 54 (7·5%) culture–positive, and 672 specimens were processed in 2014 with 35 (5·2%) culture–positive.

Chloride was requested on 711 (34·2%) of the 2081 specimens. Of these, 44 (6%) were TB culture–positive. ADA was requested on 152 (7·3%) specimens, of which 20 (13%) were TB culture–positive. Forty-seven (2·3%) specimens had both chloride and ADA measured; only two (4·3%) of these were TB culture–positive. HIV results were available for two thirds of patients. In the CSF chloride group 215 (30%) patients were HIV positive, as were 70 (46%) in the CSF ADA group. Among HIV positive patients in the chloride group, median $CD4^+$ T–cell count was 194 x 10^6/L and median viral load was 10508 RNA copies/mL; in the ADA group these were 174 x 10^6/L and 189 RNA copies/mL (Table 1).

Among patients 0–12 years of age, chloride was measured on 276 (38·7%) specimens; 20 (7%) of these were TB culture–positive. Among patients 13 years and older 435 (61·2%) specimens had chloride measurements. There were 24 (6%) TB culture–positive results in this group. Only two patients (1·3%) younger than 13 had ADA measured on their CSF. Neither of these were TB culture–positive. A summary of true and false positive and negative results, as were used to calculate diagnostic accuracy measures, is presented in Table 2.

Normal CSF chloride demonstrated very good negative predictive value across all age groups (Table 3), with poor positive predictive value noted. The same is true for CSF ADA. The prevalence of CSF culture–positive TBM was low across all the groups studied. Analyses of diagnostic accuracy in HIV–positive and HIV–negative subsets (Table 4 and Table 5) similarly show high negative predictive values and low positive predictive values for both tests, across all the groups. Other parameters are also similar between both subsets and the parent groups.

ROC curve analysis for CSF chloride (Figure 2) yielded an AUC value of 0·86 (95% CI 0·83–0·89). At the lower limit of the reference interval (120 mmol/L) the test had a good sensitivity of 93% but only a modest specificity of 55%, compared to TB culture. ROC analysis for CSF ADA (Figure 2) showed an AUC value of 0·91 (0·85–0·95). At an ADA level of 6 U/L (the upper limit of the reference interval) test sensitivity at 75% was less than that of chloride, but specificity at 89% was better. As can be seen on the ADA ROC curve, decreasing the upper limit of the reference interval from 6 to 3 U/L would improve test sensitivity from 75% to ~100%; this would also improve the test's negative predictive value.

Xpert MTB/RIF testing of CSF specimens commenced in the laboratory in May 2014. By the end of 2014, 115 CSF specimens had both TB culture and Xpert MTB/RIF results. Eleven (9·6%) of these were TB culture–positive, of which only five (45·5%) were Xpert MTB/RIF positive. Of 104 (90·4%) TB culture–negative specimens, two (1·9%) were Xpert MTB/RIF positive.

Positive yield on ZN stain microscopy was low. In the CSF chloride TB culture–positive group (n=44) only two stains (4·5%) showed AFB; in the ADA TB culture–positive group (n=20) there were also two positive ZN stains (10%). Median time–to–positivity (TTP) for TB culture–positive specimens in the MGIT automated liquid culture system was 19 days (IQR 15–25 days). The shortest specimen TTP was seven days; the longest was 45 days.

Discussion

In this study setting, a patient with a normal CSF chloride level was more than 99% likely not to have TBM; while a normal CSF ADA level conferred a probability of more than 95% of the absence of TBM. Stated differently, less than one case of TBM would be missed in every 100 patients who had normal CSF chloride levels; similarly, measuring normal CSF ADA (≤6 U/L) in 100 patients would have missed less than five cases of TBM (decreasing this cut-off to 3 U/L would improve sensitivity and negative predictive value, and increase ADA utility even more). Additionally, all diagnostic accuracy measures appeared very similar across HIV–positive and HIV–negative subsets of patients in all the groups which were studied. Previous studies looking at the utility of these analytes in the context of TBM diagnosis assessed the ability of both to prove the presence of TBM, in other words, their value as rule–in tests. Findings vary along a spectrum, from some authors reporting the tests as being very useful,[7,8,12–14] to others reporting them as having no value.[9,10,15,17] The data presented here shows poor positive predictive values for both tests in this patient population. This means that it cannot be assumed that a low CSF chloride or a high CSF ADA is diagnostic of TBM. These findings are significant because they do not support the continued use of CSF chloride and CSF ADA as markers for the presence of TBM. However, the high negative predictive values found for both tests indicate that they could be used, as part of the initial patient assessment, to identify patients unlikely to have TBM. Guidelines for the management of acute meningitis[18] advise against the measurement of chloride and ADA as these would add little value to patient management. This may remain true in the acute setting, with a clear history and clinical impression, where TBM would be a less likely cause. While the data presented here support not using CSF chloride and ADA as proxies of TBM, it is proposed that they be measured in all patients where TBM is suspected, as they can be used to identify the absence of disease with a high degree of certainty.

Mycobacterium tuberculosis causes most tuberculous CNS infections. Haematogenous spread from foci elsewhere in the body leads to entry of the organism into the CNS. Small tuberculous lesions called Rich's foci[19] develop which may be located on the meninges, the subpial, or subependymal surfaces of the brain or spinal cord. Subsequent growth or rupture of these cause different types of CNS tuberculosis. Apart from TBM, other CNS manifestations include encephalopathy, vasculopathy, miliary disease, space–occupying lesions, and various forms of spinal pathology. TBM is a very serious complication with significant morbidity and mortality in adults and children. It remains a challenge to diagnose due to the existence of varying case definitions, variable clinician experience, and few diagnostic laboratory tests with results not always immediately available to clinicians. In South Africa many patients initially present to primary– or secondary–level health care

facilities where they are usually seen by interns or community–service medical officers who lack experience in diagnosing TBM. This may lead to misdiagnosis or missed diagnoses. Data presented here show that almost 20% of all laboratory requisition forms contain no clinical information on cases (Table 1). Of the forms that do contain information, in 60–80% of cases there was no evidence that any form of TB (even non–CNS TB) was being considered clinically. Diagnostic uncertainty may be an important cause of this. Use of CSF chloride and ADA as first–line tests here may have prevented many of these cases progressing to the point of requests for expensive, time–consuming TB culture; or, as molecular assays are assuming more prominence, requests for expensive molecular tests. It may also have allowed more rational use of empiric therapy, and more appropriate referral of patients requiring specialist management.

Rapid progress in nucleic acid amplification test (NAAT) technology has shifted the paradigm in TB diagnostics. The Xpert MTB/RIF is a desktop instrument which is based on hemi–nested real–time polymerase chain reaction (PCR) technology which detects the presence of MTB in clinical specimens. The assay also determines susceptibility to rifampicin (RIF) based on the presence of common resistance mutations. The WHO recently assessed the performance of Xpert MTB/RIF in the detection of MTB in CSF.[5] Combined sensitivity across 16 studies was 79·5% (95% CI 62·0–90·2) while combined specificity was 98·6% (95·8–99·6). Although WHO strongly recommends Xpert MTB/RIF in preference to conventional microscopy and culture as the initial diagnostic test in patients with presumed TBM, given the urgency of the diagnosis, it does admit that current evidence for this recommendation is of poor quality.[5,20,21] A major limitation of Xpert MTB/RIF is that a negative result does not rule out TBM, due to relatively poor sensitivity. In fact, there are no rule–out tests in use in TBM diagnosis at present. The authors therefore recommend that CSF chloride and CSF ADA be measured at first presentation in all cases of suspected TBM in order to identify patients who are highly unlikely to have the disease (additionally, decreasing the ADA cut–off from 6 to 3 U/L would improve the sensitivity and negative predictive value of this test). This should prompt consideration and appropriate investigation for other diagnoses in the differential, unless a compelling reason or high clinical index of suspicion exist to suggest otherwise. An algorithm for the diagnosis of suspected TBM in adults and children incorporating CSF chloride and ADA measurement is presented in Figure 3.

The authors of this study have proposed several limitations. It was performed in a retrospective format and yielded a small number of positive CSF TB culture results for analysis. Predictive values as calculated apply to the local setting and patient population, so

caution is advised in extrapolating results to other settings. Also, as sensitivity and specificity are influenced by the spectrum of disease (typically higher in patient populations with advanced disease, and lower in children) results in studies elsewhere may differ from these study results. The same applies to likelihood ratios as these are derived from sensitivity and specificity. Performance of the index tests were assessed exclusively against the current gold standard for TBM diagnosis – CSF culture for MTB. A composite reference standard was not used. Investigation of test utility[22–24] in the setting of presumed TBM based on application of the uniform case definition criteria[6] was not done. Positive predictive value for both chloride and ADA may in fact improve if these criteria are also applied. CSF cell count and CSF protein concentration were not examined in this study. These could possibly become topics in future projects investigating laboratory parameters in TBM diagnosis. Deployment of these tests at all secondary level health care facilities would be required in order to decrease result turn–around times and thus provide timely decision support to clinicians working at the coalface.

In conclusion, these findings support a decision to continue offering CSF chloride and CSF ADA to clinicians as part of the diagnostic work–up of a patient with presumed TBM. However, it calls for a fundamental change in the reason why clinicians would request these tests in the first instance – not to prove the presence of TBM, but to provide a highly likely probability for its absence. [3778 words]

Contributors

HS, JW and FO designed the study. HS performed the literature review. JW extracted the data. HS cleaned, analysed, and interpreted the data, and prepared the initial draft of the manuscript. HS, JW and FO reviewed the draft, suggested amendments, and approved the final version.

Conflicts of interest

All authors declare no conflicts of interest.

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PANEL: RESEARCH IN CONTEXT

Evidence before this study

The PubMed digital archive was searched up to 17 December 2015 for papers published in any language. For cerebrospinal fluid chloride the search strategy used combinations of key terms as follows: “tuberculous meningitis” OR “TB meningitis” AND “cerebrospinal fluid chloride” OR “CSF chloride”. The search identified 25 papers. For cerebrospinal fluid adenosine deaminase the search strategy used the following combinations of key terms: “tuberculous meningitis” OR “TB meningitis” AND “cerebrospinal fluid adenosine deaminase” OR “CSF adenosine deaminase” OR “cerebrospinal fluid ADA” OR “CSF ADA”. This search identified 112 papers. Further papers of interest were identified via manual searching of the reference lists of primary papers identified through the database search. Papers were retrieved via the Health Sciences Library of the University of Cape Town. The abstracts of all papers were reviewed to critically assess the subject matter for content, results presented, and applicability to the topic of this research study. Suitable papers were then retrieved and read in full. Quality criteria applied during appraisal included consideration of the study methodology and whether it was adequate for the research question, statistical significance of results, number of papers published on the subject, number of study participants, choice and recruitment of participants, sample size, control of confounding variables, method of data analysis, and ethics considerations. The main aim was to identify studies reporting on the utility of one or both of CSF chloride and CSF ADA measurement in the context of TBM diagnosis. Preference was given to work performed in Southern Africa. There were no time limits applied as to when papers were published.

Added value of this study

This study describes in detail the diagnostic accuracy of CSF chloride and CSF ADA measurement in the presumptive diagnosis of TBM in a patient population in a developing country with a high burden of HIV, TB, and HIV/TB co–infection. Additionally, it confirms the value of the current reference interval for CSF chloride, and it suggests that changing the current cut–off used for CSF ADA may in fact improve the clinical utility of this test.

Implications of all the available evidence

There remains clinical utility in measuring chloride and ADA in CSF in patients with presumed TBM. However, the value of these two tests really lies in identifying patients who are unlikely to have the disease. Continued use of both as markers for the presence of TBM is not supported by the current available evidence.

Table 1: Patient characteristics and baseline data

Test	CSF chloride	CSF chloride	CSF chloride	CSF ADA
Age group	0–12 years	≥13 years	All ages	All ages
Median age[a]	276, 2 (1–5)	435, 37 (29–46)	711, 26 (3–39)	152, 37 (30–45)
Sex[b]				
Male	174 (63%)	204 (47%)	378 (53%)	76 (50%)
Female	102 (37%)	231 (53%)	333 (47%)	76 (50%)
Clinical data[b,c]				
TB mentioned	75 (27%)	32 (7%)	107 (15%)	15 (10%)
TB not mentioned	166 (60%)	334 (77%)	500 (70%)	109 (72%)
No data provided	35 (13%)	69 (16%)	104 (15%)	28 (18%)
CSF chloride[b,d]				
Low (<120 mmol/L)	135 (49%)	157 (36%)	292 (41%)	–
Normal	141 (51%)	278 (64%)	419 (59%)	–
CSF ADA[b,e]				
High (>6 U/L)	–	–	–	28 (18%)
Normal	–	–	–	124 (82%)
ZN stain for AFB[b]				
Positive	1 (0·4%)	1 (0·2%)	2 (0·3%)	2 (1·3%)
Negative	250 (90·6%)	381 (87·6%)	631 (88·7%)	130 (85·5%)
No result found	25 (9·0%)	53 (12·2%)	78 (11·0%)	20 (13·2%)
TB culture[b]				
Positive	20 (7%)	24 (6%)	44 (6%)	20 (13%)
Negative	256 (93%)	411 (94%)	667 (94%)	132 (87%)
CSF Xpert MTB/RIF[b]	n=11	n=17	n=28	n=11
Positive	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Negative	11 (100%)	17 (100%)	28 (100%)	11 (100%)
HIV status[b]				
Positive	39 (14%)	176 (40%)	215 (30%)	70 (46%)
Negative	133 (48%)	125 (29%)	258 (36%)	33 (22%)
No result found	104 (38%)	134 (31%)	238 (34%)	49 (32%)
$CD4^+$ T–cell count[a,f]	31, 463 (184–1007)	177, 171 (59–333)	208, 194 (78–380)	51, 174 (60–352)
Viral load[a,g]	30, 91308 (1793–670000)	46, 1160 (40–180000)	76, 10508 (69–220000)	23, 189 (40–110000)

[a]Data shown are: number of patients, median (interquartile range).

[b]Data shown are: number of patients (%).

[c]Any consideration of TB written on the laboratory requisition form was recorded as such.

[d]Laboratory reference interval for CSF chloride: 120–130 mmol/L (all ages).

[e]Laboratory reference interval for CSF ADA: ≤ 6 U/L (all ages).

[f]Laboratory reference interval for $CD4^+$ T–cell count: 500–2010 x 10^6/L (all ages).

[g]Units for viral load: RNA copies/mL.

Table 2: Summary of results used to calculate diagnostic accuracy measures

Test	Group	Subset	Number of patients	True positive	True negative	False positive	False negative
CSF chloride	0–12 years	All patients	n = 276	20	141	115	0
		HIV positive patients	n = 39	1	22	16	0
		HIV negative patients	n = 133	6	73	54	0
CSF chloride	≥13 years	All patients	n = 435	21	275	136	3
		HIV positive patients	n = 176	10	105	58	3
		HIV negative patients	n = 125	5	81	39	0
CSF chloride	All ages	All patients	n = 711	41	416	251	3
		HIV positive patients	n = 215	11	127	74	3
		HIV negative patients	n = 258	11	154	93	0
CSF ADA	All ages	All patients	n = 152	14	118	14	6
		HIV positive patients	n = 70	7	55	5	3
		HIV negative patients	n = 33	3	25	5	0

Table 3: Measures of diagnostic accuracy for CSF chloride and CSF ADA

Test	CSF chloride	CSF chloride	CSF chloride	CSF ADA
Age group	0–12 years (n=276)	≥13 years (n=435)	All ages (n=711)	All ages (n=152)
Prevalence[a,b]	7·2% (4·5–11)	5·5% (3·6–8·1)	6·2% (4·5–8·2)	13% (8·2–19·6)
Sensitivity[b]	100% (83·2–100)	87·5% (67·6–97·3)	93·2% (81·3–98·6)	70% (45·7–88·1)
Specificity[b]	55·1% (48·8–61·3)	66·9% (62·1–71·4)	62·4% (58·6–66·1)	89·4% (82·8–94·1)
PPV[b,c]	14·8% (9·29–21·9)	13·4% (8·47–19·7)	14% (10·3–18·6)	50% (30·6–69·4)
NPV[b,c]	100% (97·4–100)	98·9% (96·9–99·8)	99·3% (97·9–99·9)	95·2% (89·8–98·2)
LR+[d,e]	2·23 (1·94–2·55)	2·64 (2·16–3·24)	2·48 (2·18–2·81)	6·6 (3·72–11·7)
LR–[d,e]	0	0·187 (0·065–0·54)	0·109 (0·037–0·326)	0·336 (0·171–0·657)
Diagnostic efficiency	58·3%	68%	64·3%	86·8%
DOR[d,e]	∞	14·2 (4·41–45·2)	22·7 (7·36–69·7)	19·7 (6·66–57·9)
Area under the ROC curve[f,g]	–	–	0·86 (0·83–0·89)	0·91 (0·85–0·95)

[a]Prevalence of CSF TB culture–positive TBM.

[b]Data shown are: % (95% confidence interval).

[c]PPV: positive predictive value; NPV: negative predictive value.

[d]LR+: positive likelihood ratio; LR–: negative likelihood ratio; DOR: diagnostic odds ratio.

[e]Data shown are: ratio (95% confidence interval).

[f]Data shown are: fraction (95% confidence interval).

[g]See Figure 2 for the ROC curves for chloride and ADA.

Table 4: Accuracy of CSF chloride and CSF ADA in HIV–positive subsets

Test	CSF chloride	CSF chloride	CSF chloride	CSF ADA
Age group	0–12 years (n=39)	≥13 years (n=176)	All ages (n=215)	All ages (n=70)
Prevalence[a,b]	2·6% (0·07–13·5)	7·4% (4–12·3)	6·5% (3·6–10·7)	14% (7·1–24·7)
Sensitivity[b]	100% (2·5–100)	76·9% (46·2–95)	78·6% (49·2–95·3)	70% (34·8–93·3)
Specificity[b]	57·9% (40·8–73·7)	64·4% (56·6–71·7)	63·2% (56·1–69·9)	91·7% (81·6–97·2)
PPV[b,c]	5·88% (0·15–28·7)	14·7% (7·28–25·4)	12·9% (6·64–22)	58·3% (27·7–84·8)
NPV[b,c]	100% (84·6–100)	97·2% (92·1–99·4)	97·7% (93·4–99·5)	94·8% (85·6–98·9)
LR+[d,e]	2·38 (1·64–3·45)	2·16 (1·5–3·11)	2·13 (1·54–2·96)	8·4 (3·31–21·3)
LR–[d,e]	0	0·36 (0·13–0·97)	0·34 (0·12–0·93)	0·33 (0·13–0·85)
Diagnostic efficiency	59%	65·3%	64·2%	88·6%
DOR[d,e]	∞	6·03 (1·71–21·1)	6·29 (1·82–21·6)	25·7 (5·34–123)

[a]Prevalence of CSF TB culture–positive TBM.

[b]Data shown are: % (95% confidence interval).

[c]PPV: positive predictive value; NPV: negative predictive value.

[d]LR+: positive likelihood ratio; LR–: negative likelihood ratio; DOR: diagnostic odds ratio.

[e]Data shown are: ratio (95% confidence interval).

Table 5: Accuracy of CSF chloride and CSF ADA in HIV–negative subsets

Test	CSF chloride	CSF chloride	CSF chloride	CSF ADA
Age group	0–12 years (n=133)	≥13 years (n=125)	All ages (n=258)	All ages (n=33)
Prevalence[a,b]	4·5% (1·7–9·6)	4% (1·3–9·1)	4·3% (2·1–7·5)	9·1% (1·9–24·3)
Sensitivity[b]	100% (54·1–100)	100% (47·8–100)	100% (71·5–100)	100% (29·2–100)
Specificity[b]	57·5% (48·4–66·2)	67·5% (58·3–75·8)	62·3% (56–68·4)	83·3% (65·3–94·4)
PPV[b,c]	10% (3·8–20·5)	11·4% (3·8–24·6)	10·6% (5·4–18·1)	37·5% (8·52–75·5)
NPV[b,c]	100% (95·1–100)	100% (95·5–100)	100% (97·6–100)	100% (86·3–100)
LR+[d,e]	2·35 (1·92–2·88)	3·08 (2·38–3·98)	2·66 (2·26–3·12)	6 (2·7–13·4)
LR–[d,e]	0	0	0	0
Diagnostic efficiency	59·4%	68·8%	64%	84·8%
DOR[d,e]	∞	∞	∞	∞

[a]Prevalence of CSF TB culture–positive TBM.

[b]Data shown are: % (95% confidence interval).

[c]PPV: positive predictive value; NPV: negative predictive value.

[d]LR+: positive likelihood ratio; LR–: negative likelihood ratio; DOR: diagnostic odds ratio.

[e]Data shown are: ratio (95% confidence interval).

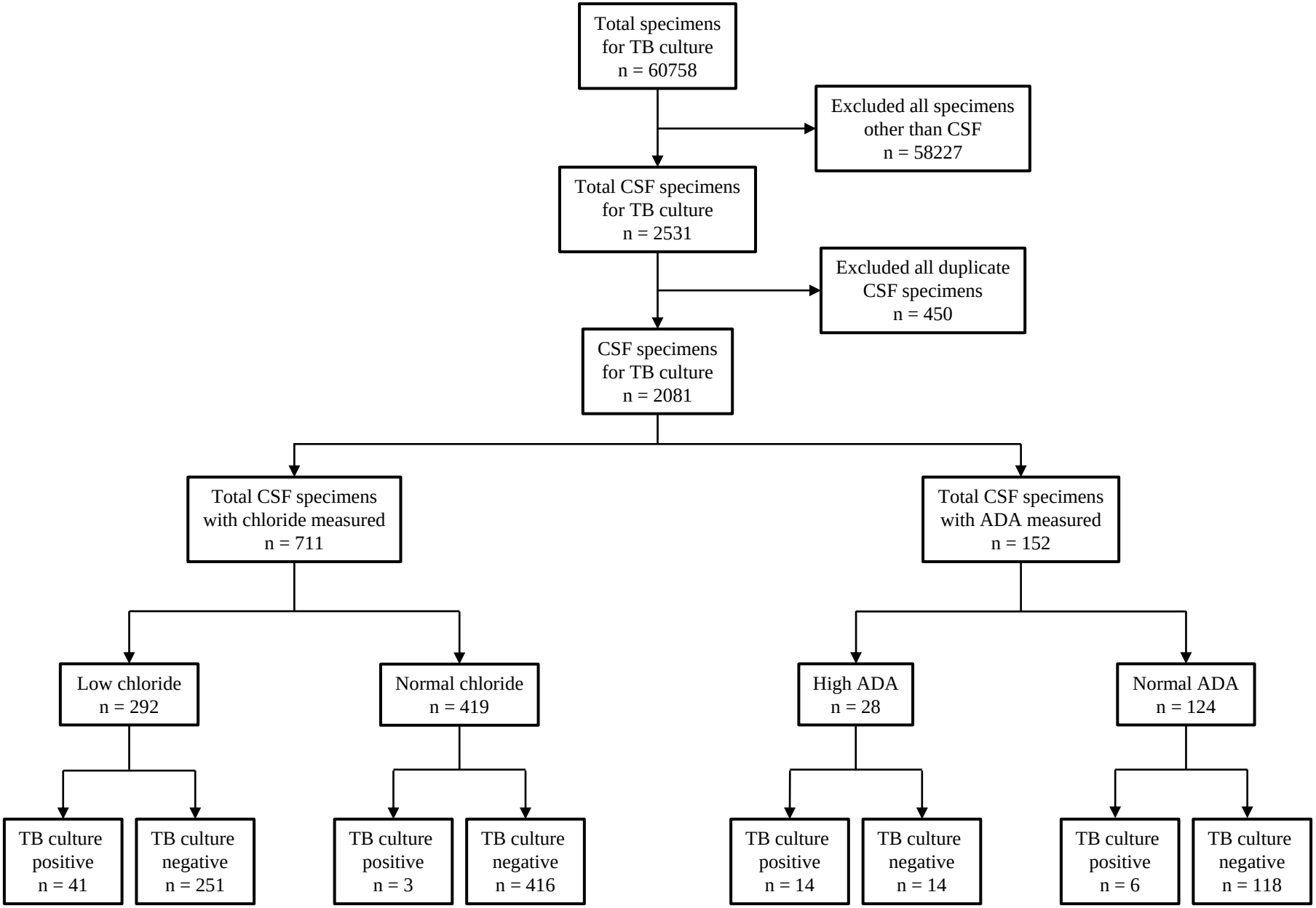

Figure 1: Study profile

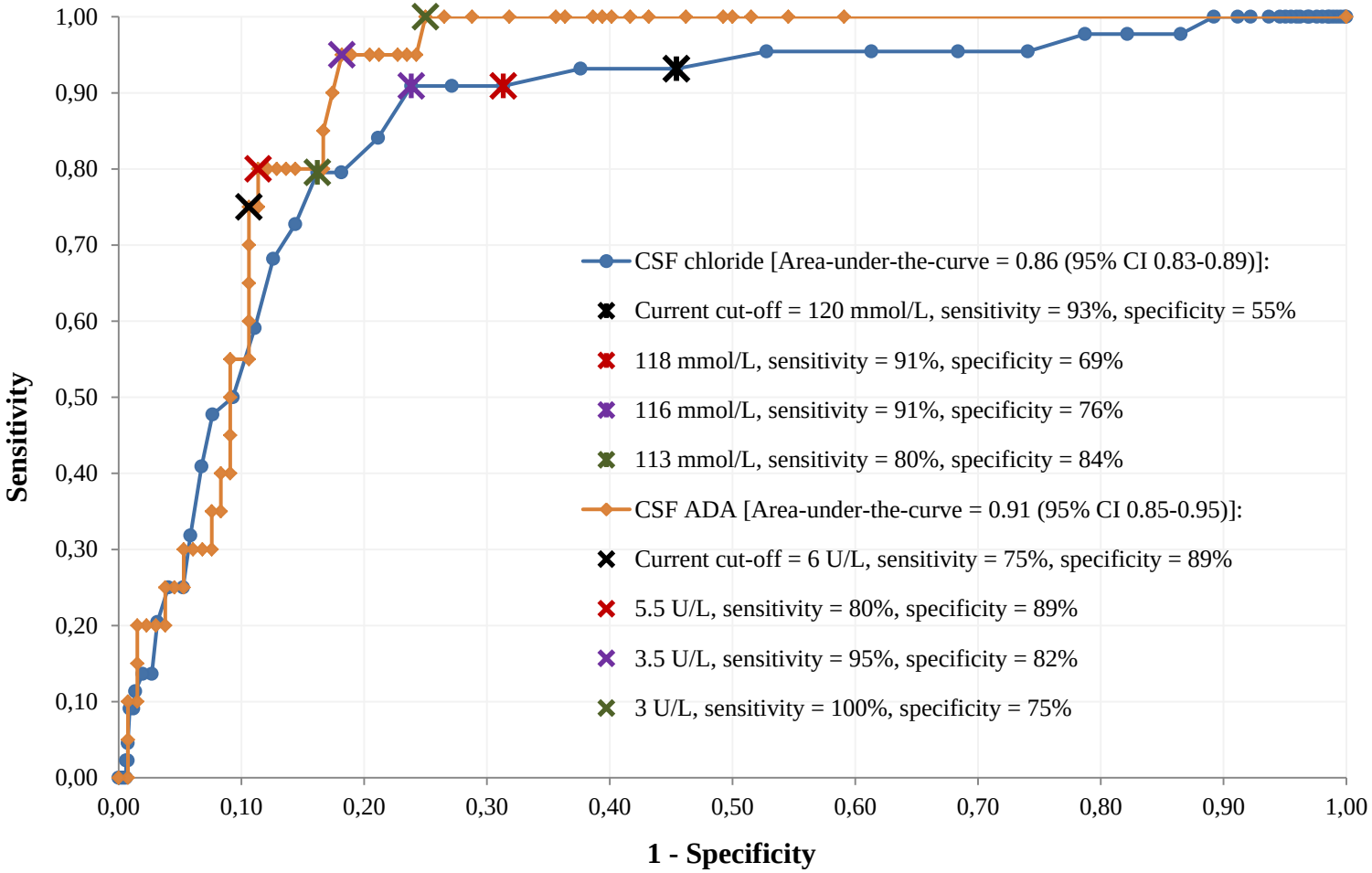

Figure 2: ROC curves for CSF chloride and CSF ADA

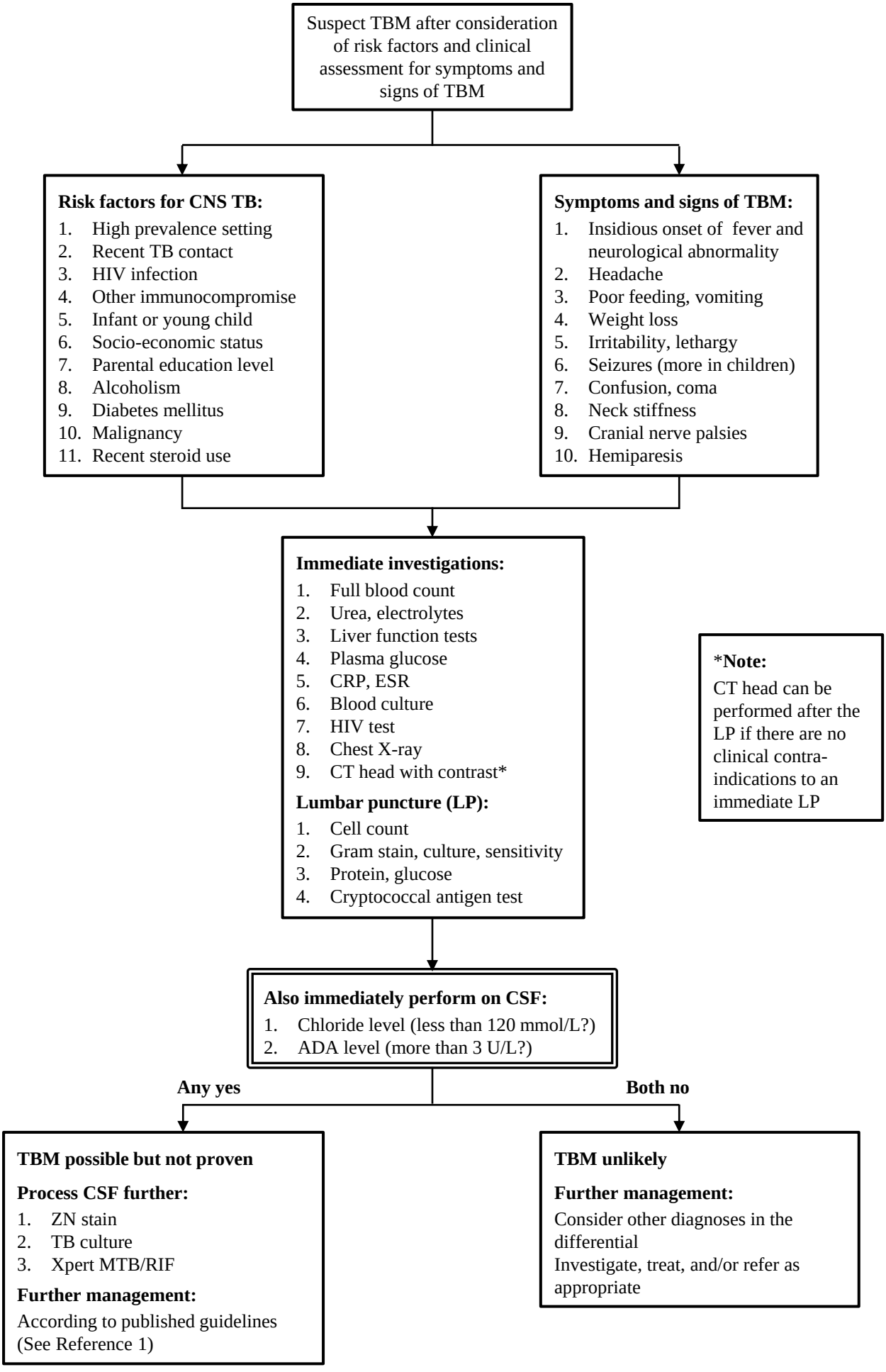

Figure 3: Diagnostic algorithm for suspected TBM in adults and children (Adapted from Reference 1)

APPENDIX A

University of Cape Town Master of Medicine Guidelines 2015

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES

MMed Part III (minor dissertation)
Guidelines for candidates, supervisors and examiners

The MMed minor dissertation is one of three examination components of the MMed degree. This minor dissertation carries one third of the weight of a full master's dissertation in terms of its credit weighting, i.e. 60 credits which approximate 600 hours of work. In order to register as a specialist in South Africa, the Health Professions Council of South Africa (HPCSA) requires all specialist trainees who register for training after 1 January 2011 to have completed a relevant research study.

The dissertation must be the result of independent work of the candidate conducted under the guidance and direction of a supervisor(s) and should demonstrate evidence of an ability to undertake research, to interpret results adequately and to review the relevant literature comprehensively and critically. Although the research need not necessarily be original, the findings must be seen to advance scientific understanding. A case report is not acceptable for the dissertation, as it cannot meet these requirements but an unusual case series may, in some circumstances be accepted. A full systematic review following the format recommended by the Cochrane Collaboration is acceptable. The topic, study design and scope of research will depend on the particular discipline and must be agreed on in consultation with the supervisor(s).

The dissertation may be presented in one of two formats:

I: Publication-ready format;
II: Monograph format.

As disciplines differ in their requirements, it is important that the format chosen is acceptable to the discipline and appropriate College within the CMSA.

Research protocol

Candidates intending to register for the MMed Part III are required to submit a full research protocol for approval to their respective Departmental Research Committees (DRC). The candidate must then obtain approval from the UCT Faculty of Health Sciences Research Ethics Committee (HREC) prior to conducting their research. Studies that involve the audit of clinical records or services also require formal REC approval. Any primary research that is taking place in a provincial or local authority health facility, such as public sector hospitals or clinics, must also be submitted to the provincial government for approval, after the UCT Research Ethics Committee approval has been obtained. **Approval to access public sector facilities for research is needed for all provincial and local authority facilities.** There are five points where approval for research can be applied for; Groote Schuur Hospital, Red Cross War Memorial Children's Hospital, Tygerberg Hopsital), the local authorities and "all other province". Teaching hospitals and the local authorities approve research projects in-house. "All other province" approvals are done via the Directorate: Health Impact Assessment (Sub-directorate: Research) at provincial head office. If research crosses these boundaries, up to five approvals may be needed. Further details can be found at http://www.capegateway.gov.za/other/2011/3/phrc_approval_guidelines_november_2010.pdf. The Provincial Health Research Committee does not approve research proposals itself, but oversees this approval process by reviewing difficult applications on referral.

The research protocol should specifically and accurately outline the scope and content of the dissertation and must include the title of the proposed dissertation, name of the supervisor(s) and their brief curriculum vitae. The protocol should be structured according to the guidelines in Form FHS015, available at http://www.health.uct.ac.za/research/humanethics/forms. This full research protocol together with a copy of the REC approval letter and completed Form D1 must be submitted to the postgraduate administration office, for approval by the Professional Masters Committee Chair and the Board of the Faculty of Health Sciences, prior to

commencement of the research. If the title, aims, objectives or any other aspect of the research change following initial submission, an ammendment must be submitted to HREC.

Timelines

Submission of the research protocol for approval should generally be made within the first 18 months of the registrar programme (this varies between disciplines). Heads of Departments or Divisions should meet with their registrars at least annually to review progress towards their research project. Unless otherwise stipulated by your Division / Department or constituent College of the CMSA, the research project should generally be completed by the end of Year 2. For a number of constituent Colleges, the dissertation must be submitted 6-months before writing the Part II examination. Often the research component of specialist training is only initiated after successful completion of the Part I examination.

Supervisors

The importance of identifying a dissertation supervisor as early as possible cannot be overemphasized. The supervisor should be an individual who can relate to the candidate's research project, be available for frequent and regular discussion and advice, and someone with whom the candidate can develop a good working relationship. Where specialised equipment and/or laboratory work is required for the study, the supervisor should assist in facilitating access to appropriate facilities.

The primary supervisor may be based outside the candidate's home department, faculty or university. In such a case, an internal (co-)supervisor will also be required in addition to the primary supervisor, to serve as a guide and link to UCT faculty and discipline-specific procedures. Primary supervisors retain responsibilities to the candidate and the university until the dissertation process is complete. The supervisor and student must complete form D3 (supervisor appointment form) and D2a which describes the contractual memorandum of agreement (MOU) between supervisor and student.

In order to assist a candidate with a master's research topic the supervisor should hold a master's degree or equivalent (such as a Fellowship of one of the constituent Colleges of the CMSA), and have relevant research experience. If the primary supervisor does not hold such a higher qualification, then a secondary supervisor who has a higher degree will need to be appointed in addition to the primary supervisor.

The dissertation

Submission of the dissertation should include the following:

The title page should contain the candidate's name, dissertation title and the name of the university. It must also state the degree, e.g. Master of Philosophy (MPhil) in, Pulmonology, Cardiology, etc.

The Table of contents

The declaration page should include a statement to the effect that the research reported is based on independent work performed by the candidate and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. It must also state that this work has not been reported or published *prior to registration* for the abovementioned degree.

The abstract should summarise the study rationale, methods, results, discussion and conclusion in fewer than 500 words.

Acknowledgements. This section should acknowledge the support or input from supervisors and briefly describe the role of potential co-authors. In a dissertation derived from work started by others, e.g. analysis of data collected for another project, the candidate's contribution must have been made after his/her registration for the degree and therefore under supervision. In a manuscript from a

potentially multi-authored project, the candidate must be first author

List of Tables
List of Figures
Abbreviations

The remainder of the dissertation may be presented in one of two formats:

I: Publication-ready format;
II: Monograph format.

I: Publication format

The dissertation must include a manuscript in publication-ready format. A manuscriot that has already been published can be included if the candidate's contribution was made during his/her registration for the degree and under supervision. The body of the dissertation must be structured as follows:

Chapter 1: Introduction and Literature review

This section must contain a structured and comprehensive review of the literature relevant to the subject matter and methods of the study. The literature review must show that the student is sufficiently acquainted with, and is able to conduct a critical appraisal of the relevant literature. Where relevant, the student should demonstrate a good understanding of evidence-based medicine. The review should summarise and interpret the existing knowledge in the field with relevance to the research setting and should identify knowledge gaps and hence the rationale for the dissertation. This chapter should end with a clear statement reflecting the aims and objectives of the research reported in the publication-ready manuscript. References quoted in this chapter should appear at the end of the chapter, not at the end of the thesis. This chapter should be between 3 000 and 4 000 words.

Chapter 2: Publication-ready Manuscript

The method and results of the study must be presented in the form of a manuscript of an article for a named peer reviewed journal, meeting all the requirements set out in the "Instructions for Authors" of that journal, including the word count and referencing style. Unless specially motivated, the journal chosen will need to allow for at least 3000 words excluding abstract, tables, figures and references. The "Instructions to Authors" of the journal must be appended. The co-authors should be listed in the appropriate order, and each of their contributions to the manuscript stated. The journal chosen for publication must be appropriate to the subject matter of the dissertation and listed in the citation index of the Institute for Scientific Information (ISI) or accredited by the Department of Education: (http://www.lib.uct.ac.za/medical/index.php?html=/libs/accredjnls.htm&libid=24)

Important note: The candidate need not have submitted the article for publication, nor is the acceptance of the article for publication a requirement for passing the degree. However, the norm is to publish the study with the supervisor(s) as co-author(s), and candidates are strongly encouraged to submit their manuscript for publication either before or shortly after examination of the minor dissertation. Submitting the manuscript for publication before submitting the minor dissertation has the advantage that addressing the peer reviewers' comments improves the standard of the manuscript included in the dissertation. A candidate who fails to submit a manuscript for publication within one year of examination of the minor dissertation must accept that their supervisor(s) may publish their data with him/her as co-author.

For a full systematic review, Chapters 1 and 2 are combined in the publication-ready manuscript.

Appendices

Append all supporting documents including:

- Questionnaire/data capture instrument(s)
- Consent forms and any related participant information sheets

- Technical appendices, including, if considered necessary, any additional tables not included in the main manuscript for the examiner to have available. These should be accompanied by a brief narrative.
- Official Ethics approval letter from the Faculty Research Ethics Committee and any other approvals required (e.g. Provincial Government).
- Instructions to Authors of the chosen journal

II: Standard monograph format

Some disciplines and constituent Colleges of the CMSA require a standard monograph format, which should be 16 000 to 20 000 words in length, and presented in a comprehensive and scholarly style.

A recommended structure for the body of the dissertation is as follows;

Chapter 1: Introduction and Literature review
(see guidelines above)

Chapter 2: Methods
Material and methods of the study must be fully described and factually presented and must evidence familiarity with the laboratory and/or clinical methods used

Chapter 3: Results

Chapter 4: Discussion and conclusions

Appendices

(see guidelines above - omit the instructions to authors)

Language and writing

Clear, grammatically correct English is essential.

Supervisors may assist candidates in developing scientific communication skills but they are not required to do detailed editing or correction of spelling, grammar, or style. They may refer candidates elsewhere for this, at the candidate's own expense. Candidates who may have difficulties are encouraged to seek help from the writing support facilities on main campus (see: http://www.ched.uct.ac.za/adp/writing/).

Candidates should refer to the document D4, Guidelines on the Layout and Style of the Dissertation or Thesis. As long as the dissertation is readable and internally consistent, any of a number of styles is acceptable. For a publication-ready manuscript, references should be formatted according to the instructions to authors for the journal selected, and candidates should use the same style throughout their dissertation. For a monograph format manuscript, the Harvard style for referencing is recommended. In this style, referencing is by first author in parentheses in the text and the bibliography is listed alphabetically (rather than using numerical superscripts in the text)For reference management, Refworks can be downloaded from the ICTS or UCT library websites.

It is suggested that candidates look at previous examples of Master's dissertations in the library for appealing layouts. Master's dissertations are available in the Health Sciences Library. A search will need to be done to obtain a list of titles and authors. This search can be done using search words (e.g. dissertation, health, health sciences, etc.). The librarian should be asked for assistance.

Some of these dissertations are available online at:
http://srvrhldig001.uct.ac.za/R/R3CAKV8FM3PHV23A363D7J4F947AN4AXGRBTHIPM2L62RSUXDM-02943?func=collections&collection_id=1526 but this site does not yet differentiate MMed, MPhil and MSc dissertations within the faculty of Health Sciences, so candidates will have to open each dissertation to identify whether it is relevant to their minor dissertation.

Submission of dissertations

On completion, the dissertation should be submitted to the Faculty Postgraduate Office. The candidate should inform the Faculty Officer one month in advance of the intention to submit, using **Form D8 (Intention to submit)**. Supervisors will be requested by the Faculty Postgraduate Officer to submit a letter supporting submission, and clearly specifying whether the dissertation will be submitted in a "Publication-ready" or "Monograph" format, so that the appropriate instructions are sent to the examiners. This letter should be supplied by the primary supervisor. If this supervisor is external, the internal supervisor must be kept informed at every stage of the process.

The candidate must submit 2 copies of the dissertation, in temporary binding (e.g. plastic ring) and an electronic copy in a universally readable format (e.g. pdf) on a compact disc. The candidate must **clearly** state which of the formats has been chosen ("Publication-ready" or "Monograph"), so that the appropriate instructions are sent to the examiners. Specific submission requirements may be set by individual disciplines or constituent Colleges of the CMSA, and registrars are obliged to ensure that their research projects and dissertations meet these specific requirements.

UCT Dissertation Submission deadlines:

1. March 15th for June graduation
2. August 15th for December graduation

Note on fees: To avoid attracting fees, dissertations need to be submitted before the beginning of the first quarter (first day of academic year), and before the start of the second semester (mid July) to qualify for a 50% fee rebate.

Examiners

The full dissertation will be submitted for examination through the Postgraduate Office of our Faculty to two external examiners (nominated by the supervisors and HOD).

It is the supervisors' responsibility to submit names of three potential examiners to the Faculty Officer when the candidate is ready to submit. Of the three examiners nominated, two are invited to examine, and one is held as an alternate. All examiners must all be external to UCT, and appointment of examiners from outside South Africa is encouraged. These nominations need to be approved by the Deputy Dean: Postgraduate Affairs on behalf of the Faculty Board and submitted to the Faculty Board for ratification via a Dean's Circular.

The examiners will be well briefed regarding the specific requirements and criteria for submission and examination of the minor dissertation. Such criteria will clearly explain the difference between the minor dissertation and a Master's degree by dissertation alone, and between the monograph and the "publication-ready" format of dissertation. Details required for each examiner are: academic qualifications, postal and/or physical address, telephone and fax numbers and e-mail address, and one paragraph description of their standing in the relevant field (drawn from their CV if need be.)

The candidate may not be informed of the identity of the examiners. After the outcome of the minor dissertation has been finalised, the examiners' identities are made known if the examiners have indicated that they do not object to this.

Publication agreement

The university has a moral responsibilty to publish all research undertaken when publication is stated as an anticipated output. A candidate who fails to submit a manuscript to a journal for publication within 1 year of submission of their thesis, must accept that their supervisor(s) are entitled to publish their data on their behalf, with the student as co-author as long as this is noted in the MOU.

APPENDIX B

Post Graduate Office Approval of Candidature

Swanepoel : Confirmation of Approval of Study Proposal

Jackie Cogill [jackie.cogill@uct.ac.za]
Sent:15 October 2015 12:38 PM
To: Hendre Swanepoel
Cc: justyna.wojno@nhls.ac.za

Dear Dr Swanepoel

Candidature Approval (SWNHEN002)

Degree	MMed in Clinical Pathology
Title	Utility of chloride and adenosine deaminase measurement in cerebrosphinal fluid for the early presumptive diagnosis of tuberculous meningitis
Department	Pathology
Supervisor	Dr J Wojno
Ethics Approval	497/2015

I am pleased to advise that the Chair of the Dissertations/Doctoral & Masters Committee has approved your candidature for the above degree on behalf of the Committee. Formal approval was obtained by publication in the Dean's Circular, PG-Med Aug2015.

Yours sincerely

Jackie Cogill

APPENDIX C

Human Research Ethics Committee Study Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

13 July 2015

HREC REF: 497/2015

Dr J Wojno
Medical Microbiology
5th Floor
Falmouth Building

Dear Dr Wojno

PROJECT TITLE: UTILITY OF CHLORIDE AND ADENOSINE DEAMINASE MEASUREMENT IN CEREBROSPINAL FLUID FOR THE EARLY PRESUMPTIVE DIAGNOSIS OF TUBERCULOUS MENINGITIS (Masters-candidate-Dr H Swanepoel)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th July 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Dr Hendre Swanepoel will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signed

pp

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

APPENDIX D

The Lancet Infectious Diseases Information for Authors

The Lancet Infectious Diseases **considers any original research contribution that advocates change in or illuminates infectious disease clinical practice and informative reviews on any topic connected with infectious diseases. Because the journal has an international readership from a wide range of specialties, it is vital that articles should be written clearly and should not assume a level of knowledge above that of, say, a reasonably well-read, recently qualified, doctor in training. One way to find out if your article is understandable to those reading outside their immediate field of interest is to show the manuscript to colleagues in other specialties. If they find it difficult to follow, so will a good proportion of the readership. Wherever possible, figures and good quality photographs (colour or black and white) should be used to supplement and to enhance the text. Further details on the different sections of *The Lancet Infectious Diseases*, and how to submit to the journal, are provided below. If you require further clarification, the journal's editorial staff will be pleased to help (email IDeditorial@lancet.com).**

Manuscripts must be solely the work of the author(s) stated, must not have been previously published elsewhere, and must not be under consideration by another journal. *The Lancet* journals are signatories of the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, issued by the International Committee of Medical Journal Editors (ICMJE Recommendations), and to the Committee on Publication Ethics (COPE) code of conduct for editors. We follow COPE's guidelines.

Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals
http://www.icmje.org

COPE Code of Conduct
http://publicationethics.org/files/u2/New_Code.pdf

How to submit your paper

Manuscript submission

Manuscript submission to all *Lancet* journals is free. Manuscripts should be submitted online via the *The Lancet Infectious Diseases*' online submission and peer review website (known as EES) at http://ees.elsevier.com/thelancetid

- Simply log on to EES and follow the onscreen instructions for all submissions
- If you have not used EES before, you will need to register first. In EES, the corresponding author is the person who enters the manuscript details and uploads the submission files
- Inclusion of illustrations (photographs, graphs, diagrams, etc) is a prerequisite for publication. Submission of original and editable artwork files is encouraged. Digital photography files should have a resolution of at least 300 dpi and be at least 107 mm wide
- In almost all cases, if you have a finished manuscript, you should submit it, rather than contacting *The Lancet Infectious Diseases* to enquire whether an unseen manuscript is likely to be accepted. Unless you have been asked by the Editor to submit by email, you should use the online system for all types of submission, including Correspondence
- If you have any technical problems or questions, please contact our dedicated customer support (available 24 h a day, 365 days a year):

  For the Americas: +1 888 834 7287 (toll-free in USA and Canada)
  For Asia and Pacific: +81 3 5561 5032
  For Europe and rest of the world: +353 61 709190
  Email: IDeditorial@lancet.com

Covering letter

- You should upload your covering letter at the "Enter Comments" stage of the online submission process
- Use the covering letter to explain why your paper should be published in *The Lancet Infectious Diseases*—the leading international infectious diseases journal—rather than elsewhere
- It is helpful to indicate what could shorten your paper—the full paper can be reviewed and a shorter version published; a table or figure, details of a DNA sequence, or further references, for example, can be published on our website or made available from the authors

First submissions to *The Lancet Infectious Diseases* should include:

1. Covering letter
2. Manuscript including tables and panels
3. Figures
4. Author statement form (see next section)
5. Declaration of interests and source of funding statements (see next section)
6. In-press papers—one copy of each with acceptance letters
7. Protocols and CONSORT details for randomised controlled trials (see Articles)
8. We encourage disclosure of correspondence from other journals and reviewers, if previously submitted, and we might contact relevant editors of such journals
9. Research in context panel, for all primary research Articles excluding meta-anlayses

Statements, permissions, and signatures

Authors and contributors

- Designated authors should meet all four criteria for authorship in the ICMJE Recommendations
- All authors, and all contributors (including medical writers and editors), should specify their individual contributions at the end of the text
- *The Lancet Infectious Diseases* will not publish any paper unless we have the signatures of all authors
- We suggest you use the author statement form and either upload the signed copy with your submission, or fax to +44 (0) 1865 853 016
- Please include written consent of any cited individual(s) noted in acknowledgments or personal communications

ICMJE Recommendations
http://www.icmje.org

Author statement form
http://download.thelancet.com/flatcontentassets/authors/tlid-author-signatures.pdf

Declaration of interests

A conflict of interest exists when professional judgement concerning a primary interest (such as patients' welfare or validity of research) may be influenced by a secondary interest (such as financial gain).

Financial relationships are easily identifiable, but conflicts can also occur because of personal relationships and rivalries, academic competition, or intellectual beliefs. A conflict can be actual or potential, and full disclosure to the Editor is the safest course. Failure to disclose conflicts might lead to publication of a correction or even to retraction. All submissions to *The Lancet Infectious Diseases* must include disclosure of all relationships that could be viewed as presenting a potential or actual conflict of interest (see *Lancet* 2001; **358:** 854–56 and *Lancet* 2003; **361:** 8–9). The Editor may use such information as a basis for editorial decisions, and will publish such disclosures if they are believed to be important to readers in judging the manuscript. Agreements between authors and study sponsors that interfere with authors' access to all of a study's data, or that interfere with their ability to analyse and interpret the data and to prepare and publish manuscripts independently, may represent conflicts of interest, and should be avoided.

- At the end of the text, under a subheading "Declaration of interests", all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of financial conflicts include employment, consultancies, stock ownership, honoraria, paid expert testimony, patents or patent applications, and travel grants, all within 3 years of beginning the work submitted. If there are no conflicts of interest, authors should state that
- All authors are required to provide a Conflict of Interest Statement and should complete a standard form, which is available at http://download.thelancet.com/flatcontentassets/authors/icjme-coi-form.pdf. This form can be uploaded with the manuscript at submission or faxed to +44 (0)1865 853017. The form has been modified by the ICMJE following consultation with authors and editors. Further information is available in a joint ICMJE statement published on July 1, 2010. For more information see *Lancet* 2009; **374:** 1395–96
- For Comment and Reviews, *The Lancet Infectious Diseases* will not publish if an author, within the past 3 years, and with a relevant company or competitor, has any stocks or shares, equity, a contract of employment, or a named position on a company board; or has been asked by any organisation other than *The Lancet Infectious Diseases* to write, be named on, or to submit the paper (see *Lancet* 2004; **363:** 2–3)

ICMJE COI form
http://download.thelancet.com/flatcontentassets/authors/icmje-coi-form.pdf

Joint ICMJE statement
http://download.thelancet.com/flatcontentassets/authors/icmje-statement.pdf

Role of the funding source

- All sources of funding should be declared as an acknowledgment at the end of the text
- At the end of the Methods section, under a subheading "Role of the funding source", authors must describe the role of the study sponsor(s), if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication
- If there is no Methods section, the role of the funding source should be stated as an acknowledgment. If the funding source had no such involvement, the authors should so state
- The corresponding author should confirm that he or she had full access to all the data in the study and had final responsibility for the decision to submit for publication

Role of medical writer or editor

- If a medical writer or editor was involved in the creation of your manuscript, we need a signed statement from the corresponding author to include their name and information about funding of this person
- This information should be added to the Acknowledgments or Contributors section
- We require signed statements from any medical writers or editors declaring that they have given permission to be named as an author, as a contributor, or in the Acknowledgments section

Patient and other consents

- Appropriate written consents, permissions, and releases must be obtained where you wish to include any case details, personal information, and/or images of patients or other individuals in *The Lancet* journals in order to comply with all applicable laws and regulations concerning privacy and/or security of personal information. Studies on patients or volunteers need approval from an ethics committee and informed consent from participants. These should be documented in your paper
- Since the consent form needs to comply with the relevant legal requirements of your particular jurisdiction, we do not provide sample forms; this is your responsibility. Your affiliated institution should be able to provide an appropriate form
- For the purposes of publishing in *The Lancet* journals, a consent, permission, or release should include, without limitation, publication in all formats (including print, electronic, and websites), in sublicensed and reprinted versions (including translations), and in other works and products
- To respect your patient's and any other individual's privacy, please do not send signed forms to *The Lancet Infectious Diseases*. Please instead complete the patient consent section of the Author statements while retaining copies of the signed forms in the event they should be needed
- If consent, permission, or release is made subject to any conditions, *The Lancet Infectious Diseases* must be made aware in writing of all such conditions before publication
- For more information about our policy, please visit http://cdn.elsevier.com/assets/pdf_file/0007/111400/patient-consent-policy.pdf

Signatures

At the external peer review stage you will need to send signed copies of the following statements:

- Authors' contributions
- Conflicts of interest statements
- Statements of role, if any, of medical writer or editor
- Acknowledgments—written consent of cited individual
- Personal communications—written consent of cited individual
- Use of copyright-protected material—signed permission statements from author and publisher

These statements can be scanned and submitted electronically to eesTheLancetID@lancet.com. To minimise delays, we strongly advise that you prepare signed copies of these statements before you submit your manuscript.

Types of article and manuscript requirements

Please ensure that anything you submit to *The Lancet Infectious Diseases* follows the guidelines provided for each article type. For instruction on how to format the text of your paper, including tables, figures, panels, and references, please see our **Formatting guidelines**.

Red section (Articles)

Articles

- *The Lancet Infectious Diseases* prioritises reports of original research that are likely to change clinical practice or thinking about a disease
- We invite submission of all clinical trials, whether phase 1, 2, 3, or 4. For phase 1 trials, we especially encourage those of a novel substance for a novel indication, if there is a strong or unexpected beneficial or adverse response, or a novel mechanism of action
- We require the registration of all interventional trials, whether early or late phase, in a primary register that participates in WHO's International Clinical Trial Registry Platform (see *Lancet* 2007; **369:** 1909–11). We also encourage full public disclosure of the minimum 20-item trial registration dataset at the time of registration and before recruitment of the first participant (see *Lancet* 2006; **367:** 1631–35). The registry must be independent of for-profit interest
- Reports of randomised trials must conform to CONSORT 2010 guidelines and should be submitted with their protocols
- All reports of randomised trials should include a section entitled Randomisation and masking, within the Methods section. Please refer to *The Lancet*'s formatting guidelines for randomised trials
- Cluster-randomised trials must be reported according to CONSORT extended guidelines
- Randomised trials that report harms must be described according to extended CONSORT guidelines
- Studies of diagnostic accuracy must be reported according to STARD guidelines
- Observational studies (cohort, case-control, or cross-sectional designs) must be reported according to the STROBE statement, and should be submitted with their protocols
- Studies of molecular epidemiology in infectious diseases must be reported according to the STROME-ID statement (see *Lancet Inf Dis* 2014; **14:** 341–52)
- We encourage the registration of all observational studies on a WHO-compliant registry (see *Lancet* 2010; **375:** 348)
- Genetic association studies must be reported according to STREGA guidelines
- Meta-analyses must be reported according to PRISMA guidelines
- To find reporting guidelines see: http://www.equator-network.org

All Articles should, as relevant:

- Be up to 3000 words with 30 references
- Include an abstract (semistructured summary), with five paragraphs (Background, Methods, Findings, Interpretation, and Funding), not exceeding 250 words. Our electronic submission system will ask you to copy and paste this section at the "Submit Abstract" stage
- For randomised trials, the abstract should adhere to CONSORT extensions: abstracts (see *Lancet* 2008; **371:** 281–83)
- For intervention studies, the abstract should include the primary outcome expressed as the difference between groups with a confidence interval on that difference (absolute differences are more useful than relative ones). Important secondary outcomes can be included as long as they are clearly marked as secondary
- Use the SI system of units and the recommended international non-proprietary name (rINN) for drug names. Ensure that the dose, route, and frequency of administration of any drug you mention are correct
- Use gene names approved by the Human Gene Organisation. Novel gene sequences should be deposited in a public database (GenBank, EMBL, or DDBJ), and the accession number provided. Authors of microarray papers should include in their submission the information recommended by the MIAME guidelines. Authors should also submit their experimental details to one of the publicly available databases: ArrayExpress or GEO
- Include any necessary additional data as part of your EES submission
- All accepted Articles should include a link to the full study protocol published on the authors' institutional website (see *Lancet* 2009; **373:** 992 and *Lancet* 2010; **375:** 348)

Putting research into context

- From Jan 1, 2015, all research papers (apart from meta-analyses) submitted to any journal in *The Lancet* family must include a panel putting their research into context with previous work, with an enhanced structure and subheadings compared with papers submitted before this date (see *Lancet* 2014; **384**: 2176–77, and panel below for guidance). Editors will use this information at the first assessment stage and peer reviewers will be specifically asked to check the content and accuracy

Research in context

Evidence before this study

This section should include a description of all the evidence that the authors considered before undertaking this study. Authors should state: the sources (databases, journal or book reference lists, etc) searched; the criteria used to include or exclude studies (including the exact start and end dates of the search), which should not be limited to English language publications; the search terms used; the quality (risk of bias) of that evidence; and the pooled estimate derived from meta-analysis of the evidence, if appropriate.

Added value of this study

Authors should describe here how their findings add value to the existing evidence (including an updated meta-analysis, if appropriate).

Implications of all the available evidence

Authors should state the implications for practice or policy and future research of their study combined with existing evidence.

Human Gene Organisation
http://www.genenames.org/

WHO's International Clinical Trial Registry Platform
http://www.who.int/ictrp/network/trds/en/index.html

MIAME guidelines
http://www.mged.org/Workgroups/MIAME/miame_checklist.html

CONSORT 2010 guidelines
http://www.consort-statement.org/consort-statement/overview0/

Array and GEO
http://www.ebi.ac.uk/microarray-as/ae/
http://www.ncbi.nlm.nih.gov/geo

CONSORT extended guidelines
http://www.consort-statement.org/extensions/extensions/

STARD guidelines
http://www.stard-statement.org/

STROBE statement
http://www.strobe-statement.org/

STREGA guidelines
http://www.medicine.uottawa.ca/public-health-genomics/web/eng/strega.html

PRISMA guidelines
http://www.prisma-statement.org/

To find reporting guidelines, see
http://www.equator-network.org

- The Discussion section should contain a full description and discussion of the context. Authors are also invited to either report their own, up-to-date systematic review or cite a recent systematic review of other trials, putting their trial into context of the review

Blue section (Comment, Correspondence, Newsdesk, Media Watch, etc)

Editorial

- Editorials are the voice of *The Lancet Infectious Diseases*, and are written in-house by the journal's editorial-writing team and signed "The Lancet Infectious Diseases"

Comment

- Commentaries may discuss articles in *The Lancet Infectious Diseases* or in other journals
- Commentaries should be about 700 words and 12 references
- The place to respond to something we have published is in our Correspondence section
- See **Conflicts of Interest** guidelines

Correspondence

- Letters should be written in response to previous content published in *The Lancet Infectious Diseases*
- Letters for publication in the print journal must reach us within 6 weeks of publication of the original item and should be no longer than 400 words
- Only one table or figure is permitted, and there should be no more than five references and five authors
- All accepted letters are edited, and proofs will be sent out to authors before publication

Newsdesk

- Most of the writers of Newsdesk articles are professional journalists, but an important event in your country that might be of wider interest can be brought to the attention of our Newsdesk editors via IDeditorial@lancet.com

Media Watch

- Readers with an interest in contributing book, film, TV, or web reviews should contact the Editor via IDeditorial@lancet.com. In general, these submissions should be between 350 and 400 words

Corrections

- Any substantial error in any article published in *The Lancet Infectious Diseases* should be corrected as soon as possible. Blame is not apportioned; the important thing is to set the record straight
- *The Lancet* journals have a policy for types of errors that we do and do not correct. We will always correct any error affecting a non-proprietary drug name, dose, or unit, any numerical error in the results, or any factual error in interpretation of results

For *The Lancet* journals' policy on correction of errors see http://download.thelancet.com/flatcontentassets/authors/correction-policy.pdf

Green section (Reviews, Historical Reviews, Personal Views, Grand Rounds, Clinical Pictures, etc)

From July 1, 2015, papers submitted for the Green section of the journal will be eligible only for online publication, with the exception of Clinical Pictures (see *Lancet Inf Dis* 2015; **15:** 760)

Reviews

- Reviews may be commissioned or submitted unsolicited, although in the latter case it would be wise to send the Editor a one-page outline first (IDeditorial@lancet.com) to ensure that a review on the same subject has not already been commissioned. If you have already written the paper, please submit it for consideration via our online system
- Complete transparency about the choice of material included is important to any Review paper. Therefore, all Reviews should include a brief section entitled "Search strategy and selection criteria" stating the sources (including databases, MeSH and free text search terms and filters, and reference lists from journals or books) of the material covered, and the criteria used to include or exclude studies. Citations to papers published in non-peer-reviewed supplements are discouraged. Since these papers should be comprehensive, we encourage citation of publications in non-English languages. An example is shown below:

Search strategy and selection criteria

References for this review were identified through searches of PubMed for articles published from January, 1971, to June, 2010, by use of the terms "Guillain-Barré syndrome", "influenza", "H1N1", "immunization", and "vaccination". Relevant articles published between 1918 and 1920 were identified through searches in the authors' personal files, in Google Scholar, and Springer Online Archives Collection. Articles resulting from these searches and relevant references cited in those articles were reviewed. Articles published in English, French, and German were included.

- Systematic reviews that do not include meta-analysis will be considered under the Review heading and must be reported according to the PRISMA guidelines
- Reviews should be 3000–5000 words, with a maximum of 150 references. A 150-word unstructured summary should be included. These papers should include about five illustrations to aid the reader

Historical Reviews

- These should follow the same rules and guidelines as for Reviews, but should cover the chronological developments in an important or interesting area of infectious diseases

Personal Views

- These should be around 1500–3000 words in length, with a maximum of 75 references
- These opinion pieces are thought provoking essays on an infection-related subject and must be prepared in a similar way to a Review article
- Unsolicited contributions are welcome, but it is best to contact the Editor (IDeditorial@lancet.com) before submission to ensure that the proposed topic is suitable for the journal

Grand Rounds

- These use a brief case report as the starting point for a review of the patient's diagnosis. Rather than rarity, we are looking for

single cases that address common problems and evidence-based review of the implications of the case
- The case report part of the text should be no longer than 800 words and the review part no longer than 3000. Up to 75 references are allowed
- Consent for publication in print and electronically must be obtained from the patient or, if this is not possible, the next of kin before submission. See **Patient and other consents**

Clinical Pictures

- The ideal Clinical Picture provides visual information that will be useful to other clinicians
- Clinical Pictures should be interesting, educational, and respectful of the patient. *The Lancet Infectious Diseases* is less interested in pictures that simply illustrate an extreme example of a medical condition
- Authors must obtain signed informed consent for publication (see Patient and other consents). Do not use "blackout" bars or similar devices to anonymise patients: if you have taken consent appropriately, masking is not necessary
- Use no more than 300 words, with no references

Formatting guidelines

Language

- Manuscripts should be submitted in English. Authors writing in Chinese, Portuguese, or Spanish may wish to use the Webshop (http://webshop.elsevier.com/languageservices) to provide an English translation of their manuscript for submission

Title page

- A brief title, author name(s), preferred degree (one only), affiliation(s), and full address(es) of the authors must be included. The name and address of the corresponding author should be separately and clearly indicated with email and telephone details

Formatting of text

- Type a single space at the end of each sentence
- Do not use bold face for emphasis within text
- We use a comma before the final "and" or "or" in a list of items
- Type decimal points midline (ie, 23·4, not 23.4). To create a midline decimal on a PC: hold down ALT key and type 0183 on the number pad, or on a Mac: ALT shift 9
- Numbers one to ten are written out in words unless they are used as a unit of measurement, except in figures and tables
- Use single hard-returns to separate paragraphs. Do not use tabs or indents to start a paragraph
- Do not use the automated features of your software, such as hyphenation, endnotes, headers, or footers (especially for references). You can use page numbering

References

- Cite references in the text sequentially in the Vancouver numbering style, as a superscripted number after any punctuation mark. For example:
  "...as reported by Saito and colleagues.[15]"
- Two references are cited separated by a comma, with no space. Three or more consecutive references are given as a range with an en rule. To create an en rule on a PC: hold down CTRL key and minus sign on the number pad, or on a Mac: ALT hyphen
- References in tables, figures, and panels should be in numerical order according to where the item is cited in the text
- Here is an example for a journal reference (note the use of tab, bold, italic, and the en rule or "long" hyphen):
  "...15[tab]Saito N, Ebara S, Ohotsuka K, Kumeta J, Takaoka K. Natural history of scoliosis in spastic cerebral palsy. *Lancet* 1998; **351:** 1687–[en rule]92."
- Give any subpart to the title of the article. Journal names are abbreviated in their standard form as in Index Medicus
- If there are six authors or fewer, give all six in the form:
  surname space initials comma
- If there are seven or more give the first three in the same way, followed by et al
- For a book, give any editors and the publisher, the city of publication, and year of publication
- For a chapter or section of a book, also give the authors and title of the section, and the page numbers
- For online material, please cite the URL, together with the date you accessed the website
- Online journal articles can be cited using the DOI number
- Do not put references in the Summary

Index Medicus
http://www.nlm.nih.gov/

Guidelines for web extra material

All material should be submitted as one PDF (with numbered pages) with the paper and will be peer reviewed. Material will be published at the discretion of *The Lancet* journals' editors. All material should be provided in English.

Text

- Main heading for the web extra material should be in 12 point Times New Roman font **BOLD**
- Text should be in 10 point Times New Roman font, single spaced
- Headings should be in 10 point **BOLD**

Tables

- Main table heading should be in 10 point Times New Roman font **BOLD**
- Legends should be in 10 point, single spaced
- Tables should be in 8 point Times New Roman font, single spaced
- Headings within tables should be in 8 point **BOLD**

Data

- SI units are required
- Numbers in text and tables should always be provided if % is shown
- Means should be accompanied by SDs, and medians by IQR
- Exact p values should be provided, unless p<0·0001

Drug names

- Recommended international non-proprietary name (rINN) is required

References

- Vancouver style—eg,

Formatting guidelines for revised manuscripts

Guidelines on format for text and figures can be found at **http://download.thelancet.com/flatcontentassets/authors/artwork-guidelines.pdf**

Formatting guidelines for randomised trials
http://www.thelancet.com/pb/assets/raw/Lancet/authors/Rctguidelines.pdf

—Smith A, Jones, B, Clements S. Clinical transplantation of tissue-engineered airway. *Lancet* 2008; **372:** 1201–09.

—Hourigan P. Ankle injuries. In: Chan D, ed. Sports medicine. London: Elsevier, 2008: 230–47.

- Numbered in order of mention in Webappendix and numbered separately from references in the full paper

Figures

- All images must have a minimum resolution of 300 dpi, width 107 mm
- Main figure heading should be in 10 point Times New Roman font **BOLD**
- Legends should be in 10 point, single spaced

Audio/video material

- The paper to which the audio or video clip relates should be mentioned in the recording
- Audio clip and video files should be accompanied with brief text explaining the content of the audio, names of interviewers/interviewees, date of recording, and place of recording if relevant
- Written consent from all parties must be supplied at submission

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- Audio material submitted as an mp3 file, no larger than 50 Mb
- Your paper may be selected for a podcast. If so, the Web Editor will contact you to arrange a pre-recorded interview to discuss your paper. For more information, see **Audio**

Audio
http://www.thelancet.com/audio

Video

- Video material should preferably be submitted in .mpg (or .mov, .avi, or .gif) format with aspect ratio of 16:9, no larger than 50 Mb
- We welcome your videos and invite you to submit any video material (reports, interviews, scans, imaging) for consideration in the online journal. Please ensure that all those featured in the video have given permission for publication (see also the above section on **Patient and other consents**)
- All video files can be submitted alongside your article in EES

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- As a member journal of the International Committee for Medical Journal Editors, *The Lancet Infectious Diseases* does not regard results that are posted in the same clinical trials registry in which primary registration resides as prior publication, if the results are presented in the form of a brief structured abstract or table (<500 words; see *Lancet* 2007; **369:** 1909–11). However, presentation of results in other circumstances (eg, investors' meetings) is discouraged and could jeopardise consideration of the manuscript

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- All Articles judged eligible for consideration by the journal's staff will be peer-reviewed within 72 h and, if accepted, published in 4–8 weeks
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Acknowledgment

- Receipt of your paper will be acknowledged by an email containing a reference number, which should be used in all future communications

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- All Reviews, Personal Views, and similar non-research material that we are interested in publishing will be checked by editors using CrossCheck (see *Lancet* 2011; **377:** 281–82). We expect that such papers are written in a way that offers new thinking without recycling previously published text

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- Every Article, Review, Historical Review, Personal View, or Grand Round published in *The Lancet Infectious Diseases* has been peer reviewed. Occasional contributions (eg, commentaries) are accepted without peer review
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- Submissions that survive in-house and peer review might be referred back to authors for revision. This is an invitation to

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APPENDIX E

STARD 2015 Updated Checklist

Table 1. The STARD 2015 list

Section & Topic	No.	Item
TITLE OR ABSTRACT		
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)
ABSTRACT		
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)
INTRODUCTION		
	3	Scientific and clinical background, including the intended use and clinical role of the index test
	4	Study objectives and hypotheses
METHODS		
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)
Participants	**6**	Eligibility criteria
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)
	8	Where and when potentially eligible participants were identified (setting, location and dates)
	9	Whether participants formed a consecutive, random or convenience series
Test methods	**10a**	Index test, in sufficient detail to allow replication
	10b	Reference standard, in sufficient detail to allow replication
	11	Rationale for choosing the reference standard (if alternatives exist)
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test
	13b	Whether clinical information and index test results were available to the assessors of the reference standard
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy
	15	How indeterminate index test or reference standard results were handled
	16	How missing data on the index test and reference standard were handled
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory
	18	Intended sample size and how it was determined
RESULTS		
Participants	**19**	Flow of participants, using a diagram
	20	Baseline demographic and clinical characteristics of participants
	21a	Distribution of severity of disease in those with the target condition
	21b	Distribution of alternative diagnoses in those without the target condition
	22	Time interval and any clinical interventions between index test and reference standard
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)
	25	Any adverse events from performing the index test or the reference standard
DISCUSSION		
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability
	27	Implications for practice, including the intended use and clinical role of the index test
OTHER INFORMATION		
	28	Registration number and name of registry
	29	Where the full study protocol can be accessed
	30	Sources of funding and other support; role of funders

APPENDIX F

De–identified raw data sheets – TB culture & CSF chloride

Number	Labno	Locn	Ward	PatID	Age	Sex	Specimen Date	Specimen	Clinical Data	TB Culture	ID and TTP	CSF Cl	CSF ADA	ZN Stain	GeneXpert	HIV	CD4	Viral Load
1	SCH5625496	RED	D1	108398512	2	F	04/01/2012	CSF	Tuberculous meningitis	Positive for AFB	MTB - 16 days	108		Neg		Neg		
2	SCH5625648	GRS	G17	122812936	50	F	04/01/2012	CSF	Investigation	Negative		118		Neg		Positive	449	
3	SCH5625816	RED	D1	105871131	10	F	04/01/2012	CSF	Tuberculous meningitis	Negative		110		Neg				
4	SCH5628446	RED	S11	122951478	1	M	05/01/2012	CSF	New onset seizures	Negative		120		Neg		Neg		
5	SCH5631794	RED	D1	40441289	3	M	08/01/2012	CSF	Meningitis	Negative		124		Neg		Neg		
6	SCH5645402	RED	D1	96059605	6	F	15/01/2012	CSF	Tuberculous meningitis	Negative		126		Neg		Neg		
7	SCH5645404	RED	S11	96059613	11		15/01/2012	CSF	Tuberculous meningitis ?	Negative		122		Neg		Neg		
8	SCH5648221	RED	ICU	123144651	9	F	17/01/2012	CSF	Tuberculous meningitis ?	Negative		117		Neg		Neg		
9	SCH5660181	RED	D1	122831084	34d	F	21/01/2012	CSF	No clinical details supplied	Negative		111		Neg		Neg		
10	SCH5661163	RED	S11	115277436	1	M	23/01/2012	CSF	Meningitis ?	Negative		119		Neg		Neg		
11	SCH5674262	RED	B2	123326787	18d	M	27/01/2012	CSF	Sepsis	Negative		112		Neg				
12	SCH5675927	GRS	G5	57059578	48	F	29/01/2012	CSF	Laboratory investigation	Negative		121				Positive	121	
13	SCH5675987	RED	A9	20824223	9	F	29/01/2012	CSF	No diagnosis supplied	Negative		122		Neg		Positive		
14	SCH5677299	RED	A9	121952006	87d	F	30/01/2012	CSF	Gastro Enteritis	Negative		128		Neg		Positive		6 485
15	SCH5688032	RED	D1	122167919	5m	F	03/02/2012	CSF	Hydrocephalus empyema	Negative		105		Neg		Neg		
16	SCH5688451	GRS	G22	114568140	35	F	03/02/2012	CSF	Laboratory investigation	Negative		129		Neg		Positive	3	
17	SCH5705767	RED	S11	120620216	6m	F	11/02/2012	CSF	Sepsis	Negative		122		Neg				
18	SCH5713557	RED	S11	119100246	8m	M	15/02/2012	CSF	Meningitis ?,IRRITABLE,FEVER,VOMITING	Negative		120		Neg		Neg		
19	SCH5713702	RED	S11	115144776	1	M	15/02/2012	CSF	Meningitis ?	Negative		122		Neg		Neg		
20	SCH5718729	RED	S11	26387076	5	M	17/02/2012	CSF	Meningitis ?TBM	Negative		124		Neg		Neg		
21	SCH5718738	RED	S11	123792236	9m	M	17/02/2012	CSF	Tuberculous meningitis	Negative		119		Neg		Positive	2 256	10 000 000
22	SCH5724141	RED	S11	107163826	2	M	20/02/2012	CSF	Convulsion seizures	Negative		124		Neg		Neg		
23	SCH5729919	GRS	E7	37056041	35	M	22/02/2012	CSF	Dementia	Negative		129	2.6	Neg		Positive		
24	SCH5732163	RED	S11	115951824	1	F	23/02/2012	CSF	Convulsion SEIZURE	Negative		123		Neg		Neg		
25	SCH5732320	GRS	C15	48113989	33	M	23/02/2012	CSF	Laboratory investigation palsy	Negative		62		Neg		Positive	355	
26	SCH5733974	RED	S12	111622924	1	F	24/02/2012	CSF	seizures	Negative		125		Neg		Neg		
27	SCH5734708	GRS	G17	65425001	32	F	24/02/2012	CSF		Negative		144	<1.0	Neg				
28	SCH5735440	RED	S11	87230967	11	M	24/02/2012	CSF	Tuberculous meningitis ?	Negative		123		Neg		Neg		
29	SCH5747021	GRS	G25	20859658	39	F	01/03/2012	CSF	Laboratory investigation	Negative		151		Neg		Positive		
30	SCH5751224	GRS	C15	20027249	27	F	03/03/2012	CSF	Subarachnoid haemorrhage	Negative		122		Neg				
31	SCH5754196	RED	B1	117900126	3	M	06/03/2012	CSF	Meningitis ?	Negative		116		Neg				
32	SCH5754197	RED	B1	123530941	31d	M	06/03/2012	CSF	No diagnosis supplied	Negative		136		Neg		Neg		
33	SCH5758780	GRS	E7	110097102	57	M	07/03/2012	CSF	Investigation	Negative		121		Neg		Neg		
34	SCH5761953	RED	S11	115144123	1	M	08/03/2012	CSF	Meningitis ?	Negative		120		Neg				
35	SCH5762760	GRS	C15	123950446	24	F	09/03/2012	CSF	Pregnant	Negative		110		Neg		Positive		
36	SCH5763821	GRS	C15	102890597	50	M	09/03/2012	CSF	Investigation	Negative		127		Neg				
37	SCH5764010	RED	D1	107012551	2	M	09/03/2012	CSF	drain - HCP	Negative		122		Neg				
38	SCH5776534	GRS	E7	108391947	49	M	15/03/2012	CSF	Laboratory investigation	Negative		123		Neg		Positive		
39	SCH5778295	WYN	CAS	124112780	11	F	16/03/2012	CSF	Investigation	Positive for AFB	MTB - 13 days	107		Neg				
40	SCH5778460	GRS	C15	29314093	36	F	16/03/2012	CSF	Tuberculous meningitis	Negative		126	<1.0	Neg		Neg		
41	SCH5779751	RED	S11	16056665	11	M	16/03/2012	CSF	Meningitis	Negative		124		Neg		Neg		
42	SCH5779826	GRS	C15	82943143	44	M	16/03/2012	CSF	Illegible diagnosis/ICD10	Negative		124				Neg		
43	SCH5782270	RED	D1	117989590	1	M	18/03/2012	CSF	Hydrocephalus	Positive for AFB	MTB - 11 days	107		Neg				
44	SCH5784315	GRS	G16	124375502	15	F	19/03/2012	CSF	Meningitis	Negative		129		Neg		Neg		
45	SCH5786604	GRS	E7	120065560	24	M	20/03/2012	CSF	Investigation	Negative		130		Neg		Neg		
46	SCH5787079	RED	B1	122852239	4m	M	20/03/2012	CSF	Failure to thrive	Negative		115		Neg		Positive		
47	SCH5789470	RED	S12	117333971	1	M	22/03/2012	CSF	complex febrile seizures	Negative		125		Neg		Neg		
48	SCH5790247	WYN	PAED	108414327	2	F	22/03/2012	CSF		Negative		127		Neg		Neg		
49	SCH5790255	WYN	PAED	115044943	1	M	22/03/2012	CSF	Meningitis	Negative		125		Neg				
50	SCH5795962	RED	S11	121515126	10	F	25/03/2012	CSF	Tuberculous meningitis ?	Negative		121		Neg		Neg		
51	SCH5797103	GRS	G25	16406027	42	M	26/03/2012	CSF	Investigation	Positive for AFB	MTB - 23 days	100		Neg		Positive	230	
52	SCH5806323	RED	S12	36925014	4	M	30/03/2012	CSF	Meningitis ?	Negative		122		Neg		Neg		
53	SCH5816303	RED	S11	117049924	1	M	04/04/2012	CSF	Meningitis ?	Negative		122		Neg		Neg		
54	SCH5817559	GRS	G25	104008487	39	M	04/04/2012	CSF	Investigation	Negative		116		Neg		Positive	79	738 415
55	SCH5823405	GRS	G16	62070685	38	M	08/04/2012	CSF	Laboratory investigation	Negative		124	<1.0	Neg		Neg		
56	SCH5828253	GRS	E7	114056419	32	F	11/04/2012	CSF	Laboratory investigation	Negative		142		Neg		Positive		
57	SCH5828262	GRS	E7	53630521	49	F	11/04/2012	CSF	Laboratory investigation	Negative		85		Neg				
58	SCH5828819	RED	ICU	87129052	11	F	11/04/2012	CSF	Tuberculous meningitis	Negative		104		Neg		Positive	28	<20
59	SCH5830103	GRS	G12	112855853	33	F	12/04/2012	CSF	Investigation	Negative		120		Neg				
60	SCH5835294	GRS	C15	124065988	52	M	14/04/2012	CSF	Investigation	Negative		122		Neg		Neg		
61	SCH5842622	RED	S12	124847492	11	M	18/04/2012	CSF		Negative		117		Neg		Neg		
62	SCH5842857	GRS	E7	87158242	44	M	18/04/2012	CSF	Laboratory investigation	Negative		112		Neg		Neg		
63	SCH5865795	RED	D1	124855081	39d	M	30/04/2012	CSF	Hydrocephalus ? Meningitis	Negative		106		Neg		Neg		
64	SCH5866849	RED	ICU	125016113	2	M	01/05/2012	CSF	Encephalopathy ?cause	Negative		120		Neg				
65	SCH5869327	GRS	C15	85589299	53	F	02/05/2012	CSF	Meningitis ?	Negative		120		Neg		Neg		

66	SCH5869956	GRS	E7	102741154	33	F	03/05/2012	CSF	No clinical details supplied	Negative		125		Neg		Positive	688	
67	SCH5873852	RED	D1	117699736	1	F	04/05/2012	CSF	Hydrocephalus	Negative		107		Neg		Neg		
68	SCH5875762	RED	ICU	125142513	10m	M	05/05/2012	CSF	?subdural haematoma	Negative		118		Neg				
69	SCH5876629	WYN	CAS	37797123	6	M	06/05/2012	CSF	Investigation	Negative		127		Neg				
70	SCH5889784	RED		124434713	48d	M	11/05/2012	CSF	Investigation	Negative		119		Neg				
71	SCH5890002	RED	B1	125137430	11m	M	11/05/2012	CSF	Meningitis	Negative		131		Neg		Positive		
72	SCH5905802	RED	ICU	17474313	6	F	19/05/2012	CSF	Hydrocephalus ?tbm	Positive for AFB	MTB - 16 days	109		Neg				
73	SCH5907295	RED	S12	124365321	65d	M	20/05/2012	CSF	Meningitis uti	Negative		121		Neg		Neg		
74	SCH5917181	RED	B1	125485862	5m	M	24/05/2012	CSF	Tuberculous meningitis ?	Negative		118		Neg		Neg		
75	SCH5918769	RED	S11	34548586	12	M	25/05/2012	CSF	Meningitis ?	Positive for AFB	MTB - 18 days	106		Neg				
76	SCH5924105	GRS	G12	55623441	62	F	28/05/2012	CSF	Investigation	Negative		123		Neg				
77	SCH5925100	RED	B2	89078539	9	F	29/05/2012	CSF	Tuberculous meningitis ?	Negative		115		Neg		Neg		
78	SCH5934412	GRS	C15	125637942	45	M	01/06/2012	CSF	Investigation	Negative		132		Neg		Neg		
79	SCH5935423	RED	S12	123875700	1	M	02/06/2012	CSF	Meningitis	Negative		122		Neg		Neg		
80	SCH5936706	RED	B1	124117292	3m	F	03/06/2012	CSF	Meningitis jml	Negative		120		Neg		Neg		
81	SCH5940101	GRS	C15	16377541	20	M	05/06/2012	CSF	Meningitis	Negative		118		Neg		Neg		
82	SCH5959327	RED	ICU	43507789	3	M	14/06/2012	CSF	Meningitis	Negative		120		Neg		Neg		
83	SCH5959556	GRS	C15	20542197	33	F	14/06/2012	CSF	Meningitis	Negative		123		Neg		Positive	251	
84	SCH5959932	RED	ICU	119621902	1	M	14/06/2012	CSF	Laboratory investigation	Negative		112		Neg		Neg		
85	SCH5962675	WYN	CAS	46502019	6	M	15/06/2012	CSF	Laboratory investigation	Negative		123						
86	SCH5972967	RED	B2	107900060	2	M	20/06/2012	CSF	Meningitis	Negative		117		Neg		Neg		
87	SCH5974685	GRS	C15	105333074	37	M	21/06/2012	CSF	Laboratory investigation	Negative		118		Neg		Positive	479	
88	SCH5976853	GRS	E7	31806680	47	F	22/06/2012	CSF	Laboratory investigation	Negative		128		Neg		Neg		
89	SCH5988226	GRS	D13	27257278	42	M	28/06/2012	CSF	Laboratory investigation	Negative		118		Neg		Neg		
90	SCH5991383	GRS	E7	102138211	36	M	29/06/2012	CSF	Laboratory investigation	Negative		126		Neg		Positive		663
91	SCH5998205	RED	E2	123840852	9	F	03/07/2012	CSF	#MS	Negative		123		Neg				
92	SCH5999561	GRS	F7	102061470	50	F	04/07/2012	CSF	Investigation	Negative		104		Neg		Positive	352	
93	SCH6004607	GRS	G16	116824095	36	M	06/07/2012	CSF	Investigation	Negative		130		Neg		Positive	171	
94	SCH6005448	RED	S11	124263898	4m	M	06/07/2012	CSF	Meningitis HIV/?BCG	Negative		122		Neg		Positive		10 000 000
95	SCH6008969	GRS	K41	124761495	28	F	09/07/2012	CSF	Laboratory investigation	Negative		121		Neg		Positive	217	
96	SCH6017363	GRS	G42	23086614	33	F	12/07/2012	CSF	Investigation	Negative		120		Neg			250	
97	SCH6019831	RED	MREG	30263867	5	F	13/07/2012	CSF	Tuberculous meningitis	Negative		101		Neg		Neg		
98	SCH6024488	WYN	CUR3	123446007	6m	M	16/07/2012	CSF	Pneumonia	Negative		112		Neg		Positive	403	1 967 875
99	SCH6029391	GRS	E7	107576704	49	M	18/07/2012	CSF	Investigation	Negative		121		Neg		Positive		
100	SCH6032345	GRS	C15	13643366	28	M	19/07/2012	CSF	No clinical details supplied	Negative		118		Neg		Positive	20	
101	SCH6038791	RED	B1	103854766	11	F	23/07/2012	CSF	Tuberculosis	Negative		122		Neg		Positive	155	104 415
102	SCH6051189	GRS	C15	68968460	49	M	29/07/2012	CSF	No clinical details supplied	Negative		108		Neg		Neg		
103	SCH6051451	GRS	C15	12863486	32	F	29/07/2012	CSF	Tuberculous meningitis	Negative		105		Neg		Positive	94	
104	SCH6051553	GRS	C15	129304614	32	F	29/07/2012	CSF	Psychosis	Negative		124		Neg		Positive	121	
105	SCH6055186	GRS	E7	117122358	37	M	31/07/2012	CSF	Investigation	Negative		121		Neg		Positive	514	<LoD
106	SCH6059490	GRS	C15	129373270	23	M	02/08/2012	CSF	Laboratory investigation	Negative		121		Neg				
107	SCH6063452	GRS	E7	36331825	33	F	03/08/2012	CSF	Laboratory investigation	Negative		131		Neg		Positive	32	3 166 981
108	SCH6066155	GRS	C15	22681969	42	F	05/08/2012	CSF	Investigation	Negative		113				Positive	88	
109	SCH6071769	GRS	C15	129463352	51	M	08/08/2012	CSF	Investigation	Negative		126		Neg		Neg		
110	SCH6079505	GRS	F17	16955130	26	F	13/08/2012	CSF	Investigation	Negative		116		Neg		Positive	155	56 280
111	SCH6080251	GRS	C27	115127409	56	M	13/08/2012	CSF	Investigation	Negative		119		Neg		Neg		
112	SCH6080317	RED		114959646	1	F	13/08/2012	CSF	Meningitis	Negative		120		Neg		Positive	682	63 905
113	SCH6082292	RED	ICU	26105031	5	F	14/08/2012	CSF	Tuberculous meningitis HCP	Positive for AFB	MTB - 15 days	110		Neg				
114	SCH6083178	RED	B1	119878163	1	F	14/08/2012	CSF	Pulmonary tuberculosis	Negative		119		Neg		Neg		
115	SCH6083872	WYN	CUR3	120984356	11m	F	14/08/2012	CSF	Tuberculous meningitis ?	Negative		123		Neg		Positive	131	119 945
116	SCH6084480	SOM	KING	19744903	38	M	15/08/2012	CSF	Meningitis	Negative		128	1.5	Neg		Neg		
117	SCH6084558	SOM	1FL	35223627	57	M	15/08/2012	CSF	Laboratory investigation	Negative		120		Neg		Positive		5 868
118	SCH6085688	RED		118095546	1	M	15/08/2012	CSF	Meningitis	Negative		123		Neg				
119	SCH6101404	SOM	CAS	125850966	38	M	22/08/2012	CSF	Investigation	Negative		118		Neg		Positive	10	
120	SCH6104648	RED	D1	33218306	4	F	23/08/2012	CSF	Tuberculosis abdomen, HCP	Negative		120		Neg				
121	SCH6107147	GRS	C15	74270067	43	M	24/08/2012	CSF	Investigation	Negative		124		Neg		Positive	341	176 118
122	SCH6107153	GRS	E7	16825176	25	F	24/08/2012	CSF	Investigation	Negative		124		Neg		Neg		
123	SCH6108697	GRS	C15	108694084	20	F	25/08/2012	CSF	Laboratory investigation	Negative		131		Neg		Neg		
124	SCH6109018	SOM	CAS	126022995	38	M	25/08/2012	CSF	Investigation	Negative		127	<1.0	Neg				
125	SCH6110469	GRS	C15	86567385	43	M	27/08/2012	CSF	Laboratory investigation	Negative		119				Positive	43	
126	SCH6110736	GRS	C15	103127023	34	F	27/08/2012	CSF	Laboratory investigation	Negative		142	<1.0	Neg		Positive	188	
127	SCH6112157	RED	B2	14134407	7	F	27/08/2012	CSF	No clinical details supplied	Negative		125		Neg		Neg		
128	SCH6116751	GRS	G17	20149902	26	F	29/08/2012	CSF	Investigation	Negative		133		Neg		Positive	12	5 654 080
129	SCH6119306	RED	S11	119468429	1	F	30/08/2012	CSF	Laboratory investigation	Negative		122				Neg		
130	SCH6119572	GRS	G7	80074206	55	F	30/08/2012	CSF	Investigation	Negative		101		Neg		Neg		
131	SCH6122130	GRS	C15	129872487	34	M	31/08/2012	CSF	Investigation	Negative		124				Neg		

132	SCH6126552	GRS	G16	129814497	20	M	03/09/2012	CSF	Investigation	Negative		121		Neg		Positive	213	
133	SCH6130119	GRS	E7	102676418	52	F	04/09/2012	CSF	Meningitis	Negative		129		Neg				
134	SCH6133954	GRS	E7	19671809	30	M	05/09/2012	CSF	Cryptococcal meningitis	Negative		114		Neg		Positive	7	113 894
135	SCH6135821	GRS	G17	12962049	37	F	06/09/2012	CSF	Investigation	Negative		131		Neg				
136	SCH6137903	RED	B1	123580300	7m	M	07/09/2012	CSF	Investigation	Negative		116		Neg		Positive	1 201	24 840
137	SCH6139588	GRS	C15	124130204	25	M	07/09/2012	CSF	Laboratory investigation	Positive for AFB	MTB - 18 days	96		Positive AFB		Positive	77	1 345
138	SCH6140959	GRS	D13	20547089	49	M	08/09/2012	CSF	Investigation	Negative		120		Neg		Neg		
139	SCH6142053	SOM		102945516	36	M	09/09/2012	CSF	Illegible diagnosis/ICD10	Negative		128		Neg		Positive	34	
140	SCH6143701	RED	B1	31420391	5	M	10/09/2012	CSF	Tuberculous meningitis ?	Negative		106		Neg		Neg		
141	SCH6144303	RED	S11	119434934	1	M	10/09/2012	CSF	Tuberculous meningitis	Negative		121				Neg		
142	SCH6145422	RED	S11	47783154	9	M	11/09/2012	CSF	Laboratory investigation	Negative		123						
143	SCH6146313	MOS	C	2347292	41	F	11/09/2012	CSF		Negative		113		Neg		Positive	304	
144	SCH6150140	RED	S11	36135705	7	F	12/09/2012	CSF	Meningitis	Negative		125		Neg		Positive	431	24 621
145	SCH6150251	GRS	C13	81778664	17	F	12/09/2012	CSF	Meningitis	Negative		119		Neg		Positive	346	
146	SCH6152796	GRS	G16	129807673	30	M	13/09/2012	CSF	Laboratory investigation	Negative		121		Neg		Neg		
147	SCH6156280	GRS	C15	28653707	33	M	14/09/2012	CSF	Encephalopathy	Positive for AFB	MTB - 14 days	111		Neg				
148	SCH6157286	GRS	C15	37430766	28	F	15/09/2012	CSF	No clinical information	Negative		120	<1.0	Neg				
149	SCH6157326	FAL		117395350	25	F	15/09/2012	CSF	Meningitis	Negative		100				Positive	187	
150	SCH6157976	SOM		16922106	27	F	15/09/2012	CSF	Laboratory investigation	Negative		122						
151	SCH6159506	RED		124610155	1	M	17/09/2012	CSF	Meningitis	Negative		114		Neg		Neg		
152	SCH6160685	RED	S11	120505482	1	M	17/09/2012	CSF	No clinical details supplied	Negative		120		Neg		Neg		
153	SCH6160686	RED	MREG	32455974	10	M	17/09/2012	CSF	No clinical details supplied	Negative		119		Neg				
154	SCH6167044	GRS	E7	25349598	53	F	19/09/2012	CSF	Investigation	Negative		123		Neg		Neg		
155	SCH6167105	SOM	KING	129887741	22	M	19/09/2012	CSF	Investigation	Negative		132		Neg		Neg		
156	SCH6170773	RED	MOPD	118498831	1	F	21/09/2012	CSF	No clinical details supplied	Positive for AFB	MTB - 24 days	104		Neg				
157	SCH6172005	GRS	E7	12744470	42	F	21/09/2012	CSF	Laboratory investigation	Negative		123		Neg		Positive	344	
158	SCH6173172	WYN	CAS	14722466	11	M	21/09/2012	CSF	Meningitis ?	Negative		128						
159	SCH6173933	RED		125216101	5m	M	22/09/2012	CSF	Meningitis Atypical Febrile seizures	Negative		121		Neg		Neg		
160	SCH6174054	KNY	2	20613147	44	M	22/09/2012	CSF	Tuberculous meningitis	Negative		123		Neg		Neg		
161	SCH6175036	SOM	KING	23094774	62	M	23/09/2012	CSF	Laboratory investigation	Negative		98		Neg		Neg		
162	SCH6177936	RED	B1	111961397	4	M	25/09/2012	CSF	Sepsis TB	Negative		119		Neg		Positive	184	673 880
163	SCH6179454	RED		45519733	3	M	26/09/2012	CSF	Laboratory investigation	Negative		119				Neg		
164	SCH6187661	GRS	C15	81988859	41	F	29/09/2012	CSF	Investigation	Negative		126						
165	SCH6188969	RED	MREG	123709685	2	M	01/10/2012	CSF	Hydrocephalus meningitis	Negative		115		Neg		Neg		
166	SCH6190255	GRS	G16	26339382	46	F	01/10/2012	CSF	Investigation	Negative		117		Neg		Positive	145	
167	SCH6191446	RED	MREG	89088256	9	F	02/10/2012	CSF	Meningitis ?	Negative		122		Neg		Neg		
168	SCH6199004	SOM	BICK	130220163	29	F	04/10/2012	CSF	Investigation	Negative		119		Neg		Positive	289	<LoD
169	SCH6199220	RED	MREG	124225053	6	F	04/10/2012	CSF	Meningitis ?	Negative		121		Neg				
170	SCH6201701	SOM	BICK	88044854	64	F	05/10/2012	CSF	Investigation	Negative		141	<1.0	Neg		Neg		
171	SCH6201765	GRS	C15	61974820	48	F	05/10/2012	CSF	Laboratory investigation	Negative		123		Neg		Neg		
172	SCH6201860	RED	S11	130466915	1	M	05/10/2012	CSF	Tuberculous meningitis ?	Negative		121		Neg		Neg		
173	SCH6204414	GRS	MOPD	33718248	46	F	07/10/2012	CSF	Tuberculosis	Positive for AFB	MTB - 15 days	125		Neg		Positive	76	
174	SCH6206350	RED	ICU	15224736	11	F	08/10/2012	CSF	Meningitis ?	Negative		105		Neg				
175	SCH6206523	RED	B1	116252438	1	M	08/10/2012	CSF	Tuberculous meningitis	Negative		115		Neg		Neg		
176	SCH6218089	GRS	G16	31502461	60	M	12/10/2012	CSF	Laboratory investigation	Negative		124	<1.0	Neg		Neg		
177	SCH6218223	WYN		130608276	3	M	12/10/2012	CSF	Tuberculous meningitis	Negative		125		Neg				
178	SCH6219322	GRS	C15	44435444	34	F	13/10/2012	CSF	Laboratory investigation	Negative		123		Neg		Positive	305	975
179	SCH6219908	GRS	C15	70241468	26	F	13/10/2012	CSF	No clinical details supplied	Negative		127		Neg			333	
180	SCH6220636	RED	B1	105852099	2	F	14/10/2012	CSF	Investigation	Negative		122		Neg		Positive	1 007	78 200
181	SCH6220648	RED	S11	128915998	1	M	14/10/2012	CSF	Investigation	Negative		118		Neg		Neg		
182	SCH6226875	RED	S11	26679902	5	F	17/10/2012	CSF	No clinical details supplied	Negative		116		Neg		Neg		
183	SCH6230450	RED	S11	111421707	3	M	18/10/2012	CSF	Laboratory investigation	Negative		120		Neg		Neg		
184	SCH6234676	GRS	C15	106838089	21	F	19/10/2012	CSF	Meningitis	Negative		124		Neg				
185	SCH6236480	SOM	KING	55062285	53	M	20/10/2012	CSF	Laboratory investigation	Negative		132	<1.0	Neg				
186	SCH6237200	GRS	C15	86724382	38	F	22/10/2012	CSF	Meningitis ?	Positive for AFB	MTB - 15 days	112		Neg		Positive	45	
187	SCH6238619	RED	S12	114482938	2	M	22/10/2012	CSF	Investigation	Negative		122		Neg		Neg		
188	SCH6238935	GRS	G17	24237448	40	F	22/10/2012	CSF	Laboratory investigation	Negative		138		Neg		Positive	54	
189	SCH6240291	GRS	G17	120221221	29	M	23/10/2012	CSF	Meningitis	Negative		129		Neg		Positive	1 005	
190	SCH6241751	SOM	KING	130676836	44	M	23/10/2012	CSF	Investigation	Negative		140	1.8	Neg				
191	SCH6243451	RED	MREG	117791954	1	F	24/10/2012	CSF	Sepsis low LOC	Negative		111				Neg		
192	SCH6244002	KNY	2	20155487	32	M	24/10/2012	CSF	?MENINGITIS	Negative		120	<1.0	Neg		Positive	299	<20
193	SCH6246954	KNY		21561451	57	F	25/10/2012	CSF	Cerebro-vascular accident	Negative		121	<1.0			Neg		
194	SCH6247473	GRS	G17	129990198	38	M	25/10/2012	CSF	Laboratory investigation	Negative		131		Neg		Positive	24	
195	SCH6247798	GRS	G5	89260376	9	M	25/10/2012	CSF	Laboratory investigation	Negative		125		Neg				
196	SCH6253715	SOM	EDB	23769383	18	F	29/10/2012	CSF	Investigation	Negative		123		Neg				
197	SCH6254228	WYN	CAS	124700949	7	M	29/10/2012	CSF	Laboratory investigation	Negative		135		Neg				

198	SCH6257389	SOM	CAS	130900459	44	M	30/10/2012	CSF	Investigation	Negative		118		Neg		Neg		
199	SCH6260034	LEN		124295882	23	F	31/10/2012	CSF	Investigation	Negative		125		Neg		Neg		
200	SCH6267454	GRS	C15	17754128	65	M	03/11/2012	CSF	Investigation	Positive for AFB	MTB - 16 days	113		Neg				
201	SCH6269062	GRS	C15	12950291	33	F	05/11/2012	CSF	Investigation	Negative		109		Neg		Positive		
202	SCH6278894	WYN	CAS	11142213	13	M	08/11/2012	CSF	Meningitis	Negative		126		Neg				
203	SCH6282048	GRS	C15	130943681	21	M	10/11/2012	CSF	Laboratory investigation	Negative		125		Neg		Neg		
204	SCH6282350	RED	ICU	131100018	4m	M	10/11/2012	CSF	No clinical details supplied	Negative		120		Neg		Neg		
205	SCH6282387	SOM	CAS	131122640	31	F	10/11/2012	CSF	Investigation	Negative		>150		Neg		Positive	97	
206	SCH6285271	SOM	BARK	85425031	25	F	12/11/2012	CSF	Laboratory investigation	Negative		141		Neg				
207	SCH6288428	SOM	BICK	47842943	80	F	13/11/2012	CSF	Laboratory investigation	Negative		148		Neg				
208	SCH6290009	RED	MREG	115020646	2	F	14/11/2012	CSF	Meningitis ?,hcp	Negative		120		Neg				
209	SCH6291095	SOM	KING	131076630	34	M	14/11/2012	CSF	Laboratory investigation	Negative		118		Neg		Neg		
210	SCH6291662	GRS	G12	57822140	71	M	14/11/2012	CSF	Delirium	Negative		123		Neg				
211	SCH6299405	RED	B2	131250623	6	M	17/11/2012	CSF	Meningitis	Negative		123		Neg		Neg		
212	SCH6301104	WYN	CAS	40214769	10	F	19/11/2012	CSF	Laboratory investigation	Negative		107		Neg				
213	SCH6301934	RED	S11	39949599	8	M	19/11/2012	CSF	Tuberculous meningitis ?	Negative		120		Neg		Neg		
214	SCH6303940	GRS	E7	119272912	22	F	20/11/2012	CSF	Laboratory investigation	Negative		125	<1.0	Neg				
215	SCH6304195	RED	S11	131260598	11	M	20/11/2012	CSF	Tuberculous meningitis ?	Negative		122		Neg		Positive	226	157 082
216	SCH6304581	SOM	KING	35241447	47	M	20/11/2012	CSF	Laboratory investigation	Negative		121		Neg				
217	SCH6310323	RED	S12	37953684	4	M	22/11/2012	CSF	Laboratory investigation	Negative		119		Neg				
218	SCH6311992	RED	MREG	123330771	1	M	23/11/2012	CSF	Tuberculous meningitis	Negative		101		Neg		Neg		
219	SCH6313251	GRS	G16	13793088	29	F	23/11/2012	CSF	Laboratory investigation	Negative		120		Neg			594	
220	SCH6315745	GRS	C15	116744947	31	F	26/11/2012	CSF	No clinical details supplied	Negative		109	3.4	Neg		Positive		
221	SCH6319536	GRS	C15	10052496	33	F	27/11/2012	CSF	Investigation	Positive for AFB	MTB - 14 days	103				Positive		
222	SCH6320024	GRS	C15	40412751	34	M	27/11/2012	CSF	Laboratory investigation	Negative		122		Neg				
223	SCH6325215	GRS	G22	33490913	44	F	29/11/2012	CSF	Psychosis	Negative		123	<1.0	Neg		Positive	245	
224	SCH6325464	GRS	G25	61490827	66	M	29/11/2012	CSF		Negative		119		Neg		Neg		
225	SCH6326844	GRS	C15	43789718	27	F	30/11/2012	CSF	Meningitis TB?	Negative		121	<1.0	Neg		Positive	36	
226	SCH6326982	KNY		20205050	44	F	30/11/2012	CSF	No clinical details supplied	Negative		120		Neg				
227	SCH6327603	GRS	G16	50502236	54	M	30/11/2012	CSF		Negative		108		Neg				
228	SCH6329940	WYN	CAS	123245771	32	M	01/12/2012	CSF	Investigation	Negative		119		Neg		Positive	86	
229	SCH6340839	GRS	C15	124287244	41	M	06/12/2012	CSF	Meningitis ?	Negative		122		Neg			47	
230	SCH6345046	RED	D1	131049280	32d	F	09/12/2012	CSF	Meningitis	Negative		103		Neg				
231	SCH6347186	GRS	C15	33513102	53	M	10/12/2012	CSF	Laboratory investigation	Negative		121	5.8	Neg		Positive	123	
232	SCH6348863	GRS	F5	41090390	37	F	11/12/2012	CSF	Laboratory investigation	Negative		128	<1.0	Neg		Positive	545	
233	SCH6349678	RED	ICU	46516571	3	F	11/12/2012	CSF	Miliary TB ?	Negative		120		Neg				
234	SCH6352410	GRS	G25	63247506	68	F	12/12/2012	CSF	Laboratory investigation	Negative		120		Neg		Positive	36	
235	SCH6352480	SOM	EDB	131592818	32d	M	12/12/2012	CSF	? Meningitis	Negative		119						
236	SCH6359871	GRS	G17	19791912	61	F	16/12/2012	CSF	No clinical details supplied	Negative		130		Neg		Neg		
237	SCH6361304	GRS	C15	103084083	48	F	18/12/2012	CSF	Laboratory investigation	Negative		120		Neg				
238	SCH6361980	GRS	C15	20463055	30	F	18/12/2012	CSF	Laboratory investigation	Negative		121					270	
239	SCH6364201	GRS	C15	26352252	40	M	19/12/2012	CSF	Tuberculous meningitis	Negative		140		Neg		Positive	223	
240	SCH6365877	GRS	C15	10617280	33	M	20/12/2012	CSF	Investigation	Negative		118	<1.0	Neg		Neg	128	
241	SCH6366876	RED	B2	131770216	8m	M	20/12/2012	CSF	Meningitis	Negative		122		Neg				
242	SCH6374305	RED	B2	36467405	9	F	27/12/2012	CSF	Meningitis ?	Negative		118		Neg		Neg		
243	SCH6375471	GEOSA		109734459	47	F	28/12/2012	CSF	Pulmonary tuberculosis	Negative		107		Neg				
244	SCH6375908	WYN	CAS	130167380	40	M	28/12/2012	CSF	Laboratory investigation	Negative		126						
245	SCH6375911	GRS	C15	25505991	66	M	28/12/2012	CSF	Laboratory investigation	Negative		122				Positive	114	
246	SCH6377865	RED	MREG	103887683	3	F	31/12/2012	CSF	Hydrocephalus	Negative		115		Neg				
247	SCH6378225	GRS	C15	131870289	80	M	31/12/2012	CSF	Laboratory investigation	Negative		128		Neg		Neg		
248	SCH6378545	RED	MREG	46075933	3	M	31/12/2012	CSF	Investigation	Negative		125		Neg		Neg		
249	SCH6380520	RED	E2	85998375	13	F	02/01/2013	CSF	Post renal transplant	Negative		128		Neg		Neg		
250	SCH6382598	KNY	1	20140059	48	M	04/01/2013	CSF		Negative		111		Neg				
251	SCH6382601	KNY	4	20863361	38	F	04/01/2013	CSF	Meningitis	Negative		111		Neg			14	
252	SCH6382602	KNY	2	20415402	36	M	04/01/2013	CSF	?CONFUSION	Negative		123		Neg		Positive		100
253	SCH6382797	KNY		20411617	37	F	04/01/2013	CSF	Diagnosis not stated	Negative		116		Neg		Positive		
254	SCH6383937	GRS	G16	72289358	47	F	04/01/2013	CSF	Investigation	Negative		127		Neg		Neg		
255	SCH6385814	GRS	E12	74734526	25	M	06/01/2013	CSF	Investigation	Negative		124		Neg		Neg		
256	SCH6389426	RED	A9	131549198	35d	M	08/01/2013	CSF	Sepsis ?meningitis	Negative		120		Neg		Neg		
257	SCH6395584	KNY	1	21445697	3	F	11/01/2013	CSF	SEIZURES	Negative		122				Neg		
258	SCH6405626	GRS	D13	54666953	35	M	16/01/2013	CSF	Laboratory investigation	Negative		124		Neg				
259	SCH6405680	GRS	G25	67876433	41	M	16/01/2013	CSF	Investigation	Negative		109		Neg		Neg		
260	SCH6414875	GRS	C15	47617212	28	M	21/01/2013	CSF	? Pulmonary TB	Negative		125		Neg				
261	SCH6416343	GRS	C15	80577372	77	M	21/01/2013	CSF	Laboratory investigation	Negative		125		Neg				
262	SCH6418245	GRS	G17	56219322	61	M	22/01/2013	CSF	Investigation	Negative		131		Neg		Neg		
263	SCH6421616	KNY	4	21580758	28	F	23/01/2013	CSF	Tuberculous meningitis	Negative		117						

264	SCH6424832	GRS	C15	86924446	37	M	24/01/2013	CSF	Investigation	Negative		125		Neg		Neg		
265	SCH6433656	KNY		20976155	10	F	29/01/2013	CSF	Diagnosis not stated	Negative		123		Neg				
266	SCH6439283	WEF	ARV	46557765	37	F	31/01/2013	CSF	Investigation	Negative		124	<1.0			Positive		
267	SCH6440810	KNY		20760583	39	F	01/02/2013	CSF	?TB MENINGITIS	Negative		117		Neg			200	
268	SCH6451776	RED	S11	115686420	2	M	06/02/2013	CSF	Meningitis ?	Negative		117		Neg		Neg		
269	SCH6451778	RED	MOPD	14525042	7	M	06/02/2013	CSF	Meningitis	Negative		119		Neg		Neg		
270	SCH6456981	KNY	OPD	20125365	35	M	08/02/2013	CSF		Negative		103		Neg				
271	SCH6458775	GRS	G8	62115209	64	M	08/02/2013	CSF	Investigation	Negative		111		Neg		Neg		
272	SCH6461059	GRS	G17	63922926	55	M	10/02/2013	CSF	No clinical details supplied	Negative		121		Neg		Neg		
273	SCH6465174	KNY	4	21317698	20	F	12/02/2013	CSF		Negative		121		Neg				
274	SCH6465912	GRS	G17	112493911	15	F	12/02/2013	CSF	Tuberculous meningitis	Negative		115		Neg		Positive	400	
275	SCH6466615	RED		131944225	46d	M	12/02/2013	CSF	Meningitis ?	Negative		116		Neg				
276	SCH6469153	LEN	16A	125328815	37	F	13/02/2013	CSF	Illegible diagnosis/ICD10	Negative		123		Neg			388	
277	SCH6472498	RED	B1	45155934	4	M	14/02/2013	CSF	TBM ?	Negative		104		Neg		Neg		
278	SCH6474231	RED	S11	119316099	8	F	15/02/2013	CSF	Investigation	Negative		121		Neg		Neg		
279	SCH6477269	RED	B1	123118937	1	M	16/02/2013	CSF	Tuberculous meningitis ?	Negative		127		Neg		Positive	169	
280	SCH6478217	RED	MREG	132734534	1	F	17/02/2013	CSF	No clinical details supplied	Negative		120		Neg		Neg		
281	SCH6479666	GRS	F7	129182739	17	M	18/02/2013	CSF	Investigation	Negative		121		Neg				
282	SCH6480555	SOM	CAS	103232252	31	F	18/02/2013	CSF	Meningitis	Negative		122		Neg				
283	SCH6482031	SOM		111070009	25	M	19/02/2013	CSF	Meningitis	Negative		122						
284	SCH6483540	BROCH	B	121374946	1	F	19/02/2013	CSF	Laboratory investigation	Negative		122		Neg				
285	SCH6484836	RED	S11	43650654	4	M	20/02/2013	CSF	Meningitis ?	Negative		122		Neg				
286	SCH6486360	SOM		42574228	15	M	20/02/2013	CSF	Laboratory investigation	Negative		123		Neg				
287	SCH6490358	KNY		21600028	9	F	22/02/2013	CSF	Diagnosis not stated	Negative		111		Neg		Neg		
288	SCH6491430	GRS	G25	132754979	72	F	22/02/2013	CSF	Investigation	Negative		105		Neg		Neg		
289	SCH6493824	GRS	E7	32829475	43	M	23/02/2013	CSF	Laboratory investigation	Negative		127		Neg		Positive		<LoD
290	SCH6495169	GRS	C15	58854118	38	M	24/02/2013	CSF	Laboratory investigation	Negative		116		Neg		Neg		
291	SCH6498600	GRS	D13	19401736	40	F	26/02/2013	CSF	Investigation	Positive for AFB	MTB - 14 days	108		Neg				
292	SCH6499584	KNY	2	21588314	41	M	26/02/2013	CSF	Retroviral Disease	Negative		102		Neg				
293	SCH6499879	GRS	C15	89024475	42	F	26/02/2013	CSF	TBM on Rx	Positive for AFB	MTB - 20 days	95		Neg		Positive	402	
294	SCH6500779	RED	D1	124690363	10m	M	27/02/2013	CSF	Hydrocephalus	Negative		114		Neg				
295	SCH6501819	RED	B1	13253331	12	M	27/02/2013	CSF	large vessel vasculopathy	Negative		122		Neg		Neg		
296	SCH6502634	SOM	BAIL	41029729	36	F	27/02/2013	CSF	Investigation	Positive for AFB	MTB - 34 days	128		Neg		Positive	13	
297	SCH6510580	RED	S11	37322575	6	M	03/03/2013	CSF	delirius	Positive for AFB	MTB - 17 days	107		Neg		Positive		<LoD
298	SCH6511372	RED	S11	131975948	56d	F	04/03/2013	CSF	seizures	Negative		123		Neg		Neg		
299	SCH6514477	RED	S11	132948365	1	M	05/03/2013	CSF	Investigation	Negative		122		Neg		Neg		
300	SCH6515726	RED	D1	123148454	7	F	05/03/2013	CSF	Tuberculous meningitis	Negative		110		Neg				
301	SCH6517589	SOM	CAS	15885262	36	F	06/03/2013	CSF	Laboratory investigation	Negative		127		Neg				
302	SCH6518316	GRS	G17	85997419	36	F	06/03/2013	CSF	Laboratory investigation	Negative		131		Neg		Positive	51	233 654
303	SCH6518594	GRS	E7	20822052	37	M	06/03/2013	CSF	Laboratory investigation	Negative		124		Neg		Positive	230	<LoD
304	SCH6518990	GRS	G17	125416438	43	M	06/03/2013	CSF	Meningitis	Negative		112		Neg		Positive	116	
305	SCH6520156	RED	S12	13683776	8	M	07/03/2013	CSF	Meningitis	Negative		115		Neg				
306	SCH6521964	FAL		124497793	1	F	07/03/2013	CSF	Laboratory investigation	Negative		130		Neg		Positive	463	10 000 000
307	SCH6523072	RED	ICU	88607734	10	M	08/03/2013	CSF	Encephalitis	Negative		124		Neg				
308	SCH6523341	KNY	4	20195467	29	F	08/03/2013	CSF	Tuberculous meningitis	Negative		126		Neg			552	
309	SCH6524657	SOM	EDB	133088328	12	F	08/03/2013	CSF	Meningitis Infarct	Negative		120		Neg		Positive	35	138 555
310	SCH6528001	GRS	G17	65696940	28	F	11/03/2013	CSF	Laboratory investigation	Negative		118	2.1	Neg		Neg		
311	SCH6532816	KNY		21603287	26	F	13/03/2013	CSF	TB Investigation	Negative		121		Neg				
312	SCH6535210	RED	ICU	133166306	2	M	14/03/2013	CSF	Meningitis TB	Positive for AFB	MTB - 14 days	112		Neg				
313	SCH6536521	RED	E2	47775978	4	F	14/03/2013	CSF	No clinical details supplied	Negative		123		Neg		Neg		
314	SCH6537027	RED	A9	118435601	1	M	14/03/2013	CSF	Tuberculous meningitis	Negative		115		Neg		Positive	297	
315	SCH6538196	RED	S12	41392283	11	F	15/03/2013	CSF	Meningitis	Negative		121		Neg				
316	SCH6538264	KNY	4	20153789	44	F	15/03/2013	CSF	Diagnosis not stated	Negative		117		Neg				
317	SCH6547330	KNY	1	20129698	43	M	19/03/2013	CSF	Laboratory investigation	Negative		117		Neg		Positive		
318	SCH6547331	GRS	G12	61251773	39	F	19/03/2013	CSF	Laboratory investigation	Negative		122		Neg		Positive	89	
319	SCH6556500	RED	MREG	125124651	11m	F	23/03/2013	CSF	Tuberculous meningitis ?	Negative		121				Neg		
320	SCH6561264	RED	A9	111196002	3	M	26/03/2013	CSF	?MENINGITIS	Negative		118		Neg				
321	SCH6561374	KNY	4	21045893	37	F	26/03/2013	CSF	?TOXO	Negative		122		Neg				
322	SCH6561871	GRS	G25	59973560	47	F	26/03/2013	CSF	Laboratory investigation	Negative		122	1.2	Neg		Neg		
323	SCH6562616	GRS	E7	129993473	39	F	26/03/2013	CSF	Investigation	Negative		117		Neg				
324	SCH6565401	RED	S11	125350264	1	M	28/03/2013	CSF	Laboratory investigation	Negative		122		Neg		Neg		
325	SCH6573238	RED	D1	133500744	13	M	01/04/2013	CSF	Hydrocephalus	Negative		105		Neg		Neg		
326	SCH6577146	OUD	4	20234662	16	M	03/04/2013	CSF	Laboratory investigation	Negative		116		Neg				
327	SCH6579507	KNY	1	21606306	55	M	04/04/2013	CSF	Laboratory investigation	Negative		129		Neg		Positive	300	
328	SCH6584475	GRS	C15	45743184	39	F	06/04/2013	CSF	Meningitis	Negative		120				Neg		
329	SCH6585105	GRS	C15	73220824	76	M	06/04/2013	CSF	Delirium	Negative		122		Neg		Neg		

330	SCH6588059	GRS	G17	76779859	65	F	08/04/2013	CSF	Meningitis	Negative		126				Neg		
331	SCH6589350	GRS	G42	45713344	22	F	09/04/2013	CSF	Laboratory investigation	Negative		118		Neg				
332	SCH6590868	WYN	SMF	59343806	67	F	09/04/2013	CSF	Meningitis	Negative		125		Neg			320	
333	SCH6592891	GRS	C15	86869781	53	F	10/04/2013	CSF	Investigation	Negative		125	<1.0	Neg		Positive	159	84
334	SCH6596930	RED	B2	39227095	5	M	12/04/2013	CSF	Laboratory investigation	Negative		117		Neg				
335	SCH6600761	GRS	C15	70389275	25	F	14/04/2013	CSF	Investigation	Negative		123		Neg				
336	SCH6602527	GRS	C15	84631019	37	F	15/04/2013	CSF	No clinical information	Negative		125	<1.0	Neg				
337	SCH6614637	KNY		20403192	28	F	19/04/2013	CSF	Investigation	Negative		120						
338	SCH6621477	KNY	2	20060083	63	M	23/04/2013	CSF		Negative		119	2.5			Positive	352	
339	SCH6622770	RED	B2	41528456	8	M	23/04/2013	CSF	already on TB Rx/septic	Negative		125		Neg		Neg		
340	SCH6625111	GRS	G12	16021818	29	F	24/04/2013	CSF	Laboratory investigation	Negative		132		Neg		Neg		
341	SCH6630695	GRS	G17	56104284	33	M	26/04/2013	CSF	Pulmonary tuberculosis	Negative		122	<1.0	Neg		Neg		
342	SCH6640324	KNY	2	20614491	32	M	02/05/2013	CSF	Pulmonary tuberculosis	Positive for AFB	MTB - 18 days	111	4.1	Neg		Neg		
343	SCH6645690	GRS	G17	121619019	71	M	04/05/2013	CSF	Laboratory investigation	Negative		121		Neg		Neg		
344	SCH6648110	GEO	A2	116474834	51	F	06/05/2013	CSF	Tuberculosis	Negative		102		Neg				
345	SCH6652189	WYN	CAS	131467664	45	M	07/05/2013	CSF	Meningitis ?	Negative		117		Neg			301	
346	SCH6653868	GRS		12754552	37	F	08/05/2013	CSF	Laboratory investigation	Negative		109		Neg		Positive		282 287
347	SCH6654523	RED	S11	134177013	16d	M	08/05/2013	CSF	Meningitis ?/seizures	Negative		118		Neg				
348	SCH6657584	RED	D1	131871535	4m	M	09/05/2013	CSF	ventriculitis	Negative		113		Neg				
349	SCH6661620	RED	ICU	120076856	1	M	11/05/2013	CSF	Meningitis ?	Negative		144		Neg		Positive	667	2 568 200
350	SCH6662427	RED	S12	131822520	5m	M	12/05/2013	CSF	Laboratory investigation	Negative		119		Neg		Neg		
351	SCH6667277	KNY	4	21082763	20	F	14/05/2013	CSF	Investigation	Negative		110		Neg				
352	SCH6669634	RED	MREG	123386302	2	F	15/05/2013	CSF	Encephalopathy ?meningitis	Negative		125		Neg				
353	SCH6672770	RED	S11	134306125	9d	M	16/05/2013	CSF	Sepsis neonatal	Negative		120		Neg				
354	SCH6673257	GRS	G17	10830859	46	M	16/05/2013	CSF	Laboratory investigation	Negative		134		Neg		Neg		
355	SCH6686729	GRS	G12	46437679	37	M	22/05/2013	CSF	Retroviral Disease	Negative		94		Neg		Positive	10	
356	SCH6687617	RED	MREG	120344916	1	M	23/05/2013	CSF	Meningitis ?	Negative		119						
357	SCH6693576	RED	S11	39971841	4	M	25/05/2013	CSF	No clinical information	Negative		111		Neg		Neg		
358	SCH6702119	RED	S11	48241673	4	M	29/05/2013	CSF	Meningitis ?	Positive for AFB	MTB - 26 days	96		Neg		Neg		
359	SCH6711419	SOM	BICK	134475656	53	F	03/06/2013	CSF	Laboratory investigation	Negative		115		Neg				
360	SCH6718646	HER		692058086	42	M	06/06/2013	CSF	Retroviral Disease	Negative		115		Neg		Positive		
361	SCH6718802	GRS	G25	76973916	40	F	06/06/2013	CSF	Laboratory investigation	Negative		126		Neg		Positive		
362	SCH6718803	GRS	G25	31928419	31	M	06/06/2013	CSF	Laboratory investigation	Negative		124		Neg		Positive	25	
363	SCH6721136	GRS	G42	117205773	28	F	07/06/2013	CSF	Investigation	Negative		121				Positive		<LoD
364	SCH6726618	GRS	G25	119419877	54	F	10/06/2013	CSF	Investigation	Negative		112		Neg		Positive	282	
365	SCH6727547	RED	D1	132825712	5m	M	10/06/2013	CSF	Tuberculous meningitis	Negative		116		Neg				
366	SCH6729390	RED	B1	134702612	3m	F	11/06/2013	CSF	Tuberculous meningitis ?	Positive for AFB	MTB - 13 days	103		Positive AFB				
367	SCH6729416	WYN	CUR3	125264796	1	M	11/06/2013	CSF	Investigation	Negative		127				Neg		
368	SCH6737213	RED	MREG	134743921	7	M	14/06/2013	CSF	Investigation	Negative		121				Neg		
369	SCH6744078	RED	B2	113443899	6	F	18/06/2013	CSF	Meningitis ?	Negative		126		Neg		Neg		
370	SCH6746329	GRS	G16	81426223	36	M	19/06/2013	CSF	Investigation	Negative		131		Neg		Positive	377	948
371	SCH6750275	GEO	TR	125119883	33	F	21/06/2013	CSF	Laboratory investigation	Negative		107		Neg		Positive	58	
372	SCH6750281	GEOSA		67094508	31	F	21/06/2013	CSF	Laboratory investigation	Negative		120		Neg				
373	SCH6750285	KNY	2	21124870	15	M	21/06/2013	CSF	Laboratory investigation	Negative		115		Neg				
374	SCH6756610	GRS	G17	35805241	65	F	24/06/2013	CSF	Laboratory investigation	Negative		129		Neg		Neg		
375	SCH6758311	GRS	G22	118322569	35	F	25/06/2013	CSF	Investigation	Negative		139		Neg				
376	SCH6765683	RED	D1	135031078	6m	F	28/06/2013	CSF	Hydrocephalus complicated meningitis	Negative		120		Neg				
377	SCH6769398	GRS	C15	133997510	22	F	30/06/2013	CSF	Laboratory investigation	Negative		119		Neg		Positive	210	
378	SCH6771016	RED	MREG	135055861	4	F	01/07/2013	CSF	Investigation	Negative		118		Neg		Neg		
379	SCH6774628	SOM	CAS	110906930	40	F	03/07/2013	CSF	No clinical details supplied	Negative		136		Neg			230	
380	SCH6774635	SOM		35496751	30	F	03/07/2013	CSF	Meningitis	Negative		145		Neg		Positive	91	
381	SCH6774734	RED	D1	105791628	14	M	03/07/2013	CSF	Meningo encephalitis	Negative		117		Neg		Neg		
382	SCH6776207	GRS	G16	113482954	20	F	03/07/2013	CSF	Investigation	Negative		142		Neg		Positive	80	
383	SCH6779052	GRS	C15	15168792	32	F	04/07/2013	CSF	Laboratory investigation	Negative		126		Neg		Positive	569	
384	SCH6782192	RED	S11	133264416	3m	F	06/07/2013	CSF	No clinical details supplied	Negative		122		Neg				
385	SCH6783801	RED	S12	15902075	7	M	07/07/2013	CSF	Meningitis ?	Negative		120		Neg		Neg		
386	SCH6784022	RED	S12	132173600	5m	M	07/07/2013	CSF	Sepsis presumed	Negative		119		Neg		Neg		
387	SCH6784936	RED	S11	133616813	3m	M	08/07/2013	CSF	Meningitis ?	Negative		120		Neg		Neg		
388	SCH6790821	GRS	G17	135150209	27	F	10/07/2013	CSF	Laboratory investigation	Negative		126					280	
389	SCH6791259	SOM	CAS	27611565	39	M	10/07/2013	CSF	Laboratory investigation	Negative		128		Neg		Neg		
390	SCH6802028	GRS	G16	16208944	25	F	15/07/2013	CSF	Laboratory investigation	Negative		113		Neg		Positive	59	
391	SCH6809850	SOM	1FL	48346951	29	M	18/07/2013	CSF	? Myelopathy	Negative		124		Neg		Positive	127	
392	SCH6810325	RED	S12	104227590	25	F	18/07/2013	CSF	Meningitis ?	Negative		121		Neg				
393	SCH6812735	GRS	E7	135206415	37	M	19/07/2013	CSF	Investigation	Negative		124		Neg		Positive	337	
394	SCH6814512	RED	ICU	131006850	8m	M	20/07/2013	CSF	Tuberculous meningitis	Positive for AFB	MTB - 23 days	106		Neg				
395	SCH6815692	RED	D1	135321966	5	F	21/07/2013	CSF	Tuberculous meningitis	Negative		116		Neg				

396	SCH6817299	GRS	F17	117506964	47	M	22/07/2013	CSF	Investigation	Positive for AFB	MTB - 21 days	116		Neg		Neg			
397	SCH6820186	RED	B2	111623526	3	M	23/07/2013	CSF	Meningitis ?/?HLH	Negative		121		Neg		Positive	89	228	
398	SCH6825315	GRS	C15	103191912	37	M	25/07/2013	CSF	Investigation	Positive for AFB	MTB - 11 days	115		Neg			89		
399	SCH6825324	GRS	C23	31385735	31	M	25/07/2013	CSF	Investigation	Negative		124		Neg		Neg			
400	SCH6830769	RED	B2	135516128	10m	M	27/07/2013	CSF	Sepsis ?	Negative		120		Neg					
401	SCH6832572	GRS	E7	26130443	40	M	29/07/2013	CSF	Investigation	Negative		128		Neg		Positive	179		
402	SCH6834209	RED	S12	121838247	1	F	30/07/2013	CSF	Meningitis ?	Negative		120		Neg		Neg			
403	SCH6840440	SOM	CAS	122692049	37	F	01/08/2013	CSF	Tuberculous meningitis	Negative		110		Neg					
404	SCH6842020	RED	ICU	40504888	5	M	02/08/2013	CSF	Investigation	Negative		122		Neg					
405	SCH6842750	GRS	C15	70087952	25	M	02/08/2013	CSF	Laboratory investigation	Negative		114		Neg		Neg			
406	SCH6842769	GRS	F7	135158194	18	F	02/08/2013	CSF	Laboratory investigation	Negative		130		Neg		Positive	512		
407	SCH6844102	RED	A9	132469032	6m	F	03/08/2013	CSF	Meningitis ?	Negative		125		Neg		Neg			
408	SCH6852895	RED	B2	129567897	11m	M	07/08/2013	CSF	Meningitis ?,on cef d2	Negative		103		Neg		Neg			
409	SCH6854887	GRS	C15	79306288	47	F	08/08/2013	CSF	Laboratory investigation	Negative		123		Neg		Neg			
410	SCH6856580	RED	S11	104141585	4	M	09/08/2013	CSF	Meningitis ?	Positive for AFB	MTB - 34 days	107		Neg		Neg			
411	SCH6858540	RED	S11	134865195	55d	M	10/08/2013	CSF	Pyrexia	Negative		119		Neg					
412	SCH6860124	WYN	CAS	86432283	39	M	11/08/2013	CSF	Laboratory investigation	Negative		128		Neg		Positive	426		
413	SCH6862518	SOM	BAIL	135656205	36	M	12/08/2013	CSF	Investigation	Positive for AFB	MTB - 24 days	103		Neg		Positive	36	2 968 923	
414	SCH6866654	GRS	G16	135746022	18	M	14/08/2013	CSF	Laboratory investigation	Negative		123		Neg		Positive	506		
415	SCH6867968	RED	D1	135700631	3m	F	14/08/2013	CSF	thalamic lesion	Negative		111		Neg					
416	SCH6874885	WYN	SMF	13606603	37	F	16/08/2013	CSF	Retroviral Disease ? CVA	Negative		124		Neg		Positive	233		
417	SCH6875759	RED	S11	121307862	4	F	17/08/2013	CSF	Meningitis ?	Negative		122		Neg					
418	SCH6876025	GRS	C15	135854628		F	17/08/2013	CSF	No clinical details supplied	Negative		122		Neg		Positive	153		
419	SCH6881350	MOS	CAS	2205045	28	F	20/08/2013	CSF	Meningitis	Negative		122	<1.0			Positive			
420	SCH6895028	RED	MREG	33858283	9	M	26/08/2013	CSF	Meningitis ?	Negative		110		Neg					
421	SCH6895304	SOM		25134248	38	M	26/08/2013	CSF	Meningitis	Negative		109		Neg		Positive	56		
422	SCH6897750	RED	S12	130340771	1	F	27/08/2013	CSF	Vomiting ? meningitis, temp 39	Negative		123		Neg		Neg			
423	SCH6904753	RED	D1	125094953	1	M	30/08/2013	CSF	Hydrocephalus	Negative		106		Neg					
424	SCH6905557	RED	S11	136108198	4	F	30/08/2013	CSF	No clinical details supplied	Negative		112				Neg			
425	SCH6907266	SOM	BICK	35069517	31	F	31/08/2013	CSF	meningism?	Negative		122		Neg		Positive	186		
426	SCH6918889	GRS	C12	58251323	49	M	05/09/2013	CSF	Laboratory investigation	Negative		125							
427	SCH6922339	SOM	KING	136206000	51	M	06/09/2013	CSF	No clinical details supplied	Negative		122		Neg					
428	SCH6922485	GRS	E7	33311887	53	M	06/09/2013	CSF	No clinical details supplied	Negative		138		Neg		Neg			
429	SCH6922902	SOM	CAS	121730295	28	F	06/09/2013	CSF	Illegible diagnosis/ICD10	Negative		128		Neg					
430	SCH6923150	SOM	CAS	67156000	28	F	06/09/2013	CSF	Tuberculous meningitis	Negative		114		Neg		Positive	5	212 571	
431	SCH6924252	GRS	C15	83357608	16	F	07/09/2013	CSF	Encephalitis	Positive for AFB	MTB - 45 days	111		Neg		Neg			
432	SCH6925059	GRS	C24	12830287	31	F	08/09/2013	CSF	Meningitis ?	Negative		130		Neg		Positive			
433	SCH6928282	GRS	G25	79707519	20	M	10/09/2013	CSF	Meningitis	Negative		113		Neg		Positive	142		
434	SCH6928893	GRS	C15	51399111	36	M	10/09/2013	CSF	Meningitis	Negative		121		Neg		Neg			
435	SCH6938855	RED	TH	135433993	1	M	13/09/2013	CSF	Hydrocephalus	Negative		119		Neg		Neg			
436	SCH6941034	RED	ICU	125519934	1	M	14/09/2013	CSF	Meningitis ?, seizures	Negative		122		Neg		Positive			
437	SCH6946853	MITDH		134797133	21	F	17/09/2013	CSF	Meningitis RVD	Negative		122		Neg		Positive			
438	SCH6954757	SOM		37128451	31	F	20/09/2013	CSF	HIV	Negative		126		Neg					
439	SCH6956590	KNY	2	20924015	45	M	21/09/2013	CSF	Laboratory investigation	Negative		105		Neg			439		
440	SCH6958810	RED	D1	134834167	4m	M	23/09/2013	CSF	Hydrocephalus	Negative		112		Neg					
441	SCH6959015	GRS	C15	71036560	27	F	23/09/2013	CSF	Laboratory investigation	Negative		127		Neg			328		
442	SCH6960801	GRS	C15	107776940	17	M	24/09/2013	CSF	Laboratory investigation	Negative		127		Neg		Neg			
443	SCH6961847	RED	MREG	130292816	1	M	25/09/2013	CSF	Tuberculous meningitis ?	Positive for AFB	MTB - 26 days	110		Neg		Neg			
444	SCH6962507	MITDH		113406086	29	F	25/09/2013	CSF	Laboratory investigation	Negative		128		Neg		Neg			
445	SCH6963816	GFJ	CAS	131485161	37	M	25/09/2013	CSF	No clinical details supplied	Negative		121		Neg					
446	SCH6967788	RED	S12	129060463	1	F	27/09/2013	CSF	Meningitis ?,?tbm	Negative		116		Neg		Neg			
447	SCH6969778	GFJ		136574217	31	M	28/09/2013	CSF	Meningitis	Negative		113		Neg					
448	SCH6969884	GFJ		73605099	24	M	28/09/2013	CSF	Meningitis ?	Positive for AFB	MTB - 18 days	113		Neg		Positive	51		
449	SCH6972022	SOM	CAS	51667467	37	M	30/09/2013	CSF	Laboratory investigation	Negative		115		Neg			569		
450	SCH6972251	RED	TH	136570918	6	M	30/09/2013	CSF	HCP	Negative		127		Neg					
451	SCH6976699	GFJ	CAS	118738764	35	M	02/10/2013	CSF	No clinical details supplied	Negative		133		Neg		Positive	39		
452	SCH6977414	GFJ	CAS	136630563	27	F	02/10/2013	CSF	Laboratory investigation	Positive for AFB	MTB - 33 days	106				Positive	151		
453	SCH6978331	GRS	C15	136612355	33	M	02/10/2013	CSF	Laboratory investigation	Negative		119		Neg		Positive	42		
454	SCH6979997	RED	S12	118054220	2	M	03/10/2013	CSF	Laboratory investigation	Negative		120		Neg		Neg			
455	SCH6984975	GRS	G17	29048816	35	F	04/10/2013	CSF	Laboratory investigation	Negative		127		Neg		Positive	16 783		
456	SCH6985544	GRS	C15	17288069	56	F	04/10/2013	CSF	Delirium	Negative		127		Neg					
457	SCH6986973	GRS	C15	25343658	32	F	05/10/2013	CSF	Retroviral Disease Program	Negative		127		Neg		Positive	127		
458	SCH6986983	GRS	C15	32374720	54	M	05/10/2013	CSF	SEIZURES	Negative		126		Neg		Neg			
459	SCH6992456	SOM	CAS	77776318	49	M	08/10/2013	CSF		Negative		118		Neg		Positive			
460	SCH6994940	GRS	C15	65660920	47	F	09/10/2013	CSF	Laboratory investigation	Negative		124		Neg		Neg			
461	SCH7000684	RED	D1	44231587	5	F	11/10/2013	CSF	Sepsis	Negative		117		Neg					

462	SCH7001017	GRS	C27	86597853	47	M	11/10/2013	CSF	Investigation	Negative		117				Neg		
463	SCH7001102	RED	S12	119646503	2	M	11/10/2013	CSF	Meningitis SUSPECTED,FEVER,LETHARGY	Negative		125						
464	SCH7008067	GRS	C15	69188837	26	F	15/10/2013	CSF	Meningitis	Negative		118		Neg		Positive	108	
465	SCH7008605	RED	B1	119996379	2	M	15/10/2013	CSF	Tuberculous meningitis ?	Negative		116		Neg		Positive	813	279 465
466	SCH7008745	GRS	K41	33393323	33	F	15/10/2013	CSF	Laboratory investigation	Negative		123		Neg		Positive		2 090
467	SCH7010987	GRS	E7	59486977	49	F	16/10/2013	CSF	Laboratory investigation	Negative		127				Neg		
468	SCH7016211	RED	D1	119494136	2	F	18/10/2013	CSF	No clinical details supplied	Negative		98		Neg				
469	SCH7017287	RED	B1	112915673	3	F	18/10/2013	CSF	Tuberculous meningitis ?	Negative		101		Neg		Neg		
470	SCH7019238	GFJ	CAS	129248829	16	F	20/10/2013	CSF	Meningitis	Negative		115		Neg			193	
471	SCH7027715	GRS	G17	71163687	25	F	23/10/2013	CSF	? Meningitis	Negative		131		Neg		Positive	538	
472	SCH7029858	GRS	C27	104881958	45	M	24/10/2013	CSF	Laboratory investigation	Negative		96	14.1	Neg				
473	SCH7030180	SOM	CAS	136652344	30	F	24/10/2013	CSF	Investigation	Negative		130		Neg		Positive		
474	SCH7030373	SOM		18179614	29	M	24/10/2013	CSF	Tuberculous meningitis	Negative		123		Neg		Positive	4	107
475	SCH7037004	RED	B1	133083238	8m	M	28/10/2013	CSF	Meningitis ?	Negative		115		Neg		Neg		
476	SCH7038188	SOM		38482840	38	F	29/10/2013	CSF	Meningitis	Negative		125				Positive	263	<LoD
477	SCH7044990	GRS	C15	137413340	29	M	31/10/2013	CSF	Laboratory investigation	Negative		125		Neg				
478	SCH7045876	RED	B1	121250344	3	F	31/10/2013	CSF	No diagnosis supplied	Negative		124		Neg				
479	SCH7050134	RED	S11	136002888	70d	M	02/11/2013	CSF	Meningitis ?	Negative		118		Neg				
480	SCH7052132	SOM	CAS	136498268	49	F	04/11/2013	CSF	Investigation	Positive for AFB	MTB - 30 days	110		Neg		Positive		<LoD
481	SCH7054568	SOM	CAS	137537189		F	05/11/2013	CSF	Investigation	Negative		125						
482	SCH7055600	VAL		14129175	36	F	05/11/2013	CSF	Retroviral Disease	Negative		133		Neg		Positive	452	<LoD
483	SCH7056091	SOM	ANC	113071021	15	F	05/11/2013	CSF	Pyelonephritis ?	Negative		117		Neg		Positive	221	
484	SCH7059672	GRS	C15	137352852	35	M	07/11/2013	CSF	No clinical details supplied	Negative		114					191	
485	SCH7062712	GRS	C15	78531704	21	F	07/11/2013	CSF	Illegible diagnosis/ICD10	Negative		125		Neg				
486	SCH7064927	GRS	G17	137567251	44	F	08/11/2013	CSF	Laboratory investigation	Negative		118		Neg		Positive	534	
487	SCH7066551	GEO	B2	109831008	42	M	09/11/2013	CSF	Laboratory investigation	Negative		135	1.9			Neg		
488	SCH7067319	RED	B2	129170684	1	F	10/11/2013	CSF	Tuberculous meningitis	Negative		119		Neg		Positive		
489	SCH7067365	GRS	C15	137638433	37	M	10/11/2013	CSF	Investigation	Negative		143	2.2	Neg			81	
490	SCH7068163	RED	B2	108806613	8	M	11/11/2013	CSF	Tuberculous meningitis ?	Negative		121		Neg				
491	SCH7068176	FAL	CAS	89343115	14	M	11/11/2013	CSF	Laboratory investigation after request	Negative		122		Neg				
492	SCH7077264	GRS	E32	32062986	49	F	14/11/2013	CSF	?Meningitis	Negative		109		Neg			476	
493	SCH7077851	GRS	D13	16035172	32	F	14/11/2013	CSF	Meningitis	Negative		115		Neg		Positive	142	
494	SCH7077941	GRS	G16	89034771	49	M	14/11/2013	CSF	YOUNG STROKE NO RISK FACTOR	Negative		120		Neg		Neg		
495	SCH7078742	GRS	C15	15985989	40	M	14/11/2013	CSF	Meningitis	Positive for AFB	MTB - 25 days	116		Neg			269	
496	SCH7088435	GRS	C15M	138802079	31	F	19/11/2013	CSF	Laboratory investigation	Negative		123		Neg		Positive	803	14 531
497	SCH7093673	GRS	C15	125357368	38	F	21/11/2013	CSF	Laboratory investigation	Positive for AFB	MTB - 15 days	121		Neg		Positive	29	45
498	SCH7094382	GRS	G17	43572122	49	F	21/11/2013	CSF	Laboratory investigation	Negative		127		Neg		Positive	54	511 110
499	SCH7104389	GRS	C27	35969427	36	F	26/11/2013	CSF	Laboratory investigation	Negative		122	1.5	Neg		Positive	156	
500	SCH7104658	GRS	C15	35157197	42	M	26/11/2013	CSF	Laboratory investigation	Negative		124		Neg		Positive		
501	SCH7106454	GRS	C14	71170617	25	M	27/11/2013	CSF	Meningitis ?	Negative		89		Neg		Neg		
502	SCH7113098	GRS	G25	134996156	54	M	29/11/2013	CSF	Investigation	Negative		123		Neg		Neg		
503	SCH7115750	GRS	C15	67940155	65	F	30/11/2013	CSF	Retroviral Disease +	Negative		129		Neg				
504	SCH7120380	GRS	C23	80532179	19	F	03/12/2013	CSF	Laboratory investigation	Negative		126				Neg		
505	SCH7126586	GRS	E16	33466624	28	F	05/12/2013	CSF	Laboratory investigation	Negative		123		Neg		Positive	142	
506	SCH7129469	GRS	C15	119183861	33	F	06/12/2013	CSF	Laboratory investigation	Negative		117		Neg				
507	SCH7135416	RED	S12	124828732	1	F	10/12/2013	CSF	Pyrexia ?meningitis	Negative		127		Neg				
508	SCH7135417	RED	MREG	96271564		M	10/12/2013	CSF	No clinical details supplied	Negative		120		Neg				
509	SCH7136651	RED	ICU	105414437	4	M	10/12/2013	CSF	Meningitis ?	Negative		134		Neg		Neg		
510	SCH7145141	GRS	G25	137656682	34	M	13/12/2013	CSF	Laboratory investigation	Negative		117		Neg			91	
511	SCH7146937	GRS	G25	29742848	39	M	14/12/2013	CSF	?MENINGITIS	Negative		129		Neg		Neg		
512	SCH7147561	GRS	C15	139228514	33	M	15/12/2013	CSF	No clinical details supplied	Negative		125		Neg				
513	SCH7148762	SOM	CAS	139233498	33	M	17/12/2013	CSF	Laboratory investigation ? Meningitis	Negative		117		Neg		Neg		
514	SCH7150195	GRS	G17	139208342	24	M	17/12/2013	CSF	Laboratory investigation	Negative		128		Neg		Neg		
515	SCH7152432	RED	D1	137150686	64d	M	18/12/2013	CSF	Hydrocephalus	Negative		110		Neg				
516	SCH7161952	RED	ICU	40495624	5	M	24/12/2013	CSF	No clinical details supplied	Negative		118		Neg		Neg		
517	SCH7162343	RED	E2	29544178	7	F	24/12/2013	CSF	Guillian Syndrome	Negative		127		Neg		Neg		
518	SCH7167653	GRS	C15	20394789	39	M	30/12/2013	CSF	?tb menengitis	Negative		124		Neg		Neg		
519	SCH7169815	GRS	G8	60964251	60	M	31/12/2013	CSF	Illegible diagnosis/ICD10	Negative		116		Neg		Neg		
520	SCH7171924	KNY	2	21001656	37	M	02/01/2014	CSF	Meningitis	Negative		120					36	
521	SCH7172126	GRS	E7	108757618	26	F	02/01/2014	CSF	Cerebro-vascular accident	Negative		122		Neg		Positive	289	
522	SCH7182393	GRS	G25	117590331	41	F	08/01/2014	CSF	No clinical details supplied	Negative		127		Neg				
523	SCH7190254	GRS	C15	113169866	32	F	12/01/2014	CSF	Investigation	Negative		117		Neg		Positive	321	
524	SCH7196991	GRS	D13	122507965	40	M	15/01/2014	CSF	infection	Negative		119		Neg			140	
525	SCH7208310	RED	S11	113347488	3	M	20/01/2014	CSF	Meningitis ?	Negative		113		Neg				
526	SCH7212592	RED	MREG	122257645	2	M	22/01/2014	CSF	Meningitis ?	Negative		122		Neg				
527	SCH7216114	RED	S12	123513186	2	M	23/01/2014	CSF	Meningitis	Negative		120		Neg		Neg		

528	SCH7219467	GRS	G17	114727084	46	M	24/01/2014	CSF	No clinical details supplied	Negative		145				Positive	59	
529	SCH7227603	GRS	C22	31100191	35	M	28/01/2014	CSF	Psychosis	Negative		120		Neg		Positive	137	62 023
530	SCH7231668	GRS	C15	46774642	43	F	29/01/2014	CSF	Meningitis	Negative		129	2.4	Neg		Positive	514	22 006
531	SCH7232541	GRS	C15	14846851	41	M	30/01/2014	CSF	Meningitis	Negative		104		Neg				
532	SCH7243382	RED	MREG	111454427	3	M	04/02/2014	CSF	No clinical details supplied	Negative		102		Neg		Neg		
533	SCH7257507	RED	S12	135853216	7m	M	09/02/2014	CSF	Tuberculous meningitis ?	Negative		104		Neg		Neg		
534	SCH7259904	GRS	C15	48030712	47	M	10/02/2014	CSF	No clinical details supplied	Negative		121		Neg		Positive	17	73 038
535	SCH7265827	GRS	G25	139712210	26	F	12/02/2014	CSF	Meningitis	Negative		116		Neg			308	
536	SCH7268749	GRS	C15	74407305	54	M	13/02/2014	CSF	No clinical information	Negative		127		Neg				
537	SCH7271582	GRS	C15	140541756	22	F	14/02/2014	CSF	Laboratory investigation	Negative		126		Neg			510	
538	SCH7273992	RED	D1	139313126	54d	M	15/02/2014	CSF	No clinical details supplied	Negative		138		Neg		Neg		
539	SCH7274169	GRS	G12	84445527	45	F	15/02/2014	CSF	Laboratory investigation	Negative		131		Neg		Positive	167	<20
540	SCH7281835	RED	ICU	115655243	3	F	19/02/2014	CSF	Tuberculous meningitis ?	Negative		113		Neg				
541	SCH7286029	GRS	G16	140382474	24	M	20/02/2014	CSF	Tuberculosis ?meningitis	Negative		108		Neg		Neg		
542	SCH7292952	GRS	G17	82578071	71	M	24/02/2014	CSF	Meningitis	Negative		127		Neg		Neg		
543	SCH7304690	RED	B1	139354427	63d	F	28/02/2014	CSF	Tuberculous meningitis tbm	Negative		114		Neg		Neg		
544	SCH7308916	SOM	CAS	133785774	35	F	03/03/2014	CSF	Headache	Negative		128		Neg		Neg		
545	SCH7316428	GRS	G17	84948421	54	M	05/03/2014	CSF	Meningitis ?	Negative		126		Neg				
546	SCH7322448	GRS	C12	82837758	44	F	07/03/2014	CSF	? RVD	Negative		132		Neg		Positive	127	<LoD
547	SCH7324490	RED	MREG	139020143	3m	M	08/03/2014	CSF	Sepsis	Negative		159				Neg		
548	SCH7324659	GRS	C15	39641642	52	F	08/03/2014	CSF	TB MENENGITIS	Negative		105		Neg			25	
549	SCH7327273	GRS	C15	107955064	53	M	10/03/2014	CSF	LOWER LIMB WEAKNESS	Negative		121		Neg				
550	SCH7339560	GRS	C12	23741598	79	F	14/03/2014	CSF	?MENINGITIS	Negative		126		Neg				
551	SCH7342523	GRS	C14	141947671	16	M	16/03/2014	CSF	No clinical details supplied	Negative		124	3.3	Neg		Neg		
552	SCH7350078	GRS	E26	33161373	47	F	19/03/2014	CSF	Tuberculosis ? MENINGITIS	Negative		121		Neg		Positive	664	
553	SCH7359135	RED	MREG	134884063	10m	F	24/03/2014	CSF	Tuberculous meningitis ?	Negative		119		Neg		Positive	2 188	2 165
554	SCH7359188	GRS	G17	122331309	20	F	24/03/2014	BCSF	Illegible diagnosis/ICD10	Negative		120		Neg				
555	SCH7367821	GRS	C22	131289506	27	M	27/03/2014	CSF	Retrovirus positive	Negative		126				Positive	197	
556	SCH7371136	GRS	G25	66605114	45	F	28/03/2014	CSF	No clinical details supplied	Negative		140		Neg			27	
557	SCH7377128	GRS	K41	57466591	34	F	01/04/2014	CSF	No clinical details supplied	Negative		118		Neg		Neg		
558	SCH7386472	RED	B2	137351755	5m	M	04/04/2014	CSF	No clinical details supplied	Negative		113		Neg		Positive	359	173 814
559	SCH7387807	GRS	C15	69115558	27	F	04/04/2014	CSF	CRANIAL NERVE	Negative		128		Neg		Neg		
560	SCH7390344	RED	MREG	113031264	3	F	06/04/2014	CSF	Meningitis ?, seizures	Negative		116		Neg				
561	SCH7391018	GRS	C15	112820915	29	M	07/04/2014	CSF	No clinical details supplied	Negative		117		Neg		Positive	9	
562	SCH7391985	GRS	C15	142456573	43	M	07/04/2014	CSF	No clinical details supplied	Negative		148		Neg		Neg		
563	SCH7396816	GRS	C15	45484151	39	M	09/04/2014	CSF	No clinical details supplied	Positive for AFB	MTB - 15 days	93		Neg				
564	SCH7398072	GRS	G17	122693021	28	F	09/04/2014	CSF	No clinical information	Negative		113		Neg		Neg		
565	SCH7407299	GRS	C15	26321083	31	F	12/04/2014	CSF	Laboratory investigation	Negative		123		Neg			652	
566	SCH7409529	GRS	D13	14068043	17	M	14/04/2014	CSF	?Ventriculitis	Negative		121		Neg		Neg		
567	SCH7412228	RED	B1	142577014	11	M	15/04/2014	CSF	Tuberculous meningitis ?	Negative		119		Neg		Neg		
568	SCH7414963	RED	D1	45528916	5	F	16/04/2014	CSF	Tuberculous meningitis	Negative		107		Neg		Neg		
569	SCH7419461	GRS	F12	109300517	23	F	17/04/2014	CSF	Meningitis ? TB	Negative		124		Neg		Neg		
570	SCH7421064	GRS	G25	115463572	26	M	18/04/2014	CSF	Illegible diagnosis/ICD10	Negative		119		Neg		Positive	306	1 296 398
571	SCH7429730	RED	S11	36368769	8	M	24/04/2014	CSF	Tuberculous meningitis ?	Negative		102		Neg		Neg		
572	SCH7430598	RED	D1	103823613	5	F	24/04/2014	CSF	Patient with ventriculitis	Negative		123		Neg				
573	SCH7433914	RED	S11	130319916	1	F	25/04/2014	CSF	Tuberculous meningitis vs JC virus	Negative		115				Positive	3 509	1 793
574	SCH7436369	SOW		30575724	32	M	27/04/2014	CSF	Delirium	Negative		122				Positive	888	
575	SCH7436461	GRS	G17	142607837	42	M	27/04/2014	CSF	No clinical information	Negative		119		Neg		Positive	7	
576	SCH7437259	GRS	C13	142769918	39	M	28/04/2014	CSF	Illegible diagnosis/ICD10	Negative		125				Positive	195	
577	SCH7437455	RED	D1	132141177	1	M	28/04/2014	CSF	Tuberculous meningitis hydrocephalus	Negative		114		Neg		Neg		
578	SCH7442180	RED	ICU	142783919	1	M	30/04/2014	CSF	Tuberculous meningitis	Negative		129		Neg				
579	SCH7442217	GRS	G16	141960021	29	F	30/04/2014	CSF	Meningitis	Negative		128					20	
580	SCH7476313	RED		116132887	3	M	16/05/2014	CSF	Laboratory investigation	Negative		124		Neg		Neg		
581	SCH7476315	RED	S12	121853659	2	M	16/05/2014	CSF	Laboratory investigation	Negative		123		Neg		Neg		
582	SCH7476318	GRS	G16	130711336	26	F	16/05/2014	CSF	Laboratory investigation	Negative		141		Neg		Positive	6	
583	SCH7477520	RED	S11	122844491	2	F	16/05/2014	CSF	Tuberculosis +	Negative		124				Neg		
584	SCH7487819	GRS	G12	142949460	55	F	21/05/2014	CSF	Meningitis	Negative		109			Neg	Positive	409	
585	SCH7493302	GRS	G25	87939336	69	F	23/05/2014	CSF	No clinical details supplied	Negative		122	<1.0	Neg	Neg	Neg		
586	SCH7497872	RED	D1	142797026	27d	M	26/05/2014	CSF	No clinical details supplied	Positive for AFB	AFB - 7 days	115		Neg				
587	SCH7500692	GRS	G16	32827271	33	F	27/05/2014	CSF	No clinical information	Negative		115		Neg		Positive	157	<20
588	SCH7503046	GEO	TR	25806738	33	F	28/05/2014	CSF	No diagnosis supplied	Negative		123						
589	SCH7503299	GRS	C15	54199369	38	M	28/05/2014	CSF	Laboratory investigation	Negative		122		Neg	Neg	Neg		
590	SCH7516004	GRS	E7	138797360	41	F	03/06/2014	CSF	Investigation	Negative		45		Neg		Neg		
591	SCH7516313	GRS	G25	34806182	30	F	03/06/2014	CSF	No clinical details supplied	Negative		119		Neg		Positive		3 449 862
592	SCH7519316	GRS	C15	17138058	62	F	04/06/2014	CSF	Illegible diagnosis/ICD10	Negative		127		Neg				
593	SCH7521120	GRS	D13	78792116	21	M	05/06/2014	CSF	Illegible diagnosis/ICD10	Negative		124		Neg		Neg		

594	SCH7525081	GRS	G16	35779602	54	F	06/06/2014	CSF	Tuberculous meningitis ?	Negative		121		Neg		Positive	383	<LoD
595	SCH7525390	GRS	G8	139570881	79	M	06/06/2014	CSF	Illegible diagnosis/ICD10	Negative		116		Neg		Neg		
596	SCH7526092	GRS	G16	72470248	29	M	06/06/2014	CSF	Meningitis	Negative		116		Neg		Positive		
597	SCH7528103	RED	MREG	111886099	4	M	08/06/2014	CSF	No clinical details supplied	Positive for AFB	MTB - 29 days	108		Neg	Neg			
598	SCH7528994	GRS	G12	81878472	38	M	09/06/2014	CSF	No clinical details supplied	Negative		121		Neg		Positive	234	5 610
599	SCH7534994	GRS	C12	85679603	47	F	11/06/2014	CSF	? TBM	Negative		118		Neg		Neg		
600	SCH7541540	GRS	G17	29110210	31	F	13/06/2014	CSF	No clinical details supplied	Negative		104		Neg		Positive	351	53
601	SCH7543369	GRS	C15	15138399	57	M	14/06/2014	CSF	Laboratory investigation	Negative		119		Neg				
602	SCH7545642	GRS	G16	54347125	68	F	17/06/2014	CSF	Investigation	Negative		121				Neg		
603	SCH7549208	GRS	G16	28326270	32	F	18/06/2014	CSF	Cryptococcal meningitis	Negative		124		Neg		Positive	31	
604	SCH7551429	RED	S12	143517670	2	M	19/06/2014	CSF	Hydrocephalus	Negative		122		Neg		Neg		
605	SCH7552492	RED	S12	118909464	3	M	19/06/2014	CSF	Meningitis	Negative		115		Neg		Positive		
606	SCH7557162	RED	S11	143142750	61d	M	21/06/2014	CSF	Tuberculous meningitis ?,meningitis	Negative		120		Neg		Neg		
607	SCH7559088	GRS	C15	144089869	25	F	23/06/2014	CSF	?MENINGITIS	Negative		121		Neg		Positive	3	228 628
608	SCH7560103	RED	S12	139295471	7m	F	23/06/2014	CSF	Meningitis ?	Negative		124		Neg		Neg		
609	SCH7561696	RED	B2	142359348	82d	F	24/06/2014	CSF	FTT vomiting	Negative		112		Neg				
610	SCH7571797	RED	B1	143533578	26d	M	27/06/2014	CSF	Sepsis	Negative		123		Neg		Neg		
611	SCH7573545	RED	D1	125909176	2	M	28/06/2014	CSF	VP shunt dysfunction	Negative		105		Neg				
612	SCH7577781	RED	S12	134702687	2	M	01/07/2014	CSF	No diagnosis supplied	Positive for AFB	MTB - 20 days	111		Neg		Neg		
613	SCH7581283	GRS	G12	144185246	42	F	02/07/2014	CSF	Stroke young	Negative		124		Neg		Positive	124	
614	SCH7581596	GRS	C15	17413576	17	M	02/07/2014	CSF	No clinical details supplied	Negative		114		Neg		Neg		
615	SCH7584829	GRS	G25	78827839	52	M	03/07/2014	CSF	No clinical information	Negative		123		Neg		Neg		
616	SCH7584889	RED	S11	144239282	2	M	03/07/2014	CSF	Tuberculous meningitis	Negative		110		Neg				
617	SCH7592121	RED	D1	107272171	4	M	07/07/2014	CSF	Hydrocephalus	Negative		111		Neg		Neg		
618	SCH7597985	GRS	G16	20606679	34	F	09/07/2014	CSF	Tuberculosis	Negative		140		Neg				
619	SCH7613434	RED	D1	43819101	7	M	16/07/2014	CSF	Tuberculous meningitis ventricular	Negative		113		Neg		Neg		
620	SCH7619696	GRS	G12	144261765	41	F	18/07/2014	CSF	No clinical information	Negative		109		Neg		Neg		
621	SCH7622783	RED	ICU	46528006	12	F	20/07/2014	CSF	Tuberculosis disseminated	Negative		114		Neg		Neg		
622	SCH7627393	GRS	G25	86257417	42	M	22/07/2014	CSF	Laboratory investigation	Negative		117		Neg		Positive	34	182 400
623	SCH7627661	GRS	G12	24782344	34	F	22/07/2014	CSF	No clinical details supplied	Negative		119		Neg		Neg		
624	SCH7638321	GRS	C15	86267705	49	F	26/07/2014	CSF	Laboratory investigation RVD POSITIVE AND HEAD	Negative		120		Neg		Positive		
625	SCH7641992	GRS	C15	124907205	18	F	29/07/2014	CSF	Meningitis ?	Positive for AFB	MTB - 13 days	101		Neg		Neg		
626	SCH7643971	GRS	C15	15293491	71	F	29/07/2014	CSF	Laboratory investigation	Negative		127		Neg		Neg		
627	SCH7649869	GRS	G25	16223802	38	M	31/07/2014	CSF	No clinical details supplied	Negative		118		Neg		Positive	299	
628	SCH7652950	GRS	F7	56070964	61	M	01/08/2014	CSF	No clinical details supplied	Negative		112						
629	SCH7655540	GRS	G22	116896424	24	F	03/08/2014	CSF	Encephalitis	Negative		121		Neg	Neg	Positive	4	
630	SCH7668941	RED	D1	115376352	3	M	08/08/2014	CSF	No clinical details supplied	Negative		119		Neg				
631	SCH7671372	SOM	CAS	116480559	29	F	09/08/2014	CSF	Tuberculous meningitis Suspect	Positive for AFB	MTB - 16 days	119		Neg	Neg	Neg		
632	SCH7672215	GRS	D12	68080829	28	M	09/08/2014	CSF	Meningitis ?	Negative		109	15.1	Neg		Neg		
633	SCH7672668	GRS	C13	55689699	67	F	10/08/2014	CSF	Headache Fever, neck stiffness	Negative		126						
634	SCH7673022	GRS	C15	108824376	29	M	11/08/2014	CSF	Meningitis	Negative		132		Neg	Neg	Neg		
635	SCH7674395	RED	S11	119086197	3	M	11/08/2014	CSF	Tuberculous meningitis ?	Negative		123		Neg		Positive	933	<20
636	SCH7679117	KNY	2	20622940	41	M	13/08/2014	CSF	Diagnosis not stated	Negative		135				Neg		
637	SCH7683886	RED	ICU	135536795	1	M	14/08/2014	CSF	No clinical details supplied	Negative		123		Neg				
638	SCH7686671	MIT	EC	144542438	33	F	15/08/2014	CSF	Investigation FACIAL PALSY	Negative		122			Neg	Positive	82	
639	SCH7688239	RED	MOPD	108298266	4	M	16/08/2014	CSF	Tuberculosis	Negative		114		Neg		Neg		
640	SCH7688241	RED	MREG	40018954	7	F	16/08/2014	CSF	Meningitis hydrochapalus	Negative		110		Neg				
641	SCH7700440	RED	B2	131431165	1	F	21/08/2014	CSF	Tuberculosis disseminated	Negative		124		Neg		Positive	626	
642	SCH7705355	RED	ICU	133932079	1	M	24/08/2014	CSF	No clinical details supplied	Negative		121		Neg				
643	SCH7718098	GRS	D15	75071936	23	M	29/08/2014	CSF	? MENINGITIS	Negative		119		Neg		Neg		
644	SCH7721382	GRS	C15	60595899	48	M	31/08/2014	CSF	Meningitis	Negative		119		Neg		Neg		
645	SCH7722589	GRS	G16	115840324	26	M	01/09/2014	CSF	Cryptococcal meningitis ?	Negative		110		Neg		Positive		
646	SCH7722910	RED	D1	13393079	1	M	01/09/2014	CSF	Meningitis ?	Negative		123		Neg	Neg			
647	SCH7727279	RED	MREG	113444830	4	F	03/09/2014	CSF	Hydrocephalus TBM	Negative		102		Neg	Neg	Neg		
648	SCH7728996	RED	B2	144810611	69d	M	03/09/2014	CSF	Meningitis	Negative		129		Neg		Positive	1 024	6 436
649	SCH7730356	GRS	C15	57077232	57	F	04/09/2014	CSF	No clinical information	Positive for AFB	MTB - 32 days	110	7.3	Neg	Neg	Positive	390	
650	SCH7734173	GRS	C15	125570374	36	F	05/09/2014	CSF	Meningitis	Negative		119	8	Neg	Neg	Positive	414	
651	SCH7755356	GRS	C15	40327173	39	F	15/09/2014	CSF	Tuberculous meningitis ?	Negative		120			Neg			
652	SCH7767779	GRS	G8	145596342	44	M	19/09/2014	CSF	Burkitt's lymphoma	Negative		115		Neg				
653	SCH7769751	RED	S11	135231462	1	M	20/09/2014	CSF	Pulmonary tuberculosis	Negative		120			Neg	Neg		
654	SCH7776964	GRS	C15	10437143	55	M	24/09/2014	CSF	No clinical details supplied	Negative		123		Neg		Positive	155	
655	SCH7780386	RED	ICU	130104052	2	M	26/09/2014	CSF	Meningitis ?	Negative		138		Neg				
656	SCH7783533	RED	A9	131561862	1	M	27/09/2014	CSF	Sepsis ?	Negative		119		Neg	Neg	Positive	1 302	112 291
657	SCH7791790	GRS	C15	29163599	31	F	01/10/2014	CSF	No clinical details supplied	Negative		124	<1.0	Neg		Positive		154
658	SCH7796981	GEO	TR	145783759	64	M	03/10/2014	CSF	Laboratory investigation	Negative		121				Positive	189	467
659	SCH7797139	GEO	TR	25638495	28	F	03/10/2014	CSF	Laboratory investigation	Negative		119					72	

660	SCH7797564	RED	ICU	145772620	3	M	03/10/2014	CSF	Encephalitis SUSPECTED	Negative		127			Neg			
661	SCH7799431	GRS	C15	74732744	48	M	04/10/2014	CSF	Meningitis	Negative		109			Neg	Positive	107	
662	SCH7800770	GRS	G25	142820570	30	F	06/10/2014	CSF	? TB MENINGITIS	Negative		118	2.6	Neg	Neg	Neg		
663	SCH7806289	RED	D1	132044520	1	F	08/10/2014	CSF	Tuberculous meningitis	Negative		112		Neg				
664	SCH7806293	RED	B1	106341308	4	F	08/10/2014	CSF	Tuberculous meningitis	Negative		107		Neg		Positive	346	<LoD
665	SCH7813315	GRS	G16	73311755	63	F	10/10/2014	CSF	Delirium septic workup	Negative		121			Neg			
666	SCH7822989	GRS	G8	145639605	41	M	15/10/2014	CSF	No clinical information	Negative		111		Neg		Positive	595	
667	SCH7827136	RED	D1	131870354	1	M	16/10/2014	CSF	ventriculitis	Negative		109		Neg		Neg		
668	SCH7830246	RED	D1	144427515	3m	M	17/10/2014	CSF	No clinical details supplied	Negative		110		Neg				
669	SCH7830436	RED	B1	137446191	1	F	17/10/2014	CSF	Meningitis ?	Negative		127		Neg		Neg		
670	SCH7839384	RED	D1	143308732	4m	M	22/10/2014	CSF	Tuberculous meningitis hcp	Negative		114		Neg		Neg		
671	SCH7843311	GRS	G25	51662021	38	M	23/10/2014	CSF	Laboratory investigation	Negative		121		Neg		Neg		
672	SCH7845892	RED	D1	142539113	1	M	24/10/2014	CSF	No clinical details supplied	Negative		123		Neg			1 806	
673	SCH7846283	GRS	G16	83194084	39	F	24/10/2014	CSF	No clinical details supplied	Negative		115		Neg		Neg		
674	SCH7847633	RED	B1	29741311	9	M	25/10/2014	CSF	Tuberculous meningitis ?	Positive for AFB	MTB - 27 days	113			Neg	Neg		
675	SCH7857707	RED	S12	103801502	5	M	30/10/2014	CSF	Meningitis	Negative		121			Neg	Neg		
676	SCH7858914	GRS	G7	36450518	57	F	30/10/2014	CSF	No clinical details supplied	Negative		112		Neg		Neg		
677	SCH7859564	RED	B2	133898718	1	F	30/10/2014	CSF	Meningitis ?	Negative		127		Neg	Neg	Positive	55	10 000 000
678	SCH7859570	RED	S11	29487758	7	M	30/10/2014	CSF	Meningitis ?	Negative		120		Neg		Neg		
679	SCH7863980	GRS	G16	119270312	37	F	01/11/2014	CSF	Meningitis CRYPTOCOCCAL	Negative		108		Neg		Positive	47	
680	SCH7864090	GRS	C15	52512415	51	M	01/11/2014	CSF	Tuberculosis ?/	Negative		122		Neg		Neg		
681	SCH7873237	WYN	CAS	139347934	14	F	06/11/2014	CSF	Meningitis	Negative		120	3.6	Neg				
682	SCH7884276	RED	S12	123110280	2	F	11/11/2014	CSF	Tuberculous meningitis ?	Negative		106		Neg		Neg		
683	SCH7885155	GRS	C13	54827605	40	M	11/11/2014	BCSF	Meningitis ?	Negative		126			Neg	Neg		
684	SCH7885484	GRS	G12	50034214	53	F	11/11/2014	CSF	No clinical details supplied	Negative		128		Neg		Positive	509	
685	SCH7887315	RED	E2	85647618	15	M	12/11/2014	CSF	Liver transplant	Negative		119		Neg				
686	SCH7887389	RED	ICU	116895491	3	M	12/11/2014	CSF	No clinical details supplied	Positive for AFB	MTB - 19 days	104		Neg				
687	SCH7899361	RED	D1	146595715	3m	M	17/11/2014	CSF	Hydrocephalus	Negative		114		Neg				
688	SCH7899362	RED	D1	145845848	43d	F	17/11/2014	CSF	Hydrocephalus meningitis	Negative		119		Neg				
689	SCH7901903	RED	ICU	115362899	4	F	18/11/2014	CSF	Encephalitis	Negative		127			Neg	Neg		
690	SCH7914300	GRS	D13	86159712	18	M	24/11/2014	CSF	No clinical details supplied	Negative		110		Neg		Neg		
691	SCH7915118	GRS	C5	146531405	39	M	24/11/2014	CSF	?IRIS	Negative		129	1.8	Neg		Positive	27	
692	SCH7915333	GRS	E7	140913120	33	F	24/11/2014	CSF	Laboratory investigation	Negative		124		Neg		Positive	312	
693	SCH7918077	RED	S12	132890419	1	F	25/11/2014	CSF	Tuberculous meningitis ?	Negative		123		Neg		Neg		
694	SCH7921840	GRS	G16	147822159	75	M	26/11/2014	CSF	No clinical details supplied	Negative		134			Neg	Neg		
695	SCH7927742	RED	S12	32030223	7	F	28/11/2014	CSF	No clinical details supplied	Negative		108		Neg		Neg		
696	SCH7932632	GRS	C15	103318176	23	F	02/12/2014	CSF	Retroviral Disease	Negative		122		Neg		Positive	222	
697	SCH7941745	GRS	C15	137531174	43	M	05/12/2014	CSF	Meningitis ?	Negative		121		Neg				
698	SCH7941901	RED	B1	119579050	3	M	05/12/2014	CSF	Tuberculosis ?Disseminated	Negative		122		Neg		Positive	827	1 557
699	SCH7943856	GRS	C15	19886597	30	F	06/12/2014	CSF	Meningitis ?	Negative		122		Neg			338	
700	SCH7952998	GRS	G17	137621272	45	F	10/12/2014	CSF	No clinical details supplied	Negative		119		Neg				
701	SCH7953281	GRS	C15	145300562	33	M	10/12/2014	CSF	No clinical details supplied	Negative		123		Neg		Positive	17	
702	SCH7954543	RED	S12	140658808	11m	F	11/12/2014	CSF	Tuberculous meningitis ?	Negative		124			Neg	Positive	649	10 000 000
703	SCH7957605	GRS	E7	147965800	43	F	12/12/2014	CSF		Negative		126		Neg	Neg			
704	SCH7964078	RED	B2	16027484	9	F	16/12/2014	CSF	No clinical details supplied	Negative		113		Neg		Positive	335	553
705	SCH7965423	GRS	G17	139570261	18	F	17/12/2014	CSF	Meningitis	Negative		120		Neg		Neg		
706	SCH7967630	GRS	C5	69738482	34	M	18/12/2014	CSF	No clinical details supplied	Negative		108	<1.0	Neg				
707	SCH7977214	GRS	E7	146707070	40	F	24/12/2014	CSF	No clinical information	Negative		125			Neg	Neg		
708	SCH7978225	KNY	5	140910696	10m	M	25/12/2014	CSF	Meningitis	Negative		118	<1.0					
709	SCH7979079	RED	E2	136181302	3	M	26/12/2014	CSF	Tuberculous meningitis	Positive for AFB	MTB - 24 days	116		Neg				
710	SCH7979903	HEIEC	EC	148272198	34	F	28/12/2014	CSF	Meningitis	Negative		125		Neg		Positive	499	
711	SCH7982461	RED	D1	148172596	4	M	30/12/2014	CSF	Hydrocephalus	Negative		120		Neg				

APPENDIX G

De–identified raw data sheets – TB culture & CSF ADA

Number	Labno	Locn	Ward	PatID	Age	Sex	Specimen Date	Specimen	Clinical Data	TB Culture	ID and TTP	CSF Cl	CSF ADA	ZN Stain	GeneXpert	HIV	CD4	Viral Load
1	SCH5647192	GRS	G12	20804035	28	F	16/01/2012	CSF	Investigation	Negative			<1.0	Neg				
2	SCH5692856	GRS		81212219	49	F	06/02/2012	CSF		Negative			1.2	Neg		Neg		
3	SCH5729919	GRS	E7	37056041	35	M	22/02/2012	CSF	Dementia	Negative		129	2.6	Neg		Positive		
4	SCH5734708	GRS	G17	65425001	32	F	24/02/2012	CSF		Negative		144	<1.0	Neg				
5	SCH5764180	GRS	E7	17079278	35	F	09/03/2012	CSF	Investigation	Positive for AFB	MTB - 17 days		48.3	Neg				
6	SCH5778460	GRS	C15	29314093	36	F	16/03/2012	CSF	Tuberculous meningitis	Negative		126	<1.0	Neg		Neg		
7	SCH5796304	GRS	C12	57908469	60	M	26/03/2012	CSF	Investigation	Negative			1.1	Neg				
8	SCH5799740	GRS	G17	124179730	52	F	27/03/2012	CSF	Sepsis	Negative			<1.0	Neg		Positive	5	
9	SCH5806967	GRS	G16	80670524	44	M	30/03/2012	CSF	Laboratory investigation	Negative			1.5	Neg		Positive		108 628
10	SCH5815360	GRS	C15	15255979	35	F	03/04/2012	CSF	No clinical details supplied	Negative			<1.0	Neg				
11	SCH5823405	GRS	G16	62070685	38	M	08/04/2012	CSF	Laboratory investigation	Negative		124	<1.0	Neg		Neg		
12	SCH5857402	GRS	C15	13736186	70	M	25/04/2012	CSF	Investigation	Negative			<1.0	Neg				
13	SCH5891194	WYN	CAS	108169301	25	F	12/05/2012	CSF	Investigation	Positive for AFB	MTB - 19 days		9.6	Neg		Positive	52	
14	SCH5898314	GRS	C15	73173122	28	M	16/05/2012	CSF	Meningitis	Negative			7	Neg		Neg		
15	SCH5936346	WYN	CAS	65206732	38	F	03/06/2012	CSF	Meningitis	Negative			<1.0	Neg				
16	SCH5975757	GRS	C15	78464484	21	M	21/06/2012	CSF	Free text diagnosis ?meningitis	Negative			<1.0	Neg		Neg		
17	SCH5979512	GRS	G17	125750406	66	M	24/06/2012	CSF	Investigation	Negative			<1.0	Neg				
18	SCH5992159	GRS	C15	22805857	27	F	29/06/2012	CSF	?sepsis	Negative			<1.0	Neg		Positive	662	
19	SCH5992251	WYN	SMM	115836736	55	M	29/06/2012	CSF	CONFUSION	Negative			<1.0	Neg		Neg		
20	SCH6001006	GRS	C15	75615351	45	F	05/07/2012	CSF	ACUTE CONFUSIONAL STATE	Positive for AFB	MTB - 26 days		6.4	Neg		Neg		
21	SCH6021902	GRS	C15	117621664	52	M	15/07/2012	CSF	Tuberculosis	Negative			<1.0	Neg				
22	SCH6034665	GRS	C15	111024204	32	F	20/07/2012	CSF	Laboratory investigation	Positive for AFB	MTB - 18 days		6.3	Neg		Positive		
23	SCH6049806	GRS	C15	84893437	15	M	27/07/2012	CSF	Meningitis ?	Negative			2.3	Neg				
24	SCH6061693	WYN	CAS	30169502	20	F	03/08/2012	CSF	Suspect TB	Negative			<1.0	Neg		Neg		
25	SCH6082419	GFJ		70883343	33	F	14/08/2012	CSF	No clinical details supplied	Positive for AFB	MTB - 23 days		24.5	Neg				
26	SCH6084480	SOM	KING	19744903	38	M	15/08/2012	CSF	Meningitis	Negative		128	1.5	Neg		Neg		
27	SCH6088786	SOM	CAS	129615423	19	F	16/08/2012	CSF	Pneumonia	Negative			1.1	Neg				
28	SCH6088914	SOM	BICK	33226879	23	F	16/08/2012	CSF	Laboratory investigation	Negative			2.4	Neg		Positive		1 530
29	SCH6092224	WYN	CAS	117432682	35	M	18/08/2012	CSF	Meningitis	Negative			9.7	Neg		Positive	27	
30	SCH6098390	GRS	C15	52334711	52	M	21/08/2012	CSF	Investigation	Negative			3	Neg		Neg		
31	SCH6099513	GRS	C15	40509077	24	F	22/08/2012	CSF	Investigation	Negative			3.3	Neg		Positive	12	
32	SCH6101017	SOM	BICK	74287210	44	F	22/08/2012	CSF	Laboratory investigation	Negative			10.5	Neg				
33	SCH6109018	SOM	CAS	126022995	38	M	25/08/2012	CSF	Investigation	Negative		127	<1.0	Neg				
34	SCH6110736	GRS	C15	103127023	34	F	27/08/2012	CSF	Laboratory investigation	Negative		142	<1.0	Neg		Positive	188	
35	SCH6123534	GRS	D15	123131450	31	M	31/08/2012	CSF	Laboratory investigation	Positive for AFB	MTB - 38 days		3.6	Neg		Positive	60	
36	SCH6125833	GRS	C15	43147776	44	M	02/09/2012	CSF	Laboratory investigation	Positive for AFB	MTB - 15 days		4.3	Neg		Positive	51	
37	SCH6126727	SOM	KING	27949338	42	M	03/09/2012	CSF	Investigation	Negative			<1.0	Neg				
38	SCH6130014	GFJ		13518030	60	M	04/09/2012	CSF	Sepsis	Positive for AFB	MTB - 21 days		5.7	Neg				
39	SCH6134695	SOM	CAS	71108435	46	M	06/09/2012	CSF	Laboratory investigation	Negative			<1.0					
40	SCH6139709	GRS	C15	72089360	50	F	07/09/2012	CSF	Laboratory investigation	Negative			7	Neg		Positive	352	
41	SCH6143741	GRS	G7	32358798	64	M	10/09/2012	CSF	Investigation	Negative			1.2	Neg		Positive	20	123
42	SCH6147546	BROCH	D	113683643	31		11/09/2012	CSF	Investigation	Negative			<1.0	Neg		Positive		132
43	SCH6157286	GRS	C15	37430766	28	F	15/09/2012	CSF	No clinical information	Negative		120	<1.0	Neg				
44	SCH6189979	WYN	CAS	103596383	57	M	01/10/2012	CSF	Investigation	Negative			<1.0	Neg				
45	SCH6201701	SOM	BICK	88044854	64	F	05/10/2012	CSF	Investigation	Negative		141	<1.0	Neg		Neg		
46	SCH6202306	GRS	K41	58004508	32	F	06/10/2012	CSF	No clinical details supplied	Negative			8.8	Neg		Neg		
47	SCH6218089	GRS	G16	31502461	60	M	12/10/2012	CSF	Laboratory investigation	Negative		124	<1.0	Neg		Neg		
48	SCH6236480	SOM	KING	55062285	53	M	20/10/2012	CSF	Laboratory investigation	Negative		132	<1.0	Neg				
49	SCH6236485	SOW	CAS	81992778	37	F	20/10/2012	CSF	Laboratory investigation	Positive for AFB	MTB - 24 days		2.7	Neg				
50	SCH6241751	SOM	KING	130676836	44	M	23/10/2012	CSF	Investigation	Negative		140	1.8	Neg				
51	SCH6244002	KNY	2	20155487	32	M	24/10/2012	CSF	?MENINGITIS	Negative		120	<1.0	Neg		Positive		40
52	SCH6246954	KNY		21561451	57	F	25/10/2012	CSF	Cerebro-vascular accident	Negative		121	<1.0					
53	SCH6256333	GRS	C15	130219991	39	M	30/10/2012	CSF	Investigation	Negative			1.1	Neg		Positive	11	
54	SCH6272304	WYN	CAS	56194657	50	M	06/11/2012	CSF	Laboratory investigation	Positive for AFB	MTB - 20 days		6.8	Positive AFB		Neg	462	
55	SCH6295461	GRS	C15	70908645	24	M	15/11/2012	CSF	Meningitis ?	Negative			<1.0	Neg		Neg		
56	SCH6303940	GRS	E7	119272912	22	F	20/11/2012	CSF	Laboratory investigation	Negative		125	<1.0	Neg				
57	SCH6315745	GRS	C15	116744947	31	F	26/11/2012	CSF	No clinical details supplied	Negative		109	3.4	Neg		Positive		
58	SCH6325215	GRS	G22	33490913	44	F	29/11/2012	CSF	Psychosis	Negative		123	<1.0	Neg		Positive	245	
59	SCH6326844	GRS	C15	43789718	27	F	30/11/2012	CSF	Meningitis TB?	Negative		121	<1.0	Neg		Positive	36	
60	SCH6347186	GRS	C15	33513102	53	M	10/12/2012	CSF	Laboratory investigation	Negative		121	5.8	Neg		Positive	123	
61	SCH6348863	GRS	F5	41090390	37	F	11/12/2012	CSF	Laboratory investigation	Negative		128	<1.0	Neg		Positive	545	
62	SCH6365877	GRS	C15	10617280	33	M	20/12/2012	CSF	Investigation	Negative		118	<1.0	Neg		Neg	128	
63	SCH6372219	KNY	4	21103049	29	F	25/12/2012	CSF	ENCEPHILITIS?	Negative			<1.0					
64	SCH6380829	WYN		131910531	33	M	02/01/2013	CSF	Meningitis	Negative			1.7			Positive	210	204 600
65	SCH6382663	GRS	C15	44138691	40	M	04/01/2013	CSF		Negative			1.6	Neg				
66	SCH6386938	SOM	CAS	89395859	39	F	07/01/2013	CSF	Meningitis	Negative			2.4	Neg				
67	SCH6439283	WEF	ARV	46557765	37	F	31/01/2013	CSF	Investigation	Negative		124	<1.0					
68	SCH6469149	SOM	CAS	40632697	35	F	13/02/2013	CSF	No clinical details supplied	Negative			5.5	Neg		Positive	250	40
69	SCH6510418	GRS	C15	34663476	43	M	02/03/2013	CSF	Delirium	Negative			1.4	Neg		Neg		
70	SCH6528001	GRS	G17	65696940	28	F	11/03/2013	CSF	Laboratory investigation	Negative		118	2.1	Neg		Neg		
71	SCH6561871	GRS	G25	59973560	47	F	26/03/2013	CSF	Laboratory investigation	Negative		122	1.2	Neg		Neg		
72	SCH6592891	GRS	C15	86869781	53	F	10/04/2013	CSF	Investigation	Negative		125	<1.0	Neg		Positive	159	84
73	SCH6602527	GRS	C15	84631019	37	F	15/04/2013	CSF	No clinical information	Negative		125	<1.0	Neg				
74	SCH6621477	KNY	2	20060083	63	M	23/04/2013	CSF		Negative		119	2.5			Positive		
75	SCH6628759	GRS	C15	38086740	62	M	25/04/2013	CSF	No clinical details supplied	Positive for AFB	MTB - 18 days		48.1	Neg		Neg		
76	SCH6630695	GRS	G17	56104284	33	M	26/04/2013	CSF	Pulmonary tuberculosis	Negative		122	<1.0	Neg		Neg		

77	SCH6640324	KNY	2	20614491	32	M	02/05/2013	CSF	Pulmonary tuberculosis	Positive for AFB	MTB - 18 days	111	4.1	Neg					
78	SCH6701224	GRS	G12	73418113	33	F	29/05/2013	CSF	Laboratory investigation	Negative			<1.0	Neg		Positive	7		
79	SCH6730014	GRS	K41	129099842	24	F	11/06/2013	CSF	Investigation	Negative			2.1	Neg					
80	SCH6796673	SOM	BAIL	132461567	41	M	12/07/2013	CSF	Laboratory investigation	Negative			1.3	Neg					
81	SCH6810263	GRS	G5	76209139	50	F	18/07/2013	CSF	Investigation	Negative			<1.0	Neg		Positive			
82	SCH6881350	MOS	CAS	2205045	28	F	20/08/2013	CSF	Meningitis	Negative		122	<1.0			Positive			
83	SCH6916489	SOM	KING	103267118	40	M	04/09/2013	CSF	No clinical details supplied	Negative			1.1	Neg		Positive			
84	SCH6922532	DPMSA	4A	54909791	34	M	06/09/2013	CSF	Pulmonary tuberculosis seizers	Negative			2.3	Neg		Positive			189
85	SCH6924288	WYN		73936866	44	F	07/09/2013	CSF	Investigation	Negative			2.8	Neg		Positive	67		
86	SCH6939302	SOM	BAIL	131399792	31	M	13/09/2013	CSF	No clinical information	Negative			7.8	Neg		Positive			
87	SCH6948665	GRS	F26	85621431	45	F	18/09/2013	CSF	No clinical information	Negative			9.4	Neg		Positive			10 000 000
88	SCH7029858	GRS	C27	104881958	45	M	24/10/2013	CSF	Laboratory investigation	Negative		96	14.1	Neg					
89	SCH7066551	GEO	B2	109831008	42	M	09/11/2013	CSF	Laboratory investigation	Negative		135	1.9						
90	SCH7067365	GRS	C15	137638433	37	M	10/11/2013	CSF	Investigation	Negative		143	2.2	Neg		Positive	81		
91	SCH7104389	GRS	C27	35969427	36	F	26/11/2013	CSF	Laboratory investigation	Negative		122	1.5	Neg		Positive	156		
92	SCH7160344	SOM	CAS	139303838	22	F	23/12/2013	CSF	No clinical details supplied	Negative			4.4	Neg		Neg			
93	SCH7163009	WYN	CAS	117966564	29	F	24/12/2013	CSF	Meningitis	Negative			2.4	Neg		Positive			
94	SCH7163445	SOM	CAS	119239499	28	M	25/12/2013	CSF	No clinical details supplied	Negative			<1.0	Neg		Neg			
95	SCH7168120	SOM	CAS	139366348	33	M	30/12/2013	CSF	NEW ONSET SEIZURES	Negative			<1.0	Neg		Neg			
96	SCH7173446	SOM	CAS	73745325	57	F	03/01/2014	CSF	Laboratory investigation Arachnoiditis	Negative			44.7	Neg		Neg			
97	SCH7179954	GRS	F8	19580760	45	M	07/01/2014	CSF	Investigation	Negative			<1.0	Neg		Positive	174		
98	SCH7187161	GRS	D12	132443763	39	F	10/01/2014	CSF	No clinical details supplied	Negative			1.6	Neg		Positive	208	40	
99	SCH7187494	GRS	C15	61094116	44	M	10/01/2014	CSF	Investigation	Negative			4.5	Neg		Positive	215	40	
100	SCH7202389	SOM	CAS	117212951	33	M	17/01/2014	CSF	Meningitis ?	Negative			<1.0	Neg					
101	SCH7213862	WYN	SMM	119836955	24	M	22/01/2014	CSF	Investigation	Positive for AFB	MTB - 8 days		8.5	Positive AFB		Positive	4		
102	SCH7231668	GRS	C15	46774642	43	F	29/01/2014	CSF	Meningitis	Negative		129	2.4	Neg		Positive	514	22 006	
103	SCH7236812	SOM	CAS	88741111	40	F	31/01/2014	CSF	No clinical details supplied	Negative			2.3	Neg					
104	SCH7288743	GRS	C15	136013901	49	M	21/02/2014	CSF	Laboratory investigation	Negative			2.9	Neg		Positive	88		
105	SCH7308719	WYN		72312689	46	M	02/03/2014	BCSF	Meningitis ?	Positive for AFB	MTB - 16 days		7.5	Neg		Positive	157		
106	SCH7342523	GRS	C14	141947671	16	M	16/03/2014	CSF	No clinical details supplied	Negative		124	3.3	Neg		Neg			
107	SCH7447878	GRS	C15	142854553	18	F	03/05/2014	CSF	Meningitis	Negative			4.4	Neg		Positive	68		
108	SCH7463442	GRS	C15	57835860	68	F	12/05/2014	CSF	No clinical details supplied	Negative			19.3		Neg				
109	SCH7470440	GRS	D13	85369601	17	M	14/05/2014	CSF	Laboratory investigation	Positive for AFB	MTB - 12 days		5.9	Neg		Positive	554		
110	SCH7479831	GRS	C15	25652215	27	F	18/05/2014	CSF	Illegible diagnosis/ICD10	Negative			2.5				499		
111	SCH7493302	GRS	G25	87939336	69	F	23/05/2014	CSF	No clinical details supplied	Negative		122	<1.0	Neg	Neg	Neg			
112	SCH7503148	FAL	CAS	142364421	53	F	28/05/2014	CSF	?Meningitis	Negative			2.3	Neg			209		
113	SCH7519957	GRS	C15	135182921	68	M	04/06/2014	CSF	CEREBRILE	Negative			1.1	Neg		Neg			
114	SCH7557918	GRS	C15	144082948	45	M	22/06/2014	CSF	?MENINGITIS	Positive for AFB	MTB - 19 days		7.6	Neg					
115	SCH7561405	MOS	C	1105022	34	F	24/06/2014	CSF	Retroviral Disease	Negative			4.1			Positive			3 834
116	SCH7562978	WYN	SMM	87330486	13	M	24/06/2014	CSF	Tuberculous meningitis	Negative			70.6		Neg	Neg			
117	SCH7576168	WYN	SMF	64880222	30	F	30/06/2014	CSF	Gastro Enteritis HIV	Negative			2		Neg	Positive	162		
118	SCH7590134	GRS	C15	124638024	38	M	06/07/2014	CSF	Pyrexia of unknown origin	Negative			<1.0	Neg		Positive	193	40	
119	SCH7590306	GRS	C15	26863357	36	M	06/07/2014	CSF	No clinical details supplied	Positive for AFB	MTB - 15 days		7.9	Neg					
120	SCH7590496	GRS	C15	45232287	24	M	06/07/2014	CSF	Tuberculous meningitis	Positive for AFB	MTB - 15 days		25.3			Positive			
121	SCH7612489	GRS	C15	144445368	32	F	16/07/2014	CSF	?MENINGITIS	Negative			<1.0	Neg		Positive			
122	SCH7627110	2MIL	LAB	94875549PE	41	M	22/07/2014	CSF	Tuberculosis	Negative			3.2	Neg		Positive			83 612
123	SCH7639139	SOM	CAS	103309910	27	F	27/07/2014	CSF	Meningism / Confusion	Negative			1.7	Neg		Positive			1 902 683
124	SCH7646819	GRS	G8	39837505	28	M	30/07/2014	CSF	Illegible diagnosis/ICD10	Negative			1.6	Neg		Positive	215		
125	SCH7647072	GRS	G8	112351739	50	F	30/07/2014	CSF	BURKITTIS LYMPHOMA	Negative			2.5	Neg		Positive	160		
126	SCH7669531	GRS	C15	144618097	33	M	08/08/2014	CSF	Tuberculosis	Negative			1.6	Neg		Neg			
127	SCH7672215	GRS	D12	68080829	28	M	09/08/2014	CSF	Meningitis ?	Negative		109	15.1	Neg		Neg			
128	SCH7696765	GRS	E7	37610995	31	F	20/08/2014	CSF	Tuberculous meningitis ?	Negative			<1.0	Neg		Positive	735		
129	SCH7705030	SOW	C2	21924402	41	M	23/08/2014	CSF	Meningitis	Negative			4.7	Neg		Positive	353		
130	SCH7730356	GRS	C15	57077232	57	F	04/09/2014	CSF	No clinical information	Positive for AFB	MTB - 32 days	110	7.3	Neg	Neg	Positive			
131	SCH7734173	GRS	C15	125570374	36	F	05/09/2014	CSF	Meningitis	Negative		119	8	Neg	Neg	Positive	414		
132	SCH7734771	SOM	ICU	144547833	40	M	05/09/2014	CSF	Cerebro-vascular accident Pulmonary oedema / hy	Negative			1.2			Positive			
133	SCH7738449	2MIL	SURG	86752508DB	8	M	08/09/2014	CSF	Laboratory investigation	Negative			<1.0	Neg					
134	SCH7745366	GRS	C15	46336954	26	F	10/09/2014	CSF	Meningitis	Negative			3	Neg			614		
135	SCH7766156	GRS	C15	23627078	39	M	19/09/2014	CSF	Illegible diagnosis/ICD10	Negative			4.4		Neg	Positive	285	40	
136	SCH7768857	WYN		14417414	37	F	20/09/2014	CSF	Tuberculous meningitis	Negative			<1.0	Neg		Positive	260		
137	SCH7791790	GRS	C15	29163599	31	F	01/10/2014	CSF	No clinical details supplied	Negative		124	<1.0	Neg		Positive			154
138	SCH7800770	GRS	G25	142820570	30	F	06/10/2014	CSF	? TB MENINGITIS	Negative		118	2.6	Neg	Neg	Neg			
139	SCH7817731	2MIL	LAB	98141062MC	36	F	13/10/2014	CSF	Delirium	Negative			1.3	Neg		Positive			12 902
140	SCH7866147	GRS	C15	146441845	66	F	03/11/2014	CSF	Meningitis	Negative			5.6	Neg		Positive	289	40	
141	SCH7873237	WYN	CAS	139347934	14	F	06/11/2014	CSF	Meningitis	Negative		120	3.6	Neg					
142	SCH7876812	WYN		102879392	24	F	07/11/2014	CSF	Laboratory investigation	Negative			2.1	Neg					
143	SCH7882290	WYN	SMF	146514518	42	F	10/11/2014	CSF		Negative			2.3		Neg	Positive	620		
144	SCH7894798	2MIL	DERM	94845377DA	43	F	14/11/2014	CSF	Laboratory investigation ?meningitis	Negative			1.1	Neg		Positive			10 000 000
145	SCH7897178	FAL	CAS	124813387	29	M	15/11/2014	CSF	Meningitis ?	Negative			8.6		Neg		134		
146	SCH7905071	2MIL	LAB	99040057MC	31	M	19/11/2014	CSF	Headache	Negative			1.5	Neg					
147	SCH7915118	GRS	C5	146531405	39	M	24/11/2014	CSF	?IRIS	Negative		129	1.8	Neg		Positive	27		
148	SCH7926079	SOM	BICK	147879332	62	M	28/11/2014	CSF	No clinical details supplied	Negative			<1.0		Neg	Neg			
149	SCH7926792	WYN	CAS	75749176	29	F	28/11/2014	CSF	Laboratory investigation	Negative			2.7	Neg		Positive	395	407 656	
150	SCH7967630	GRS	C5	69738482	34	M	18/12/2014	CSF	No clinical details supplied	Negative		108	<1.0	Neg					
151	SCH7978225	KNY	5	140910696	10m	M	25/12/2014	CSF	Meningitis	Negative		118	<1.0						
152	SCH7978410	SOM	CAS	44300523	30	F	25/12/2014	CSF	Retrovirus positive ? TBM	Positive for AFB	MTB - 18 days		10.8	Neg		Positive	36		

APPENDIX H

STATA – Results of statistical calculations

CSF chloride diagnostic accuracy in 0-12 year age group: All patients / HIV+ / HIV-

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                                                     ___  ____  ____  ____  ____(R)
                                                    /__    /   ____/   /   ____/
                                                   ___/   /   /___/   /   /___/
                                                     Statistics/Data Analysis


      ___  ____  ____  ____  ____ (R)
     /__    /   ____/   /   ____/
    ___/   /   /___/   /   /___/   12.0   Copyright 1985-2011 StataCorp LP
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                                          979-696-4601 (fax)

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    Notes:

  1 . capture log close

  2 . clear

  3 . clear matrix

  4 . set more off

  5 . diagti 20 0 115 141

           True |
        disease |      Test result
         status |      Neg.       Pos. |     Total
    ------------+----------------------+----------
         Normal |       141        115 |       256
       Abnormal |         0         20 |        20
    ------------+----------------------+----------
          Total |       141        135 |       276

                                                       [95% Confidence Interval]
    ---------------------------------------------------------------------------
    Prevalence                          Pr(A)      7.2%      4.5%        11%
    ---------------------------------------------------------------------------
    Sensitivity                       Pr(+|A)      100%     83.2%      100%
    Specificity                       Pr(-|N)     55.1%     48.8%     61.3%
    ROC area                  (Sens. + Spec.)/2    .775      .745      .806
    ---------------------------------------------------------------------------
    Likelihood ratio (+)      Pr(+|A)/Pr(+|N)      2.23      1.94      2.55
    Likelihood ratio (-)      Pr(-|A)/Pr(-|N)         0         .         .
    Odds ratio                      LR(+)/LR(-)       .      6.33         .
    Positive predictive value         Pr(A|+)     14.8%     9.29%     21.9%
    Negative predictive value         Pr(N|-)      100%     97.4%      100%
    ---------------------------------------------------------------------------

      Missing values or confidence intervals may be estimated
      using the -sf- or -sf0- options.

  6 . diagti 1 0 16 22

           True |
        disease |      Test result
         status |      Neg.       Pos. |     Total
    ------------+----------------------+----------
         Normal |        22         16 |        38
       Abnormal |         0          1 |         1
    ------------+----------------------+----------
          Total |        22         17 |        39
```

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			[95% Confidence Interval]	
Prevalence	Pr(A)	**2.6%**	**.065%**	**13.5%**
Sensitivity	Pr(+|A)	**100%**	**2.5%**	**100%**
Specificity	Pr(-|N)	**57.9%**	**40.8%**	**73.7%**
ROC area	(Sens. + Spec.)/2	**.789**	.	**1**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**2.38**	**1.64**	**3.45**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**0**	.	.
Odds ratio	LR(+)/LR(-)	.	**0**	.
Positive predictive value	Pr(A|+)	**5.88%**	**.149%**	**28.7%**
Negative predictive value	Pr(N|-)	**100%**	**84.6%**	**100%**

Missing values or confidence intervals may be estimated
using the -sf- or -sf0- options.

```
7 . diagti 6 0 54 73
```

True disease status	Test result Neg.	Pos.	Total
Normal	**73**	**54**	**127**
Abnormal	**0**	**6**	**6**
Total	**73**	**60**	**133**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**4.5%**	**1.7%**	**9.56%**
Sensitivity	Pr(+|A)	**100%**	**54.1%**	**100%**
Specificity	Pr(-|N)	**57.5%**	**48.4%**	**66.2%**
ROC area	(Sens. + Spec.)/2	**.787**	**.744**	**.831**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**2.35**	**1.92**	**2.88**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**0**	.	.
Odds ratio	LR(+)/LR(-)	.	**2.05**	.
Positive predictive value	Pr(A|+)	**10%**	**3.76%**	**20.5%**
Negative predictive value	Pr(N|-)	**100%**	**95.1%**	**100%**

Missing values or confidence intervals may be estimated
using the -sf- or -sf0- options.

```
8 .
```

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```
                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
                                                    ___/   /   /___/   /   /___/
                                                      Statistics/Data Analysis
```

```
      ___  ____  ____  ____  ____ (R)
     /__    /   ____/   /   ____/
    ___/   /   /___/   /   /___/   12.0   Copyright 1985-2011 StataCorp LP
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                                           College Station, Texas 77845 USA
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                                           979-696-4601 (fax)

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           Licensed to:  Hendre Swanepoel
                         UCT_HS

  Notes:

1 . capture log close

2 . clear

3 . clear matrix

4 . set more off

5 . diagti 21 3 136 275
```

True disease status	Test result Neg.	Pos.	Total
Normal	**275**	**136**	**411**
Abnormal	**3**	**21**	**24**
Total	**278**	**157**	**435**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**5.5%**	**3.6%**	**8.1%**
Sensitivity	Pr(+|A)	**87.5%**	**67.6%**	**97.3%**
Specificity	Pr(-|N)	**66.9%**	**62.1%**	**71.4%**
ROC area	(Sens. + Spec.)/2	**.772**	**.701**	**.843**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**2.64**	**2.16**	**3.24**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**.187**	**.0647**	**.54**
Odds ratio	LR(+)/LR(-)	**14.2**	**4.41**	**45.2**
Positive predictive value	Pr(A|+)	**13.4%**	**8.47%**	**19.7%**
Negative predictive value	Pr(N|-)	**98.9%**	**96.9%**	**99.8%**

```
6 . diagti 10 3 58 105
```

True disease status	Test result Neg.	Pos.	Total
Normal	**105**	**58**	**163**
Abnormal	**3**	**10**	**13**
Total	**108**	**68**	**176**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**7.4%**	**4%**	**12.3%**
Sensitivity	Pr(+|A)	**76.9%**	**46.2%**	**95%**
Specificity	Pr(-|N)	**64.4%**	**56.6%**	**71.7%**
ROC area	(Sens. + Spec.)/2	**.707**	**.582**	**.831**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**2.16**	**1.5**	**3.11**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**.358**	**.132**	**.973**
Odds ratio	LR(+)/LR(-)	**6.03**	**1.71**	**21.1**
Positive predictive value	Pr(A|+)	**14.7%**	**7.28%**	**25.4%**
Negative predictive value	Pr(N|-)	**97.2%**	**92.1%**	**99.4%**

7 . diagti 5 0 39 81

True disease status	Test result Neg.	Pos.	Total
Normal	**81**	**39**	**120**
Abnormal	**0**	**5**	**5**
Total	**81**	**44**	**125**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**4%**	**1.3%**	**9.09%**
Sensitivity	Pr(+|A)	**100%**	**47.8%**	**100%**
Specificity	Pr(-|N)	**67.5%**	**58.3%**	**75.8%**
ROC area	(Sens. + Spec.)/2	**.838**	**.795**	**.88**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**3.08**	**2.38**	**3.98**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**0**	**.**	**.**
Odds ratio	LR(+)/LR(-)	**.**	**2.61**	**.**
Positive predictive value	Pr(A|+)	**11.4%**	**3.79%**	**24.6%**
Negative predictive value	Pr(N|-)	**100%**	**95.5%**	**100%**

Missing values or confidence intervals may be estimated
using the -sf- or -sf0- options.

8 .

```
                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
                                                    ___/   /   /___/   /   /___/
                                                      Statistics/Data Analysis
```

```
  ___  ____  ____  ____  ____ (R)
 /__    /   ____/   /   ____/
___/   /   /___/   /   /___/   12.0   Copyright 1985-2011 StataCorp LP
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                                       College Station, Texas 77845 USA
                                       800-STATA-PC        http://www.stata.com
                                       979-696-4600        stata@stata.com
                                       979-696-4601 (fax)

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         Licensed to:  Hendre Swanepoel
                       UCT_HS

Notes:

1 . capture log close

2 . clear

3 . clear matrix

4 . set more off

5 . diagti 41 3 251 416
```

True disease status	Test result Neg.	Pos.	Total
Normal	**416**	**251**	**667**
Abnormal	**3**	**41**	**44**
Total	**419**	**292**	**711**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**6.2%**	**4.5%**	**8.22%**
Sensitivity	Pr(+|A)	**93.2%**	**81.3%**	**98.6%**
Specificity	Pr(-|N)	**62.4%**	**58.6%**	**66.1%**
ROC area	(Sens. + Spec.)/2	**.778**	**.736**	**.82**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**2.48**	**2.18**	**2.81**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**.109**	**.0366**	**.326**
Odds ratio	LR(+)/LR(-)	**22.7**	**7.36**	**69.7**
Positive predictive value	Pr(A|+)	**14%**	**10.3%**	**18.6%**
Negative predictive value	Pr(N|-)	**99.3%**	**97.9%**	**99.9%**

```
6 . diagti 11 3 74 127
```

True disease status	Test result Neg.	Pos.	Total
Normal	**127**	**74**	**201**
Abnormal	**3**	**11**	**14**
Total	**130**	**85**	**215**

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			[95% Confidence Interval]	
Prevalence	Pr(A)	**6.5%**	**3.6%**	**10.7%**
Sensitivity	Pr(+|A)	**78.6%**	**49.2%**	**95.3%**
Specificity	Pr(-|N)	**63.2%**	**56.1%**	**69.9%**
ROC area	(Sens. + Spec.)/2	**.709**	**.592**	**.825**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**2.13**	**1.54**	**2.96**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**.339**	**.124**	**.93**
Odds ratio	LR(+)/LR(-)	**6.29**	**1.82**	**21.6**
Positive predictive value	Pr(A|+)	**12.9%**	**6.64%**	**22%**
Negative predictive value	Pr(N|-)	**97.7%**	**93.4%**	**99.5%**

7 . diagti 11 0 93 154

True disease status	Test result Neg.	Pos.	Total
Normal	**154**	**93**	**247**
Abnormal	**0**	**11**	**11**
Total	**154**	**104**	**258**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**4.3%**	**2.1%**	**7.5%**
Sensitivity	Pr(+|A)	**100%**	**71.5%**	**100%**
Specificity	Pr(-|N)	**62.3%**	**56%**	**68.4%**
ROC area	(Sens. + Spec.)/2	**.812**	**.781**	**.842**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**2.66**	**2.26**	**3.12**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**0**	**.**	**.**
Odds ratio	LR(+)/LR(-)	**.**	**4.68**	**.**
Positive predictive value	Pr(A|+)	**10.6%**	**5.4%**	**18.1%**
Negative predictive value	Pr(N|-)	**100%**	**97.6%**	**100%**

Missing values or confidence intervals may be estimated
using the -sf- or -sf0- options.

8 .

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```
                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
                                                    ___/   /   /___/   /   /___/
                                                      Statistics/Data Analysis
```

```
  ___  ____  ____  ____  ____ (R)
 /__    /   ____/   /   ____/
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Notes:

1 . capture log close

2 . clear

3 . clear matrix

4 . set more off

5 . diagti 14 6 14 118
```

True disease status	Test result Neg.	Pos.	Total
Normal	**118**	**14**	**132**
Abnormal	**6**	**14**	**20**
Total	**124**	**28**	**152**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**13%**	**8.2%**	**19.6%**
Sensitivity	Pr(+|A)	**70%**	**45.7%**	**88.1%**
Specificity	Pr(-|N)	**89.4%**	**82.8%**	**94.1%**
ROC area	(Sens. + Spec.)/2	**.797**	**.691**	**.903**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**6.6**	**3.72**	**11.7**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**.336**	**.171**	**.657**
Odds ratio	LR(+)/LR(-)	**19.7**	**6.66**	**57.9**
Positive predictive value	Pr(A|+)	**50%**	**30.6%**	**69.4%**
Negative predictive value	Pr(N|-)	**95.2%**	**89.8%**	**98.2%**

```
6 . diagti 7 3 5 55
```

True disease status	Test result Neg.	Pos.	Total
Normal	**55**	**5**	**60**
Abnormal	**3**	**7**	**10**
Total	**58**	**12**	**70**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**14%**	**7.1%**	**24.7%**
Sensitivity	Pr(+|A)	**70%**	**34.8%**	**93.3%**
Specificity	Pr(-|N)	**91.7%**	**81.6%**	**97.2%**
ROC area	(Sens. + Spec.)/2	**.808**	**.655**	**.962**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**8.4**	**3.31**	**21.3**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**.327**	**.127**	**.846**
Odds ratio	LR(+)/LR(-)	**25.7**	**5.34**	**123**
Positive predictive value	Pr(A|+)	**58.3%**	**27.7%**	**84.8%**
Negative predictive value	Pr(N|-)	**94.8%**	**85.6%**	**98.9%**

7 . diagti 3 0 5 25

True disease status	Test result Neg.	Pos.	Total
Normal	**25**	**5**	**30**
Abnormal	**0**	**3**	**3**
Total	**25**	**8**	**33**

			[95% Confidence Interval]	
Prevalence	Pr(A)	**9.1%**	**1.9%**	**24.3%**
Sensitivity	Pr(+|A)	**100%**	**29.2%**	**100%**
Specificity	Pr(-|N)	**83.3%**	**65.3%**	**94.4%**
ROC area	(Sens. + Spec.)/2	**.917**	**.849**	**.984**
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	**6**	**2.7**	**13.4**
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	**0**	**.**	**.**
Odds ratio	LR(+)/LR(-)	**.**	**3.18**	**.**
Positive predictive value	Pr(A|+)	**37.5%**	**8.52%**	**75.5%**
Negative predictive value	Pr(N|-)	**100%**	**86.3%**	**100%**

Missing values or confidence intervals may be estimated
using the -sf- or -sf0- options.

8 .

ROC curve calculations for CSF chloride

Notes:

```
1 . capture log close

2 . clear

3 . clear matrix

4 . set more off

5 . import excel "C:\Users\Hendre\Dropbox\MMed 2015\Data\Processed Data\TBCUL & CSF Cl - Stata import.xl

6 . gen NCSFCl=-CSFCl

7 . roctab TBCUL NCSFCl, binomial detail table graph summary
```

NCSFCl	TBCUL 0	1	Total
-159	1	0	1
-151	2	0	2
-148	2	0	2
-145	2	0	2
-144	2	0	2
-143	1	0	1
-142	3	0	3
-141	3	0	3
-140	4	0	4
-139	1	0	1
-138	4	0	4
-136	2	0	2
-135	3	0	3
-134	3	0	3
-133	3	0	3
-132	6	0	6
-131	10	0	10
-130	7	0	7
-129	13	0	13
-128	18	1	19
-127	29	0	29
-126	23	0	23
-125	31	1	32
-124	38	0	38
-123	47	0	47
-122	57	0	57
-121	49	1	50
-120	52	0	52
-119	42	1	43
-118	28	0	28
-117	22	0	22
-116	18	3	21
-115	20	2	22
-114	13	0	13
-113	12	3	15
-112	12	2	14
-111	10	4	14
-110	12	4	16

-109	**11**	**1**	**12**
-108	**6**	**3**	**9**
-107	**6**	**4**	**10**
-106	**4**	**3**	**7**
-105	**8**	**0**	**8**
-104	**6**	**2**	**8**
-103	**3**	**3**	**6**
-102	**5**	**0**	**5**
-101	**4**	**1**	**5**
-100	**1**	**1**	**2**
-98	**2**	**0**	**2**
-96	**1**	**2**	**3**
-95	**0**	**1**	**1**
-94	**1**	**0**	**1**
-93	**0**	**1**	**1**
-89	**1**	**0**	**1**
-85	**1**	**0**	**1**
-62	**1**	**0**	**1**
-45	**1**	**0**	**1**
Total	**667**	**44**	**711**

Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-
(>= -159)	**100.00%**	**0.00%**	**6.19%**	**1.0000**	
(>= -151)	**100.00%**	**0.15%**	**6.33%**	**1.0015**	**0.0000**
(>= -148)	**100.00%**	**0.45%**	**6.61%**	**1.0045**	**0.0000**
(>= -145)	**100.00%**	**0.75%**	**6.89%**	**1.0076**	**0.0000**
(>= -144)	**100.00%**	**1.05%**	**7.17%**	**1.0106**	**0.0000**
(>= -143)	**100.00%**	**1.35%**	**7.45%**	**1.0137**	**0.0000**
(>= -142)	**100.00%**	**1.50%**	**7.59%**	**1.0152**	**0.0000**
(>= -141)	**100.00%**	**1.95%**	**8.02%**	**1.0199**	**0.0000**
(>= -140)	**100.00%**	**2.40%**	**8.44%**	**1.0246**	**0.0000**
(>= -139)	**100.00%**	**3.00%**	**9.00%**	**1.0309**	**0.0000**
(>= -138)	**100.00%**	**3.15%**	**9.14%**	**1.0325**	**0.0000**
(>= -136)	**100.00%**	**3.75%**	**9.70%**	**1.0389**	**0.0000**
(>= -135)	**100.00%**	**4.05%**	**9.99%**	**1.0422**	**0.0000**
(>= -134)	**100.00%**	**4.50%**	**10.41%**	**1.0471**	**0.0000**
(>= -133)	**100.00%**	**4.95%**	**10.83%**	**1.0521**	**0.0000**
(>= -132)	**100.00%**	**5.40%**	**11.25%**	**1.0571**	**0.0000**
(>= -131)	**100.00%**	**6.30%**	**12.10%**	**1.0672**	**0.0000**
(>= -130)	**100.00%**	**7.80%**	**13.50%**	**1.0846**	**0.0000**
(>= -129)	**100.00%**	**8.85%**	**14.49%**	**1.0970**	**0.0000**
(>= -128)	**100.00%**	**10.79%**	**16.32%**	**1.1210**	**0.0000**
(>= -127)	**97.73%**	**13.49%**	**18.71%**	**1.1297**	**0.1684**
(>= -126)	**97.73%**	**17.84%**	**22.78%**	**1.1895**	**0.1274**
(>= -125)	**97.73%**	**21.29%**	**26.02%**	**1.2416**	**0.1068**
(>= -124)	**95.45%**	**25.94%**	**30.24%**	**1.2888**	**0.1752**
(>= -123)	**95.45%**	**31.63%**	**35.58%**	**1.3962**	**0.1437**
(>= -122)	**95.45%**	**38.68%**	**42.19%**	**1.5567**	**0.1175**
(>= -121)	**95.45%**	**47.23%**	**50.21%**	**1.8088**	**0.0962**
(>= -120)	**93.18%**	**54.57%**	**56.96%**	**2.0512**	**0.1249**
(>= -119)	**93.18%**	**62.37%**	**64.28%**	**2.4762**	**0.1093**
(>= -118)	**90.91%**	**68.67%**	**70.04%**	**2.9013**	**0.1324**
(>= -117)	**90.91%**	**72.86%**	**73.98%**	**3.3501**	**0.1248**
(>= -116)	**90.91%**	**76.16%**	**77.07%**	**3.8136**	**0.1194**
(>= -115)	**84.09%**	**78.86%**	**79.18%**	**3.9779**	**0.2017**
(>= -114)	**79.55%**	**81.86%**	**81.72%**	**4.3849**	**0.2499**
(>= -113)	**79.55%**	**83.81%**	**83.54%**	**4.9127**	**0.2441**
(>= -112)	**72.73%**	**85.61%**	**84.81%**	**5.0530**	**0.3186**
(>= -111)	**68.18%**	**87.41%**	**86.22%**	**5.4140**	**0.3640**
(>= -110)	**59.09%**	**88.91%**	**87.06%**	**5.3262**	**0.4601**
(>= -109)	**50.00%**	**90.70%**	**88.19%**	**5.3790**	**0.5512**
(>= -108)	**47.73%**	**92.35%**	**89.59%**	**6.2420**	**0.5660**
(>= -107)	**40.91%**	**93.25%**	**90.01%**	**6.0636**	**0.6337**
(>= -106)	**31.82%**	**94.15%**	**90.30%**	**5.4417**	**0.7242**
(>= -105)	**25.00%**	**94.75%**	**90.44%**	**4.7643**	**0.7915**
(>= -104)	**25.00%**	**95.95%**	**91.56%**	**6.1759**	**0.7816**
(>= -103)	**20.45%**	**96.85%**	**92.12%**	**6.4968**	**0.8213**

ROC curve calculations for CSF chloride

(>= -102)	**13.64%**	**97.30%**	**92.12%**	**5.0530**	**0.8876**
(>= -101)	**13.64%**	**98.05%**	**92.83%**	**6.9965**	**0.8808**
(>= -100)	**11.36%**	**98.65%**	**93.25%**	**8.4217**	**0.8985**
(>= -98)	**9.09%**	**98.80%**	**93.25%**	**7.5795**	**0.9201**
(>= -96)	**9.09%**	**99.10%**	**93.53%**	**10.1061**	**0.9173**
(>= -95)	**4.55%**	**99.25%**	**93.39%**	**6.0636**	**0.9618**
(>= -94)	**2.27%**	**99.25%**	**93.25%**	**3.0318**	**0.9847**
(>= -93)	**2.27%**	**99.40%**	**93.39%**	**3.7898**	**0.9832**
(>= -89)	**0.00%**	**99.40%**	**93.25%**	**0.0000**	**1.0060**
(>= -85)	**0.00%**	**99.55%**	**93.39%**	**0.0000**	**1.0045**
(>= -62)	**0.00%**	**99.70%**	**93.53%**	**0.0000**	**1.0030**
(>= -45)	**0.00%**	**99.85%**	**93.67%**	**0.0000**	**1.0015**
(> -45)	**0.00%**	**100.00%**	**93.81%**		**1.0000**

	ROC		Binomial Exact	
Obs	Area	Std. Err.	[95% Conf.	Interval]
711	**0.8615**	**0.0280**	**0.83462**	**0.88666**

8 .

ROC curve calculations for CSF ADA

Notes:

```
1 . capture log close

2 . clear

3 . clear matrix

4 . set more off

5 . import excel "C:\Users\Hendre\Dropbox\MMed 2015\Data\Processed Data\TBCUL & CSF ADA - Stata import.x

6 . roctab TBCUL CSFADA, binomial detail table graph summary
```

CSFADA	TBCUL 0	1	Total
.9	**54**	**0**	**54**
1.1	**6**	**0**	**6**
1.2	**4**	**0**	**4**
1.3	**2**	**0**	**2**
1.4	**1**	**0**	**1**
1.5	**4**	**0**	**4**
1.6	**4**	**0**	**4**
1.7	**2**	**0**	**2**
1.8	**2**	**0**	**2**
1.9	**1**	**0**	**1**
2	**1**	**0**	**1**
2.1	**3**	**0**	**3**
2.2	**1**	**0**	**1**
2.3	**5**	**0**	**5**
2.4	**4**	**0**	**4**
2.5	**3**	**0**	**3**
2.6	**2**	**0**	**2**
2.7	**1**	**1**	**2**
2.8	**1**	**0**	**1**
2.9	**1**	**0**	**1**
3	**2**	**0**	**2**
3.2	**1**	**0**	**1**
3.3	**2**	**0**	**2**
3.4	**1**	**0**	**1**
3.6	**1**	**1**	**2**
4.1	**1**	**1**	**2**
4.3	**0**	**1**	**1**
4.4	**3**	**0**	**3**
4.5	**1**	**0**	**1**
4.7	**1**	**0**	**1**
5.5	**1**	**0**	**1**
5.6	**1**	**0**	**1**
5.7	**0**	**1**	**1**
5.8	**1**	**0**	**1**
5.9	**0**	**1**	**1**
6.3	**0**	**1**	**1**
6.4	**0**	**1**	**1**
6.8	**0**	**1**	**1**
7	**2**	**0**	**2**
7.3	**0**	**1**	**1**

7.5	0	1	1
7.6	0	1	1
7.8	1	0	1
7.9	0	1	1
8	1	0	1
8.5	0	1	1
8.6	1	0	1
8.8	1	0	1
9.4	1	0	1
9.6	0	1	1
9.7	1	0	1
10.5	1	0	1
10.8	0	1	1
14.1	1	0	1
15.1	1	0	1
19.3	1	0	1
24.5	0	1	1
25.3	0	1	1
44.7	1	0	1
48.1	0	1	1
48.3	0	1	1
70.6	1	0	1
Total	132	20	152

Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-
(>= .9)	100.00%	0.00%	13.16%	1.0000	
(>= 1.1)	100.00%	40.91%	48.68%	1.6923	0.0000
(>= 1.2)	100.00%	45.45%	52.63%	1.8333	0.0000
(>= 1.3)	100.00%	48.48%	55.26%	1.9412	0.0000
(>= 1.4)	100.00%	50.00%	56.58%	2.0000	0.0000
(>= 1.5)	100.00%	50.76%	57.24%	2.0308	0.0000
(>= 1.6)	100.00%	53.79%	59.87%	2.1639	0.0000
(>= 1.7)	100.00%	56.82%	62.50%	2.3158	0.0000
(>= 1.8)	100.00%	58.33%	63.82%	2.4000	0.0000
(>= 1.9)	100.00%	59.85%	65.13%	2.4906	0.0000
(>= 2)	100.00%	60.61%	65.79%	2.5385	0.0000
(>= 2.1)	100.00%	61.36%	66.45%	2.5882	0.0000
(>= 2.2)	100.00%	63.64%	68.42%	2.7500	0.0000
(>= 2.3)	100.00%	64.39%	69.08%	2.8085	0.0000
(>= 2.4)	100.00%	68.18%	72.37%	3.1429	0.0000
(>= 2.5)	100.00%	71.21%	75.00%	3.4737	0.0000
(>= 2.6)	100.00%	73.48%	76.97%	3.7714	0.0000
(>= 2.7)	100.00%	75.00%	78.29%	4.0000	0.0000
(>= 2.8)	95.00%	75.76%	78.29%	3.9188	0.0660
(>= 2.9)	95.00%	76.52%	78.95%	4.0452	0.0653
(>= 3)	95.00%	77.27%	79.61%	4.1800	0.0647
(>= 3.2)	95.00%	78.79%	80.92%	4.4786	0.0635
(>= 3.3)	95.00%	79.55%	81.58%	4.6444	0.0629
(>= 3.4)	95.00%	81.06%	82.89%	5.0160	0.0617
(>= 3.6)	95.00%	81.82%	83.55%	5.2250	0.0611
(>= 4.1)	90.00%	82.58%	83.55%	5.1652	0.1211
(>= 4.3)	85.00%	83.33%	83.55%	5.1000	0.1800
(>= 4.4)	80.00%	83.33%	82.89%	4.8000	0.2400
(>= 4.5)	80.00%	85.61%	84.87%	5.5579	0.2336
(>= 4.7)	80.00%	86.36%	85.53%	5.8667	0.2316
(>= 5.5)	80.00%	87.12%	86.18%	6.2118	0.2296
(>= 5.6)	80.00%	87.88%	86.84%	6.6000	0.2276
(>= 5.7)	80.00%	88.64%	87.50%	7.0400	0.2256
(>= 5.8)	75.00%	88.64%	86.84%	6.6000	0.2821
(>= 5.9)	75.00%	89.39%	87.50%	7.0714	0.2797
(>= 6.3)	70.00%	89.39%	86.84%	6.6000	0.3356
(>= 6.4)	65.00%	89.39%	86.18%	6.1286	0.3915
(>= 6.8)	60.00%	89.39%	85.53%	5.6571	0.4475
(>= 7)	55.00%	89.39%	84.87%	5.1857	0.5034
(>= 7.3)	55.00%	90.91%	86.18%	6.0500	0.4950
(>= 7.5)	50.00%	90.91%	85.53%	5.5000	0.5500
(>= 7.6)	45.00%	90.91%	84.87%	4.9500	0.6050

(>= 7.8)	**40.00%**	**90.91%**	**84.21%**	**4.4000**	**0.6600**
(>= 7.9)	**40.00%**	**91.67%**	**84.87%**	**4.8000**	**0.6545**
(>= 8)	**35.00%**	**91.67%**	**84.21%**	**4.2000**	**0.7091**
(>= 8.5)	**35.00%**	**92.42%**	**84.87%**	**4.6200**	**0.7033**
(>= 8.6)	**30.00%**	**92.42%**	**84.21%**	**3.9600**	**0.7574**
(>= 8.8)	**30.00%**	**93.18%**	**84.87%**	**4.4000**	**0.7512**
(>= 9.4)	**30.00%**	**93.94%**	**85.53%**	**4.9500**	**0.7452**
(>= 9.6)	**30.00%**	**94.70%**	**86.18%**	**5.6571**	**0.7392**
(>= 9.7)	**25.00%**	**94.70%**	**85.53%**	**4.7143**	**0.7920**
(>= 10.5)	**25.00%**	**95.45%**	**86.18%**	**5.5000**	**0.7857**
(>= 10.8)	**25.00%**	**96.21%**	**86.84%**	**6.6000**	**0.7795**
(>= 14.1)	**20.00%**	**96.21%**	**86.18%**	**5.2800**	**0.8315**
(>= 15.1)	**20.00%**	**96.97%**	**86.84%**	**6.6000**	**0.8250**
(>= 19.3)	**20.00%**	**97.73%**	**87.50%**	**8.8000**	**0.8186**
(>= 24.5)	**20.00%**	**98.48%**	**88.16%**	**13.2000**	**0.8123**
(>= 25.3)	**15.00%**	**98.48%**	**87.50%**	**9.9000**	**0.8631**
(>= 44.7)	**10.00%**	**98.48%**	**86.84%**	**6.6000**	**0.9138**
(>= 48.1)	**10.00%**	**99.24%**	**87.50%**	**13.2000**	**0.9069**
(>= 48.3)	**5.00%**	**99.24%**	**86.84%**	**6.6000**	**0.9573**
(>= 70.6)	**0.00%**	**99.24%**	**86.18%**	**0.0000**	**1.0076**
(> 70.6)	**0.00%**	**100.00%**	**86.84%**		**1.0000**

	ROC		Binomial Exact	
Obs	Area	Std. Err.	[95% Conf.	Interval]
152	**0.9066**	**0.0242**	**0.85029**	**0.94873**

7 .

Median and IQRs for age, CD4, and viral load for CSF chloride group ages 0 to 12

```
  Thursday October 29 10:56:54 2015   Page 1

                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
                                                    ___/   /   /___/   /   /___/
                                                      Statistics/Data Analysis


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     /__    /   ____/   /   ____/
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    Notes:

  1 . capture log close

  2 . clear

  3 . clear matrix

  4 . set more off

  5 . import excel "C:\Users\Hendre\Dropbox\MMed 2015\Data\Processed Data\TBCUL & CSF Cl - Age 0 to 12 yea

  6 . univar Age CD4 ViralLoad
                                                -------------- Quantiles --------------
    Variable       n     Mean     S.D.      Min      .25      Mdn      .75      Max
    ------------------------------------------------------------------------------
         Age     276     3.26     3.32     0.08     1.00     2.00     5.00    12.00
         CD4      31   748.26   783.43    28.00   184.00   463.00  1007.00  3509.00
    ViralLoad      30  1.9e+06  3.7e+06    40.00  1793.00 91307.50  6.7e+05  1.0e+07
    ------------------------------------------------------------------------------

  7 .
```

Median and IQRs for age, CD4, and viral load for CSF chloride group ages 13 and older

```
  Thursday October 29 10:59:50 2015   Page 1

                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
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                                                      Statistics/Data Analysis


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    Notes:

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3 . clear matrix

4 . set more off

5 . import excel "C:\Users\Hendre\Dropbox\MMed 2015\Data\Processed Data\TBCUL & CSF Cl - Age 13 years an
  > trow

6 . univar Age CD4 ViralLoad
                                              -------------- Quantiles --------------
    Variable       n     Mean     S.D.      Min      .25      Mdn      .75      Max
    ------------------------------------------------------------------------------
         Age      437    38.21    13.52    13.00    29.00    37.00    46.00    80.00
         CD4      177   221.69   193.33     3.00    59.00   171.00   333.00  1005.00
    ViralLoad      46  4.2e+05  1.1e+06    40.00    40.00  1160.00  1.8e+05  5.7e+06
    ------------------------------------------------------------------------------

7 .
```

Median and IQRs for age, CD4, and viral load for CSF chloride group all ages

```
  Thursday October 29 10:53:50 2015   Page 1

                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
                                                    ___/   /   /___/   /   /___/
                                                      Statistics/Data Analysis


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    Notes:

1 . capture log close

2 . clear

3 . clear matrix

4 . set more off

5 . import excel "C:\Users\Hendre\Dropbox\MMed 2015\Data\Processed Data\TBCUL & CSF Cl - Ages recalculat

6 . univar Age CD4 ViralLoad
                                              -------------- Quantiles --------------
    Variable       n     Mean     S.D.      Min      .25      Mdn      .75      Max
    ------------------------------------------------------------------------------
         Age      713    24.68    20.16     0.08     3.00    26.00    39.00    80.00
         CD4      208   300.17   395.05     3.00    78.00   194.00   380.00  3509.00
    ViralLoad       76  1.0e+06  2.6e+06    40.00    68.50 10508.00  2.2e+05  1.0e+07
    ------------------------------------------------------------------------------

7 .
```

Median and IQRs for age, CD4, and viral load for CSF ADA group all ages

```
  Thursday October 29 11:02:49 2015   Page 1

                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
                                                    ___/   /   /___/   /   /___/
                                                      Statistics/Data Analysis


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     /__    /   ____/   /   ____/
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    Notes:

  1 . capture log close

  2 . clear

  3 . clear matrix

  4 . set more off

  5 . import excel "C:\Users\Hendre\Dropbox\MMed 2015\Data\Processed Data\TBCUL & CSF ADA - Ages recalcula

  6 . univar Age CD4 ViralLoad
                                                -------------- Quantiles --------------
    Variable       n     Mean     S.D.      Min      .25      Mdn      .75      Max
    ------------------------------------------------------------------------------
         Age     152    38.12    13.14     0.83    29.50    36.50    45.00    70.00
         CD4      51   225.31   197.55     4.00    60.00   174.00   352.00   735.00
    ViralLoad      23  9.9e+05  2.9e+06    40.00    40.00   189.00  1.1e+05  1.0e+07
    ------------------------------------------------------------------------------

  7 .
```

Median and IQRs for time-to-positivity for positive TB cultures

```
  Thursday November 12 16:16:36 2015   Page 1

                                                      ___  ____  ____  ____  ____(R)
                                                     /__    /   ____/   /   ____/
                                                    ___/   /   /___/   /   /___/
                                                      Statistics/Data Analysis


      ___  ____  ____  ____  ____ (R)
     /__    /   ____/   /   ____/
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    Notes:

1 . capture log close

2 . clear

3 . clear matrix

4 . set more off

5 . import excel "C:\Users\Hendre\Dropbox\MMed 2015\Data\Processed Data\TBCUL all positives for TTP.xlsx

6 . univar TBCULTBPOL
                                                -------------- Quantiles --------------
    Variable       n     Mean     S.D.      Min      .25      Mdn      .75      Max
    ------------------------------------------------------------------------------
    TBCULTBPOL      182    20.88     7.88     7.00    15.00    19.00    25.00    45.00
    ------------------------------------------------------------------------------

7 .
```

APPENDIX I

SOP GPL3670 – General specimen processing

STANDARD OPERATING PROCEDURE

Title: Specimen Processing

Document number: GPL3670

Version number: 5

(Changes from previous version highlighted)

Written by: K Mentoor

Checked by: C. Misland

Approved by: J. Wojno

Active date: 25-06-2015

Date of next review	*Date reviewed*	*Reviewed by*	*Action*
25-06-2016			

Date withdrawn: ……..

PURPOSE

This document addresses processing of different categories of specimens:

1. Urinary samples
2. Faecal samples
3. Cerebrospinal samples
4. Fluids and tissue from sterile sites
5. Respiratory samples
6. Mycological samples
7. Pus and pus swabs
8. Gastric Biopsies for Helicobacter
9. Hardware (e.g. Orthopeadic nails and screws)

RESPONISIBILITY

All technical staff must understand and apply the information of this document.

TURNAROUND TIMES

These are stipulated in SOP MIC 0762

MATERIALS AND REAGENTS

Anaerobic indicator strips
Blotting paper
Cell counting chamber
Cell counting fluid
Clostridium GeneXpert kit
Commercial anarobic saches
Cover slips
Crushers pestle and mortars - sterile
Cytospin funnels, filters and slide holders
DNA extraction fluid
Flat bottomed 96 well microtitre trays
Flint
Forceps and scalpel – sterile
Formal saline
Genbox anaerobic sachets and boxes
Glass slides
Glass tubes
Gloves
Masks
Graduated and automatic pipettes
Immersion oil
KOH 30%
Legionella Urinary Antigen Card Test
Loops
LPG gas
Lugol's iodine
Lysing reagent for Bordetella
Microscope cleaning fluid
Optochin and vancomycin antibiotic discs
Petri dishes
Pipette tips - sterile
Pipettes – sterile, plastic and glass
Plastic screw-topped tubes – sterile
Plastic slide holders (Blue)
Precipitating fluid

Saline tubes - sterile
Scalpel blades
Sodium hypochlorite 5% (Jik)
Sputazol (0,1% dithiothreithol)
Staining dish
Staining reagents
Sterile containers
Swabs – cotton, sterile
Tissues
Various culture media
Various media – liquid and solid agar in plates, tubes and bottles
Wax
Racks – plastic, metal and wooden.

EQUIPMENT

Adjustable air-displacement pipette with sterile tips
Anaerobic gas and cylinder
Anaerobic jars
Bio-safety cabinet class II
Bunsen burners
Centrifuge
Cytospin
Incubators (aerobic and CO_2)
Microscope
Plate rotator
Refrigerator
Sample protective cabinet (unventilated)
Slide dryer
Vacuum pump
Vortex mixer

ABBREVIATIONS

CSF – Cerebrospinal fluid
FTA – Flourescent Treponemal Antibody
VDRL – Venereal Disease Research Laboratory
CLAT – Cryptococcal Latex Agglutination Test
CRAG – Cryptococcal Antigen
ZN - Ziehl Neelsen
LIS – Laboratory information system
SNF – Supernatant fluid
NG – No Growth
MGEN.Y (After request outstanding list)
MGEN.C (Cell count outstanding list)
MTB.M (ZN outstanding list)
MGEN.T (Clostridium outstanding list)
MGEN.Q (Bordetella & PCR16s outstanding lists)

RETENTION AND DISPOSAL OF SAMPLES

1. All samples are kept for 6 days at 2-8ºC, excluding pus swabs which are kept at room temperature. These are then discarded into red plastic lined boxes.
2. Urine microtitre trays for microscopy kept for ±2 days.
3. All CSF samples are kept for two weeks.

HEALTH AND SAFETY

Observe the general laboratory safety rules as set out in the NHLS safety manual.

PROCEDURE

General procedures are listed below. Specimen specific procedures will be addressed in the different sections.

Daily duties

1) Bench senior to allocate staff to sections as per roster on bench
2) Allocated staff member is responsible for ensuring the allocated resposibilites are performed for the section.
3) One processing staff member starts at 07H00.
   Priority is given to specimen in the UCTCL rack from C17 so that microscopy results are recorded on the LIS before 08H00.
   Thereafter sputum samples are labelled for processing so that samples can be sent for TB culture ASAP.
4) Specimen receiving clerk to:

· check and label urines first at 07H00.
· check after request list and record details on Checking of after requests and cell counts FRM/GEN/14
5) Stagger tea times and lunch times.

General Sorting and Registration rules

1. C17 registration clerk to be informed of the importance of pus swab and fluid specimen type and site when registering.
2. Technologist checking the forms to indicate clearly the CORRECT specimen type code that should be used along with the site code or text if available.
3. Clinical information should always be consulted as this may provide additional information about the type and site of the specimen.
4. If no specimen type is indicated on the request form, a laboratory clerk will contact the clinician to confirm details.

General household rules

1. Keep lids on red lined boxes, except when discarding material.
2. Keep biocide discard jars' lids on at all times – to avoid fumes affecting staff.
3. Tissue/hand towels needed at following stations:
   i. -On trolley
   ii. -For urines
   iii. -For washing of cell counting chambers
4. Change 70% alcohol in coplin jar every morning.
5. Record cleaning of benches and microscope at 15H30.
6. Record cleaning of BSC after every use, and perform a smoke test once a week.
7. Discard specimens and clean trays for the next day at 15H30.
8. Stock bench with media ,stains, slides and tubes as to prepare for late shift at 15H30.
9. Pre-reduce anaerobic plates after each delivery.
10. Cut strips of parafilm.
11. Cut CSF filters for cytospin
12. Change urine wire loops Monday and Thursday and indicate on the urine worksheet.
13. Sign worksheets to indicate person responsible for processing.

General GLP (Good Laboratory Practice)

1. Remove smears from hot tray as soon as they are dry – to avoid burning.
2. Stack plates in numerical order when labeling.
3. Pack slides in specific tray when labeling, leaving a gap to start.
4. Record time of specimen arrival on bench on M/PROC/01 sheet.

Anaerobic jars

See general SOP no MIC 0738

1. Anaerobic Indicator strips are used.
2. Separate 2 day anaerobic plates (Nala & Brucella) from one day plates (4%).
3. Always indicate the date to be opened on 2 day jars.
4. Put up a 2 day anaerobic jar after every batch
5. Always use the correct lid and bottom with the same number.
6. Close Genboxes properly. See package insert.
7. Put up the jars after every delivery.
8. Pre-reduce anaerobic media after every delivery.
9. Record details on the anaerobic worksheet FRM/proc/03/.

Centrifuge and cytospin use

1. Leave lids open when not in use.
2. Always set timer when spinning.
3. Unpack balances after spinning.
4. Place balances in volumetric sequence when unloading:
   -Yellow together &
   -Green separate from yellow labeled tubes.

QUALITY CONTROL

1. All media is checked prior to being issued for routine use.
2. Check and change loops to ensure that they remain round and are free of bends, dents, corrosion or incinerated material.
3. All pipettes are calibrated
4. Control slides for the Gram Stain and ZN stain are performed daily and recorded on Stains worksheet FRM/GEN/04 according to Internal Quality Control SOP GPL1863
5. Logsheet for control of Reagents and Package Inserts FRM/GEN/12 is completed when opening a new batch / lot:
   Urine Antigen Test
   GeneXpert Closdridium Test
   Lysis Buffer
   Genbox Anaerobic
   Anaerobic indicator strips

SECTION 3: CSF PROCESSING

Responsibilties

1. Check MGEN.C for outstanding cell counts, record on FRM/GEN/14
   a) this must be performed after hours and on weekends at the end of the 20h00 shift
2. Complete move order weekly
3. Complete the Logsheet for control of Reagents and Package Inserts FRM/GEN/12 for new lots.
4. Perform IQC cell count IQCs once a week on a CSF sample from:
   C18
   C17
   RXH
   GFJ
   SOM
   a) Record details on 10% Microscopy IQC FRM/GEN/11
5. Perform a Gram stain control and a ZN control daily
   a) Record on Stains from FRM/GEN/04

Frequency

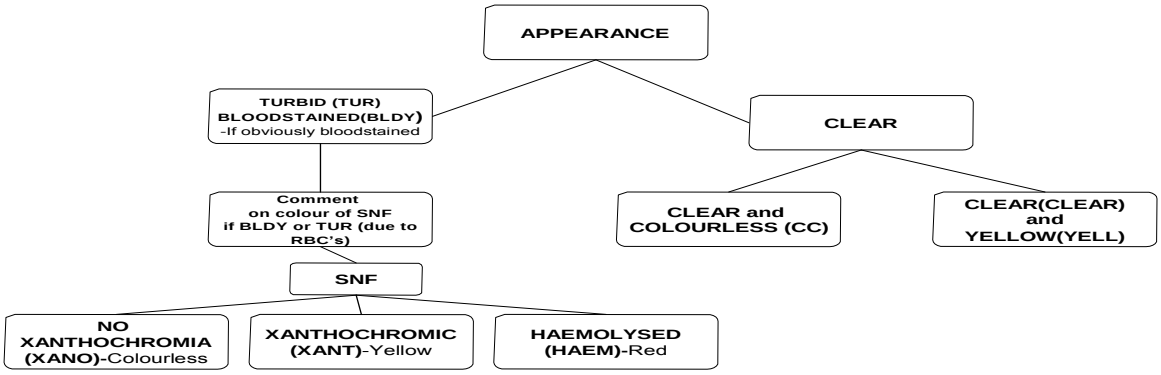
CSF's are processed as soon as possible, as the cell count is urgent.

Receipt of sample:

A guideline of a minimum of 2 ml of CSF is required for culture.

1. Correlate CSF to request form
2. Initial top left hand corner of request form
3. Date stamp request form
4. Comment on appearance of CSF according to flow diagram 1,
5. Record on request form and initial.
6. Comment on request form if CSF is clotted and continue to perform a cell count.

Flow diagram:1



Up-Country CSFs

1) CSFs referred from 'up-country' labs will not require a cell count or culture as they are referred for CLATs and or FTAs only..

Cell Counts:

Unsuitable CSFS for cell counts include:

- CSFs received in clot activating tubes
- Heavily bloodstained CSFS

Record and initial on the request form and enter on the LIS the reason the CSF was unsuitable for cell count.

Cell Count Set Up:

1) Perform cell counts on all CSFs including post mortem CSFs, clotted CSFs and referred CSFs where a cell count was NOT performed at the stat lab.
2) Use the clearest CSF if more than 1 tube is received.
3) Invert CSF 10-15 times. If conical tube is received, flick bottom of tube to mix cells.
4) Pipette 0.1ml CSF using a sterile graduated glass pipette into a glass tube.
5) Place cover slip on cell counting chamber
   NOTE: if performing more than one cell count, differentiate on the request form and cell chamber using numbers 1,2,3,4 etc.
6) Pipette 10µl cell counting fluid in glass tube and mix with CSF.
7) Pipette stained CSF on cell counting chamber, covering the mirrored surface.
8) NOTE: unstained cellcounts are performed on small volume CSFs as follows:
   a) Pipette 20µl of CSF onto cell counting chamber
   b) Place counting chamber in moist chamber for 5-10 minutes.
   c) Centrifuge the CSF for 15 minutes 4000rpm.

Registration:

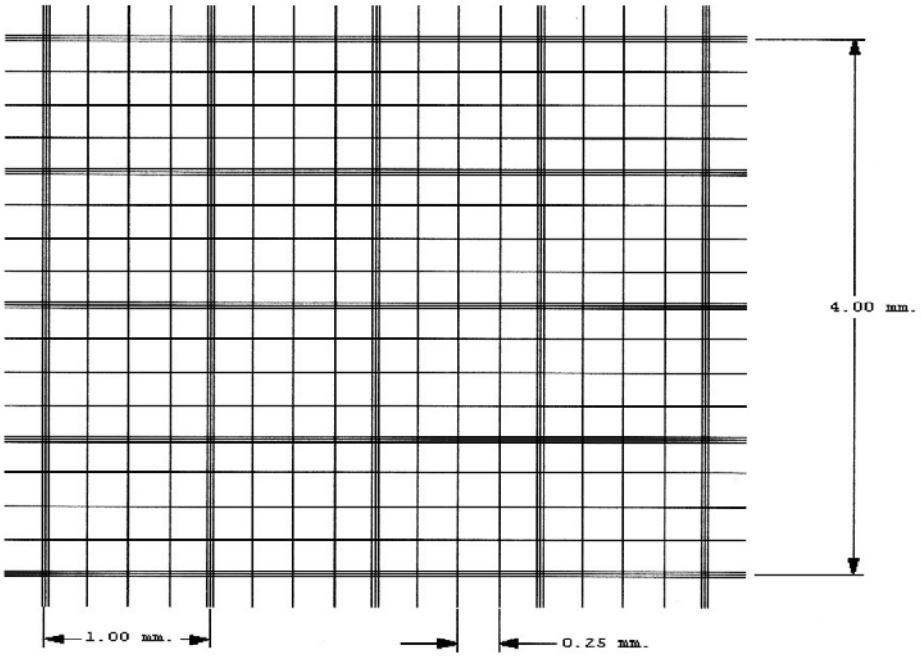
1) Register CSF on LIS
2) Ensure all test requests are ordered, excluding TB, as this will await the cell count result.
3) Routine culture – CSF, FCELL
4) CLAT - CRYP
5) Fungal – MYCO, CRYP
6) FTA / TPHA / VDRL – FTA
7) GeneXpert - PCRXC
8) Add additional test requests to CSFs already registered in C17.
9) Place form stickers on request form
10) Scan form/s

Perform Cell Count:

1) Count the number of polymorphonuclear cells and lymphocytes in 5 large blocks
2) Count the erythrocytes in 1 large block and multiply 5.
3) If many white blood cells are observed, count only one large block and multiply by 5.
4) If innumerable cells are observed, count only one small block and multiply by 80.
5) If cells are unclassifiable, count cells and report as unidentifiable cells.
6) Indicate on request form for clotted CSFs that a cell count was performed but may be inaccurate due to the presence of clots.
7) Using the cell count and table 1 determine if a TB culture is required and if a comment is applicable.
8) Record all results and comments on the request form and initial.
9) Enter cell count results on LIS under FCELL.
10) Order TB tests if required.

TB MC&S or GeneXpert on request is only performed on CSF's with a cell count of 5 or more lymphocytes or if the specimen is from an immuno-compromised patient, irrespective of the cell count. If TB is requested and not performed due to laboratory criteria, add the comment TBCSF (Specimen not sent for TB culture) on the request form and LIS.

11) Telephonically inform the requesting doctor or ward of cell count results from UCT Private Hosiptal patients.
12) If a substantial number of unidentifiable cells are observed, contact the clinician and if it is significant prepare sample for cytology:
    a) Aliquot CSF into a labelled tube.
    b) Photocopy request form
    c) Send sample and form to cytology.



Fuchs-Rosenthal Ruling

Fuschs-Rosenthal counting chamber was originally designed for counting cells in CSF specimens. The depth is 0.2mm and the ruled area consists of 16 x1mm squares divided by triple lines. These squares are subdivided to form 16 smaller squares, each with an area of 1/16th of mm².
Calculation of cell counts = 1.0mm x 1.0mm x 0.2mm x 5 = cells/ mm³.

CULTURE

- Label all media and tubes with 'SCH' labels.
- Label slides with SCH number, surname and bench
- Record supernantant fluid (SNF) appearance of bloodstained and turbid CSFs on the request form.

Routine culture:

1) Using a pipette separate SNF from deposit into sterile
2) Resuspend deposit and inoculate:
   - At least 5-7 drops for a cytospin Gram Stain, if insufficient prepare a slide for a direct Gram stain
   - 2% BA CO_2 at 35ºC
   - BBA CO_2 at 35ºC

Spin slide in cytospin for 2 minutes at 1000rpm

Brain abcesses add:

- Brucella agar – 2 day anaerobically

Nala agar – 2 day anaerobically

Post Mortem CSFs add:

- NYC - CO_2 at 35ºC
- Col-gent + optochin CO_2 at 35ºC

FTA Processing:

1) Pipette appromixmately 100µl into a blue top tube
2) Place in FTA container in the refrigerator

Fungal culture:

1) Inoculate 40µl of SNF into a glass tube and perform the CRAG Lateral Flow Assay.
2) Inoculate 2 drops of deposit onto the PDA slope ONLY if the doctor specifically request Fungal culture, and incubate the slope at 30ºC O_2

TB Processing:

1) Place red dots on tubes and form
2) Cytospin at least 5-7 drops of deposit and prepare a ZN stain
   - If insufficient for a ZN stain, report on LIS:
     'Regret, insufficient sample for TB microscopy'
3) In the case of a request for GeneXpert the remainder of the CSF is sent to the TB laboratory as soon as possible. No ZN stain is needed in this case.

- Place a label on the Processing Audit Trail Form FRM/PROC/01
- Take forms and stained slides to CSF bench

APPENDIX J

SOP MIC0699 – TB specimen processing

STANDARD OPERATING PROCEDURE

Title: TB Specimen Processing

Document number: MIC0699

Version number: 11

(Changes from previous version highlighted)

Written by: Ghebrekristos, Yonas

Checked by: N/A

Approved by: Musoni, Pascal

Active date: 12-06-2015

Date of next review	*Date reviewed*	*Reviewed by*	*Action*
12-06-2016			

Date withdrawn: ……..

PURPOSE

This protocol details the steps to be taken when processing and decontaminating clinical specimens to optimise the recovery of mycobacteria (particularly *M. tuberculosis*) while reducing the contamination rate as much as possible.

PRINCIPLE

Mycobacterial culture is an important facet of the laboratory diagnosis of tuberculosis. However, mycobacterial culture is complicated by the fact that some mycobacteria (such as *M. tuberculosis*) take longer to grow than other organisms. If there are any rapidly-growing organisms in the clinical sample, they can overgrow any mycobacteria present, contaminating the culture. The decontamination process, if not carried out correctly, can also affect the viability of mycobacteria in the clinical sample. It is thus important that appropriate specimens are decontaminated to reduce the likelihood of bacterial contamination, while still allowing mycobacteria to survive.

SCOPE

This SOP applies to all specimens in the laboratory on which mycobacterial culture has been requested, or on which myobacterial culture is deemed necessary from the clinical data supplied.
This SOP must be followed by any medical technologist / student medical technologist / technician / medical scientist / pathologist / pathology registrar who may be working in the laboratory and processing specimens for mycobacterial culture.

ABBREVIATIONS

The following abbreviations are used in this SOP:

CSF	Cerebrospinal fluid
TB	Tuberculosis
MOTT	Mycobacteria other than tuberculosis
MGIT	Mycobacterial Growth Indicator Tube
LJ	Lowenstein-Jensen medium
AUR	Auramine stain
ZN	Ziehl-Neelsen stain
PB	Phosphate buffer
PANTA	Polymixin B, Azlocillin, Nalidixic acid, Trimethoprim, Amphotericin B
OADC	Oleic acid – albumin – dextrose – catalase
NALC	N-Acetyl-L-Cysteine
NaOH	Sodium hydroxide
$Na_3C_6H_5O_7$	Sodium Citrate
EQA	External quality control
LIS	Laboratory information system

Specimens for TB culture

- TB culture is performed on all specimens when requested by the clinician or as followed by GeneXpert algorithm.
- Any specimen taken as part of an invasive procedure (e.g. sterile fluids and tissues) on which only TB microscopy has been requested will automatically get a TB culture even if not specifically requested by the clinician.
- TB culture must be performed on all specimens where the clinical data raises the possibility of multi-drug resistant TB.
- All EQA samples that require TB culture.

For specimens considered unsuitable for TB culture follow the SOP no GPL1667 for rejected specimens.

- Specimens (tissues) received in formalin are unsuitable for TB culture. MTEST and forward the specimen to Histology.
- Notify requesting doctor of all rejected trial specimens.

EQUIPMENT AND SUPPLIES

Biosafety cabinet
Refrigerated centrifuge
30 or 50 ml sterile tubes
Glass slides
Glass tissue crushers
Sterile forceps
Sterile scalpels
Petri dishes
Plastic pipettes
BD BBL MGIT package Insert

REAGENTS

Decontamination solution:

1. NALC / NaOH/ $Na_3C_6H_5O_7$
   - 4% NaOH/2.9% Sodium citrate solution – supplied in 1L aliquots by the media laboratory at NHLS Green Point.
   - Add 0,5g NALC powder to 100ml NaOH/Sodium citrate.
   - Mix well, date and initial the bottle and ready for use.
2. Phosphate buffer (PB) pH 6.8 is supplied in 20ml aliquots by the media laboratory.
3. MGIT PANTA
   - Reconstitute lyophilised MGIT PANTA with 15ml MGIT OADC supplement.
   - Record on the box the date of reconstitution, the expiry date and your initials.
   - Reconstituted PANTA is stable for 5 days and stored at 2^oC-8^oC.

FREQUENCY

- Specimens are processed daily Monday to Friday.
- No processing occurs over Saturdays, Sundays and public holidays.
- Samples are processed in batches of maximum 30 specimens.
- The cut-off time for the last batches of the day is 13H30.

SAFETY PRECAUTIONS

M. tuberculosis is spread by the airborne route. Many laboratory procedures are accompanied by the formation of aerosols that may contain bacteria but most importantly, mycobacteria. The technologist must be aware of aerosol formation and the dangers thereof. **Therefore all procedures, as far as possible, should be performed in the laminar flow cabinet.** Refer to the NHLS Safety Manual for additional information regarding Health and Safety.

NOTE: PROCESS ONE SPECIMEN AT A TIME INSIDE THE LAMINAR FLOW CABINET.

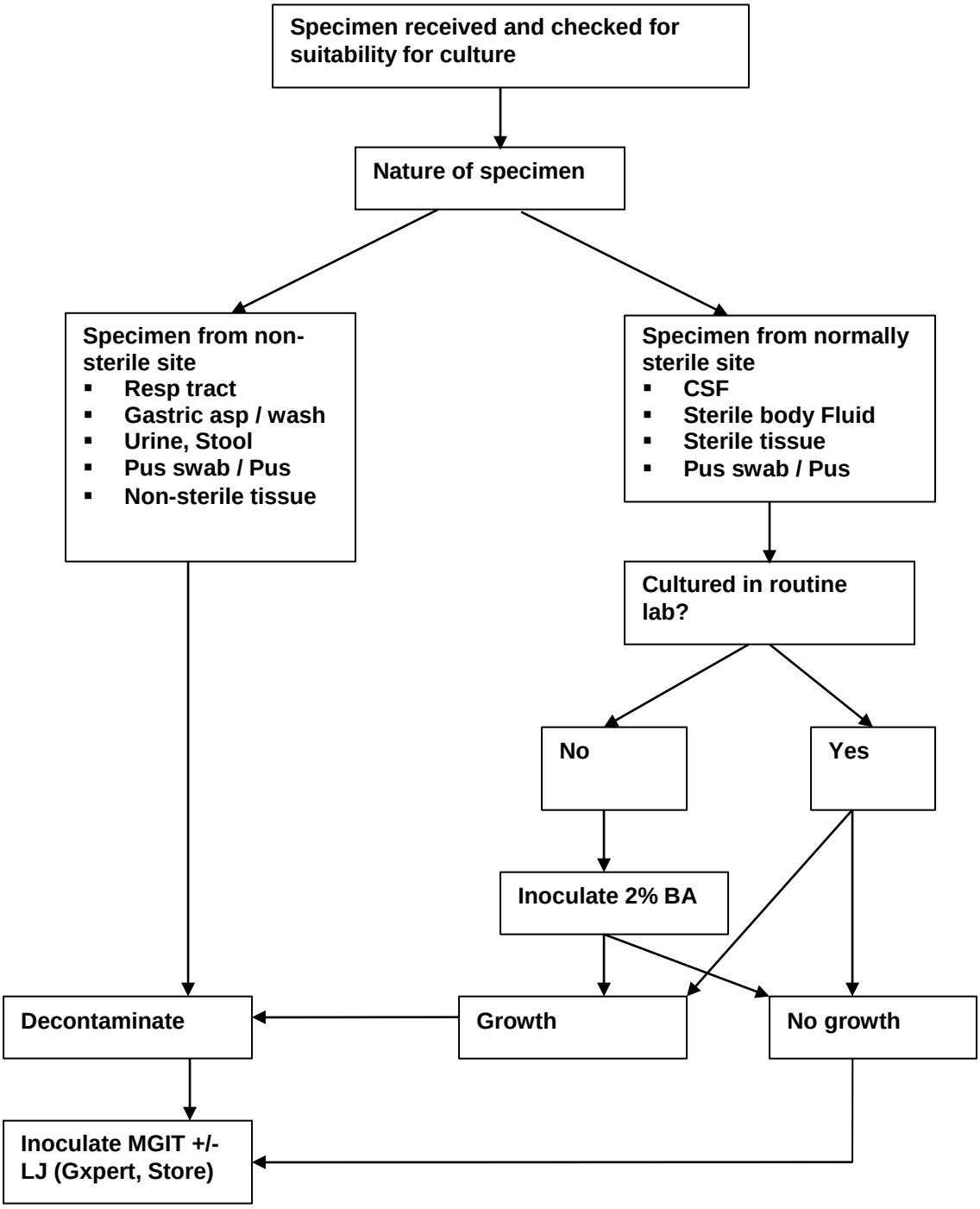
Specimens broken in the centrifuge:

i. Working under the laminar flow cabinet, decontaminate the buckets by using a suitable disinfectant.
ii. Leave the buckets to soak under the hood for at least 15 minutes before removing any broken pieces with a pair of forceps and discarding them in the sharps container. Rinse the buckets in water and leave to dry before using again.
iii. For specimens broken in the centrifuge send out a final report.

Refer also to the NHLS Safety Manual, for additional information regarding protective personal equipment, and procedures to be followed in the case of suspected exposure to blood / body fluids.

SUMMARY

The following flow chart and table provide a summary of specimen processing for mycobacterial culture, as well as which stains / media are used for different specimen types. Details will be found in the subsequent text.



SPECIMEN TYPE	Microscopy	Decontam	Culture	Media
Urines, Respiratory specs, Gastric washings	Auramine	Yes	Yes	MGIT
CSF	ZN	No*	Yes	MGIT
Fluids, curretings, pus swabs	ZN	No*	yes	MGIT
Tissue	ZN	No*	Yes	MGIT
Bone Marrow & Blood Cultures	None	No*	Yes	Myco F/Lytic inoculated at bedside
Bone Biopsies	None	No*	Yes	MGIT, tube size depending on the size of the tissue
EQA samples for culture	None	Yes	Yes	MGIT and LJ in 37°C incubator

*Decontaminate if routine culture has yielded growth
*Rectal Tissue must be decontaminated.
Table 1: Summary of processing requirements for different specimen types. Please refer to the text for full details.

Stool specimens for TB microscopy and culture:

Do direct TB microscopy.
Add coded comment “tbst” under remarks on LIS (tbst and control enter) which decodes to: “ TB culture of stool has low utility as more than 90% cultures become contaminated. Alternative specimens such as blood, bone marrow or tissue are superior. Please contact microbiology lab if culture of stool is required”.
Do not store the stool specimen.

Colonic/perianal tissue samples: Routine culture is not performed but add coded comment “colon” under remarks on LIS (colon and control enter) which decodes to: “routine bacterial culture of perianal tissue and colonic biopsies yields normal enteric flora, and is thus not performed. If infection with enteric pathogens (Salmonella, Shigella etc) is suspected, please submit a stool sample.”

Skin Biopsies: Routine culture can not always be performed due to insufficient sample. Add coded comment “skinbiopsy” under remarks on LIS (skinbiolpsy and control enter) which decodes to: “Insufficient specimen to perform all tests requested. TB culture and fungal culture requests will be prioritised. In future please submit 2 biopsy samples if bacterial, fungal and TB cultures are required.”

SPECIMEN PROCESSING

RECEPTION AREA

Specimens for TB culture are separated into 2 groups:

(a) specimens forwarded from other benches
(b) new specimens only for TB culture

The specimens are stored in the reception area fridge in the designated boxes until collected by the TB lab staff. It is extremely important to refrigerate specimens that cannot be processed on the same day in order to reduce contamination.

Each sample is accompanied by a form. Always make sure all the forms are date and time stamped.

SORTING IN THE TB LAB

1. Upon receiving:
   a) Ensure that specimens are adequately containerised. Leaking specimens are to be discarded with consent of one of the senior staff. However, as far as possible, theatre specimens must be saved.
   b) Always check that the name, folder number and any other details on the specimen correspond with that on the form.
   c) Always make sure that all the requests from the clinician are performed.
   d) Initial the top, front left hand corner of the request form.
2. Separate specimens into the following groups:
   a) Specimens NOT FOR decontamination
   b) Specimens FOR decontamination
   c) Specimens for TB culture only that needs to be checked for sterility.
   d) Specimens for Genexpet.

Specimens NOT FOR decontamination	CSF's. Fluids, Tissues, Bone biopsies, Curretings and Pus swabs which had NO GROWTH on the routine culture.	Write NO DC on the front of the request form.
Specimens FOR decontamination	Gastric washings Bronchial brushings (RXH) Urines, stools Fluids, tissues and pus swabs which had GROWTH on the routine culture	Write DC in the front of the request form
Specimens that need to be checked for sterility	Fluids, Tissues, Pus swabs which have not been cultured in routine laboratory	Fluids- spin down at 3200 rpm and use deposit. Tissues- crush. Inoculate the specimen onto a 2%BA and incubate aerobically overnight. Assess the following day if decontamination is required or not. Growth = decontaminate (D/C) No growth = No decontamination.

Speimens for Genexpert only	Sputa, Tracheal aspirates from adults	**See datasheet DAT/TB/03 for samples for Genexpert**
Specimens for decontamination (culture) and Genexpert	Sputa and tracheal aspirates from children ≤13 years.	

PREPARATION OF A BATCH

(a) Batches of specimens are prepared in numerically labelled boxes, and consist of a maximum of 30 specimens.
(b) The specimens are processed in the following order:
1. Specimens NOT requiring decontamination
2. Specimens requiring decontamination.
3. Decontamination solution QC.

REMEMBER to put specimens with a positive auramine result at the back of the batch to minimize cross-contamination.

(a) A sterile tube (e.g. Sterilin tube) is used for each specimen that requires decontamination.
(b) Pre-label the specimens and the sterile tube (1-30). After registration, proper labelling will occur.
(c) Make a note of special processing requirements:
Samples for Auramine only = yellow dot on falcon tube.
Samples not requiring microscopy = orange dot on form and falcon tube.
Samples for culture and Genexpert =blue dot on falcon tube.
Trial that requires extra e.g. Grams stains.
(d) Place all the forms of the batch in a labelled sleeve and send for registration. Use appropriate test method during registration.
- TBA/TBZ TB microscopy
- TBCUL TB culture
- TBS TB sensitivity
- PCRGX GenXpert
- LJ CULTB
- Use the profile available when registering trial forms.

(e) Each batch is accompanied by a TB processing worksheet generated electronically using "MTBRG".
This worksheet is created in the order of which the batch was processed. It also reflects reagents used as well as who is responsible for the different areas of the processing.
(f) All samples for TB culture must be received in "Receive in Work area", MTB.K.

TOPPLING

- All specimens requiring decontamination are toppled to the corresponding sterile tube.
- Topple a maximum of 5ml of specimen into the sterile tube.
- Always use the most representative portion of the sample.
- Pus swabs: transfer the swab to the sterile tube and add 2ml of sterile saline. Vortex well to dislodge any organisms that may be present on the swab.
- Stool specimens: mix 5ml of stool with 5ml Middlebrook 7H9 medium and vortex well.

- Contaminated bone: add sufficient sterile saline to cover the bone.

DECONTAMINATION

Prepare the decontamination solution.

Method:

i. Add an equal quantity of decontamination solution to the specimen using a separate pipette for each specimen. Set timer for 20 minutes once added to the 1st specimen of the batch.
ii. After adding decontamination solution to all samples in the batch, any left over decontamination solution is used as the QC. Pour 5ml of the left over decontamination solution into a sterile tube and fill the tube up with PB as soon as possible. After this follow steps iii, iv, vi and vii.
iii. MIX THOROUGHLY. SHAKE IF NECESSARY.
iv. Allow to stand on the bench for 20 minutes, vortexing the container at 5 minute intervals. Proper attention should be given to the specimen treatment time (**no more than 20 minutes**-delays have adverse effects on the number of tubercle bacilli that survive the decontamination process).
v. After 20 minutes, add pre-aliquotted phosphate buffer (PB - pH6.8) up to the 50ml mark using a separate aliquot for each specimen and mix. Do not use an aliquot of PB for more than one specimen. If there is any PB remaining in the aliquot, discard it.
vi. Centrifuge for 15 minutes at 3000 x g in a refrigerated centrifuge.
vii. After centrifugation, as each specimen is removed from the centrifuge, check for possible breakage that may have occurred.

Carefully decant supernatant fluid into discard jar containing an appropriate disinfectant. Be aware of the deposit or the swab/bone when decanting. This is an area where cross contamination can occur in the form of splash backs so please do it with caution.

PREPARATION OF SMEARS FOR MICROSCOPY

All samples received for TB culture must have a TB microscopy done.

An auramine is done on pulmonary, faecal and urine specimens.

A Zhiel-Neelsen is done on all extra-pulmonary specimens.

Microscopy is NOT done on bone marrow, blood cultures, bone biopsies and certain EQA samples (refer to the instructions for each EQA sample).

Specimens that come from the routine laboratory will usually have either a ZN or an auramine result. There is no need to repeat the microscopy unless a sputum sample had a direct (ie unconcentrated) microscopy done and the result was negative, in which case the auramine must be repeated.

Method:

i. A clean slide is labelled for each specimen, and the laboratory number is indicated on each slide.
ii. Onto each slide place a drop of precipitation fluid. This acts as a fixative as the decontamination procedure depletes the specimen of protein.
iii. The above deposit is mixed thoroughly and a drop (30ul) is added to the precipitation fluid. Do NOT use more than this as the smears will be too thick.
iv. Evenly spread the smear out about 1.5cm by 1cm.
v. Allow the smears to dry completely under the bio-safety cabinet, since mycobacteria can still be viable at this stage.

INOCULATION OF MEDIA

For each specimen label a MGIT tube with the patient's details. All media must have the patient's full name and initial and specimen number.

Check media requirements according to specimen type, clinical details or test requested.

The following specimens must have an LJ slope (green dot on MGIT) inoculated.

- Culture for MOTTs or atypical mycobacteria is requested - LJ in the 30°C incubator
- Clinical data suggests *M.marinum* (chronic soft tissue infection caused by fish hook or injury whilst cleaning a fish tank) or *M. ulcerans* infection (chronic soft tissue infection) - LJ in the 30°C incubator
- Specimens from clinical trials if requested (see front page of request form or trial SOP) - LJ in the 37°C incubator

All EQA samples – LJ in the 30 or 37°C incubator depending on clinical story provided

Method:

i. Add 800ul of MGIT PANTA to each tube just before inoculation.
ii. Resuspend the deposit with 0.5ml fresh PB (6.8). If an LJ, direct HAIN, GenXpert, or storage is needed then re-suspend with extra 0.5 ml for each test method.
iii. Inoculate the MGIT with approximately 0.5ml specimen using a sterile plastic pipette. If LJ required place 2-3 drops onto the slope.
iv. Discard the plastic pipette into the appropriate waste container inside the bio-safety cabinet.
v. Recap the MGIT tube tightly and mix by inverting the tube.
vi. Transfer pus swabs into the MGIT using a sterile forceps.

STORAGE OF SEDIMENT

Storage is performed as requested per trial.

Aliquot 0.5ml of decontaminated sample into a labeled storage tube.
Enter details on "S" drive in the sediment storage folder.
Enter details under "STORE" test method on the LIS.
Place in designated box at -70°C.

INCUBATING OF MEDIA

After inoculation, place the MGIT tubes into the MGIT machine immediately according the BACTEC MGIT™ 960 system user manual.
Place the LJ slopes, with slightly loosened caps in the aerobic incubator, sloping overnight. The next day tighten the caps and put in the appropriate tray and incubator.
All media / cultures are incubated for 42 days / 6 weeks.

Large pieces of Bone or uncrushable tissues (if not sterile)

i. Add sufficient sterile saline to cover the bone
ii. Add an equal volume of NALC/NaOH
iii. Allow to stand on the bench for 20 minutes, shaking the container at 5 minute intervals.
iv. After 20 minutes, make up to 20ml with pre-aliquotted PB (pH6.8).
v. Leave the bone to stand for 20 minutes (do not centrifuge).

Aseptically place the bone directly into a MGIT tube. If the tissue or bone is too large, place into a sterile container and decant a sufficient volume of MGIT medium to cover the specimen.

PROCESSING OF SPECIMENS NOT REQUIRING DECONTAMINATION

Bone / Tissues too small to crush

- Aseptically place the bone or small tissue directly into a MGIT tube. If the tissue or bone is too large, place into a sterile container and decant a sufficient volume of MGIT medium to cover the specimen. Incubate with the LJ slopes. TB microscopy is not required on these specimens.

Sterile fluids

i. If the specimen has not been centrifuged in the routine laboratory, then centrifuge for 15 minutes at 3000 x g in a refrigerated centrifuge.
ii. After centrifugation, prepare a smear for microscopy if this has not yet been done. The ZN stain is used for CSF, pleural and ascitic fluids.
iii. Inoculate approximately 0,5ml of the specimen into a MGIT tube, and 2-3 drops onto an LJ slope if necessary.

LIMITATIONS

- Prolonged decontamination will result in loss of viability of Mycobacteria.
- Incorrect concentration of the decontamination solution will affect the decontamination of the specimens.

MEDIA AND SOLUTIONS

Any MGIT tube not used immediately for inoculation of specimens must be stored in the dark (cupboard).

HOUSEKEEPING IN THE TB LAB

Mornings:
Unpack LJ's from the sloping tray and place in appropriate incubator.
Read the 2% plate of previous day sterility checks.
Collect all specimens and forms from the reception area.
End of the day:
Back up all MGIT machines
Stock up all workstations with pipettes, swabs, Phosphate buffers, gloves and paper towels.
Discard specimens from previous week.
Wipe down benches daily with Agglusept. On days when molecular extraction is performed, use 0.5% - 1% solution hypochlorite, followed by 70% alcohol, for affected pipettes, surfaces & cabinets in addition to Agglusept..

Sign the Daily Housekeeping logsheets after cleaning.

Quality control

Refer to Internal Quality Control in the TB Laboratory SOP MIC0733.
Refer to the BD BBL MGIT package Insert
When opening a new box of PANTA, record the lot number and expiry date on the Logsheet for Control of Reagents and Package Inserts FRM /GEN/07.

OUTSTANDING WORKLISTS

The following outstanding lists must be checked:

TBA / TBZ – MTB.M	daily
PCRGX – MTB.X	daily

PCRCU – MTB.P must be checked before and after the HAIN test has been performed.

SEND - MTB.R	weekly
MTB.T (TBCUL, TBSN1)	monthly
CADM.H (STORE)	twice a week
MTB.K (TBCUL)	3 times a week

CLINICAL APPLICATION

Any patient suspected of having TB should have an appropriate specimen sent for microscopy, which is the mainstay of diagnosis in South Africa. In some instances, it is also necessary to perform culture for mycobacteria.

These instances include:

- Repeated smear negative cases with ongoing clinical suspicion of TB
- Suspected MDR cases
- Suspected non-tuberculous mycobacterial infections

REFERENCES

Isenberg H. Clinical Microbiology Procedures Handbook, Volume 1

Kent PT, Kubica GP. Public Health Mycobacteriology - A Guide for the Level III Laboratory. 1985. Centres for Disease Control, Atlanta Georgia.

BD BBL MGIT package Insert

BACTEC MGIT 960 Sytem User's Manual

APPENDIX K

SOP MIC0700 – MGIT / LJ / MycoF/Lytic positives processing

STANDARD OPERATING PROCEDURE

Title: PROCESSING OF MGIT TUBES, LJ SLOPES and MYCO/F Lytic Bottles

Document number: MIC0700

Version number: 5
(Changes from previous version highlighted)

Written by: C Visser

Checked by: N/A

Approved by: Y. Ghebrekristos

Active date:

Date of next review	*Date reviewed*	*Reviewed by*	*Action*

Date withdrawn: ……..

PROCESSING OF MGIT TUBES, LJ SLOPES and myco/f lytic bottles

PURPOSE
This SOP serves as a guideline for placing and removing MGIT tubes / LJ slopes into and from the incubator. It also describes how to follow-up positive MGIT tubes / LJ slopes and Myco/F Lytic bottles.

PRINCIPLE
The MGIT system is an automated, liquid medium based culture system for detecting mycobacterial growth. Once specimens have been inoculated, MGIT tubes are placed into a MGIT incubator unit. Growth is automatically evaluated by the machine by measuring changes in the fluorescence of the tube, which is influenced by a change in the oxygen tension in the medium. Once a change in fluorescence is measured, the tube is “flagged positive” by the incubator.

LJ medium is a solid, egg based medium for culture of mycobacteria. It is often used as an adjunct to liquid based media, although there is some evidence that the MGIT media alone is sufficiently sensitive. Certain specimens (detailed in the specimen processing SOP) are inoculated onto LJ media as well. Growth is measured visually.

The Myco/F-Lytic bottle is liquid culture used with the BACTEC 9000 blood culture system to allow the detection of mycobacteria, Growth is automatically evaluated by the machine by measuring changes in the fluorescence sensor at the bottom of the tube, which is influenced by a change in the oxygen tension in the medium. Once a change in fluorescence is measured, the bottle is “flagged positive” by the incubator.

SCOPE
This SOP applies to all MGIT, LJ cultures and MycoF/ Lytic bottles which have been inoculated with either clinical or quality control samples.
This SOP must be followed by any medical technologist / student medical technologist / technician / medical scientist / pathologist / pathology registrar who may be working in the laboratory and following up cultures.

ABBREVIATIONS
The following abbreviations are used in this SOP:

MGIT	Mycobacterial Growth Indicator Tube
LJ	Lowenstein-Jensen medium
AUR	Auramine stain
ZN	Ziehl-Neelsen stain
LED	Light emitting diode
2% BA	2% Blood agar
LIS	Laboratory Information System

EQUIPMENT AND SUPPLIES
Bactec MGIT 960 System User’s Manual
Biosafety cabinet
Glass slides
MGIT tubes
LJ slopes
Myco/F-Lytic BACTEC culture bottles
2% Blood agar plates
Bactec 960 MGIT incubator unit
37°C aerobic incubator

REAGENTS
Precipitating fluid

FREQUENCY

Positive MGITs are unloaded Monday to Fridays excluding public holidays.
Negative MGITs are removed daily excluding Sundays and public holidays.

SAFETY PRECAUTIONS

M. tuberculosis is spread by the airborne route. Many laboratory procedures are accompanied by the formation of aerosols containing bacteria but most importantly, mycobacteria. All workers in the TB laboratory must be aware of aerosol formation and the dangers thereof. **Any work that involves opening either a MGIT tube or an LJ slope MUST be performed in biosafety cabinet.**

Refer to the NHLS Safety Manual for additional information regarding Health and Safety.

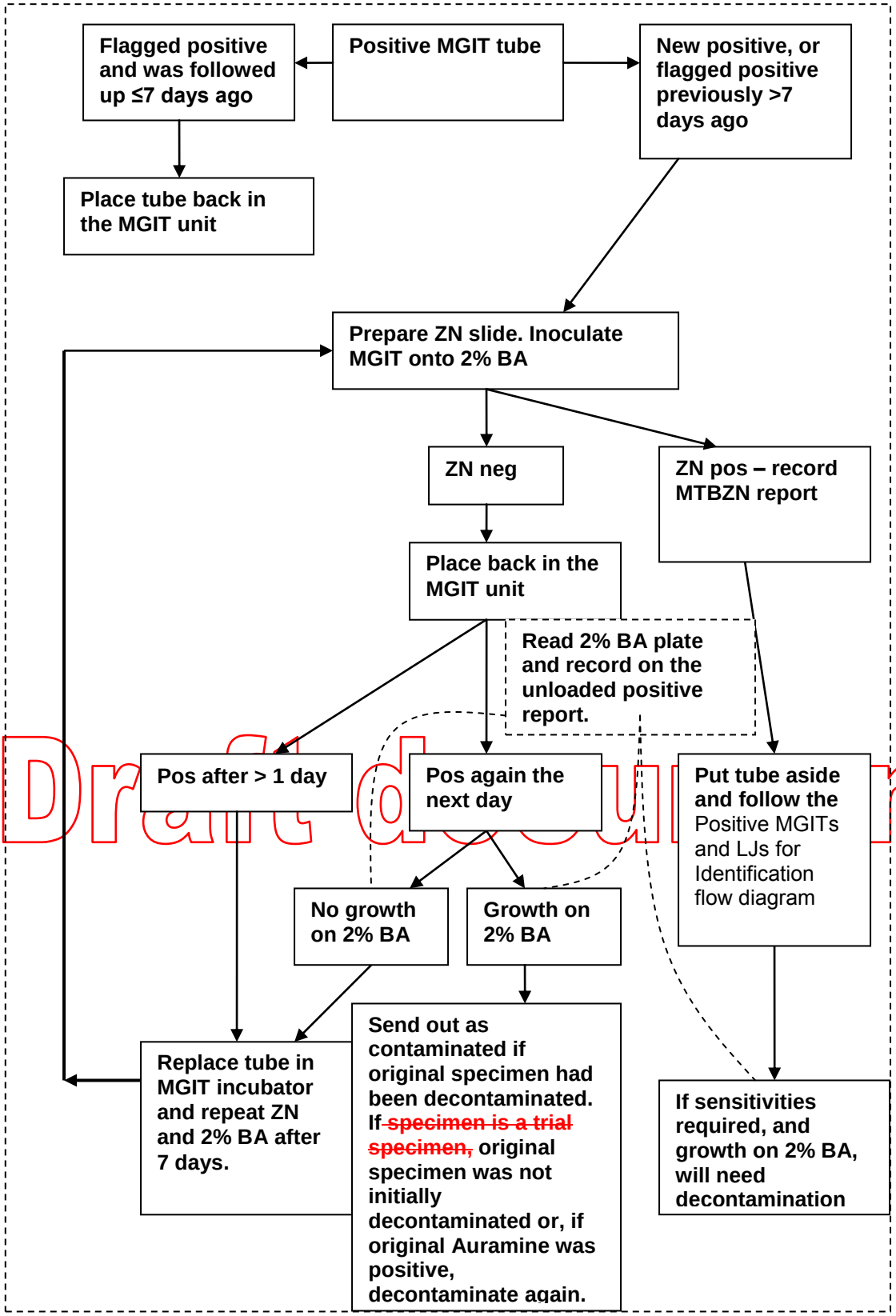
INCUBATION OF MGIT TUBES

1) Incubate MGITs according to the BACTEC MGIT 960 System User's Manual.
2) If a specimen is in a container other than a MGIT tube e.g. large bone, incubate at 37°C aerobically. This culture will be inspected manually.

PROCESSING POSITIVE MGIITS

1) Remove positive MGIT tubes according to the BACTEC MGIT 960 System User's Manual.
2) Print an 'Unloaded Positive Report' according to the BACTEC MGIT 960 System User's Manual and initial, date and file.
3) Read the 2% plate and record results on the MTBZN worksheet created the previous day.
4) Record on the LIS under notes if growth was observed on the 2% BA for any ZN positive MGITs.
5) Sort the positive MGITs:
   a) Return MGITs positive >1 day but <7 days (MGITs are dated) to the same MGIT unit it was removed from.
   b) MGITs positive the previous day and where growth is obtained on the 2% BA, place the MGIT in the contaminated box.
   c) MGITs positive the previous day and where no growth is obtained on the 2% BA, return MGIT to the same MGIT unit it was removed from.
6) Create an MTBZN worksheet per MGIT unit for all positive MGITs which are first time positive or are positive again after 7 days.
7) Label a slide and a 2% BA plate for each MGIT.
8) Place a drop of precipitating fluid onto the slide to ensure adhesion of the smear.
9) Aspirate approximately 0.1ml from the bottom of the MGIT tube and inoculate the 2% BA and slide.
10) Dry slides under the hood and perform the ZN stain according to Commonly Used Staining Techniques SOP MIC0728.
11) Incubate the 2% BA overnight aerobically at 37ºC.
12) Read and record ZN results on the MTBZN worksheet:
   a) Indicate 'C+' for cording
   b) Indicate '+' for no cording
13) If ZN is negative, date the MGIT tube and return to the same MGIT unit within 5 hours of removal.
14) Set aside smear positive MGITs and indicate on the label those where no cording was observed.
15) Enter results of the smear positive MGIT under TBCUL on the LIS.
   a) Enter 'P' is cording was observed
   b) Enter 'Q' if cording was not observed.
16) Follow the Positive MGITs and LJs for Identification flow diagram to determine what test to perform on the positive MGIT.

Flowchart 1: Summary of what to do with unloaded positive tubes.



NEW POSITIVES AND TUBES PREVIOUSLY REMOVED > 7 DAYS AGO

1) Create an MTBZN worksheet per MGIT unit.
2) Label a slide for ZN staining and a quadrant on 2% BA plate to check for contamination
3) **Work under the hood when working with the MGIT tubes.**
4) Place a drop of precipitating fluid on the slide to ensure adherence of the smear.
5) Aspirate (fish) approximately 0,1ml from the bottom of the MGIT tube, inoculate the BA quadrant and prepare a slide for a ZN.
6) Dry the slides under the hood and perform the ZN stain according Commonly Used Staining Techniques SOP MIC0728.
7) Incubate the 2% BA plates overnight, aerobically at 35ºC.
8) Once the ZN has been read, record results on the MTBZN worksheet. Slides can be discarded.
9) If the ZN is negative, date the MGIT tube and return to the appropriate MGIT unit within 5 hours of removal. Only the MGIT tube's barcode needs to be scanned.
10) If the ZN is positive, refer to the 'Positive MGITs / LJs for Identification' flow diagram.
11) Place MGIT tubes or LJs in the box for the HAIN PCR test.
12) Read the 2% BA plates and record the results on the MTBZN worksheet.
13) Growth on 2% BA from MGITs that are ZN positive must be entered on the LIS.
14) Refer to the Flow Chart or Table 1 for the appropriate course of action depending on the results of the 2% BA culture and the ZN.
15) If the culture is contaminated with an organism that may be Nocardia, consult with a pathologist or registrar to determine whether the organism should be identified and reported.
16) ALWAYS remember to check whether an LJ slope was inoculated as well. If a specimen is being discarded and an LJ was inoculated, the LJ can be incubated for the remainder of the 42 days unless it also becomes contaminated

Table 1: Summary of action to be taken for positive MGIT tubes

Original auramine	2% blood agar	Specimen type	ZN performed on MGIT	Action
Neg	Growth	Any specimen that has been decontaminated ~~and is not a trial specimen~~.	Negative	**Send out as contaminated**
~~Neg~~	~~Growth~~	~~Any specimen that~~ was NOT decontaminated ~~or a trial specimen~~.	Negative	**~~Decontaminate~~**
Pos	Growth	Any	Negative	**Decontaminate**
Neg or Pos	No growth	Any	Negative	**Re-incubate in MGIT machine.**

PROCESSING OF NEGATIVE MGIT TUBES

1) Unload negative MGITs according to the BACTEC MGIT 960 USERS Manual.
2) Print an "unloaded negatives" report and initial, date and file.
3) Ensure that the number MGITs removed equals the number of results on the unloaded negative report.
4) Negative MGIT results are reported via the interface.
5) Visually inspect all negative MGIT tubes. If there appears to be TB colonies, perform a ZN. If the ZN is positive, follow the flow diagram for positive MGITs.
6) If ZN is negative report as negative on the LIS under TBCUL.
7) The negative MGIT tubes are sent for autoclaving before being discarded.

PROCESSING OF MGIT CULTURES NOT IN THE MGIT INCUBATOR

These are specimens such as bone that were too large to fit into a normal MGIT tube.

1) Examine the culture medium weekly (usually a Monday) for any sign of visible turbidity
2) If the bottle is turbid, proceed as for a positive MGIT culture (sections A or B depending on the age of the culture)
3) If there is no visible growth after 6 weeks, perform a ZN stain on the liquid in the bottle.
4) If the ZN is negative, send out as No Growth
5) If the ZN is positive, follow the Positive MGITs / LJs for Identification' flow diagram.

LJ SLOPES

LJs in the ~~'going out' tray and in the~~ box awaiting Identification are read once a week.

1) Rotate week indicators on the LJ trays
2) Read the LJs in the 'going out' tray (week 6) and in the 30ºC and record results on the LIS under CULTB
   a) N - Negative (no growth)
   b) P – positive (growth)
   c) 'Contm' - Contaminated
3) Aerate LJ slopes in the week 3 rack and the box awaiting Identification as follows:
   a) Loosen caps – **DO NOT REMOVE** – and replace bottle in rack.
   b) Allow to stand on the bench until bottles have cooled to room temperature.
   c) Re-incubate overnight with the caps loose.
   d) Remove the rack the following day and allow to cool. Tighten the caps before replacing the rack in the incubator.

LJ slopes with visible growth

1. Find out if the corresponding MGIT has flagged positive yet. If so, work from the MGIT tube, and use the LJ as a backup if necessary.
2. If the MGIT has not flagged yet, proceed by preparing a ZN slide.
3. Using a cotton swab, remove a portion of the growth from the LJ slope, prepare a slide for ZN.
4. If the ZN is positive, follow the Positive MGITs / LJs for Identification' flow diagram.
5. If the ZN is negative, and the slope appears contaminated, enter the result on the LIS as contaminated and discard the LJ slope.
6. If the culture is contaminated with an organism that may be a Nocardia, consult with a pathologist or registrar to determine whether the organism should be identified and reported.

LJs are sent for autoclaving before being discarded.

POSITIVE TB BLOOD CULTURE BOTTLES

BD MycoF-Lytic bottles and BacT/ALERT MB bottles.
Positive TB blood culture bottles will be sent from the blood culture lab without a form. A Gram stain will have been performed by the blood culture lab and a result of 'no bacteria observed' should be resulted on the LIS

1) Add the blood culture details to the MTBZN worksheet
2) Label a slide for a ZN stain
3) Sterilise the top of the bottle with 70% Methylated spirits
4) Using a syringe inoculate the slide, dry and perform a ZN stain according to Commonly Used Staining Techniques SOP MIC 0728.
5) Read ZN slide and record results on the MTBZN worksheet
6) Report ZN results in the remarks field under the CULBA test method on the LIS.
7) If ZN negative:

a) Sub to LJ and place the bottle and LJ with the cap loose in the sloping box overnight at 37°C aerobically. The next day tighten the LJ cap and transfer the bottle and LJ to the 'identification' box.

8) If ZN positive and sensitivity is required sub to LJ and incubate the bottle and LJ as per point 7a.
a) NOTE a HAIN PCR test can not be performed directly from a blood culture. It must be subbed to an LJ and a HAIN PCR performed from a positive LJ.
9) If ZN positive and no sensitivity is required, perform the BD MTB TBc ID test according to Identification of Mycobacterium species SOP.
10) Note: Use the Positive MGITs / LJs for Identification' flow diagram if a referral can be made.

QUALITY CONTROL

Internal Quality Control in the TB Laboratory MIC0733.
Internal Quality Control GPL1863.

LIMITATIONS

Refer to Isenberg, H.D; Clinical Microbiology Procedures Handbook, Volume 2
Bactec MGIT 960 System User's Manual section

CLINICAL APPLICATION

Although microscopy is the primary means of diagnosing TB in South Africa, culture is sometimes indicated to make a diagnosis of TB. Detection of mycobacterial growth from a clinical specimen is one way of confirming a diagnosis of tuberculosis.

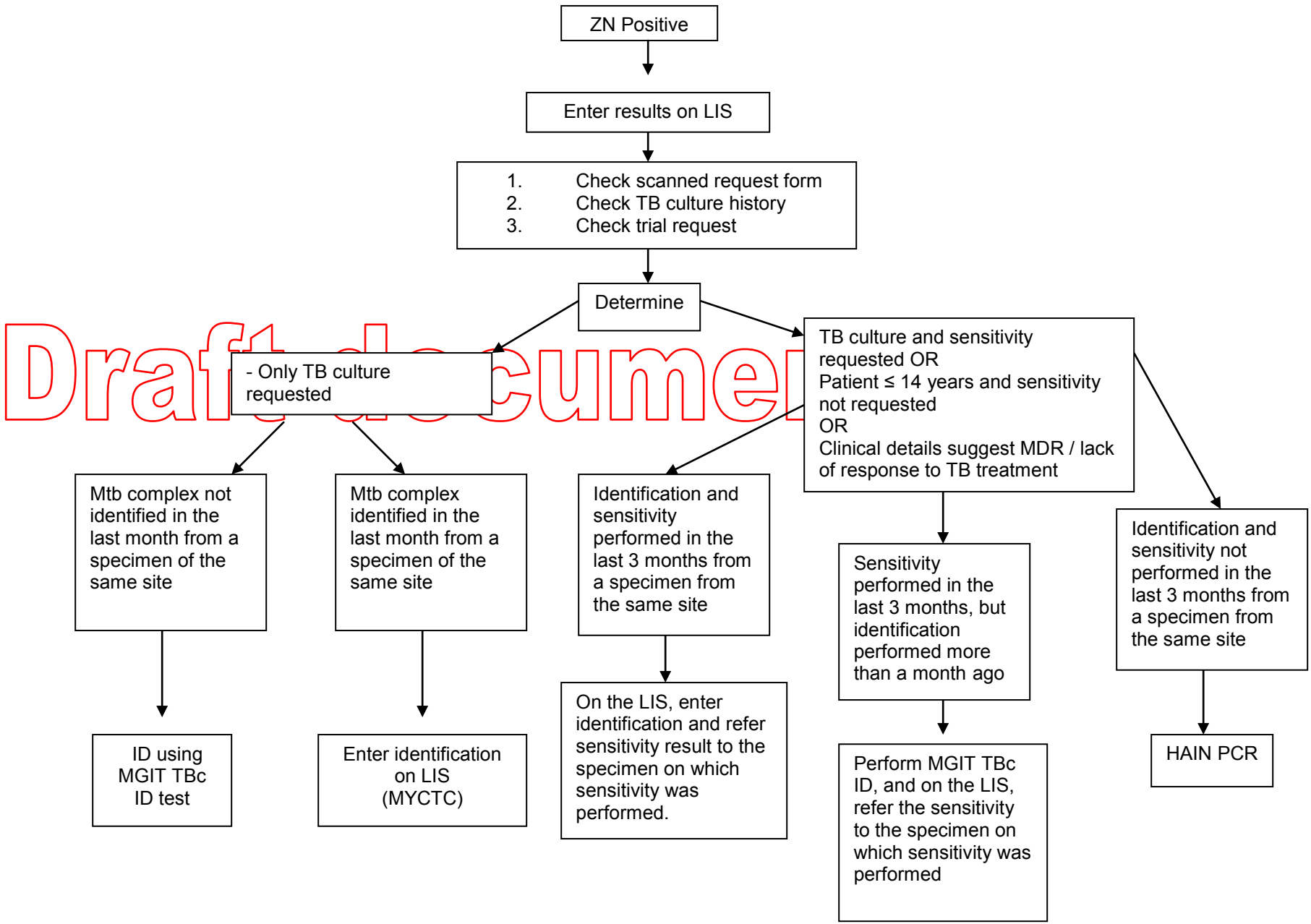
ATTACHMENTS

Appendix A (unloaded positives report) example
Appendix B (unloaded negatives report) example

REFERENCES

Bactec MGIT 960 System User Manual.
Isenberg, H.D; Clinical Microbiology Procedures Handbook, Volume 2

Positive MGITs / LJ's for Identification (Flow Diagram)



APPENDIX L

SOP MIC1645 – Identification of *Mycobacterium spp.*

Cover Page

Printed on: 11/09/2014

Details

Type	Number	Revision No.
SITE-SPECIFIC PROCEDURES\COASTAL REGION\GROOTE SCHUUR\MICRO	MIC1645	5

Title
Identification of Mycobacteria Species

Change Details

(Changes from previous revision highlighted)

Active Date	Approver	Author	Checked by
11/09/2014	Wojno, Justyna	Ghebrekristos, Yonas	

Next review date:	11/09/2015

IDENTIFICATION OF MYCOBACTERIA SPECIES

PRINCIPLE

Commercial kits are available for rapid identification and molecular identification of Mtb complex and genotypic susceptibility testing.

The HAIN Lifescience GenoType kits, uses a DNA strip for molecular identification of Mycobacterium species. A DNA strip is also available for determining genotypic resistance to Rifampicin and isoniazid. The procedure includes DNA extraction, amplification and hybridization.

MGIT TBc Identification Test is a chromogenic assay which detects the presence of a protein released by Mtb complex during culture in a MGIT.

PURPOSE

This Sop serves as a guideline for identification of Mtb complex, MOTTS, and Mycobacterium bovis BCG using a variety of commercial kits.

RESPONSIBILTY

The SOP must be followed by all Medical Technologists / Student Medical Technologists or Technicians / Medical Scientists / Registrars / Pathologists working in the TB laboratory.

ABBREVIATIONS

Mtb – Mycobacteria tuberculosis
MOTTS – Mycobacteria other than tuberculosis
PCR – Polymerase chain reaction
ZN – Ziehl Neelsen
NTM – Non-tuberculosis Mycobacteria
ID – identification
LJ – Lowenstein Jensen slope
INH – Isoniazid
AFB's – Acid fast bacilli
MDR – Multi-drug resistant

HEALTH AND SAFETY

Refer to the NHLS Health and Safety Manual.
Refer to the following package inserts:

- HAIN Genotype MTBDR plus
- HAIN Genotype Mycobacterium CM
- HAIN Genotype Mycobacterium AS
- HAIN Genotype MTBC
- BD MGIT TBc ID
- HotStarTaq PCR
- GT-Blot 48 Users Manual

All procedures, where possible, must be performed in a Class II Biosafety Cabinet.

MATERIALS AND REAGENTS

HAIN LifeScience package inserts (instructions for use) are available from www.hain-lifescience.de/ifu.html. The IFU number is found on the outside of the kit box.
HAIN Genotype MtbDR plus kit and package insert
HAIN Genotype Mycobacterium CM kit and package insert
HAIN Genotype Mycobacterium AS kit and package insert

HAIN Genotype MTBC kit and package insert
BD MGIT TBc ID test kit and package insert
HotStarTaq PCR enzyme and handbook
GT-Blot 48 users Manual
HAIN worksheets
Eppendorf tubes
Glass pipettes
Pipettes
Eppendorf 1.5ml
0.2ml PCR tubes

EQUIPMENT
Biosafety cabinet class II
Heating block
Sonicator
Thermocycler
Microcentrifuge
GT Blot
Vortex
Timer
GT-Blot 48

FREQUENCY
HAIN Genotype MtbDRplus – performed once a week
HAIN Genotype Mycobacterium CM / AS – performed once a week
HAIN Genotype MTBC – as required
BD MGIT TBc ID – performed daily
Refer to Turn-around Times for Microbiology and Virology SOP MIC0762.

SAMPLES
- Positive MGITs confirmed with a smear positive AFB result.
- LJ's with growth resembling Mycobacteria (not suitable for BD MGIT TBc ID).
- Positive BD MYCO-F-LYTIC blood cultures confirmed with a smear positive AFB result (suitable for BD MGIT TBc only).

PROCEDURE
Follow the flow diagram below to determine what test to perform on MGITs which have flagged positive.

BD MGIT TBc ID Test
Performed on Positive MGITs confirmed with a smear positive AFB result and where only TB culture has been requested.
- Label a test device with the 'SCH' number.
- Perform according to the BD MGIT TBc ID package insert.

HAIN Test
- Create a worksheet using the HAIN worksheets provided in the kits, recording the 'SCH" number and surname of the patient's culture.
- Label eppendorf tubes using the 'SCH' number.

Extraction of DNA:

Method 1:
Alternative method used for TB cultures from MGIT and LJ slopes. (In-house extraction method)

MGIT tubes:
· Aliquot 1 – 1.5ml of culture into the correctly labelled eppendorf tube.
LJ Slopes:
· Aliquot 1 -1.5ml of ultra pure water into an eppendorf tube.
· Make a heavy suspension of the colonies from the LJ slope in the water, using a sterile swab.
Positive control:
· Use a MGIT or LJ culture of H37RV strain.
Negative control:
· Use ultra pure water.

- Switch on the heating block and allow to reach a temperature of 95 – 105 °C.
- Incubate for 30 minutes in the heating block.
- Refrigerate tubes at 4°C for a minimum of 20 minuntes.
- Centrifuge for 5 mins at 10000rpm in a microfuge.
- Extracted suspensions are stored at 4°C.

Method 2:
HAIN Lifesciences method used for MGIT and LJ TB cultures.
Perform according to the HAIN package insert and Genolyse Protocol authorized by HAIN.
Note: A heating block is used instead of a waterbath.

Amplification:
Mastermix is prepared first thing in the morning in the PCR clean room.
Do not handle TB specimens prior to preparing mastermix.
No lab coats from C18 must be worn in the PCR clean room.
Wash hands before entering the PCR clean room.

- Make up amplification mix according to the HAIN package insert.

- Make up amplification mix in a polypropylene tube using the following formula per test.
  NB: multiply by the number of samples to be run.

Ultra Pure Water	1.1µl
10x Buffer	5µl
$MgCl_2$ (25mM)	3.6µl
Primer Nucleotide Mix	35µl
HotstarTaq Polymerase	0.3µl

- Aliquot 45µl into 0.2ml PCR tubes.
- Record each batch of mastermix and lot number details on the Mastermix Record Sheet FRM/MOL/03.

Addition of DNA:
This procedure is performed in the Virology section.
- Add the DNA to the mastermix according to the HAIN package insert.

Amplification profile:
- Using the thermocycler, amplify according to the profile stated in the HAIN package insert.

- Use of thermocycler:
  Turn on thermocycler
  Load tubes and close cover
  Using the arrow keys select user
  Press enter
  Press run
  Using the down arrow key select the required programme
  Press accept (F1) for selected programme
  Press accept (F1) to confirm volume

- Turn off thermocycler:
  Press stop
  Press stop to confirm stop
  Press exit (F5) to end run
  Switch off

Hybridization:

- Perform according to the HAIN package insert.
- Use the GT-BLOT 48 User Manual to operate the GT-BLOT 48.

RESULTS

MGIT TBc ID Test

- Refer to the BD MGIT TBc ID test package insert.
- Report results on the LIS.
- If the result is negative, charge using REFCH 0327.

HAIN Tests

- Cellotape the HAIN strips to the HAIN worksheet.
- Interpret results according to the appropriate package insert and record results on the HAIN worksheet:
  - HAIN Genotype MTBDR plus package insert
  - HAIN Genotype Mycobacterium CM package insert
  - HAIN Genotype Mycobacterium AS package insert
  - HAIN Genotype MTBC package insert
- Record results on the LIS.
- Record all results of HAIN tests and any additional work under ‘notes’. This excludes completed PCRCU results.
- ALL HAIN tests must be charged using REFCH 0447. This excludes completed PCRCU results.
- All HAIN results must be checked for interpretation and reporting by a second person and countersigned.
- Refer to datasheet DAT/TB/04 for additional work and comments on HAIN results.

ADDITIONAL PROCESSES

Selection of Isolates for Susceptibility Testing:

- Order phenotypic sensitivity testing:
  - Isolates identified as Mtb complex but are inconclusive to INH and / or Rifampicin
  - Isolates identified as Mtb complex but are Rifampicin monoresistant.
  - Required by a trial.
- Check the notes field for a record of any growth on 2% blood agar to ensure culture is pure for sensitivity testing.

- Subculture INH resistant isolates for Streptomycin and Ethambutol sensitivity testing when specifically requested.
- Subculture Rifamipicin resistant isolates for Greenpoint TB lab for 2nd and 3rd line sensitivity testing (Ethionamide, Amikacin, and Ofloxacin).

- If culture is contaminated and from a sterile site and can not be referred to another culture, send MGIT for re-decontamination.
- NOTE: 1st line sensitivity testing can be performed phenotypically and genotypically.

Comments:

- If awaiting 1st line phenotypic sensitivity testing on non-trial samples, add the following comment:
  "Awaiting phenotypic sensitivity results"
- If awaiting 2nd and 3rd line sensitivity testing, add the following comment:
  "Awaiting 2nd and 3rd line sensitivity results".

Subculture for MGIT DST:

- Label MGIT tube with 'SCH' number and type of sensitivity to be performed.
- Vortex culture.
- Pipette 800µl of growth supplement into a new MGIT tube.
- Pipette 500µl of the culture into the MGIT.
- Place in MGIT machine.

Subculture for LJ slope:

- Subculture isolates onto an LJ slope as determined by using the flow diagrams.
- Incubate at 37ºC for 42 days.
- Check the notes field for a record of any growth on 2% blood agar to ensure culture is pure to sub to an LJ slope.

Storage of isolates:

Store isolates as required per trial.
Order a STORE test.
Label a storage tube.
Aliquot 300µl of 50% glycerol into the storage tube
Vortex culture
Aliquot 1ml of the isolate into the storage tube
Record details in the storage folder on S drive.
Record details on DISA under store.

QUALITY CONTROL

Refer to appropriate package inserts:

- HAIN Genotype MTBDR plus package insert
- HAIN Genotype Mycobacterium CM package insert
- HAIN Genotype Mycobacterium AS package insert
- HAIN Genotype MTBC package insert
- BD MGIT TBc ID test package insert
- HotStarTaq PCR
- Record the lot number and expiry date of the strips used on the HAIN worksheets.
- Record all molecular run failures on the "Run Failure log form: Molecular FRM/MOL/05".

- Each new HAIN Kit, MGIT TBc ID kit and HotStarTaq kit is recorded on the Logsheet for Control of Reagents and Package Inserts FRM/GEN/12.

- Each new lot number for MGIT TBc ID kit is controlled by running a positive control, H37RV MGIT culture, and a negative control, 7H9 middlebrook media from an uninoculated MGIT tube.

LIMITATIONS AND REFERENCES

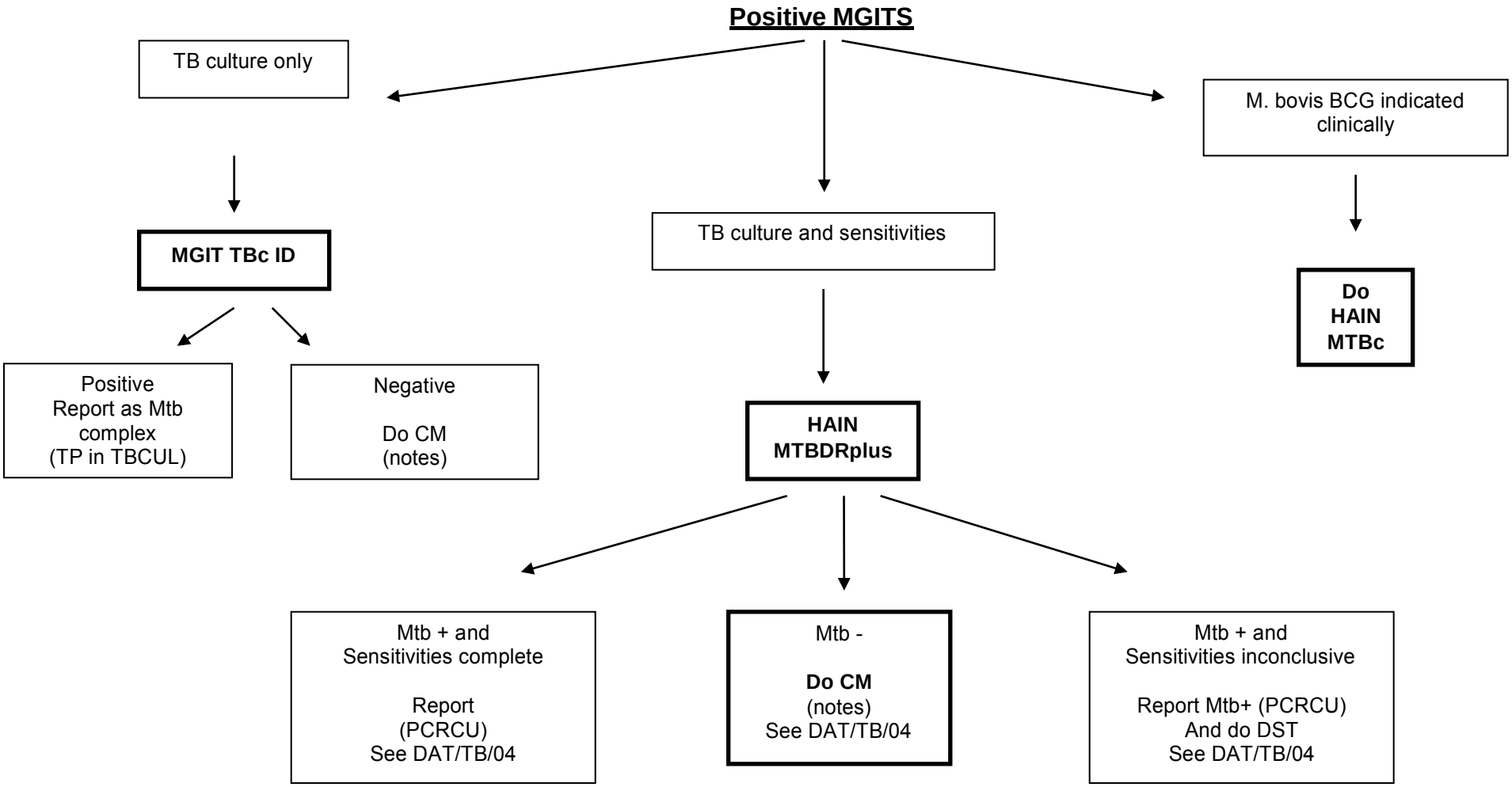
Refer to appropriate package inserts:

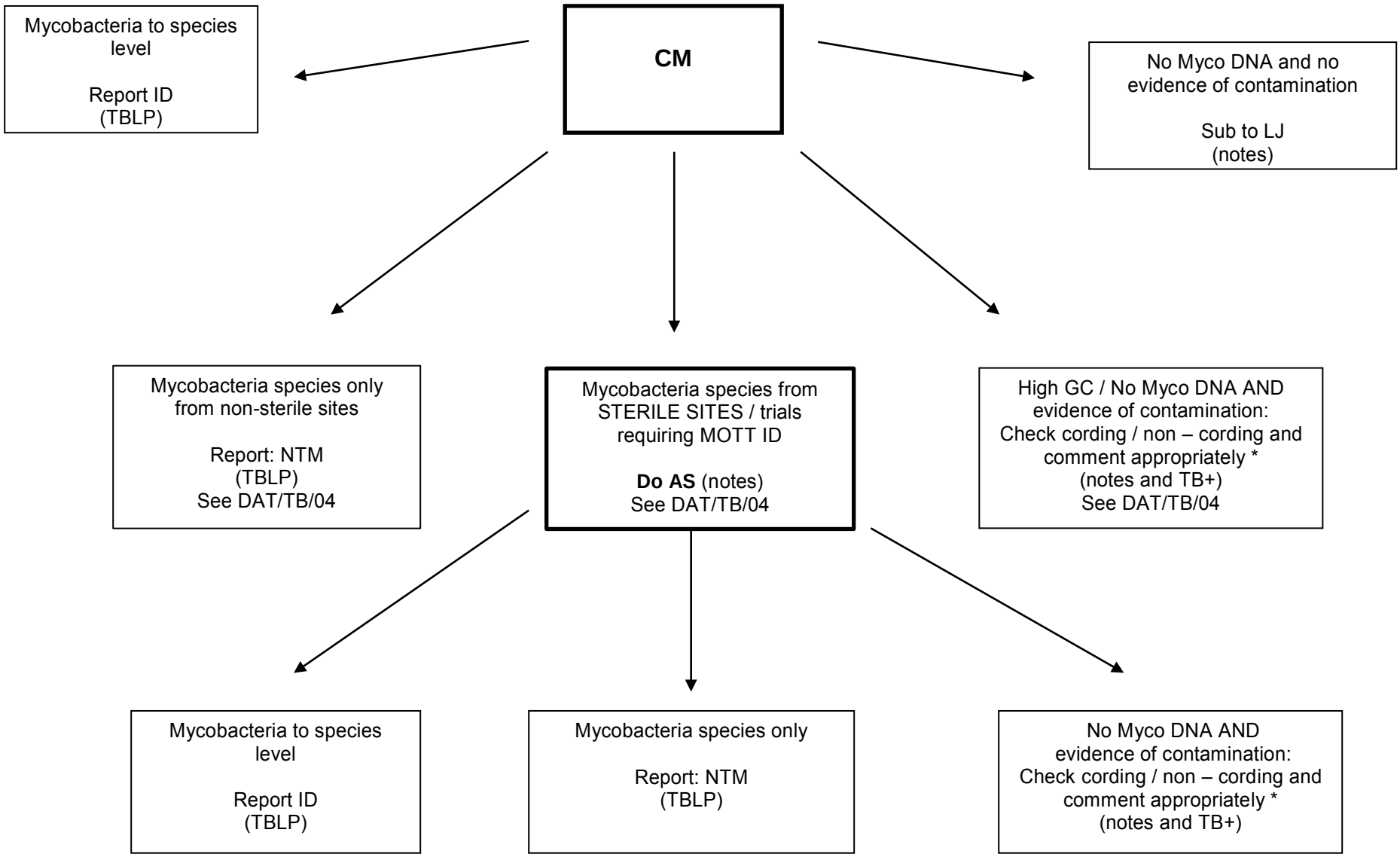
- HAIN Genotype MTBDR plus package insert
- HAIN Genotype Mycobacterium CM package insert
- HAIN Genotype Mycobacterium AS package insert
- HAIN Genotype MTBC package insert
- BD MGIT TBc ID test package insert
- HotStarTaq PCR handbook

OUTSTANDING WORKLISTS

Check the following outstanding worklists:

- MTB.P – PCRCU / PCRTB – each HAIN run
- MTB.T – All TB tests – monthly





* = Acid fast bacilli resembling / not resembling Mtb complex were observed in culture. Identification can not be confirmed due to contamination.

APPENDIX M

Roche Modular Ion–Selective Electrodes – Principles

ISE technology

This chapter provides you with an overview of ISE technology used by the **cobas** 6000.

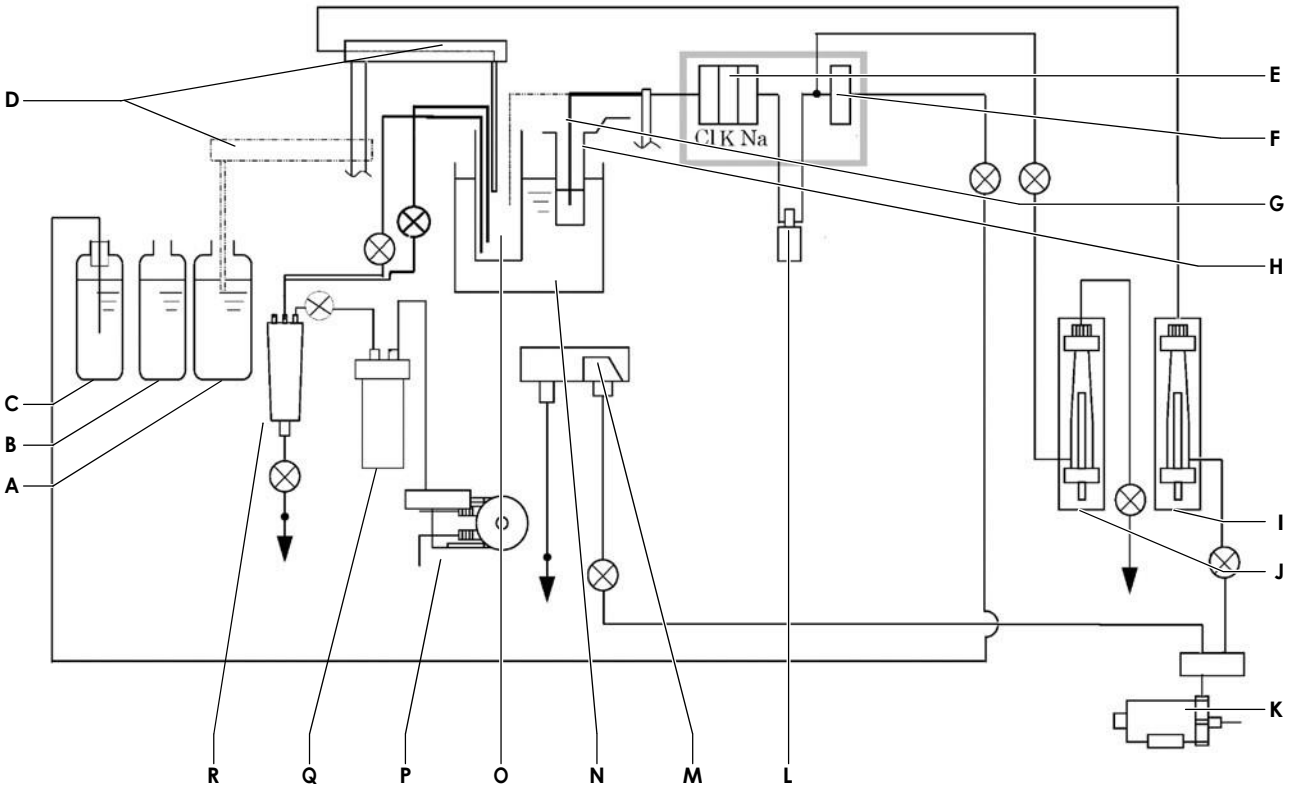
In this chapter *Chapter* 1

Components and function of the ISE unit

The figure below visualizes the liquid flow paths of the ISE unit. The following symbols are used in the figure:

Solenoid valve

To waste container



A	Internal standard (ISE IS)	**G**	Sipper nozzle	**M**	ISE rinse station
B	Diluent (ISE Dil.)	**H**	Reaction cell	**N**	Incubator bath
C	Reference solution (ISE Ref.)	**I**	ISE syringe	**O**	IS bath
D	ISE pipetter	**J**	Sipper syringe	**P**	Vacuum pump
E	Measuring electrodes	**K**	Water pump	**Q**	Vacuum tank
F	Reference electrode	**L**	Pinch valve	**R**	Vacuum reservoir

Figure A-1 ISE liquid flow path diagram

Sample probe, reaction cell, and ultrasonic mixer

The ISE unit uses the sample probe, reaction cells, and ultrasonic mixer of the **c** 501 module.

Sample probe The sample probe transports sample liquid from the sample tube to a reaction cell.

Reaction cell The reaction disk of the **c** 501 module carries the reaction cells. All reaction cells are seated in the controlled-temperature incubator bath. The incubator bath maintains the cells at the required temperature of 37°C.

Ultrasonic mixer After the sample probe has dispensed the sample into the reaction cell, the ISE pipetter adds ISE Dil.. The ultrasonic mixer of the **c** 501 module mixes the diluted sample.

ISE pipetter

The ISE pipetting system is composed of the ISE pipetter (consisting of pipetter arm and probe), the ISE syringe, and the ISE rinse station.

ISE pipetter The ISE pipetter transports ISE Dil. to the reaction cell and ISE IS to the internal standard bath.

The ISE pipetter probe is equipped with a level detector (capacitance method) which is applied to check and correct the filling volume of any bottle present in the ISE reagent compartment.

ISE syringe The ISE pipetter is connected to the ISE syringe by tubing, which controls the pipetting action.

ISE rinse station This rinse station is used for both ISE pipetter probe and ISE sipper probe.

ISE reagent compartment

The ISE reagent compartment provides five positions for reagent bottles:

- ISE IS: Two bottles
- ISE Dil.: Two bottles
- ISE Ref.: One bottle

The reagent compartment is equipped with position sensors for each reagent bottle (reflection type).

Internal standard bath

Internal standard bath (IS bath) has two chambers for heating internal standard (ISE IS) to measuring temperature (37°C). After heating, the ISE IS solution is aspirated by the sipper probe into the measuring flow path. The residual ISE IS solution is aspirated through the vacuum nozzle to empty the IS bath.

The use of two chambers allows for an optimized flow of the analysis: While the content of one chamber is ready for use, fresh ISE IS is pipetted into the other chamber where it is given time to heat up for the next measurement.

ISE sipper

The ISE sipper mechanism consists of a sipper nozzle and a sipper syringe. Between the sipper nozzle and the syringe is the ISE measuring flow path.

Sipper nozzle The sipper nozzle lowers either into ISE IS solution in the IS bath or into sample solution in a reaction cell to aspirate the respective solution into the measuring flow path.

Sipper syringe The sipper syringe provides the negative pressure for following functions:

- Aspirate sample solution or ISE IS into the measuring flow path (measurement electrodes)
- Aspirate ISE Ref. into the measuring flow path (reference electrode)
- Aspirate measured sample solution, ISE IS, and ISE Ref. from the measuring flow path into the waste container.

ISE measuring

The ISE measuring system is contained in a temperature-controlled compartment. It is composed of three ion specific electrodes and one reference electrode.

The difference between the potentials at the reference electrode and the ion-selective electrode equals the electromotive force (EMF). For every test, the EMF of both ISE IS and diluted sample solution are measured for each sort of ions (Cl^-, K^+, and Na^+). From these EMF values the results are calculated using the calibration curve.

Measurement electrodes The measurement electrodes use a special design. Membranes with ion-selective binding capacity and an open liquid junction allow the selective measurement of the ion concentrations. The electrodes are directly connected to form a flow path for the diluted sample and the ISE IS solutions.

Reference electrode The reference electrode uses the same design of the measurement electrodes. It is exclusively used as a reference for every measurement. ISE Ref. is aspirated through the electrode and a reference electrode potential is registered.

Pinch valve The pinch valve is used to control the flow of liquid that passes the electrodes.

Measurement sequence

This section describes the sequence of the ISE measurement.

Preparation

First, the sample pipetter pipettes a sample into a reaction cell. Then, into this cell, ISE Dil. is pipetted by the ISE pipetter and mixing is carried out with the ultrasonic mixing unit. Next, the ISE pipetter dispenses ISE IS solution into the IS bath where it is heated to 37°C.

ISE IS measurement

The ISE sipper aspirates ISE IS solution from the IS bath into the measuring flow path to perform an ISE IS measurement (single-point calibration). The residual ISE IS solution is aspirated through the vacuum nozzle to empty the IS bath.

The sipper syringe aspirates ISE Ref. from the ISE Ref. bottle to the reference electrode to perform ISE Ref. measurement.

Sample measurement

The ISE sipper aspirates diluted sample from the reaction cell into the measuring flow path to perform the sample measurement.

The sipper syringe aspirates ISE Ref. from the ISE Ref. bottle to the reference electrode to perform ISE Ref. measurement.

For every ISE measurement, the analyzer measures three electromotive force values (EMF); for chloride, potassium, and sodium, where EMF denotes the difference in potential between the respective ion-selective electrode and reference electrode.

Finalization

Finally, the results are calculated from the electromotive forces of ISE IS and diluted sample. The ISE system is now ready for the next analysis. If there are no more samples to be analyzed, the ISE unit performs a final ISE IS measurement and stops.

Summary This table summarizes the flow of an ISE analysis:

Step	Time	Actor	Action
		Preparation of measurement	
1	0.0 s	Sample pipetter	Pipettes sample (9.7 µl) to cell
2	12.0 s	ISE pipetter	Aspirates (348 µl) and dispenses ISE Dil. (291 µl) to cell
3	15.0 s	Ultrasonic mixing unit	Mixes sample and ISE Dil.
4		ISE pipetter	Aspirates (590 µl) and dispenses ISE IS (450 µl) to IS bath
5		IS bath	ISE IS heats to measuring temperature (37°C)
		Internal standard (ISE IS) measurement	
6	284.5 s	ISE Sipper	Aspirates ISE IS to Cl/K/Na electrodes (400 µl)
7		Sipper syringe via tubing	Aspirates ISE Ref. from the ISE Ref. bottle to reference electrodes (65 µl)
8	292.0 s	Electrodes	Measure ISE IS
		Diluted sample measurement	
9	301.0 s	ISE Sipper	Aspirates sample to Cl/K/Na electrodes (250 µl)
10		Sipper syringe via tubing	Aspirates ISE Ref. from the ISE Ref. bottle to reference electrodes (65 µl)
11	310.0 s	Electrodes	Measure sample
		Finalization of measurement	
12	315.0 s		Result calculation and output If there are more samples to be analyzed, go to step 1. If there are no more samples, repeat 6-8 and stop.

Table A-1 Flow of ISE analysis

ISE unit - Ion selective electrode principles

This chapter provides you with an overview of the ion selective electrode test principles and result calculation used by the **cobas** 6000.

In this chapter *Chapter* **4**

Introduction

The ISE unit performs indirect measurement of electromotive force (EMF) in millivolts between ion selective electrodes and the reference electrode. Indirect measurement means that all samples are diluted at a 1:31 ratio.

The EMF values of each sample are converted to mmol/L values by a calculation algorithm that uses the EMF data together with data from a two-point calibration with two primary standards.

A one-point calibration before and after each routine sample measurement is used to offset the drift between consecutive measurements. For this one-point calibration the internal standard (IS) is used.

Calculation of unknown sample concentrations

The concentration of the sodium, potassium, and chloride in a sample is calculated from the EMF of the specific electrode by the following equation, which is derived from the Nernst Equation:

Equation B-1 $$C_s = C.\text{Value} + C_{IS} \times 10^{(E_s - E_{IS})/S}$$

C_s	Concentration of the specific ion in sample
$C.\text{Value}$	Compensation value
C_{IS}	Concentration of the internal standard
E_s	Electromotive force (voltage) of the unknown sample for the specific ion
E_{IS}	Electromotive force (voltage) of the internal standard for the specific ion
S	Slope

APPENDIX N

Diazyme Adenosine Deaminase Kit – Package insert

Diazyme Laboratories
12889 Gregg Court
Poway, CA 92064, USA
Tel: 858-455-4768 / Fax: 858-455-3701
Email: support@diazyme.com
Website: www.diazyme.com

Adenosine Deaminase Assay Kit

Configuration

The Diazyme Adenosine Deaminase reagent is provided in bulk and the following kit configuration:

REF	Kit Size
DZ117A-K	R1: 1 x 50 mL R2: 1 x 25 mL

Intended Use

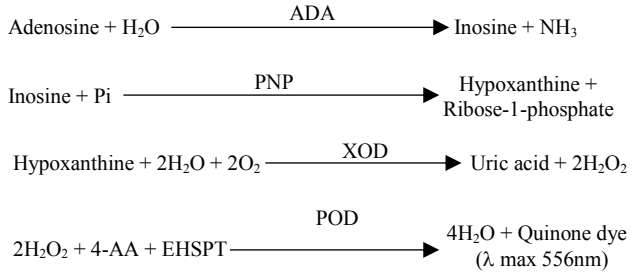
Adenosine Deaminase (ADA) Assay Kit is for determination of ADA activity in serum, plasma, pleural fluid, and cerebrospinal fluid samples.

Background

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, especially high in T lymphocytes. Published literature states that elevated serum ADA activity has been observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis and hepatoma.[1,2] Increased ADA activity was also observed in patients with tuberculous effusions.[3] These reports state that determination of ADA activity in patient serum may add unique values to the diagnosis of liver diseases in combination with ALT or γ-GT (GGT) tests and may also be useful in the diagnostics of tuberculous pleuritis.[3]

Assay Principle

The Diazyme ADA Assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.



One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per min at 37°C.

Reagent – Working Solutions

Reagent 1	
Tris HCl, pH 8.0	50 mM
4-AA	2 mM
PNP	0.1 U/mL
XOD	0.2 U/mL
Peroxidase	0.6 U/mL
Stabilizers	
Reagent 2	
Tris-HCl, pH 4.0	50 mM
Adenosine	10 mM
EHSPT	2 mM

Precautions

1. USA: For Research Use Only. Not for use in diagnostic procedures.
2. EU: For in vitro diagnostic use.
3. R1 is light-sensitive and should be stored in a dark place.
4. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
5. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
6. The reagents contain < 0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
7. Do not use the reagents after the expiration date labeled on the outer box.
8. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

Reagent Handling

ADA REAGENT comes in a liquid two-reagent system, ready-to-use for both manual method and automated chemistry analyzers (kinetics). ADA CONTROL and CALIBRATOR are in lyophilized form, and need to be reconstituted with 1.0 mL of DI water before use. The reconstituted CONTROLS and CALIBRATOR are stable for 1 week at 2-8°C. CONTROLS and CALIBRATOR sold separately.

Reagent Stability and Storage

REAGENT are stable until their expiration date when stored at 2-8°C.

Specimen Collection and Preparation

Serum, heparinized plasma, pleural fluid, or cerebrospinal fluid may be assayed. Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant.
Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered. ADA content of blood is stable for 1 week when stored at 2–4°C. Pleural fluid should be collected in a sterile or heparinized tube and processed within 2 hours at room temperature or stored at 4°C or -20°C for 2 days and up to 2.5 years at -80°C.[7,8,9] Cerebrospinal fluid (CSF) should be clear and collected in a sterile tube without anticoagulant. ADA is stable in CSF for 24 hours at 25ºC, 7 days at 4ºC and 3 months at -20ºC.[10]

Materials Provided

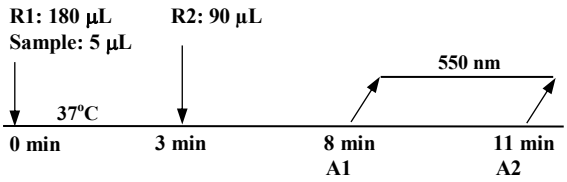
See "Reagent – Working Solutions" section for REAGENT.

Materials Required but not Provided

- Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 540nm – 550nm may be used
- Controls for validating the performance of the Diazyme Adenosine Deaminase Assay Kit (REF DZ117A-CON)
- Calibrators for the Diazyme Adenosine Deaminase Assay Kit are provided separately (REF DZ117A-CAL)
- 0.9% Saline is needed as CALIBRATOR 0
- General laboratory equipment

Assay Procedure

Test Scheme for Chemistry Analyzers



Application sheets for use of Diazyme Adenosine Deaminase Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

Calibration

0.9% saline and the Diazyme Adenosine Deaminase Calibrator (REF DZ117A-CAL) are needed for calibration. The lot specific CALIBRATOR values are stated in the Certificate of Analysis.

Quality Control

We recommend that each laboratory use the Diazyme Adenosine Deaminase Control Set, listed under Materials Required section, to validate the performance of ADA reagents. The Diazyme ADA Control Set is available from Diazyme Laboratories (REF DZ117A-CON). The CONTROL interval and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Each laboratory should follow federal, state, and local guidelines for testing QC material.

Results

The ADA results are printed out in U/L. Literature cites ADA activity tests in serum samples to be in the range of 0-15 U/L[1-4]. Literature citations show that for pleural fluid, values were found to be in the range of 0-30 U/L, and for cerebrospinal fluid (CSF), values were found to be in the range of 0-9 U/L.[4,6]

Limitations

If the sample ADA activity is greater than 200 U/L, the sample should be diluted with saline before measurement. The result should be multiplied by the dilution factor. Assay is specific for ADA and has no detectable reaction with other nucleosides. The reagent solution should be clear. If turbid, the reagent may have deteriorated.

Analytical Characteristics[5]

Results from individual laboratories may vary.

Precision

The precision of the Diazyme Adenosine Deaminase Assay was evaluated on the Cobas Mira instrument according to a modified Clinical Laboratory Standards Institute EP5-A guideline. In the study, two serum specimens containing 11 U/L and 30 U/L ADA were tested with 2 runs per day with duplicates over 15 working days.

	Within Run Precision		Run to Run Precision	
	11 U/L	30 U/L	11 U/L	30 U/L
No. of Data Points	30	30	30	30
Mean (U/L)	11.11	30.74	9.63	29.62
SD	0.16	0.45	0.47	0.59
Cv%	1.47	1.45	4.90	2.00

Linearity

The linearity of the procedure is from 0 – 200 U/L.

Interference

Assay is not affected by serum bilirubin up to 30 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 750 mg/dL, and ascorbic acid up to 4 mg/dL.

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CE EC REP **MDSS**
Schiffgraben 41
30175 Hannover,
Germany

IVD **12889 Gregg Court**
Poway, CA 92064, USA
Tel: (858) 455-4754
Fax: (858) 455-4750