# A comparison of standard C-reactive protein laboratory measurement to point of care C-reactive protein in a neonatal intensive care unit setting

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## Declaration

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## List of Abbreviations

| | |
|---|---|
| CRP | C-reactive protein |
| EONNS | early onset neonatal sepsis |
| ESBL | extended-spectrum beta-lactamases |
| IL-6 | interleukin - 6 |
| IL-8 | interleukin - 8 |
| MAS | meconium aspiration syndrome |
| MRSA | Methicillin-resistant *Staphylococcus aureus* |
| NPV | negative predictive value |
| PCT | procalcitonin |
| POCT | point of care test |
| PPV | positive predictive value |
| ROC curve | receiver operator characteristic curve |
| TNF-α | tumour necrosis factor - alpha |
| TPN | total parenteral nutrition |
| WHO | World Health Organisation |

# 1. Research Proposal

## 1.1. Background

Neonatal sepsis or septicaemia describes the systemic response to infection and/or the isolation of bacteria from the bloodstream in the first 28 days of life.[1] Neonatal sepsis contributes significantly to the morbidity and mortality burden among term and preterm neonates, particularly in developing countries. The World Health Organisation (WHO) estimates that 10% of all under-five mortality is due to neonatal sepsis.[2] Infection rates prove to be higher with decreasing birth weight and gestational age. [3] Neonatal sepsis not only presents a mortality risk but also a grave morbidity risk as it has been associated with cerebral white matter damage in term and preterm infants.[3] Culture-proven septicaemia has been shown to increase the rate of several neurodevelopmental and growth outcomes, including: delayed mental and psychomotor development; vision and hearing impairment; cerebral palsy; poor weight gain, and poor head growth.[3] In order to reduce brain injury associated with infection, interventions such as: earlier diagnosis; stabilising blood pressure; maintaining adequate oxygenation; reducing systemic inflammation and generation of proinflammatory cytokines, as well as pharmacological therapies, may reduce the impact of oxygen free radicals on vulnerable oligodendroglial precursors.[3] Not only does the infection itself pose problems for long-term growth and development, but prolonged and high drug levels of aminoglycoside therapy are known to cause ototoxicity.[3]

Neonatal sepsis has been classified into early onset and late onset sepsis. This classification assists us in identifying the most likely organisms to be implicated, the mode of transmission and guides us in instituting the appropriate antibiotic therapy.[4] Early onset neonatal sepsis is defined as infection from birth to 72 hours of life and is mostly due to transplacental infection or an ascending infection from the maternal cervix caused by microorganisms colonising the genitourinary tract. The occurrence of early onset neonatal sepsis is associated with multiple risk factors, which include but are not limited to low birth weight, prolonged rupture of maternal amniotic membranes prior to delivery, foul-smelling amniotic liquor, multiple vaginal examinations, maternal fever, difficult or

prolonged labour and aspiration of meconium.[5] Late onset neonatal sepsis occurs after 72 hours of life as a result of horizontal transmission of organisms, mostly those that thrive in the home or hospital. The presentation is usually as septicaemia, pneumonia or meningitis. Risk factors associated with late onset neonatal sepsis are infants' low birth weight or low gestational age, the need for mechanical ventilation, the use of parenteral nutrition, previous antimicrobial exposure, lack of human milk feeding, the presence of superficial infections and aspiration of feeds. Additionally, breaks in barrier defense by frequent needle pricks causing loss of skin integrity, the presence of indwelling central venous catheters or intravenous lines and inadequate hand hygiene practices of health care providers, play a significant role.[5]

Neonatal sepsis signs and symptoms are often non-specific, non-sensitive and subtle. These include: fever or hypothermia, apnoea, cyanosis, feeding difficulties, lethargy or irritability, hypotonia, seizures, bulging fontanelle, poor perfusion, coagulopathy, abdominal distension, hepatomegaly and jaundice.[4] Early warning signs and symptoms may be confused with non-infective causes, which include apnoea of prematurity, variation in environmental temperature and an acute exacerbation of chronic lung disease.[6] Even though the onset of sepsis may not immediately be apparent, the effects of not providing early diagnosis and treatment may lead to septic shock, disseminated coagulopathy and rapid progression to death.[6]

Laboratory sepsis markers alongside the evaluation of clinical signs and risk factors are important in diagnosing neonatal sepsis. Isolation of bacteria from blood is considered to be the gold standard for diagnosing sepsis.[4] Common pathogens implicated in neonatal sepsis include: gram positive organisms (*S. epidermidis*, *S. aureus* and *Enterococcus* species), gram negative bacilli (*K. pneumoniae* and *Pseudomonas aeruginosa*) as well as fungal pathogens (usually *Candida*). Multidrug resistant organisms are becoming increasing prevalent and pose antibiotic challenges. These organisms include methicillin-resistant *S. aureus*, vancomycin-resistant *enteroccoci*, pan-resistant *Acinetobacter* and extended spectrum beta-lactamase (ESBL) and cabapenemase-producing gram-negatives.[7] Blood culture results are usually only available 24 to 48 hours later and if negative do not exclude sepsis. Since sepsis may run a fulminant course if not treated

early, antibiotic therapy is usually instituted without waiting for blood culture results. 0.5 to 1 ml of blood taken for culture decreases its sensitivity, as about 60 to 70 % of infants have a low level of bacteraemia.[4] To ensure optimal results approximately 6 ml of blood would be required for inoculation, but in neonates this is not feasible.[4] A positive culture may also arise as a result of asymptomatic bacteraemia or contamination. Procedures that improve blood culture sensitivity and specificity include proper skin disinfection prior to blood sampling, appropriate volume of blood per culture, culturing early in the septic episode and if culturing through an existing intravenous device, a peripheral blood culture should also be collected.[5]

Although blood cultures are the gold standard of diagnosis in sepsis, they pose many diagnostic dilemmas, necessitating the need for a diagnostic test with both positive and negative predictive value. The ideal diagnostic test in the neonatal period should be sufficiently sensitive for infected neonates not to remain untreated but specific enough to allow rational antibiotic prescription and have predictive negative value to allow safe withholding or discontinuation of antibiotics.[8] It is important to note that the most appropriate and ideal biomarker has not yet been established. Usually broad spectrum antibiotics are prescribed due to low culture positive rates. These have the potential to disturb commensal flora and have been associated with the emergence of multidrug resistant gram negative bacilli and the development of invasive candidiasis.[9] In early onset neonatal sepsis in extremely low birth weight infants, prolonged empiric antibiotic usage has been associated with higher risk of death and necrotising enterocolitis.[9] Furthermore, the adverse effects of antibiotic therapy may be nephrotoxicity, hepatotoxicity, haematological abnormalities and blood sampling for drug level monitoring.[9] Ultimately the ideal infection diagnostic test should require a small volume of specimen, be inexpensive, easy to carry out, provide rapid results, allow differentiation between causative agents and comparisons between laboratories.[8]

The C-reactive protein (CRP) is one of the most studied and most used laboratory indicators for neonatal sepsis. It is an acute phase reactant synthesised by the liver. It rises rapidly within four to six hours of an inflammatory process and has a half life of 24 to 48 hours.[4] It takes up to 12 hours to change significantly after the onset of infection and

has a low sensitivity in the early phase of sepsis.[4,10] CRP results greater than 10 mg/L are considered positive in most literature, irrespective of gestational or postnatal age.[2] Any elevation of serum CRP in the neonate always represents endogenous synthesis as it crosses the placenta in very low quantities.[10] Studies have shown that sensitivities and specificities for CRP range from 74-98% and 71-94% respectively.[10] Serial CRP measurements have been highlighted as useful in identifying neonates who do not have bacterial infection. A repeat CRP 48 hours post antibiotic initiation has a 99% negative predictive value in accurately identifying neonates uninfected.[10] Serial measurements are useful for reviewing the response to antibiotics, the duration of antibiotic therapy, and to recognise potential complications. Unlike blood cultures, CRP measurement is unaffected by prior antibiotic therapy. It is important to note that other non-infectious conditions such as meconium aspiration syndrome, traumatic or ischaemic tissue injuries, haemolysis or chorioamnionitis, also cause elevation in CRP levels.

There are many other tests that may be performed to assist in diagnosing neonatal sepsis. The complete or full blood cell count (FBC) is one of these tests and is rapid, inexpensive and widely available. The diagnostic accuracy of the aforementioned test is not well-defined in the neonatal setting and may be affected by physiological changes, especially in very low birth weight infants.[11] High and low white blood cell counts, high absolute neutrophil counts, high immature-to-total neutrophil ratios and low platelet counts are associated with late onset neonatal sepsis.[11] However, a high proportion of infants with late onset sepsis have normal full blood counts and no full blood count index can reliably rule out infants with late onset sepsis.[11] Procalcitonin or PCT is another biomarker of neonatal sepsis. It is an acute phase reactant produced by both hepatocytes and macrophages. The serum concentrations of PCT start to increase four hours post exposure to bacterial endotoxins and peak within six to eight hours, remaining elevated for at least 24 hours.[4] At 48 hours serum concentrations of PCT return to baseline. In non-infected neonates PCT concentrations tend to vary widely and PCT has its limitations. In the absence of true neonatal infection, PCT may in fact be increased in: newborns requiring neonatal resuscitation; infants born to mothers with chorioamnionitis; maternal Group B *streptococcal* colonisation, and prolonged rupture of membranes.[4] Additionally

the use of PCT is limited by its physiological surge which reaches peak levels at 24 hours of age in preterm and term neonates. This suggests that PCT has better diagnostic accuracy for late onset sepsis in comparison to early onset sepsis.[12] A meta-analysis showed high statistical heterogeneity for all analyses of PCT with variable sensitivity and specificity across studies.[12] While PCT may be more sensitive than CRP, it is not sensitive enough to avoid antibiotic therapy.[12] It is important to note that PCT values were found to reduce more quickly with treatment than CRP values.[8] When considering diagnostic testing and expenses, PCT proves to be a more expensive test than CRP.

At Groote Schuur Hospital's neonatal unit clinical signs as well as FBC and CRP are pivotal in directing management for neonatal sepsis. C-reactive protein is requested for both early onset and late onset sepsis and is considered positive if more than 10 mg/L. Antibiotics are initiated as soon as the neonate is thought to have sepsis and once a blood culture has been performed. C-reactive protein is done in all neonates with suspected sepsis. At this neonatal unit the clinician performs blood sampling for CRP and the blood is sent to the laboratory for standard laboratory measurement by the Cobas 6000 analyser. Once antibiotics are started the duration of treatment is tailored to clinical circumstances. If the initial CRP is below 10 mg/L, a second blood sample is sent for CRP 48 hours after antibiotic initiation. Blood cultures that remain negative after 48 hours, along with the repeat CRP below 10 mg/L prompt the cessation of antibiotic therapy in patients who no longer exhibit clinical signs of sepsis. The neonatal unit protocol prohibits routine courses of antibiotic therapy in the absence of proven or strong suspicion of sepsis.[13]

C-reactive protein measurement can be performed using standard laboratory measurement or alternatively a point of care analyser. This research study will be carried out at Groote Schuur Hospital's neonatal unit, where the National Health Laboratory Service (NHLS) uses the Roche Cobas 6000 analyser for standard laboratory measurement of CRP. The Cobas 6000 system has been found to accommodate robust chemistry and immunochemistry, and has good potential for workstation consolidation in medium-sized laboratories.[14] This analyser in comparison to other leading laboratory analysers has been shown to provide excellent precision data and result equivalence for most assays.[14] This

system utilises an automated particle enhanced immunoturbidimetric assay. The reagent used contains latex spheres that are coated with monoclonal anti-CRP antibody. The antibody is raised to the CRP to agglutinate. Light is passed through the sample and may be scattered, reflected or absorbed. Immunoturbidimetry measures the absorbance of light from the sample. This determines a quantitative CRP result, as the anaylate concentration is inversely proportional to the light signal.[2] The result is considered positive if more than 10mg/L. This conventional method of obtaining CRP requires collection of blood in a 400 µl microtainer which is then sent to an offsite laboratory. The cost of each CRP test is more than R65.

Point of care testing (POCT) is defined as medical testing at or near the site of patient care. It allows for smaller blood volume sampling, rapid availability of results, earlier therapeutic decision making and may in fact be more cost effective when compared to standard laboratory measurement. This research study will be using the Alere Afinion™ point of care CRP test, which is a solid phase immunochemical assay requiring an analyser and test cartridges. This test requires only 1.5 µl of blood, has a test time of approximately four minutes and its measuring range for CRP is 5-200 mg/L.[15] Each CRP test cartridge costs approximately R50. This point of care test is a simple three step procedure that involves: 1) collecting the blood sample via clinician chosen standard sampling techniques, 2) collecting the blood using the self-contained capillary tube in the test cartridge that collects the exact volume of blood required for the test and, 3) inserting the test cartridge into the analyser to obtain a result. The test cartridge is placed in the analyser where it is automatically diluted with a liquid that lyses the blood cells. The sample mixture is passed through a membrane coated with anti-CRP antibodies. The CRP in this sample is concentrated onto this membrane. A solution containing anti-CRP antibodies conjugated with ultra-small gold particles is then passed through this membrane and the gold-antibody conjugate binds to the immobilised CRP on the membrane, which then turns red-brown. Excess gold-antibody is removed by a washing solution. The analyser measures the colour intensity of the membrane and it is this intensity that is proportional to the amount of CRP within the sample. The CRP concentration is then quantitatively displayed on the analyser screen.[16] This POCT states

that a CRP of less than 5mg/L is found in healthy individuals and can reach between 20 and 200mg/L in the presence of acute inflammation.[16]

The Afinion™ point of care test procedure requires a minimal level of user-interaction, making it more user-friendly than the NycoCard™ and the QuickRead® point of care tests. The NycoCard™ point of care system requires additional manual steps by the user, such as dilution of the sample, applying a conjugate, washing the sample and finally reading the test result.[15] Similarly the QuickRead® CRP point of care system requires a specific instrument, capillaries, cuvettes and reagents that require accurate measurement and mixing with a specific volume of blood sample to perform the test.[2] The QuickRead® CRP requires additional and potentially complicated steps to carry out the test, these include: filling the capillaries with blood; dispensing the specimen into the cuvette containing a 1ml buffer; closing the cuvette with the CRP-reagent cap containing the CRP-reagent and mixing it by gentle shaking; after mixing the cuvette is placed in the instrument-well to derive a control measure which takes 40 seconds; the cap containing the dried anti-human CRP latex reagent is depressed, resulting in the reagent being released into the sample; the cuvette is then taken out of the instrument-well and gently remixed; the final step involves the sample being replaced in the instrument-well for the measurement of the CRP concentration.[2] The choice of the Afinion™ point of care test in this study is supported by its simple use requiring just three steps and its minimal use of blood (only 1.5µl in comparison to 5µl in the NycoCard™ system and 20µl in the QuickRead® system).[17, 2] This ensures that staff training on the use of the point of care system is simple and quick to demonstrate within this busy neonatal unit, where both staff and patient turnover is high. In a paediatric and adult population the Afinion™ point of care test has shown great accuracy and precision when compared to standard laboratory measurement, however the results of this study could not be generalised to the neonatal population.[15] This suggests the need for further studies of point of care CRP testing within the neonatal population. A further study looking at the QuickRead® point of care test suggested that it may be beneficial in resource-limited settings, where it may facilitate reduction in costs and earlier patient discharge.[2]

## 1.2. Purpose and Objectives

### 1.2.1. Primary objectives

The purpose of this study is to validate the Alere Afinion™ point of care test CRP in neonates with suspected sepsis at Groote Schuur Hospital's neonatal unit using the CRP obtained from the National Health Laboratory System's Roche Cobas 6000 as the reference test

### 1.2.2. Secondary objectives

1. To measure the potential change in antibiotic dosage exposure if management decisions regarding antibiotic therapy were made using CRP results obtained from the Alere Afinion™ CRP system when compared to standard NHLS laboratory testing
2. To measure the ease of use of Alere Afinion™ by using time until test completion as a proxy measure

## 1.3. Method

### 1.3.1. Design

The study design is that of a prospective observational study and diagnostic test evaluation.

### 1.3.2. Sample

Participants eligible for inclusion in the study will be neonates with suspected sepsis who are admitted to the Groote Schuur Hospital neonatal unit. An informal and unpublished audit performed at the Groote Schuur Hospital neonatal unit showed that approximately 140 to 160 CRP laboratory tests are done per month.[18] A total of at least 138 samples will be obtained for analysis. The sample size was calculated using Epicalc2000. The sample size was estimated to be 138 in order to ensure a sensitivity and specificity of 90% with a margin of error of +/- 5%. Patients will only be recruited during normal working hours. This sampling method was selected due to workforce limitations in both clinical and laboratory staff after hours. Clinicians involved in patient care within the unit will be performing the blood sampling. Blood will be drawn at the clinician's discretion either through venepuncture or arterial puncture. Blood sampling for the standard laboratory measurement and the POCT test will be taken from the same blood sampling site. The

medical staff including the medical officers, paediatric registrars as well as the neonatology consultants will be making therapeutic decisions solely based on the standard laboratory measurement result and not on the POCT result.

Inclusion criteria: any neonate in whom the CRP will be done either as part of a septic work up or follow up test. This CRP must be analysed by both the Alere Afinion$^{TM}$ point of care CRP analyser and the standard laboratory Cobas 6000 analyser. Parental informed consent must be obtained for inclusion of the participant in the study.

Exclusion criteria: any neonate in whom the Alere Afinion$^{TM}$ CRP result or standard laboratory CRP result will not be available. Neonates will be excluded should parental consent not be granted.

#### 1.3.3. Data

Patient names and hospital numbers will be used to identify samples. Data will be collected with the use of a pre-designed data collection sheet (see addendum 1). This sheet will be completed by the doctor who has taken the blood sample for both the Alere Afinion$^{TM}$ CRP measurement and standard laboratory measurement. It is the responsibility of the clinicians caring for the patient to obtain informed consent. Doctors working within the Groote Schuur Hospital Neonatal Unit will be trained to use the point of care test and instructed as to how to complete the data collection sheet. The clinicians will be instructed to telephonically contact the primary investigator of all new cases daily and a formal record book noting the patient details and time of test will be kept within the unit. If consent cannot be obtained prior to performing the POCT then the patient will be excluded from the study. Data collection sheets will be kept within the patient's folder until the primary investigator is able to collect the sheets. CRP results will be verified by reviewing the standard laboratory measurement result on the NHLS computer results and the POCT by reviewing the records on the analyser. The POCT CRP result will not be used to determine patient management. Clinicians will obtain both results but will only base clinical decisions on the standard laboratory measurement result. The laboratory staff and technicians will be unaware of this study and will be expected to process results as usual.

Data from the data collection sheet will be captured and synthesised in Microsoft Excel spreadsheets. The hard copies of data collection sheets will be kept in a file in a lockable cabinet, which will only be accessible to the researchers. Collated data will be kept in a password protected Microsoft Excel spreadsheet.

The data collection sheet will include:

- Patient demographic details
- Risk factors for sepsis
- Signs and symptoms indicating possible sepsis
- Reason for requesting a CRP
- Whether a blood culture and other cultures were taken or not taken
- Whether empiric antibiotic therapy was initiated
- Time from blood sampling to obtaining point of care result
- Time of CRP laboratory results being released on NHLS result system
- Time from blood sampling to obtaining standard laboratory measurement result
- CRP results from both POCT and standard laboratory measurement

Blood culture results and duration of antibiotic therapy will be followed up by the researcher. The time that the clinician first becomes aware of the result will be recorded as the time to result. The time it takes to perform the Afinion$^{TM}$ test will be noted for comparison. Clinicians must always document the time that the sample was taken.

#### 1.3.4. Analysis

Standard statistical methods will be utilised through the statistical software package, Stata. The primary objective will be analysed using receiver operator characteristic curves and Bland-Altman Plot. This will provide graphical representation of the true positive rate versus the false positive rate when comparing the point of care CRP to the gold standard NHLS CRP and analyse the agreement between the two measures. The sensitivity, specificity, positive predictive values and negative predictive values will be analysed using the laboratory CRP test as a reference. The p-value will be considered statistically significant if less than or equal to 0.05. When comparing categorical variables, such as blood culture samples to the point of care test, the chi-squared or Fisher's exact test will

be used to analyse this data. Other descriptive variables will be described in terms of means, medians, interquartile ranges, proportions and 95% confidence intervals. Statistical support will be obtained from the School of Public Health and Family Medicine during data collection and analysis.

## 1.4. Timeline

The research study from start to completion involves the following process and timeframe:

- Proposal and synopsis writing - 3 months
- Submission to Departmental Research Committee (DRC) and Faculty of Health Sciences Human Research Ethics Committee (HREC) - 2 months
- Data collection - 3 to 4 months
- Data analysis - 2 months
- Thesis write up - 4 months

## 1.5. Dissemination Plan

The results of this study will primarily be included in a MMED dissertation. The research data will be presented at the School of Child and Adolescent Health Research Day. Furthermore the results of this study will be formatted as a journal article so that it may be suitable for publication in a peer-reviewed journal.

## 1.6. Costs

The cost of this study is estimated to be approximately R 16 000.

The budgetary requirements include (addendum2):

- Point of care test and control kits
- Printing of data collection sheets
- Office consumables
- Research assistant

Alere Healthcare Company will provide the Afinion$^{TM}$ AS100 Analyser for the duration of the study. The analyser will remain the property of Alere Healthcare Company and will be returned to them on completion of the study. Test and control kits will be purchased

using a registrar research grant provided by the Department of Paediatrics. No extra costs will be incurred with respect to the standard laboratory measurement, as these tests will be performed irrespective of the study.

## 1.7. Conflict of Interest

There is no conflict of interest in terms of this study. This study is designed and will be preformed independently from the Alere Healthcare Company. The researcher is not employed by this organisation, or associated organisations and in no way will gain or lose financially from the results of this study. No long-term contracts will be entered into on behalf of Groote Schuur Hospital with Alere Healthcare Company and the placement of the analyser in the neonatal unit will only be for the purposes of this study.

## 1.8. Ethical Considerations

All patients will be enrolled with parental consent (see addendum 3). The information sheet and parental consent will be available in English, Afrikaans and Xhosa. Patients will be identified by their names and folder numbers. Patient clinical information will remain private and confidential. Only the clinicians and researchers will have access to the identifying data and clinical information of the patients. There is no risk to participating in this study. Participants who are already having blood drawn for standard laboratory testing will be included and a small volume of blood (1.5 µl) will be used for point of care testing. Blood sampling and point of care testing will be performed by experienced medical clinicians involved in patients' care. Management of patients will be based on laboratory results and not point of care results, until this study is able to validate the point of care system for future use. Benefits may be access to rapid results and reduced antibiotic therapy exposure and hospital stay. Future point of care testing within the unit may benefit patients and the neonatal unit in this regard. Ethical approval will be obtained from the Faculty of Health Sciences Human Research Ethics Committee.

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# 2. Literature Review

## 2.1. Objectives

1. To compare the C-reactive protein (CRP) to other diagnostic tests and biomarkers
2. To review point of care CRP test studies' outcomes and impact on management

## 2.2. Search Strategy

PubMed database was searched online. Three searches were performed:

1. Main search:
   (((((("Infant, Newborn"[Mesh] AND Humans[Mesh] AND infant[MeSH])) OR (((newborn OR neonate)) AND Humans[Mesh] AND infant[MeSH])) AND Humans[Mesh] AND infant[MeSH])) AND ((((((("Point-of-Care Systems"[Mesh] OR "Point-of-Care Testing"[Mesh])) OR point of care)) OR ((("Clinical Laboratory Services"[Mesh] OR "Automation, Laboratory"[Mesh])) OR ((laborator* OR lab))))) AND (("C-Reactive Protein"[Mesh]) OR ((C-Reactive Protein OR CRP)))) AND Humans[Mesh] AND infant[MeSH])
   Filters included were humans and infants from birth to 23 months. This search yielded 369 articles of which 70 were applicable to the study topic. 18 studies were excluded because they were not in English. 8 studies were not obtainable in full text. 44 articles from this search were reviewed.

2. (Alere OR Afinion) AND (CRP OR C reactive protein) yielded 7 articles, 5 of which were applicable to the study topic.

3. Antibiotics AND (CRP OR c reactive protein) AND (newborn OR neonate) yielded 791 articles and 28 articles were applicable and therefore reviewed for additional information.

## 2.3 Literature

### 2.3.1. Background and Epidemiology

Neonatal sepsis is a major public health problem and an economic burden especially to the developing world. Three quarters of the 2.6 million neonatal deaths annually are early neonatal deaths occurring in the first 7 days of life - an overwhelming majority (99%) of which occur in low to middle-income countries.[1] Neonatal sepsis is responsible for 10% of all under-five mortality, being the third leading cause of neonatal deaths, after prematurity and intrapartum related deaths (such as asphyxia). [1–3] The World Health Organisation (WHO) estimates that 42% of sepsis related neonatal deaths occur in the first week of life.[1,2]

While neonatal sepsis is variably defined based on several clinical and laboratory parameters, it is essentially a clinical syndrome characterised by systemic signs of circulatory compromise caused by bacterial invasion in the bloodstream within the first month of life.[2] Wynn et al have stated that a static definition of sepsis is likely to be associated with limitations in diagnostic accuracy as sepsis is a dynamic, complex and heterogeneous condition.[4] The signs and symptoms of neonatal sepsis are non-specific and are often subtle. These clinical features include cyanosis, apnoea, grunting, lethargy or irritability, hypotonia, bulging fontanelle, poor perfusion, petechiae, purpura, hypoglycaemia or hyperglycaemia, abdominal distension, hepatomegaly, unexplained jaundice or "just not looking right".[1,5–7]

Predisposing factors for neonatal sepsis are prematurity, male gender, prolonged rupture of membranes and chorioamnionitis, maternal colonisation with Group B *Streptococcus*, maternal urinary tract infection and perinatal asphyxia.[6] Neonates, particularly preterm neonates, are at higher risk of infections than the rest of the paediatric population as they have immature immune systems. This may be as a result of poor innate immunity with impaired cytokine production, reduced expression of adhesion molecules in neutrophils and decreased response to chemotactic factors.[1] Humoral immunity may be affected as transplacental passage of antibodies starts in the second trimester of pregnancy with rapid increase in the third trimester.[1] Cytotoxic T cell activity may also be impaired and complement activity is half that of healthy adults.[1,2] Recurrent blood sampling and invasive procedures further add to the disruption of neonates' protective barriers. Advocating early initiation of breastmilk and increasing exclusive breastfeeding have been shown to

significantly reduce diarrhoea and acute respiratory tract infections.[2] Breastmilk consists of secretory IgA, lysozymes, white blood cells and lactoferrin and encourages the growth of healthy lactobacilli, while reducing the growth of *Escherichia coli* and other pathogenic gram negative organisms.[2]

Based on the timing of infection, neonatal sepsis is characterised by early onset neonatal sepsis (EONNS) and late onset neonatal sepsis (LONNS). These two classifications indicate the presumed mode of transmission, likely implicated organisms and initiation of antibiotic therapy. Epidemiologists define EONNS as culture positive infections within the first three postnatal days of life.[8] Similarly Zea-Vera et al and Shah et al define EONNS as symptoms starting before 72 hours or within the first three days of life.[1,5] Hisamuddin et al define EONNS as disease present during the first five to seven days of life.[9] The first definition, as defined by epidemiologists, is more widely accepted. EONNS arises as a result of vertical transmission from mother to infant during the intrapartum period. LONNS is defined as symptoms occurring after the EONNS period, which is after 72 hours of life or the first three days of life, alternatively after the first week of life. LONNS is usually caused by nosocomial infection related to prolonged hospitalisation, mechanical ventilation, intravenous cannulation or central line placement, parenteral nutrition and antibiotic administration as well as poor hand hygiene practices. Zea-Vera and Ochoa describe common pathogens causing sepsis in developed and developing countries in tables 1 and 2.[1]

*Table 1 Common pathogens of neonatal sepsis in developed countries*

| Early-onset | | Late-onset | |
|---|---|---|---|
| Pathogen | Frequency (%) | Pathogen | Frequency (%) |
| Group B *Streptococcus* | 43–58 | Coagulase-negative *Staphylococcus* | 39–54 |
| *E. coli* | 18–29 | *E. coli* | 5–13 |
| Other gram-negative bacteria | 7–8 | *Klebsiella* sp. | 4–9 |
| *S. aureus* | 2–7 | *S. aureus* | 6–18 |
| Coagulase-negative *Staphylococcus* | 1–5 | *Candida albicans* | 6–8 |
| *L. monocytogenes* | 0.5–6 | *Enterococcus* sp. | 6–8 |
| | | *P. aureginosa* | 3–5 |
| | | Other *Candida* species | 3–4 |

*E.coli = Escherichia coli*; *S.aureus = Staphylococcus aureus*; *L.monocytogenes = Listeria monocytogenes*; *P.aureginosa = Pseudomonas aureginosa*

Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. Journal of Tropical Pediatrics [Internet]. 2015 [cited 2016 June 2];61(1):1-13. Table 1, Common pathogens of neonatal sepsis in developed countries; p.3. Available from PubMed

*Table 2 Common pathogens of neonatal sepsis in developing countries*

| Community-acquired | | Hospital-acquired | |
|---|---|---|---|
| Pathogen | Frequency (%) | Pathogen | Frequency (%) |
| *Klebsiella* sp. | 14–21 | *Klebsiella* sp. | 16–28 |
| *S. aureus* | 13–26 | *S. aureus* | 8–22 |
| *E. coli* | 8–18 | *E. coli* | 5–16 |
| Group B *Streptococcus* | 2–8 | Coagulase-negative *Staphylococcus* | 8–28 |
| *S. pneumonia* | 2–5 | *Pseudomonas* sp. | 3–10 |
| *Salmonella* sp. | 1–5 | *Enterobacter* sp. | 4–12 |
| | | *Candida* sp. | 0.3–3 |

*S.aureus = Staphylococcus aureus*; *E.coli = Escherichia coli*; *S.pneumonia = Streptococcous pneumonia*

Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. Journal of Tropical Pediatrics [Internet]. 2015 [cited 2016 June 2];61(1):1-13. Table 2, Common pathogens of neonatal sepsis in developing countries; p.3. Available from PubMed

Gerdes stipulates that the goals of the clinician in neonatal sepsis are to 1) develop a systematic approach to the diagnosis of sepsis based on the relative importance of known symptoms and risk factors, 2) miss no cases, 3) minimise the duration of antimicrobial treatment for those high risk infants who are uninfected, and 4) provide a safe observation protocol for the low risk newborn.[6] While clinical information is usually adequate to start empiric antibiotic treatment, it is difficult to use this information alone to tailor therapy in terms of agents and duration of treatment.[10]

### 2.3.2. C-Reactive Protein

C-reactive protein (CRP) is an acute phase reactant that belongs to the pentraxins, a family of proteins structurally made up of five identical subunits to form a pentameric disc-like structure. It is synthesised in the liver and physiologically binds to phosphocholine expressed in dead and dying cells causing an initiatory burst to activate the complement system.[11] It may be produced in different cells including hepatocytes, lymphocytes, monocytes, atherosclerotic plaques, neurons and some tumours but its plasma concentration is almost exclusively due to synthesis in hepatocytes.[11] CRP was first described in 1930 by Tillet and Francis at Rockerfeller University and takes its name from the observation that it forms an insoluble complex with C- polysaccharide of Streptococcus pneumoniae.[12,13] CRP rapidly increases in 4 to 6 hours after the inflammatory process and is duplicated in 8 hours, reaching a peak in 36 hours and has a half-life of 19 hours.[11] Due to the delayed response to sepsis, the sensitivity of CRP elevation at the time of evaluation for

suspected sepsis is low, particularly in EONNS.[13] Kumar et al reported the sensitivity for early onset proven sepsis and late onset proven sepsis was 88.9% and 98.2% respectively.[14] Lower cut off values for preterm neonates have been suggested after Hofer et al found CRP to be less reliable in early onset infections in preterm infants when compared to term newborns.[15] Any elevation of CRP in the newborn is indicative of endogenous synthesis as it passes the placenta in exceedingly low quantities.[12] The normal levels of CRP in newborn infants range from 2 to 5 mg/l and cut-off values reported in the literature range from 1.5 to 20 mg/l but the consensus is that 10 mg/l is the upper limit of normal.[12,16]

Elevated measures of CRP may be associated with conditions other than bacterial infections, namely viral infections, trauma, ischaemic tissue injuries, haemolysis, meconium aspiration syndrome, complicated labour and delivery, animal-derived surfactant administration, intraventricular haemorrhage and chorioamnionitis without invasive fetal or neonatal disease.[13,16] Ohlin and colleagues determined that the five clinical signs of feeding intolerance, distended abdomen, low blood pressure, bradycardia and apnoea, together with CRP were statistically significantly associated with a positive blood culture.[17] Both single measurements and serial measurements of CRP have been studied.

Lacaze-Masmonteil et al conducted a study that explored the value of a single CRP measurement at 18 hours of age. They suggested that an elevated 18 hour CRP in isolation should not be used as a reason to prolong antibiotics as an 18 hour CRP was ≥10 mg/l in 85.8% of uninfected newborns.[16] Furthermore, for proven or possible EONNS there was a low CRP sensitivity possibly because of intrapartum maternal antibiotics and delayed rise in CRP in infected preterm neonates. Boonkasidecha and colleagues reviewed CRP levels obtained at the time of initial sepsis work up and 12 to 24 hours later and found the use of a serial CRP to be better than a single measurement.[18] The second CRP had a higher sensitivity and negative predictive value (NPV) than the first CRP. The cut off points for CRP in this study were notably much lower than the universally accepted cut off of ≥10 mg/l. Budget constraints or lack of blood culture facilities promote the use of a single CRP measurement 24 to 48 hours after the suspected sepsis.[18] A decrease of CRP within 48 hours after birth indicates that infection is unlikely and antibiotics can be discontinued. This would reduce health care costs, facilitate earlier discharge, prevent complications of therapy and avoid family anxiety.[19,20] CRP is a valuable tool for objective monitoring of the success and length

of treatment as it declines in response to effective antibiotic therapy.[21] Defensive medical practice, lack of experience, uncertainty, "routine practice", lack of awareness of the associated costs and use of protocols and guidelines has led to overutilisation of laboratory testing.[22]

Two main technological advancements in diagnostic testing have been:

1) the development of point of care or bedside tests, with availability of miniaturised and portable machines for rapid testing even for complex measurements usually performed by sophisticated instruments in specialised laboratories, and

2) the development of new methods including the study of genomics, the production of proteins, lipids and metabolites.[10]

A significant advantage of CRP over other biomarkers is that point of care testing is commercially available providing bedside measurements that are performed and obtained within minutes.

### 2.3.3. Diagnostic tests and biomarkers for neonatal sepsis other than C Reactive Protein

***Blood culture***

The gold standard for the definitive diagnosis of sepsis is the blood culture test for bacteria. While this is the most reliable test available it is flawed. Wang et al note that the blood culture test can be negative for one in five subjects with sepsis.[23] Kayange et al note that the neonatal blood culture positive rate ranges from 25-54%.[24] Mishra et al reviewed studies that showed positive culture rates from 8-73% in the diagnosis of potential neonatal sepsis.[25] However, the sensitivity and specificity of blood culture is dependent on timing of sampling, adequate disinfection of the phlebotomy site or indwelling catheter, sufficient blood volume sampling and inoculation of culture medium, which poses significant challenges in extreme preterm infants. Furthermore it may take 48 hours or longer to obtain a positive result.[13] For this reason initiation of empiric treatment is a necessity and other diagnostic tests or biomarkers may be employed for guidance.

Accurate biomarkers that aim to diagnose sepsis or indicate the evolution of the infectious processes may strengthen the clinician's decisions regarding therapeutic management. According to the National Institute of Health a biomarker is defined as biological

characteristics objectively measured and used as a marker either of a normal or pathological biological pathway, or pharmacological response to a specific intervention.[10] There are two categories of biomarkers: prognostic biomarkers, which stratify a patient's risk of having a specific outcome independent of treatment and predictive biomarkers, which allow prediction of the potential benefits and risks of treatment.[10] The ideal biomarker should be accurate and reproducible. This ensures that 100% of all patients with the disease are detected by the test (sensitivity) and 100% of all patients that do not have the disease have a negative test result (specificity).[25] However, finding the ideal biomarker for neonatal sepsis has been difficult. Interpreting a biomarker can be problematic as measurements are affected by many factors namely a lack of standardisation between different methods and biological factors such as blood collection tubes and transport media, time from sampling to analysis, precision, reproducibility and threshold of measurement.[10]

***Haematological tests***

Haematological tests are frequently used to assist with diagnosing sepsis but may be weaker diagnostic tests than the previously mentioned biomarkers.[26] The most utilised of these haematological tests include the white cell count, neutrophil count, immature to total neutrophil ratio (I:T ratio) and platelet count, which are quick, easy and inexpensive to perform. In the presence of normal haematological findings in newborns with clinical features of sepsis, it is not sufficiently reassuring to defer initiation of antimicrobial therapy.[13] The performance of these tests was evaluated by Benitz in 298 neonates in the first month of life, his findings are summarised in table 3.[13]

*Table 3 Performance of haematological markers*

| Haematological finding | Sensitivity | Specificity | Positive predictive value (PPV) | Negative predictive value (NPV) |
|---|---|---|---|---|
| **White cell count** | 44% | 92% | 36% | 94% |
| **Platelet count ≤ 150 000mm$^3$** | 22% | 99% | 60% | 93% |
| **Increased I:T ratio** | 96% | 71% | 25% | 99% |
| **Increased or decreased neutrophil count** | 96% | 61% | 20% | 99% |

***PCT***

Procalcitonin (PCT) is an amino acid protein, which is a precursor to calcitonin produced by the C-cells of the thyroid gland. [27] It is produced by the liver and macrophages with serum levels rising 4 to 6 hours after exposure to bacterial products.[13] A spontaneous increase in PCT in the first day of life with peak at 24 to 36 hours after birth in healthy newborns and decrease thereafter have been documented.[13] PCT concentrations rise in blood in accordance with the intensity of inflammatory reaction due to infection without increasing the level of calcitonin and have the potential to differentiate between sepsis and systemic inflammatory response syndrome (SIRS) resulting from sterile inflammation.[27,28] PCT levels also increase in uninfected neonates with birth asphyxia, intracranial haemorrhage, pneumothorax or after resuscitation.[28] PCT's role as a biomarker in the diagnosis and prognosis of sepsis is ambiguous as the initial PCT may not be reliable but instead serial measurements may assist in monitoring sepsis outcomes and guide therapy to reduce antibiotic exposure without increasing mortality.[27] In a study conducted by Kordeck, it was found that elevations in PCT were associated with gram positive, predominantly coagulase-negative *staphylococcus*, and gram negative bacterial infections.[29] This may be useful in determining those newborns infected with coagulase-negative *staphylococcus* as oppose to newborns with samples contaminated by the organism. While cost and availability may hinder utility of PCT in resource-limited countries, other limitations are establishing detailed age specific normative ranges, low sensitivity in EONNS and lack of specificity.[13]

***Cytokines***

Cytokines such as Il-6, IL-8 and TNF-alpha, are intracellular messengers that act via specific receptors on target cells and mediate the inflammatory response.[30] Acute phase reactants are produced in response to these proinflammatory cytokines, therefore high levels of cytokines occur promptly after exposure to bacterial products, preceding the increase in acute phase reactants.[28] IL-6 has a very short half-life with concentrations declining rapidly with therapy, becoming undetectable in most newborns within 24 hours.[28] It has been proposed that the combination of measurements, for example IL-6 and CRP, may enhance the likelihood of an abnormal result when uncertain about the stage of illness at which the neonate is being evaluated.[13] Limitations of the utility of cytokines in routinely diagnosing neonatal sepsis include methodological difficulties in their detection, absence of their

routine usage in all centres and increase in their levels in all non-specific infections and in shock.[28]

***CD64***

CD64 is a leucocyte surface antigen with a high affinity Fc receptor, which binds to IgG. These receptors are involved in the innate and adaptive immune response to stimulate phagocytosis or antibody-mediated cytotoxicity.[27] CD64 only has highest diagnostic accuracy in neonates until 24 hours after suspected sepsis and is able to differentiate between sepsis and SIRS in various patient populations.[27] Wang et al report CD64 to have superior diagnostic accuracy to the I:T ratio and CRP when detecting EONNS.[23] CD64 testing can be done on the sample sent for the complete blood count, only requires 50 µl of blood for detection and can be made available within hours of performing the complete blood count.[23] Laboratories in developed countries and in only some developing countries have flow cytometry to perform the test.

***A comparison of biomarkers***

Meem and colleagues reviewed PubMed, EMBASE and HINARI sources to conduct a structured literature search on existing biomarkers for the diagnosis of neonatal infections. They reviewed 65 articles for statistical analysis with sensitivity, specificity and cut off values as noted in table 4 below.[31] CRP is shown to be the most specific but least sensitive test.

*Table 4 Performance of biomarkers*

| Biomarker | Cut-off range | Cut-off sub-ranges | Percentage of papers | Sensitivity ranges (%) | Specificity ranges (%) | Cut-off | Sensitivity | Specificity |
|---|---|---|---|---|---|---|---|---|
| CRP | 0.2–95 mg/L | 0.2–10 | 70 | 41–96 | 72–100 | mean=17. 1median=10 | mean=66.53 median=69 (IQR=26.3) | mean=86.14 median=90 (IQR=13.9) |
| | | 11–30 | 15 | 33–56 | 74–96 | | | |
| | | 31–95 | 15 | 23–87 | 46–98 | | | |
| PCT | 0.34–100 ng/mL | 0.34–1.0 | 48 | 58–100 | 50–100 | mean=8.92 median=1.17 | mean=77.93 median=8 (IQR=14.8) | mean=81.84 median=82.5 (IQR=16.6) |
| | | 2–10 | 39 | 59–95 | 50–100 | | | |
| | | 11–100 | 13 | 21–95 | 87–100 | | | |
| IL-6 | 3.6–500 pg/mL | 2–10 | 15 | 88–96 | 66–89 | mean=76.49 median=30 | mean=77.87 median=80 (IQR=29.9) | mean=78.61 median=78 (IQR=18.9) |
| | | 11–30 | 34 | 61–90 | 56–90 | | | |
| | | 31–100 | 31 | 57–100 | 43–100 | | | |
| | | 101–500 | 20 | 74–97 | 70–100 | | | |
| IL-8 | 1–1000 pg/mL | 0.6–100 | 74 | 34–92 | 52–96 | mean=220.53 median=70 | mean=72.48 median=80 (IQR=15.5) | mean=80.57 median=82 (IQR=21.7) |
| | | 101–1000 | 26 | 36–92 | 65–96 | | | |
| CD64 | different units used | – | – | 79–100 | 81–96.8 | not analyzable | mean=82.42 median=92 (IQR=17) | mean=82.79 median=88 (IQR=15) |
| TNF-α | 1.7–70 pg/mL | – | – | 54–100 | 43–96.6 | mean=18.94 median=7.5 | mean=78.72 median=80.4 (IQR=22.7) | mean=81.4 median=93 (IQR=14.9) |

CRP = C-reactive protein; PCT = procalcitonin; IL 6 = interleukin 6; IL-8 = interleukin 8; TNF- α = tumour necrosis factor-alpha

Meem M, Joyanta K, Mortuza R, et al. Biomarkers for diagnosis of neonatal infections : A systematic analysis of their potential as a point-of-care diagnostics. Journal of Global Health [Internet]. 2011 [cited 2016 June 2];1(2):201-209. Table 2, Sensitivity, specificity and cutt-off values of biomarkers in reviewed studies; p.204. Available from: Pubmed

### 2.3.4. Point of care CRP studies

Thirteen studies reviewing CRP point of care diagnostics were identified in this literature search. Only one of these studies took place in a developing country and only three studies focused specifically on neonates. The paediatric population was studied in the emergency departments of various hospitals. Table 5 reviews all thirteen studies and table 6 compares variables of the three most commonly used point of care CRP tests.

Of the thirteen studies tabulated:

- Studies 1 to 8 reviewed CRP point of care tests against a reference laboratory CRP test
- Studies 9 and 10 focused on using point of care CRP tests to distinguish between viral and bacterial infections without a reference test
- Studies 11 and 12 compared various point of care CRP tests against each other
- Study 13 focused on the cost-effectiveness of the point of care CRP test.

These studies show that point of care CRP tests correlate well with standard laboratory CRP tests. However, for certain point of care tests, such as the QuikRead method, sensitivity and specificity may improve at higher cut off CRP values. Semi-quantitative point of care CRP methods are less costly, but not as precise or reliable as quantitative methods. The Afinion

analyser, a quantitative method, was practically more suitable and user-friendly than other point of care tests. The Afinion POCT was not specifically studied in the neonatal population. Most of these studies took place in the paediatric emergency medicine department where the point of care CRP was useful in distinguishing viral from bacterial infections in order to decide on whether or not to prescribe antibiotics. Although CRP is useful for discontinuing antibiotics in neonates with low risk of infection, studies have not quantified the impact of point of care testing on reducing antibiotic exposure.

*Table 5 Point-of-care CRP test studies*

| No. | Authors | Study population | Aim | POCT CRP test/s | Conclusion |
|---|---|---|---|---|---|
| **1** | Diar et al [32] | Neonates | To validate a bedside CRP test against the laboratory CRP test | QuikRead | • QuikRead CRP can be used as a bedside tool<br>• At cut off value of 16.2 mg/l QuikRead gives optimal agreement between it and the laboratory test<br>• QuikRead CRP may improve availability of CRP results, reduce costs and facilitate earlier patient discharge in resource limited settings |
| **2** | Makhoul et al [33] | Neonates | To test whether fast complete blood count (CBC) and CRP significantly differ from conventionally measured CBC and CRP | ABX-Micros analyser | • ABX-Micros is equivalent to conventional methods only in measuring serum CRP levels but not CBC in suspected late onset sepsis<br>• The mean difference between conventional test and point of care test was not statistically significant<br>• Advantages of ABX-Micros: requires small volume of blood for testing and measures CRP within 4 minutes<br>• Disadvantages of ABX-Micros: expensive and needs frequent calibration |

| No. | Authors | Study population | Aim | POCT CRP test/s | Conclusion |
|---|---|---|---|---|---|
| **3** | Zecca et al [34] | Neonates | To compare the diagnostic accuracy between 2 rapid bedside tests for CRP with the laboratory method and their reliability | Quick-Read NycoCard | • Both rapid tests have excellent correlation against the reference laboratory method<br>• The accuracy of both rapid tests is affected only by very high CRP concentrations, more evident with the NycoCard CRP test<br>• Advantages of both tests: easy to use, require small volumes of blood for testing, and provide highly reliable results in under 5 minutes<br>• Useful for serial CRP measurements |
| **4** | Verbakel et al [35] | Children 0-18 years | To determine the analytical accuracy and user-friendliness of the Afinion CRP test | Afinion | • Even at high CRP levels there was high agreement and precise measurements<br>• Few differences between methods with low CRP levels were not statistically significant<br>• Deemed user-friendly |
| **5** | Papaevangelou et al [36] | Children under 14 years | To use the QuickRead CRP in the paediatric emergency department and compare it to the standard laboratory method | QuickRead | • QuickRead was reliable for rapid diagnosis<br>• CRP was useful for distinguishing between bacterial and viral infections<br>• A useful test to determine whether or not to admit a febrile child and whether or not to |

| No. | Authors | Study population | Aim | POCT CRP test/s | Conclusion |
|---|---|---|---|---|---|
| | | | | | prescribe antibiotics for those discharged<br>• QuickRead CRP was useful for deciding on the type of antibiotics in those with urinary tract infection, pneumonia or acute otitis media |
| **6** | Esposito et al [37] | Children under 14 years | To compare the results of a rapid test for the bedside assay of CRP with those of standard laboratory assay samples in those attending the emergency department for acute respiratory infections | QuikRead | • Two methods showed good concordance<br>• In children with respiratory tract infections, CRP concentrations greater than 70 mg/l are often due to bacteria and if less than 20 mg/l are usually viral in origin |
| **7** | Galetto-Lacour et al [38] | Infants 7 days to 36 months | To assess the value of bedside tests for predicting the occurrence of severe bacterial infections (SBI) in children with fever without source | Not specified | • CRP and PCT performed better than IL-6<br>• PCT less than 0.5 ng/l or CRP less than 40 mg/l almost rules out SBI<br>• PCT has a slight advantage over CRP as it increases earlier after stimulation and has a better NPV |
| **8** | Ivaska et al [39] | Children 0-16 years | To investigate the analytical accuracy and feasibility of | HemoCue WBC Afinion AS 100 | • Agreement between point of care test and laboratory methods for both WBC and CRP |

| No. | Authors | Study population | Aim | POCT CRP test/s | Conclusion |
|---|---|---|---|---|---|
| | | | point of care tests for WBC and CRP at the paediatric emergency department | CRP | were sufficient for identifying children at risk of SBI<br>• Precision of point of care test was less than laboratory test possibly due to blood sampling time differences and sampling methods (capillary versus venous sampling) |
| **9** | Marcus et al [40] | Children 4-17 years | To investigate the validity and feasibility of the 2 minute Quick Read CRP test in the prediction of bacterial pneumonia in children in the emergency department | Quick Read | • Quick Read CRP test was a useful predictor of bacterial pneumonia especially within 96 hours from onset of symptoms<br>• Mean CRP levels for bacterial pneumonia 121.3 ± 122 mg/l<br>• Mean CRP levels for viral pneumonia 27.2 ± 26 mg/l |
| **10** | Marcus et al [41] | Children 0-17 years | To determine the clinical usefulness of the bedside Quick-Read CRP test for predicting bacterial gastroenteritis in the paediatric emergency department | Quick-Read | • CRP levels of ≥ 95 mg/l during the first 48 hours of presentation suggest bacterial gastroenteritis |

| No. | Authors | Study population | Aim | POCT CRP test/s | Conclusion |
|---|---|---|---|---|---|
| **11** | Brouwer et al [42] | Not specified | To review the assay performance and necessary pre-analytical handling of various available point of care CRP tests | Semi-quantitative methods – Actim CRP strips; Cleartest CRP strips<br><br>Quantitative methods – QuikRead go; smart analyser; Afinion AS 100 analyser; iChroma analyser; Microsemi; AQT90 Flex | • In terms of analytical validation and practical evaluation Afinion and Smart CRP were most suitable<br>• Afinion underestimated that CRP concentration by more than 10%, therefore Smart CRP was the preferred method<br>• Quantitative methods favoured as an actual result is obtained, there is less hands-on time and the result does not have to be read-out immediately |
| **12** | Minnaard et al [43] | Not specified | To evaluate the analytical performance, agreement and user-friendliness of | Afinion As 100 NycoCard Reader II | • Afinion, NycoCard Reader II, QuikRead go and QuikRead 101 showed better agreement with the laboratory test than the Eurolyser Smart |

| No. | Authors | Study population | Aim | POCT CRP test/s | Conclusion |
|---|---|---|---|---|---|
| | | | several point of care CRP tests with a laboratory CRP test as the reference standard | Eurolyser Smart 700/340 CRP QuikRead go QuikRead 101 | 700/340<br>• With high CRP values agreement with the laboratory standard systematically decreased for all points of care tests<br>• The Afinion test kit had the least chance of flaws in blood application<br>• The Afinion and Eurolyser Smart 700/340 analysers required the least separate actions |
| **13** | Hunter [44] | Not specified | To evaluate the cost effectiveness of CRP point of care test for respiratory tract infections in primary care | Afinion | • Additional cost per patient of the point of care CRP test outweighs the cost saving and quality-adjusted life years associated with decrease in infections in the long-term |

*Table 6 Most commonly used point of care CRP tests*

| Test | Method | Blood volume required | Steps for procedure | Time to result |
|---|---|---|---|---|
| **Afinion**[35] | immunoturbidimetry | 1.5 µl | 3 steps | 4 minutes |
| **QuikRead**[43] | immunoturbidimetry | 20 µl | 7 steps | 3 minutes |
| **NycoCard**[34] | immunoturbidimetry | 5 µl | 5 steps | 7 minutes |

It is estimated that between 11 and 23 uninfected neonates have received antibiotics for each documented bacterial infection within the neonatal intensive care unit.[45] An ideal diagnostic test for neonatal sepsis may reduce antibiotic exposure. Several studies recommend the use of CRP to curtail antibiotic exposure. Low CRP levels 24 or 48 hours after presentation supports the discontinuation of antibiotics.[45–47] Broad spectrum and long-term antibiotic exposure is linked to production of multi-drug resistant gram negative bacilli and invasive candidiasis.[48] The consequences of this may be high costs, unnecessary intravenous catheterisation or cannulation, separation of mother and infant, prolonged hospitalisation, high risk of colonisation by pathogenic organisms, emergence of drug-resistant organisms, prescription and medication administration errors, intravenous infiltrates, alteration of gut flora, necrotising enterocolitis and death.[45,49] The adverse effects of antibiotics also include nephrotoxicity, hepatotoxicity, ototoxicity, haematological abnormalities and iatrogenic anaemia due to frequent blood sampling.[48]

### 2.3.5. Conclusion

Antibiotic stewardship ensures patient safety and quality of treatment, to combat the emergence of resistance and preserve the activity of our current antimicrobial agents. Some of the unique challenges facing neonatologists are the inability to obtain positive blood cultures in newborns as a result of antibiotics administered to mothers during the intrapartum period, the inability to obtain adequate blood culture samples, the difficulty in distinguishing between infection, colonisation or contamination by organisms and the establishment of appropriate treatment regimens for preterm neonates. Rational prescribing, employing antibiotic stewardship programs and ensuring that all members of

society take responsibility for the effective use of antibiotics are necessary. A reliable biomarker for neonatal sepsis forms one of the cornerstones of a successful antibiotic stewardship program.

CRP as POCT is a quick, reliable and user-friendly method of obtaining a CRP result. This may assist in reducing antibiotic exposure and costs especially in resource limited settings. This may be especially useful in settings where significant delays in obtaining laboratory results occur.

Most CRP POCT validity and prediction studies have been prospective observational or diagnostic test evaluation studies. Small sample sizes were evident in half of the reviewed studies. This may not have provided biologically or statistically representative groups to achieve study aims. The use of convenience sampling may have also introduced selection bias. Only three POCT studies focused specifically on the neonatal population. Most studies were performed in the paediatric emergency department, where point of care testing was used to differentiate between bacterial and viral infections for clinical management. Two studies did not compare each POCT to the gold standard but instead relied upon information provided by the manufacturer and comparison of findings of several random samples with the laboratory's measurements. Studies predominantly relied on paediatricians or neonatologists trained to perform the POCT, to undertake testing. In some studies the operators of the POCT analysers were not stipulated. Only one POCT study was previously performed in South Africa, therefore the results of the other studies may not be generalised to the South African population. In this review only one study has specifically measured the cost-effectiveness of point of care testing. Studies that formally quantify the limitation of unnecessary antibiotic exposure by the utilisation of point of care testing are limited in the published literature especially in the neonatal population and none were found in this review. Future studies looking at this aspect are recommended.

This research study offers specific evaluation of the Afinion CRP POCT in the South African neonatal intensive care unit setting against the standard laboratory measure. The use of junior clinicians in performing the rapid bedside tests ensures a more realistic outcome. The study also focuses on the use of CRP point of care testing to reduce antibiotic exposure by using time to results as a proxy measure.

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# 3. Manuscript in publication ready format

## 3.1 Title page

Title: A comparison of standard C-reactive protein laboratory measurement to point of care C-reactive protein test in a neonatal intensive care unit setting

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## 3.2 Abstract


**Background**

Laboratory biomarkers are important adjuncts to clinical data in diagnosing neonatal sepsis. Available diagnostic tests often provide results 6 to 48 hours later. A bedside C-reactive protein (CRP) test may be able to exclude or diagnose sepsis within minutes.

**Objectives**

The objectives were to validate the Alere Afinion™ point of care test (POCT) CRP in a tertiary neonatal unit against the gold standard CRP assay in use by the National Health Laboratory service and to determine the difference in time to obtaining a result between the two systems.

**Methods**

A prospective observational study was conducted between February 2015 and June 2015. Neonates who were clinically indicated to undergo CRP testing were simultaneously tested using the POCT and laboratory assays. The sensitivities, specificities and predictive values for the POCT, with the laboratory test as the reference test were determined. The time to results between the two tests was compared.

**Results**

There were 139 measured CRP sample pairs from patients with suspected or proven neonatal sepsis. Using 10 mg/L as the cutoff value for both CRP tests, the sensitivity, specificity, positive predictive value and negative predictive value were 97.4%, 99%, 97.4% and 99% respectively. The area under the receiver operating characteristic curve was 0.99 ($p<0.001$). The time to POCT result was 4 minutes. Laboratory results were registered at a mean of 4.7 hours but only checked after a mean of 6.8 hours.

**Conclusions**

The POCT CRP and laboratory CRP test have excellent correlation in neonates and may be a useful, quick, reliable method to rationalise antibiotic usage, reduce costs and allow for earlier patient discharge.

## 3.3 Background

Neonatal sepsis is a clinical syndrome secondary to bloodstream invasion by bacteria resulting in circulatory compromise in the first 28 days of life. Neonatal sepsis is the third leading cause of neonatal deaths and is responsible for 10 % of all under-five mortality.[1,2] Predisposing factors for neonatal sepsis are prematurity, male gender, prolonged rupture of membranes and chorioamnionitis, maternal colonisation with Group B *Streptococcus*, maternal urinary tract infection and birth asphyxia.[3] The clinical features of neonatal sepsis are often subtle and non-specific making diagnosis challenging. These features may be difficulty in feeding, convulsions, movement only when stimulated, bulging fontanelle, lethargy or irritability, hypotonia, tachypnoea, cyanosis, apnoea, severe chest indrawing, grunting, temperature instability, hypoglycaemia or hyperglycaemia, poor perfusion, petechiae or purpura, abdominal distension, hepatomegaly or unexplained jaundice.[1,3–5]

Neonatal sepsis are classified as either early onset neonatal sepsis (EONNS), widely accepted as sepsis presenting in the first 72 hours of life and late onset neonatal sepsis (LONNS) which occurs after 72 hours of life. The distinction exists to assist in diagnosing the mode of transmission and most likely organisms causing the infection to focus antibiotic therapy. EONNS is as a result of intrapartum infection where LONNS are mostly hospital acquired infections associated with prolonged hospital stay, mechanical ventilation, indwelling catheters, parenteral nutrition and inadequate hand hygiene.

Clinical information is mostly used to initiate treatment but is difficult to use to confirm sepsis or to tailor antibiotic therapy. The gold standard for diagnosing neonatal sepsis remains blood culture for bacteria, however blood culture positive rates vary widely and it often takes 24 to 48 hours to obtain a result.[6,7] Biomarkers may be used to assist with diagnosis and management strategies for neonatal sepsis. While none are ideal, C-reactive protein (CRP) remains the preferred biomarker in many neonatal units due to it being the most extensively studied biomarker and its availability, rapidity and cost effectiveness.[8]

CRP is an acute phase reactant synthesised in the liver and rapidly rises in 4 to 6 hours after the inflammatory process, reaching a peak in 36 hours and has a half life of 19 hours.[9] The consensus for the upper limit of normal levels for CRP is 10 mg/l.[8] A point of care test (POCT) is commercially available to provide bedside CRP measurements to be obtained

within minutes. This may assist with the reduction of antibiotic exposure as antibiotic stewardship is pivotal for patient safety, quality of treatment and reduction in antibiotic resistance however data of its application in the neonatal population is limited.

## 3.4 Objectives

The purpose of this study is to validate the Alere Afinion$^{TM}$ POCT CRP in neonates with suspected sepsis at Groote Schuur Hospital's neonatal unit using the CRP from the gold standard laboratory assay as the reference test. The secondary objective is to measure the potential change in antibiotic dosage exposure if antibiotic therapeutic decisions were made using the CRP results from the Alere Afinion$^{TM}$ CRP system rather than the standard laboratory NHLS CRP test.

## 3.5 Methods

This was a prospective observational study and diagnostic test evaluation conducted from February 2015 to June 2015 at Groote Schuur Hospital neonatal unit, Cape Town, South Africa. Ethical approval for the study was approved by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town (HREC REF: 763/2014).

Patients were recruited during normal working hours due to workforce limitations in both clinical and laboratory staffing after hours.

We included any neonate in whom the CRP was performed between 8 am and 4 pm on weekdays as part of a septic work up or follow-up test and parental consent obtained. CRP measurements for both the Alere Afinion$^{TM}$ analyser and the standard laboratory analyser had to be available for eligibility.

Neonates in whom parents denied consent or in whom the POCT CRP or standard laboratory result were not available were excluded.

Patient names and hospital numbers were used to identify samples and data were recorded with the use of pre-designed data collection sheets. Patient demographics, risk factors for sepsis, clinical features of sepsis, reasons for requesting the CRP, blood culture results, use of empiric antibiotics, times of sampling and time to results as well as the POCT CRP and laboratory CRP were captured on the data collection sheets. Patients were noted to have suspected EONNS if clinical features of sepsis were found within the first 72 hours of life and

suspected LONNS if such features were noted after 72 hours of life. Clinicians involved in patient care within the neonatal unit were responsible for performing the tests and completing data collection sheets. The clinicians included medical interns, medical officers and registrars who received training to use the POCT analyser. Blood sampling was done at the clinician's discretion through either venepuncture or arterial puncture. Blood for both CRP measurements were taken from the same sampling site and occasion. Laboratory CRP measurements were done by trained technicians based at the National Health Laboratory Service on the hospital's premises using the Roche Cobas 6000 analyser and were blinded to the study and results. Therapeutic decisions were based solely on the standard laboratory CRP and not the POCT CRP result.

The Alere Afinion$^{TM}$ POCT was utilised for this study as it only required 3 steps to perform the test, 1.5 µl of blood for testing and provides a CRP results within 4 minutes. The 3 steps for the point of care test are 1) collect the blood sample, 2) use the self-contained capillary tube in the test cartridge to collect the blood, and 3) insert the test cartridge into the analyser to obtain a CRP result. Both the POCT analyser and the laboratory analyser use immunoturbidimetry to obtain measurements. Immunoturbidimetry measures the absorbance of light from a sample where agglutination between latex particles coated with antibody and corresponding antigens on the CRP molecules take place. A quantitative CRP result is determined as the analyte concentration is inversely proportional to the light signal. The Alere Afinion$^{TM}$ control testing was performed as per manufacturer's recommendations. The Alere Afinion$^{TM}$ has a CRP detection reference range of <5 to 200 mg/l, while the laboratory CRP has a range of <1 to 350 mg/l. A positive CRP result was noted to be >10 mg/l for both tests.

Data captured on hard copy data collection sheets were transferred to digital data collection sheets in EpiData® Manager (v2.0.0.25) and Entry Client (v2.0.1.11). Data were exported to Microsoft ® Excel ® 2013 (15.0.4841.1000) IBM ® SPSS R Statistics®23.0 and Stata® Statistical Software: *Release 14* for analysis.

Patient demographics are presented as either percentages of the total sample or medians with interquartile ranges, and minimum and maximum values. Risk factors and clinical features of sepsis are expressed through percentages and one or more variable per patient

may have been reported. Reasons for CRP testing are documented as percentages of the total sample. CRP test data are compared between the POCT CRP and the laboratory CRP though medians, ranges and quartiles. A scatter plot with appropriately constructed regression line indicates the degree of linearity between the two CRP test methods. The Bland-Altman plot was used to assess the absolute differences and means between the two CRP test methods. Sensitivities, specificities, negative predictive values (NPV), positive predictive values (PPV) for the POCT method and the laboratory and positive blood cultures were determined and their confidence intervals calculated. The sensitivity and specificity were combined in the receiver operating characteristic curves (ROC curves) and reported as the area under the curve with standard error of 95% confidence interval and p value. The statistical significance was taken as $p < 0.05$. Time to POCT CRP and laboratory CRP results were compared using mean values. The time to results were used as a proxy measure for determining duration of antibiotic exposure.

## 3.6 Results

During the four month study period from February 2015 to June 2015, 158 paired CRP samples were taken for the study. 17 tests performed by the POCT analyser did not yield a CRP result and a further 2 CRP tests performed by the laboratory were insufficient for testing. Therefore 19 samples were excluded from the study and 139 CRP samples were included. More than one sample may have been obtained per patient during the study period on separate occasions, but each sample was regarded individually. A chart illustrating the study population is noted in Figure 1. Of the total sample population 61 (44%) had suspected EONNS and 78 (56%) had suspected LONNS.

The demographic details are reported in table 1. About half of the study population were male (50.4%) with a preponderance for suspected LONNS (56.4%). The median age at CRP test was 5 days (interquartile range of 19 days) with minimum of 0 days and maximum of 68 days. The median gestational age was 30 weeks (interquartile range of 6 weeks) with minimum of 24.7 weeks and maximum of 40.6 weeks. The median birth weight was 1340 g (interquartile range of 1091.5 g) with minimum of 620 g and maximum of 3795 g. 82.7% of the study population consisted of preterm newborns and 63% of the study population comprised of newborns with very low birth weight (≤1500g).

The reasons for requesting CRP tests were perinatal infection risk in 18%, neonatal signs and symptoms in 29.5%, follow-up CRP test for serial measurements in 51.5%, and in only one case was the reason not recorded. The top predisposing factors (table 2) selected in the entire study population include low birth weight (80.6%), preterm labour (33.8%), HIV exposure (15.1%), maternal fever (12.2%) and the presence of central or percutaneous invasive catheters (10.7%). Meconium aspiration syndrome (MAS) featured in the top five risk factors for suspected EONNS as it was documented in 13.1% of all noted risk factors. Parenteral nutrition (TPN) featured highly in suspected LONNS as it was selected in 6.4% of the risk factors.

The clinical features of suspected sepsis (table 3) were variable depending on timing of suspected infection. In suspected EONNS the predominant clinical features were respiratory concerns (55.7%) and hyperglycaemia or hypoglycaemia (14.8%), but in a large group the patients were in fact asymptomatic (31.2%). In suspected LONNS the predominant clinical features were apnoea (38.5%), feeding issues (30.8%) and lethargy or irritability (20.5%).

In terms of bacteriology, 127 blood cultures were performed, one of which was rejected, the reason for which was not recorded. The culture positivity rate was 7.1%. The blood cultures in EONNS and LONNS and isolated organisms are as documented in table 4.

The median result of the POCT CRP and the laboratory test was <5 mg/l and 1.8 mg/l respectively. The range for the POCT CRP was <5 to 200 mg/l with first quartile <5 mg/l and third quartile 11.5 mg/l. The range for the laboratory CRP test was <1 to 247.2 mg/l with first quartile <1 mg/l and third quartile 10.8 mg/l. In looking at the agreement between the studied POCT and the reference test the simple regression plot is shown in figure 2 with y= -0.0548 + 0.956x and 95% confidence intervals. The adjusted regression coefficient of determination ($R^2$) was 0.97 with p <0.001. This implies that 97% of variability between the POCT and laboratory test has been accounted for. The estimated slope coefficient was 0.96 with 95% confidence interval and p value <0.0001.

The difference between the means of the two CRP tests, illustrated in the Bland-Altman plot (figure 3), is - 0.682 with 95% confidence interval for the mean difference -11.35 to 9.987. This indicates that the POCT is less than the laboratory test by 0.682 on average. At the cut off CRP level of 10 mg/l for both test methods the sensitivity, specificity, PPV and NPV were

97.4%, 99%, 97.4% and 99% respectively. The 95% confidence intervals for the aforementioned sensitivity, specificity, PPV and NPV were 84.6% to 99.9%, 93.8% to 99.9%, 84.6% to 99.9% and 93.8% to 99.9% respectively. The POCT with cut off of 10 mg/l for CRP and positive blood cultures as a reference test showed that the sensitivity, specificity, PPV and NPV were 12.1%, 94.6%, 44.4% and 75.2% respectively and their confidence intervals were 4.5% to 29.1%, 87.3% to 98%, 15.4% to 77.3% and 66.2% to 82.5% respectively.

The ROC curve (figure 4) assesses the accuracy of the POCT with the laboratory test as the reference test. The area under the curve is 0.993 ($p < 0.0001$).

Considering time to results, the Alere Afinion$^{TM}$ CRP test took 4 minutes to perform the test and obtain a result. In comparison the mean time to registering the laboratory result was 4.7 hours with range from half an hour to 31.2 hours and the mean time to checking the laboratory result was 6.8 hours with range of 1.5 to 31 hours.

## 3.7 Discussion

Almost every text regarding neonatal sepsis will focus on describing it as a major health problem posing diagnostic, prognostic and management dilemmas. CRP is useful in confirming and excluding bacterial infection as a cause of neonatal illness. This study highlights a possible method to address some of the management and diagnostic dilemmas with specific focus on the reduction of antibiotic exposure in neonates. Between 11 and 23 uninfected neonates are estimated to receive antibiotics for each documented bacterial infection within the neonatal intensive care unit.[10] While empiric antibiotics are usually initiated in light of clinical findings, they are not without adverse effects or consequences relating to the patient, the family, the hospital and the wider community. They include toxic drug effects such as nephrotoxicity, hepatotoxicity, ototoxicity, haematological abnormalities, unnecessary blood sampling and venous cannulation, risk of colonisation by pathogenic organisms, alteration of gut flora, risk of necrotising enterocolitis and death, separation of infant from mother, prolonged hospitalisation, high health care costs and emergence of drug-resistant organisms.[10,11]

CRP is useful, easily accessible in most settings, extensively studied and a cheaper test than most other biomarkers for infection. The Alere Afinion$^{TM}$ POCT CRP can be performed by the clinician, near the bedside with minimal blood volumes required for sampling and allowing

for rapid CRP determination. The linear regression and Bland-Altman plots showed good agreement between the two CRP test methods. The estimated slope coefficient of linear regression indicated that for every increase of 1 mg/l of the laboratory CRP measurement, there would be a corresponding 0.96 mg/l increase in the POCT measurement. When considering a cut off value of 10 mg/l for both CRP methods the sensitivity, specificity, PPV and NPV were all >95%. These results suggest that the CRP testing by the Alere Afinion ™ analyser may be used as an alternative to the laboratory CRP analyser. It must be noted there were 17 episodes where the POCT CRP measurements were not available due to processing or machine errors. The error codes were displayed and grouped. In eight cases the haematocrit was too high or too low, in four cases there were capillary filling errors, in three cases there were test cartridge or analyser failures and two errors were not documented.

The majority of these errors were due to human error or equipment failure. This suggests that more training sessions for staff and more frequent calibration of the analyser may be required.

There are very few studies focusing on POCT CRP in the neonatal population and in particular the Alere Afinion™ CRP test in neonates. Often neonatal studies have small sample sizes and are European-based, which may not fit the South African context. The results of this study correlate well with previous studies but the Alere Afinion™ showed the best results against the reference test when compared to other point of care systems. Zecca et al reported that for cut off values of ≥10 mg/l for the bedside and laboratory CRP tests, sensitivities for the Quick-Read CRP and NycoCard CRP were 97.2% and 94.4% respectively and specificities for the Quick-Read and NycoCard CRP were 80.5% and 83.3% respectively.[12] Diar and colleagues used a cut off value of 8 mg/l for the QuikRead CRP and 10 mg/l for the laboratory CRP to determine sensitivity, specificity, PPV and NPV of 84%, 80%, 30% and 97% respectively.[13] The Alere Afinion™ CRP test may be favoured above other point of care tests as it has fewer steps or procedures to perform and requires less blood volume for testing than the Quick Read and NycoCard POCT CRP.

Time to laboratory results were not specifically measured in any of the previous studies. In busy laboratories and neonatal units results are not made available or checked in optimal

time. Despite the National Health Laboratory Service being on the premises of the hospital where the study took place, it was apparent that the mean times to registering the result and the clinicians checking the result were almost 5 and 7 hours respectively. During this time the neonate may have received a dose of antibiotics that may otherwise have been discontinued in a newborn that had been tested by POCT CRP.

The limitations of this study include reliance on doctors who regularly rotate through the department to record data and perform tests that may have led to information bias. Doctors may not have recorded all the correct predisposing factors, clinical features or measurements accurately. We used “time to obtaining result” as a proxy measure for potential reduction in antibiotic exposure when CRP was done to monitor antibiotic treatment. We did not record whether this translated into actual unnecessary antibiotic exposure. Furthermore this study took place within working hours when staff numbers are optimal and systems more efficient, which may have introduced selection bias. After hour emergency cases may mean sicker neonates, busier nights for fewer members of staff and longer time to laboratory results being registered and checked.

## 3.8 Conclusion

In resource limited settings with offsite laboratories, delayed time to laboratory CRP results, lack of blood culture result availability and financial constraints, the Alere Afinion$^{TM}$ POCT CRP may be a suitable alternative to the standard laboratory measure. Serial point of care CRP measuring are likely to assist with earlier discontinuation of antibiotic therapy in support of antibiotic stewardship.

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## 3.10 Figures and Tables

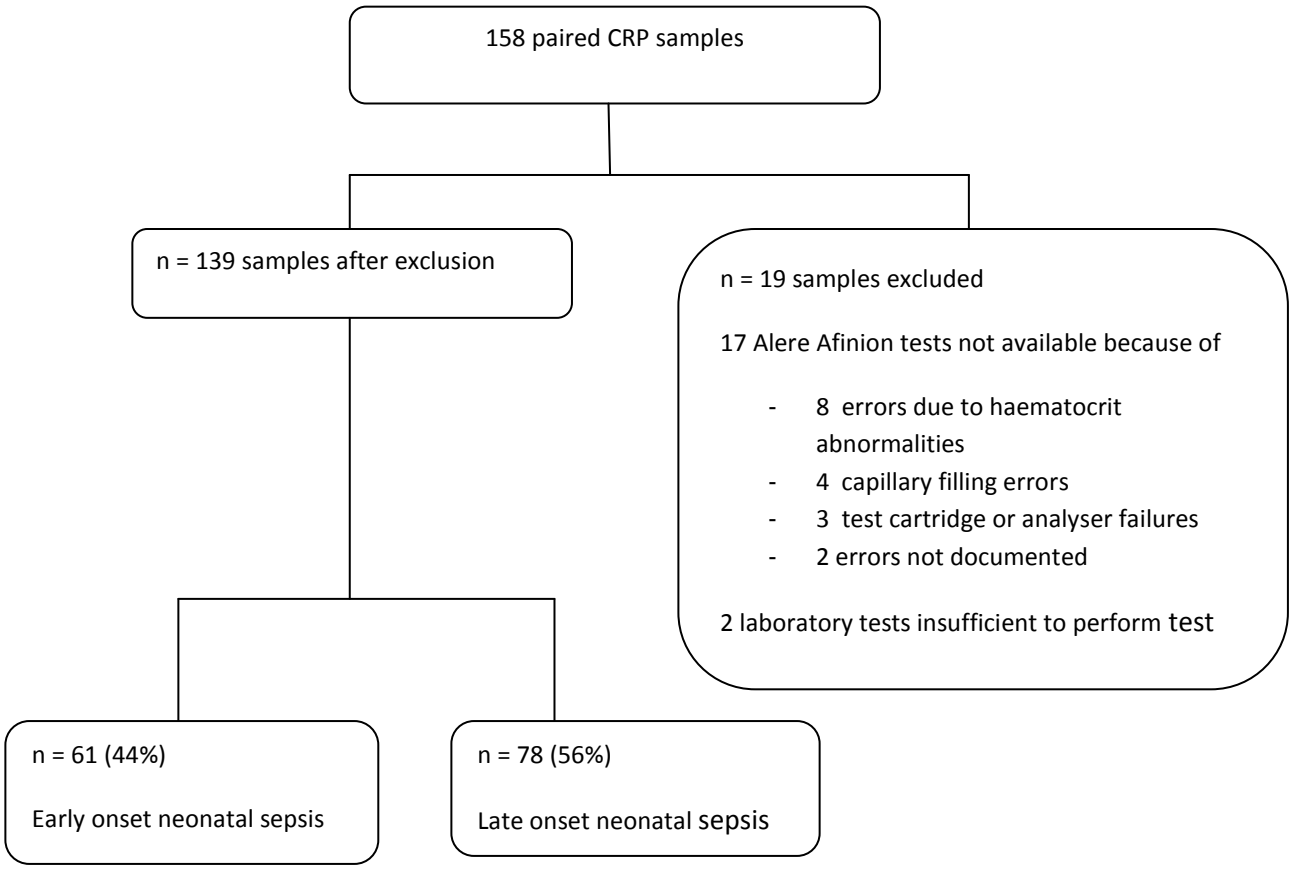


**Figure 1: Study population**

## Table 1: Patient Demographic Data

| Variable | All patients | Patients with suspected EONNS | Patients with suspected LONNS |
|---|---|---|---|
| | **n= 139** | **n=61 (44%)** | **n= 78 (56%)** |
| Sex | | | |
| Male | 70 (50.4%) | 26 (42.6%) | 44 (56.4%) |
| Female | 69 (49.6%) | 35 (57.4%) | 34 (43.6%) |
| Age at CRP test (days) | | | |
| Median (IQR) | 5 (19) | 2 (3) | 20 (25) |
| Range (min - max) | 0-68 | 0-3 | 4-68 |
| Birth gestational age (weeks) | | | |
| Median (IQR) | 30 (6) | 33 (8.6) | 29 (3) |
| Range (min - max) | 24.7-40.6 | 24.7-40.6 | 25-40 |
| Preterm | 115 (82.7%) | 44 (72.1%) | 71 (91%) |
| Term | 24 (17.3%) | 17 (27.9%) | 7 (9%) |
| Birth weight (grams) | | | |
| Median (IQR) | 1340 (1091.5) | 1320 (1360) | 1340 (523.75) |
| Range (min - max) | 620-3795 | 620-3795 | 630-3500 |
| <1000g | 36 (25.9%) | 6 (9.8%) | 30 (38.5%) |
| 1001-1500g | 51 (36.7%) | 17 (27.9%) | 34 (43.6%) |
| 1501-2000g | 17 (12.2%) | 12 (19.7%) | 5 (6.4%) |
| 2001-2500g | 11 (7.9%) | 8 (13.1%) | 3 (3.8%) |
| > 2500g | 24 (17.3%) | 18 (29.5%) | 6 (7.7%) |

*IQR = interquartile range

**Table 2: Positive risk factors for presumed sepsis in study population**

| Risk factor | Total patients | Patients with suspected EONNS | Patients with suspected LONNS |
|---|---|---|---|
| | **n=139** | **n=61 (44%)** | **n=78 (56%)** |
| BW <2500g | 112 (80.6%) | 41 (67.2%) | 71 (91%) |
| PROM | 8 (5.7%) | 5 (8.25%) | 3 (6%) |
| Difficult / prolonged labour | 7 (5%) | 5 (8.2%) | 2 (2.6%) |
| Maternal fever | 17 (12.2%) | 16 (26.2%) | 1 (1.3%) |
| Chorioamnionitis | 4 (2.9%) | 1 (1.6%) | 3 (3.9%) |
| Pre-term labour | 47 (33.8%) | 24 (39.3%) | 23 (29.5%) |
| MAS* | 9 (6.5%) | 8 (13.1%) | 1 (1.28%) |
| TPN* | 5 (3.4%) | 0 | 5 (6.4%) |
| Formula feeds | 4 (2.9%) | 0 | 4 (5.1%) |
| Central / long lines | 14 (10.7%) | 6 (9.8%) | 8 (10.3%) |
| HIV exposure | 21 (15.1%) | 10 (16.4%) | 11 (14.1%) |
| Post surgery | 1 (0.7%) | 0 | 1 (1.3%) |

MAS* = meconium aspiration syndrome; TPN* = total parenteral nutrition

**Table 3: Clinical signs and symptoms for presumed sepsis**

| Sign / Symptom | Total patients | Patients with suspected EONNS | Patients with suspected LONNS |
|---|---|---|---|
| | **n=139** | **n=61 (44%)** | **n=78 (56%)** |
| Temperature instability | 2 (1.4%) | 1 (1.6%) | 1 (1.3%) |
| Hyper / hypoglycaemia | 14 (10.1%) | 9 (14.8%) | 5 (6.4%) |
| Apnoea | 35 (25.2%) | 5 (8.2%) | 30 (38.5%) |
| Cyanosis | 7 (5%) | 1 (1.6%) | 6 (7.7%) |
| Feeding issues | 26 (18.7%) | 2 (3.3%) | 24 (30.8%) |
| - abdominal distension | | | |
| - vomiting | | | |
| Lethargy / irritability | 19 (13.7%) | 3 (4.9%) | 16 (20.5%) |
| Neurological concerns | 8 (5.8%) | 5 (8.2%) | 3 (3.9%) |
| - seizures | | | |
| - bulging fontanelle | | | |
| Poor perfusion | 0 | 0 | 0 |
| Coagulopathy | 1 (0.7%) | 0 | 1 (1.3%) |
| Jaundice / hepatomegaly | 11 (7.9%) | 5 (8.2%) | 6 (7.7%) |
| Respiratory concerns | 60 (43.2%) | 34 (55.7%) | 26 (33.3%) |
| - increased oxygen requirements | | | |
| - respiratory distress | | | |
| Skin integrity | 1 (0.7%) | 0 | 1(1.3%) |
| - wound dehiscence | | | |
| - skin infection | | | |
| Asymptomatic | 24 (17.3%) | 19 (31.2%) | 5 (6.4%) |

**Table 4: Bacteriology of blood cultures**

| Culture | N (% of total cultures) |
|---|---|
| **EONNS** | |
| Total Positive | 1 (0.8%) |
| - Extended spectrum β-lactamase producing *K. pneumoniae* | |
| Total Negative | 55 (43.3%) |
| Rejected | 1 (0.8%) |
| Not done | 4 |
| | |
| **LONNS** | |
| Total Positive | 8 (6.3%) |
| - *E.coli* | 2 |
| - Extended spectrum β-lactamase producing *K. pneumoniae* | 2 |
| - Coagulase-Negative *Staphylococcus* | 2 |
| - Methicillin-resistant *S. aureus* | 1 |
| - *Bacillus Cereus* | 1 |
| Total Negative | 62 (48.8%) |
| Rejected | 0 |
| Not done | 8 |

*K.pneumoniae = Klebsiella pneumoniae*; *E.coli = Escherichia coli; S. aureus = Staphylococcus aureus*

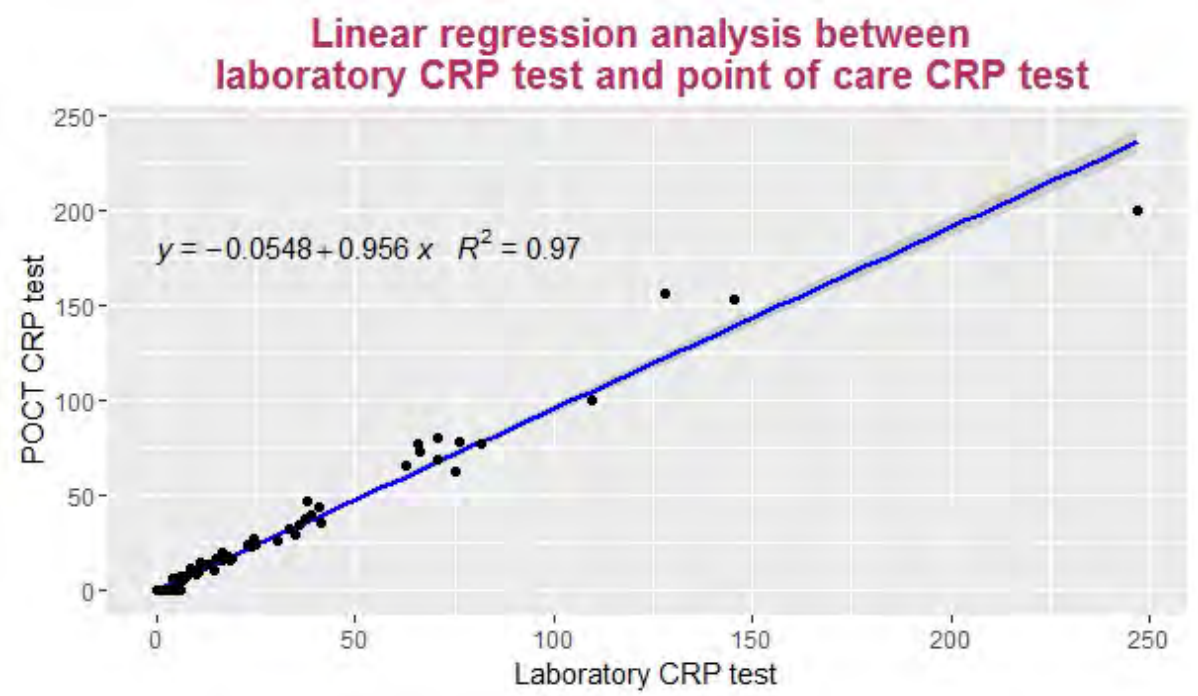


**Figure 2: Linear regression**

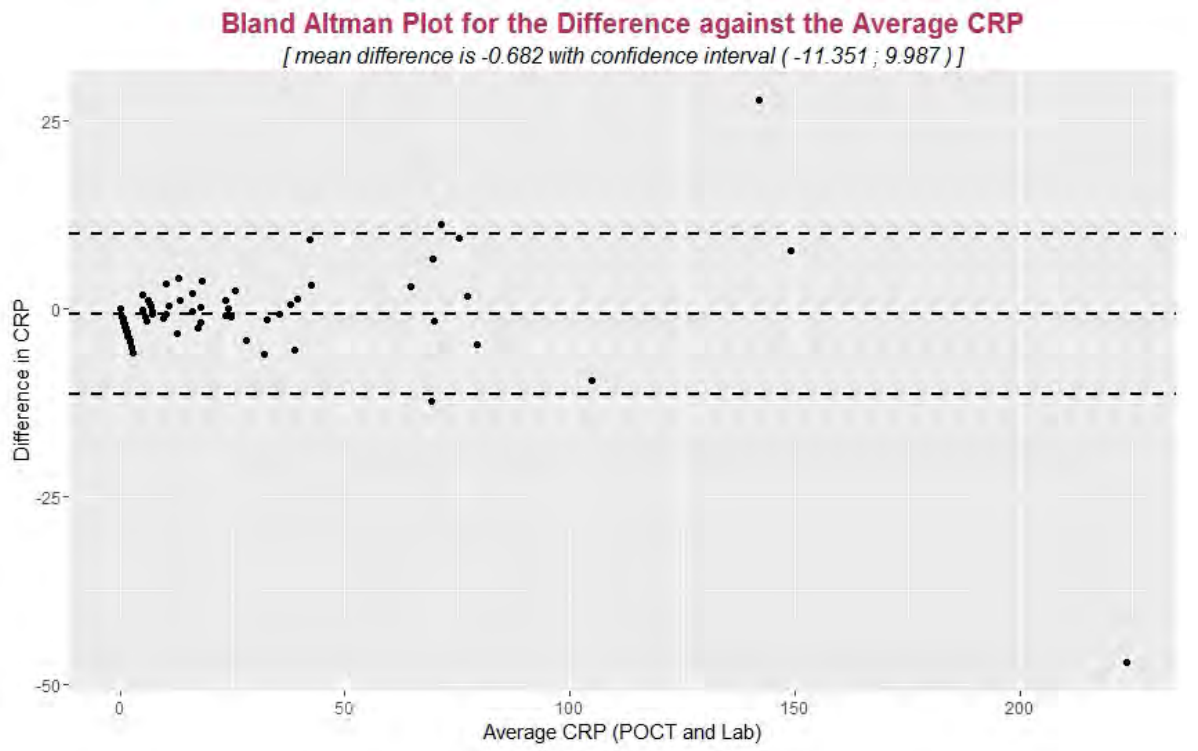


**Figure 3: Bland-Altman plot**

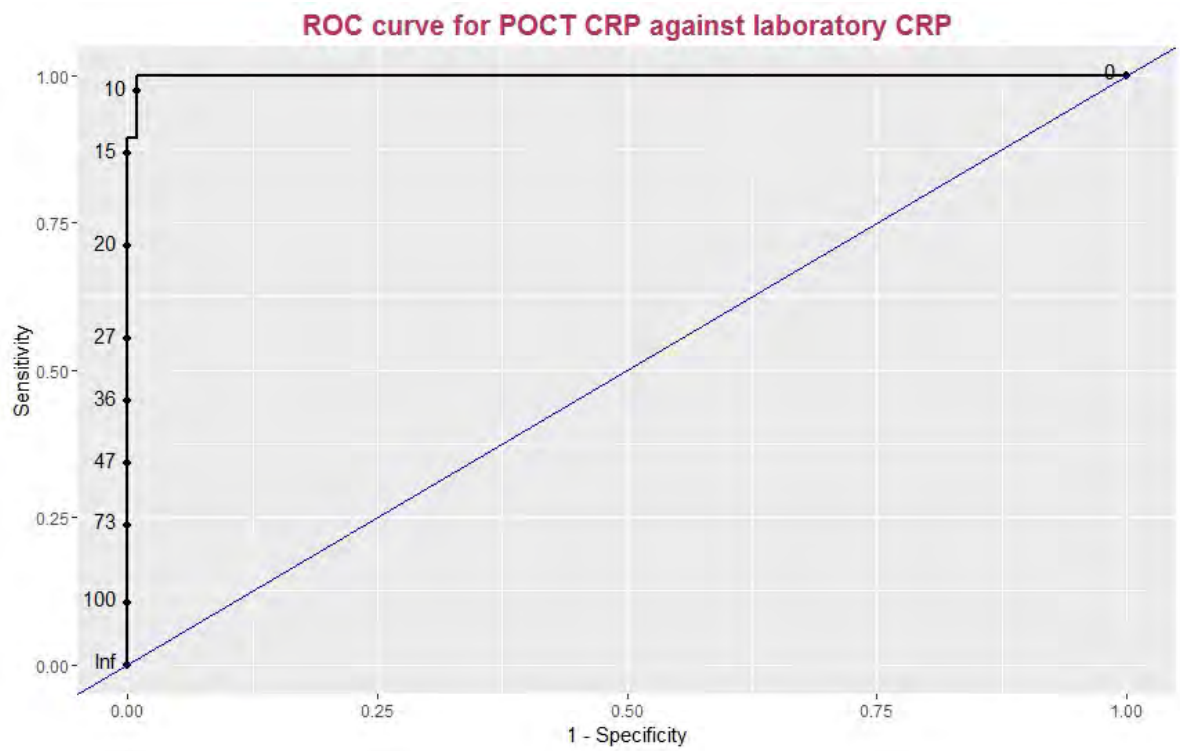


**Figure 4: ROC curve**

# 4. Appendices

## Addendum 1: Data collection sheet

## CRP POINT OF CARE RESEARCH PROJECT

### A) PATIENT DEMOGRAPHICS

Date:

Patient sticker

| |
|---|
| Name of patient: |
| Folder number: |
| Sex: Date of birth: |

Gestational age:

Chronological age:

Birth weight:

Current weight:

### B) RISK FACTORS FOR SEPSIS

1) LBW
2) PROM
3) Difficult or prolonged labour
4) Maternal fever
5) Chorioamnionitis
6) Pre-term labour
7) Meconium aspiration
8) TPN
9) Formula feeds
10) Central /long lines
11) HIV exposure

### C) SIGNS AND SYMPTOMS OF SEPSIS

1) Temperature instability
2) Hyper/hypoglycaemia
3) Apnoea

4) Cyanosis

5) Feeding difficulties / abdominal distension / vomiting

6) Lethargy / irritability

7) Seizures / bulging fontanelle

8) Poor perfusion

9) Coagulopathy

10) Jaundice / hepatomegaly

11) Increased oxygen requirements / respiratory distress

12) Wound dehiscence or skin infection

13) Asymptomatic

**D) <u>REASON FOR REQUESTING CRP</u>**

1) Perinatal risk for early neonatal sepsis

2) Neonatal signs/symptoms of sepsis

3) Follow up CRP (hours after CRP initiation: _____)

**E) <u>BLOOD CULTURE TAKEN AND RESULT</u>**

| | **RESULT** |
|---|---|
| **YES** | |
| **NO** | |

**<u>OTHER CULTURES:</u>**

**F) <u>EMPIRIC ANTIBIOTIC THERAPY</u>**

1) Yes 2) No

**G) <u>TIME FROM SAMPLING TO OBTAINING RESULTS</u>**

| **POCT** | **NHLS** |
|---|---|
| (in min) | (in min) |

**TIME OF SAMPLING: _____ TIME AT RESULT:**

**POCT _____**

**NHLS result released_____ result obtained____**

**H) <u>CRP RESULT</u>**

| **POCT** | **NHLS** |
|---|---|
| | |

## Addendum 2: Budget

| Item | Cost |
|---|---|
| Test and control kits | R10 000 |
| Printing and office consumables | R1 500 |
| Research assistant | R4 500 |
| **Total** | **R16 000** |

## Addendum 3:

## Parental information: C-reactive protein as a tool for identifying infection

We are doing a study on C-reactive protein. This is a special protein made by the liver and found in the blood when there is infection or inflammation in the body.

Infection in newborns is very serious and should be diagnosed and treated early. Newborn babies don't always show classic signs and symptoms of infection. Subtle clinical clues and blood tests are helpful in diagnosing infection and starting and stopping antibiotics.

**What is this study all about?**

This study is comparing two ways to calculate this special protein. The first way is a test done by the laboratory. The second way is a test done by an analyser in the nursery.

Usually when we are concerned about infection we take blood from your baby and send it to the laboratory for tests, including the C-reactive protein test. In our study the blood will not only be sent to the laboratory but will be analysed in the nursery within a few minutes. This quick test will give us a value which we can compare to the laboratory value.

The blood for both the laboratory and nursery analyser tests will be taken at the same time. The nursery analyser needs a small volume of additional blood (1.5 µl) to run the test.

Participation in the study is completely voluntary. Medical information recorded will be kept confidential. The results from this study may be published in a medical journal. If you no longer wish to participate in this study, you may withdraw your baby from it at anytime. This will not at all affect how we care for your baby.

**How will this study help my baby and others?**

The quick test done by the analyser in the nursery will be able to give us a result within minutes. For the duration of this study only the result from the laboratory will be used to determine further management of your child. We hope to use our results from this study to make future testing quicker, simpler and possibly cheaper and improve quality of care to infants

**Who is doing this study?**

Dr Yaseen Joolay and Dr Kim Prince will be responsible for carrying out this study. Should you have any queries about the study, you may contact us on 021 404 6069.

Please note that the Human Research Ethics Committee of the University of Cape Town has granted approval for this research. Should you have any ethical concerns, do not hesitate to contact the committee on 021 406 6492.

## Consent form

I ……., the parent of …… have read and discussed this form with the researchers and understand what the research project entitled: “A comparison of standard CRP laboratory measurement to point of care testing CRP in a neonatal intensive care unit setting” is about.

My questions have been answered.

I freely agree (tick the appropriate block):

☐ To take part in the study

☐ I confirm that I have read and understand the information on the sheet provided. I have had the opportunity to ask questions

☐ I understand that my participation in this study is voluntary and I may withdraw at any time

☐ I agree that the data collected may be stored and used for future research

| | | |
|---|---|---|
| Name of parent | Signature | Date |
| Name of researcher | Signature | Date |
| Name of witness | Signature | Date |

## Letter of Ethical 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 5411
**Email:** shuretta.thomas@uct.ac.za
**Website**: www.health.uct.ac.za/fhs/research/humanethics/forms

28 October 2014

**HREC REF: 763/2014**

**Dr Y Joolay**
Neonatology
H46
OMB

Dear Dr Joolay

**PROJECT TITLE: A COMPARISON OF STANDARD C-REACTIVE PROTEIN LABORATORY MEASUREMENT TO POINT OF CARE C-REACTIVE PROTEIN TEST IN A NEONATAL INTENSIVE CARE UNIT SETTING (MMed-candidate- Dr K Prince)**

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 27 October 2014.

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 October 2015.**

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 Kim Prince 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

**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) and Declaration of Helsinki guidelines.

HREC 763/2014

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC /63/2014

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**Scientific measurements** must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dl). Litres is denoted with a lowercase 'l' e.g. 'ml' for millilitres). Units should be preceded by a space (except for %), e.g. '40 kg' and '20 cm' but '50%'. Greater/smaller than signs (> and 40 years of age'. The same applies to ± and º, i.e. '35±6' and '19ºC'.

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**Journal references:** Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. [http://dx.doi.org/10.1000/hgjr.182] [PMID: 2764753]

**Book references:** Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101. ***Chapter/section in a book:*** Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA jun, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.

**Internet references:** World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: World Health Organization, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).

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# 5. Acknowledgements

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I wish to thank Mr Robert Prince for his invaluable statistical support, education and incredible mathematical mind.

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I am thankful for the support of my family and friends, particularly my father Robert Prince and mother Rachel Prince who helped me realise my potential in order to carry out my dream career. Their support throughout my career has been extraordinary.