# Retrospective review of urinary lipoarabinomannan (ULAM) in the diagnosis of disseminated tuberculosis (TB) in medical inpatients at a district level hospital in Cape Town

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**Declaration:**

I, Christie Erwee, declare that this dissertation/thesis is my own original work (except where acknowledgement indicate otherwise). It is being submitted for the degree of Masters of Medicine (MMed) in Medicine. Neither the whole work nor part of it has been or is being submitted for another degree in this or any other university. This work has not been reported or published prior to registration for the above-mentioned degree. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any matter whatsoever. Full ethics approval has been granted by the local ethics committee.

Signed

Signed by candidate

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**Abbreviations:**

| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| ART | Antiretroviral Treatment |
| cells/uL | cells per microlitre |
| CI | Confidence Intervals |
| CT | Computer Tomography |
| CXR | Chest X-ray |
| ELISA | Enzyme-linked immunosorbent assay |
| HIV | Human Immunodeficiency Virus |
| IQR | Interquartile Range |
| IRIS | Immune Reconstitution Inflammatory Syndrome |
| LOS | Length of Stay |
| MPDH | Mitchells Plain District Hospital |
| MTB | Mycobacterium tuberculosis |
| NHLS | National Health Laboratory System |
| PLWH | People living with HIV |
| RCT | Randomised Control Trial |
| SA | South Africa |
| SD | Standard deviation |
| SOP | Standard operating procedure |
| TB | Tuberculosis |
| ULAM | Urinary lipoarabinomannan |
| VL | Viral Load |
| U/S | Ultrasound |
| WHO | World Health Organisation |

# Retrospective review of urinary lipoarabinomannan (ULAM) in the diagnosis of disseminated tuberculosis (TB) in medical inpatients at a district level hospital in Cape Town

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**Abstract:**

**Background:**

Mycobacterium tuberculosis (MTB) and Human Immunodeficiency Virus (HIV) remain a major public health concern in South Africa (SA) with high mortality rates in patients with MTB and HIV co-infection. In 2016 Urinary Lipoarabinomannan (ULAM) became available in SA to expedite the diagnosis and treatment of Tuberculosis (TB) in this vulnerable population.

**Objectives**

To describe the experience at a district level hospital following the availability of ULAM testing for investigating HIV positive TB suspects. Firstly, to define the population and clinical outcomes in this cohort and secondly to assess if the hospital's standard operating procedure (SOP) regarding ULAM testing was followed and what additional MTB investigations were performed.

**Methods**

An observational, retrospective cohort study of patients who underwent ULAM testing at Mitchells Plain District Hospital (MPDH) was conducted from 2016 until 2018. The following data was collected: age, gender, CD4 count, viral load (VL), whether Antiretroviral Treatment (ART) naïve or interrupted and duration of ART if on treatment. Additionally, MTB diagnostic tests were performed and finally, patient clinical outcomes with regards to length of stay (LOS) and 30-day mortality and whether TB treatment was initiated. Descriptive statistics were used to analyse the data.

**Results**

324 participants were included in the study. 54.6% of participants were female and the median age of the entire cohort was 36 (IQR 30-34). Median CD4 count was 41 (IQR 16-76) with high viral load of log 4.9 (IQR 3.9-5.4). 44.1% of participants were ART naïve, 36.1% of participants were on treatment and 19.8% had interrupted treatment. The median duration of ART was 0.6 years (IQR 0.1-2). 750 MTB specific tests were performed in the cohort, 48% of the participants initiated on TB treatment had positive microbiological results proving MTB infection. Overall 30-day mortality was 12.7%.

**Conclusions**

This study showed participants who were severely immunocompromised with little ART exposure and a mortality rate of 12.7%. Patients were frequently initiated on empiric MTB treatment with a proportion of participants not being adequately investigated, particularly in the LAM negative group. Sputum was the most frequent MTB specific test performed. Clinicians adhered to the SOP put in place to guide the use of ULAM.

**Background**

Globally around 10 million people annually develop Mycobacterium tuberculosis (MTB) infection [1]. South Africa (SA) ranks 7th highest in the world, with an incidence of 520 per 100 000 population. A significant number, 59%, are co-infected with Human Immunodeficiency Virus (HIV). The HIV positive population in SA has one of the highest mortality rates from MTB [1].

Mitchells Plain District Hospital (MPDH) is a 230-bed facility located in Cape Town. The District Hospitals in the Cape Metropole area serve a population with a high burden of disease, particularly communicable [2]. Of those requiring admission to general medical wards, almost half of patients are severely or critically ill [3]. In addition, the burden of HIV and MTB co-infection is significant with a third of patients requiring hospital admission being confirmed HIV positive and, of these, 17% having concurrent MTB [3]. Of those admitted with advanced HIV (CD4 < 150 cells/uL) a third of admissions were MTB related with an overall mortality of 55% [4]. Given this high prevalence, it becomes critical to expedite the diagnosis and treatment of patients with MTB and HIV co-infection to improve clinical outcomes.

In 2014 the World Health Organization (WHO) called for the development of a non-sputum-based point of care test to identify all forms of MTB. The test required would need to have a high specificity to initiate immediate treatment on receiving the result [5]. The urinary lipoarabinomannan (ULAM) test was initially developed in 2003 as an enzyme-linked immunosorbent assay (ELISA) urine test, but in 2010 the first point-of-care lateral flow ULAM test was developed by Alere [6].

Confirming the diagnosis of MTB can be difficult if relying only on sputum testing, especially in people living with HIV (PLWH) where extrapulmonary MTB and paucibacillary pulmonary disease is more common [7-9]. Considering these challenges, multiple studies have shown that the addition of ULAM to testing has significantly improved case finding [8,10-14]. In a recent systematic review by the WHO, the pooled sensitivity and specificity for ULAM antigen testing in symptomatic HIV-infected inpatients with a CD4 cell count < 100 cells/uL was 56% and 90% respectively [15].

In 2015 the WHO recommended the use of ULAM in symptomatic patients with a CD4 < 100 cells/uL and in all severely ill HIV positive patients regardless of CD4 count [16]. There was initial evidence to suggest mortality benefit in patients initiated on MTB treatment utilising ULAM in a diagnostic algorithm, presumably based on reduced delay in treatment initiation [16, 17]. Subsequent randomised control trials (RCT) have shown no significant effect of the addition of ULAM testing on mortality [18, 19]. The WHO guidelines have since been updated in 2019 to include all symptomatic patients and patients with a CD4 < 200 cells/uL [15].

When assessing mortality most studies show a higher mortality rate in patients with a positive ULAM [8, 20, 21]. This relationship is likely related to the patients in the ULAM positive group representing patients with lower CD4 counts with clinical indicators of increased disease severity [8, 20-22].

This study described the initial two-year experience at a District Level hospital following the inclusion of ULAM testing in medical inpatients with suspected disseminated MTB from 2016 -2018.

At this stage ULAM was a novel test with no South African guidelines available at the time of the study, hence, a standard operating procedure (SOP) was developed to guide this at a hospital level to ensure appropriate use in the right clinical context. This included the need to review which additional tests were being performed together with the ULAM, to ensure that both necessary laboratory and radiological investigations were being done in the correct clinical settings. Key points were to define the patient population in terms of immune status [CD4 count, ART status and viral load (VL)] as well as clinical outcomes relating to mortality, length of stay (LOS) and initiation of TB treatment.

**Methods**

We conducted an observational, retrospective cohort study of patients who underwent ULAM testing during their hospital admission at MPDH, a secondary level hospital in Cape Town. These patients were identified by reviewing the ULAM register, which was designed to ensure that the ULAM was being used in the correct clinical context, as guided by the hospital SOP (created with guidance from the Division of Infectious Diseases at Groote Schuur Hospital). All ULAMs performed at MPDH during the period from 2016 to 2018 was entered into the register.

The indications for the use of ULAM as per the SOP were as follows:

1) All HIV infected inpatients with CD4 counts <150 cells/uL or stage 3 or 4 disease with suspected MTB at any site with negative initial investigations or inability to provide samples for investigation (failed sputum induction)

2) HIV infected inpatients with CD4 counts <150 cells/uL or stage 3 or 4 disease with severe adverse effects after recently starting MTB drugs (within two weeks) in whom indication for MTB treatment is in doubt.

Ethics approval was obtained from the Faculty of Health Sciences Human Research Ethics Committee at the University of Cape Town (HREC REF 449/2021). Given the retrospective nature of this study informed consent was waivered.

For each participant the following data was obtained:

1.) Demographic data, including age and gender.

2.) The immune status including recent CD4 count and viral load and whether ART naïve, on treatment or treatment interrupted.

3.) Associated recent MTB diagnostics including all MTB cultures, microscopy and molecular testing obtained from the National Health Laboratory system (NHLS). Radiological investigations including recent chest X-ray (CXR), computer tomography (CT) and ultrasound (U/S) obtained from the electronic radiology system – Xero viewer. U/S was considered positive if reported as in keeping with abdominal TB (including either lymphadenopathy, ascites or splenic microabscesses with no other clear diagnosis defined on the ultrasound report) or pericardial effusion noted. CXR

was considered positive if interpreted by the investigator as suggestive of MTB. All tests were performed at the discretion of the primary treating clinician.

4.) Patient outcomes: including length of stay (LOS) and 30-day mortality obtained using the Clinicom data base.

5.) Information regarding initiation of TB treatment was obtained from the electronic medical records of prescriptions and discharge medication issued on the discharge summary.

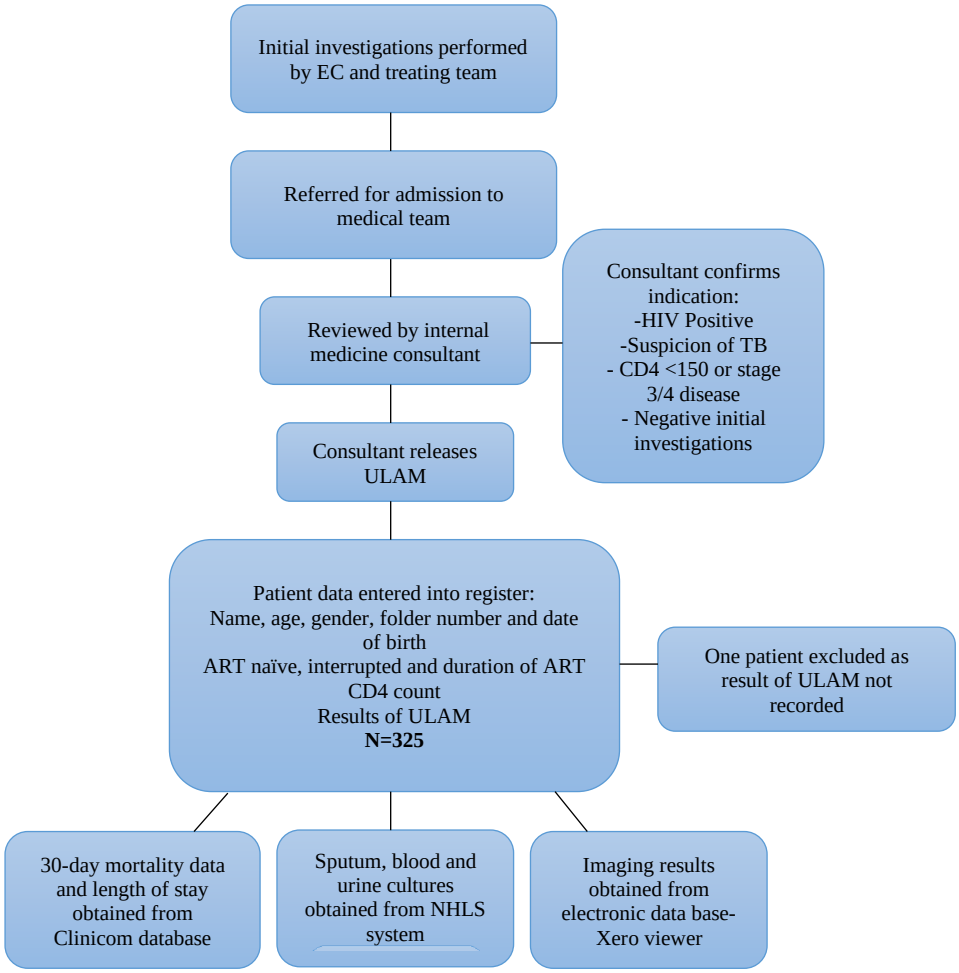


***Diagram 1: The flow diagram outlining the study process***

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY. Demographic and clinical characteristics were summarised by descriptive statistics. Mean ± standard deviation (SD) was used for normally distributed data and median with interquartile ranges (IQR) for skewed data.

Continuous variables were compared using either independent sample t-tests (parametric) or Mann—Whitney U tests (non-parametric). Categorical variables are presented as frequencies and percentages were compared using Pearson's X2 or Fisher's exact test as appropriate.

Univariate analysis was used to identify risk factors associated with increased mortality. Risk factors for death including age and sputum culture positivity were further analysed using logistic regression. Factors associated with time to death were analysed using Cox Proportional Hazards regression. All regression estimates are presented with 95% confidence intervals (CI). All p-values were considered significant at $p \leq 0.05$.

**Results**

A total of 325 participants were entered into the register from 2016-2018. One participant was excluded from the study as the result of the ULAM was not recorded in the register. Only 89 participants had results for VL for the period included in the study and only 116 had available information regarding ART duration.

Almost half of participants (n =144) were found to be ULAM positive (44.1%) with a further 16.1% in the ULAM negative group having microbiological evidence of MTB. In the cohort of 324 participants 750 MTB specific tests (Sputum GeneXPert, auramine staining, sputum-, urine-, or blood TB culture) were performed.

In total for the entire cohort (n = 324) 14.8% (n=48) were sputum GeneXpert positive, 15.4% (n=50) sputum MTB culture positive and 13.5% (n=44) were MTB blood culture positive.

***Table 1: Demographics and clinical variables****

| | Whole sample n = 324 | ULAM positive n = 144 | ULAM negative n = 180 | *p* |
|---|---|---|---|---|
| Age | 36 (30 – 43) | 36 (29 – 43) | 36 (30 – 42) | 0.895 |
| Sex | | | | 0.410 |
| Male | 147 (45.4%) | 69 (47.9%) | 78 (43.3%) | |
| Female | 177 (54.6%) | 75 (52%) | 102 (56.7%) | |
| CD4 | 41 (16 – 76) | 37 (15.8 – 71) | 44.5 (16 – 80) | 0.386 |
| VL (log) | 4.9 (3.9 – 5.4) | 5.1 (3.9 – 5.6) | 4.7 (3.8 – 5.2) | 0.039 |
| ART | | | | 0.895 |
| - Naïve | 143 (44.1%) | 62 (43.1%) | 81 (45%) | |
| - On treatment | 117 (36.1%) | 52 (36.1%) | 65 (36.1%) | |
| - Interrupted | 64 (19.8%) | 30 (20.8%) | 34 (18.9%) | |
| ART duration (years) | 0.6 (0.1 – 2) | 0.3 (0.1 – 2) | 0.9 (0.1 – 2) | 0.499 |
| Length of stay (days) | 9 (6 – 17) | 10 (6 – 20) | 8 (5 – 14.8) | 0.065 |
| Started MTB treatment | 229 (71.3%) | 144 (100%) | 87 (48.3%) | < .001 |
| 30-day Mortality | 41 (12.7%) | 16 (10.4%) | 26 (14.4%) | 0.279 |

*Data expressed as median and interquartile range (IQR)

Demographics and between group differences:

A total of 324 participants were enrolled in the study. The median age was 36 (IQR 30 -43) for the whole cohort. 54% (n=177) were female with an overall median CD4 of 41 (IQR 16-76) and VL of log 4.9 (IQR 3.9 -5.4). Participants who were ULAM positive had a significantly higher VL (p = 0.039). The median LOS was 9 days (IQR 6 – 17). There was no statistically significant difference in hospital stay between the two groups (p = 0.065). There were no other between-group differences for demographic or clinical variables. 63% (n=207) of all participants were not receiving ART at the time of presentation. 44% (n=143) were ART naïve and 19.8% (n=64) had interrupted treatment. The median duration of ART at time of presentation was 6 months (IQR 1 – 24 months) with a median of 3 months in the ULAM positive group and 9 months in the ULAM negative group.

Clinical outcomes:

Overall 30-day mortality rate was 12.7% with no significant 30 day mortality difference noted between the ULAM positive and negative groups (p=0.279). Patients in the LAM negative group who were initiated on TB treatment had a mortality rate of 17% versus 10% in patients who were not initiated on TB treatment. Univariate analysis showed that age (p = 0.046), LOS (p = 0.044), and a positive sputum culture (p = 0.011) were independently significantly associated with mortality. However, after logistical regression analysis, only age remained a significant predictor of mortality (p = 0.007), with older patients being more likely to die.

Age, gender, CD4, VL, starting TB treatment, ART status and duration, LOS and ULAM result were entered into Cox Regression analysis. No demographic or clinical characteristics were associated with time to death.

***Table 2: Predictors of mortality***

| | **p value** | **95% CI** |
|---|---|---|
| **Age** | 0.007 | [-0.14, -0.02] |
| **Length of stay** | 0.235 | [-0.02, 0.08] |
| **Positive sputum culture** | 0.994 | [-4548.16, 4548.5] |

All 144 participants in the ULAM positive group were initiated on TB treatment with 87 (48.3%) participants in the ULAM negative group also being initiated on TB treatment. Of these, 29 (33.3%) participants started on treatment had positive microbiological results, 38 (43.7%) had positive radiological evidence and 20 (23%) had no positive results and were empirically initiated on TB treatment.

***Figure 1: Investigations performed and positive results:***

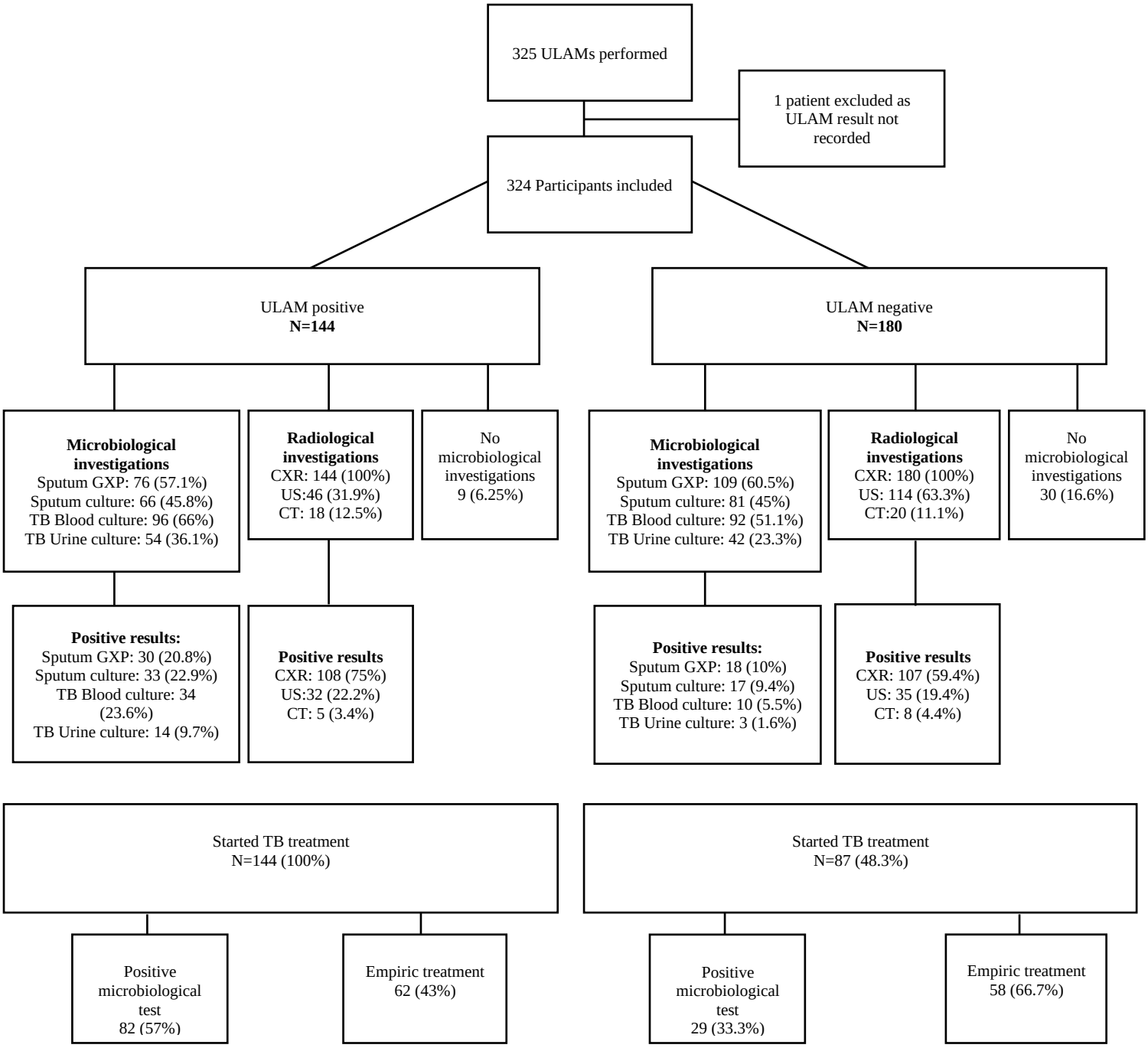

**Figure 1** summarises the microbiological and radiological investigations done in both the ULAM positive and negative group. In total 57.1% (n = 185) of participants had a sputum GeneXpert, 41.7% (n = 135) had a sputum auramine stain and 45.3% (n = 147) had sputum culture done. 40.1% (n = 130) of participants were sputum scarce (no sputum sample obtained for GeneXpert, auramine stain or culture) with no difference noted in the ULAM positive and negative group (40.9% and 39.4% respectively). 49.3% (n=160) had an U/S, 31.9% (n=46) participants in the ULAM positive group and 63.6% (n=116) participants in the ULAM negative group. 16% (n = 30) of participants in the ULAM negative group and 6.25% (n = 9) of participants in the ULAM positive group had no microbiological investigations (sputum, blood, urine, pleural fluid, CSF or lymph node aspirates) performed.

Though a similar amount of sputum samples were sent in both groups, patients in the ULAM positive group had more MTB urine and MTB blood cultures sent, with the ULAM negative group having more U/S performed.

**Discussion**

Our findings retrospectively describe the initial two-year experience after the rollout of ULAM testing at a district level hospital in Cape Town. The patient population described was profoundly immunocompromised as evidenced by low median CD4 count of < 50 cells/uL with unsuppressed VLs. Our patient cohort was young and female predominant in keeping with the known HIV epidemiology in SA [23]. Only 36% of patients were accessing ART, despite the "test and treat" policy for PLWH. This highlights the ongoing challenge of curbing new HIV infections and overcoming barriers to testing and treatment initiation as well as reducing new infections in SA. For many patients this was the index presentation of their HIV diagnosis, presenting unwell with advanced disease and established opportunistic infections.

Of note is the duration of ART in this cohort. The ULAM positive group had a mean exposure to ART of only 3 months, compared to the ULAM negative group of 11 months. This raises the question if a proportion of these patients were, in fact, presenting with unmasking immune reconstitution inflammatory syndrome (IRIS) in the three months after initiation on ART. Given the median CD4 of < 50 cells/uL in this cohort, it is possible many clinical presentations were in keeping with unmasking TB

IRIS [24]. The latest WHO recommendation of performing ULAM in all patients with a CD4<200 cells/uL may likely prevent some of these admission in future [15].

A 2016 systematic review analysed outcomes in Sub-Saharan African patients where ULAM was used and found a mortality rate of 7-53% irrespective of ULAM result. When only looking at SA inpatient studies included in this review, the mortality rate ranges between 13 and 22.3% [22]. We report a 30-day mortality rate at 12.7%, only marginally lower. This difference could be explained by our shorter follow-up period of only 1 month, as compared to 2-6 months in the other SA studies included in the review [22].

Our study noted no statistically significant difference in the 30-day mortality rate between ULAM positive and ULAM negative participants, with a slightly higher absolute mortality of 14.4% in the ULAM negative versus 10.45% in the ULAM positive group. In the outpatient setting ULAM was shown to be associated with a higher mortality rate [22]. Since our study only included inpatients, we compared our findings to those in the systematic review conducted in inpatient settings [8, 25]. The first inpatient study only showed a trend toward a higher mortality in ULAM positive patients whereas in the second study a significant difference in CD4 count was noted in the ULAM positive and negative groups. (42 cells/uL vs 140 cells/uL). Our study had a non-significant difference (p=0.386) in CD4 count when comparing ULAM positive and ULAM negative patients (37 cells/uL vs 44 cells/uL ). Our study more closely resembles what was found in a 2020 systematic review, which found no statistical difference in mortality rate between those having a positive vs negative ULAM [26]. The notable difference in this review was, they only included participants with positive MTB blood cultures thus selecting for a more critically ill study population with a median CD4 count of 76 cells/uL [26]. Similarly, our study population consisted of critically ill patients with very low median CD4 counts. Thus, perhaps ULAM is of limited prognostic value in a critically ill patient population and may be more useful in an outpatient setting [26].

Of note, although not statistically significant (p=0.279), the ULAM negative group did have a higher absolute mortality rate of 14.4% vs 10.4% in the ULAM positive group. Despite the negative ULAM result, 40% of participants in this group had other microbiological or radiological evidence for MTB. We postulate that participants were not able to be initiated on treatment based on the negative ULAM result, the diagnosis and treatment of MTB or other opportunistic infections might have been delayed while awaiting further investigation and a delay of four or more days of TB treatment relates to an increase in mortality [26].

As expected, all the patients in the ULAM positive group were appropriately initiated on TB treatment. In the ULAM negative group however 48.3% of participants were also initiated on treatment. Of these only 33.3% had microbiological evidence for MTB. If we interrogate the investigations performed in these participants, we find that the most frequent investigations performed in the ULAM negative group were radiological: CXR and U/S imaging. Both of which have a low sensitivity and specificity for MTB and does not provide sensitivity details, thus not negating the need for further investigation [27, 28]. 40% of patients in the ULAM negative group were not able to produce any sputum sample, further highlighting the challenges of obtaining sputum samples in severely ill HIV positive patients at the district hospital level where sputum induction and bronchial alveolar lavage is not readily available [8, 9]. However, of note is that MTB blood and urine cultures were also infrequently performed (51.1% had blood cultures and 23.3% had urine cultures) leading to the possible opportunities to be missed in patients in this group who were empirically initiated on MTB treatment. 8% of the patients empirically initiated on TB treatment had no further investigations performed to microbiologically identify TB.

Though the two groups were comparable in many aspects, there are notable differences in how participants were investigated. In the ULAM positive group more blood (66% vs 51.1%) and urine cultures (36.1% vs 23.3%) were performed, possibly highlighting the recognition on behalf of the clinicians to ensure some microbiological cultures were sent for sensitivity testing. The ULAM negative group had almost twice the number of U/S performed (63.7% vs 31.9%). Though the addition of ULAM to the TB diagnostic algorithm might not alter the need for ultrasound, we postulate that the result of ULAM might influence the need for ultrasound [29]. Patients who are ULAM negative will

require further investigation to prove MTB and to assess for other diagnoses, thus explaining the higher number of ultrasounds performed in this group. Though some patients in the ULAM positive group likely required ultrasounds for indications other than to diagnose MTB it may be that this number is somewhat inflated by the SOP that was in place at the time of this study. As ULAM was consultant released as per the SOP, ULAM testing was not available after hours and might have been delayed until the next day prompting clinicians to initiate investigations without the benefit of the ULAM result. Rather than performing tests consecutively, an admission ultrasound might have been requested in conjunction with other investigations to fast-track patient movement through the system and unintentionally not cancelled if the ULAM was subsequently positive.

On review of the implementation of the department's SOP on the usage of ULAM testing, the SOP was adhered to as only 15 (4.6%) participants had a CD4 count of more than 150 and only 6 (1.9%) participants had a positive test prior to the ULAM being done. All ULAM positive participants were appropriately initiated on TB treatment as directed by the SOP.

Study Limitations:

The strength of this study's findings is limited by the small sample size (324 participants) included in the cohort. Not all results were available for all participants, including VL. Death statistics on Clinicom may not capture all deaths if they occurred at home or in other provinces within SA. The SOP in place at the time resulted in some delay from admission to ULAM being performed. Though the delay was not more than 24 hours, it might have had a minor impact on mortality and decision making by managing teams on how to further investigate participants. There were no statistically significant differences noted in the ULAM positive and negative group with regards to mortality, length of stay and clinical parameters. It is likely that this study was not sufficiently powered in terms of sample size to make such deductions.

Conclusion:

The cohort of participants in our study were severely immunocompromised with minimal exposure to ART despite a current national "test and treat" ARV strategy. Participants were frequently initiated on empiric TB treatment with a proportion of these patients not being adequately investigated. Some participants did not have any other MTB investigations performed apart from the ULAM. Urine and MTB blood culture, both easily accessible, were least frequently used. Sputum, though more difficult to obtain, remained the most frequent MTB specific test performed. Clinicians adhered to the SOP put in place to guide ULAM use, suggesting if a SOP were put in place to guide investigation of patients empirically treated for TB it would be effective.

Future research:

This study was performed in a very strictly controlled setting where ULAM was not freely available to clinicians to use. In the current setting it would be interesting to note how the population having ULAM testing performed has changed and how clinicians are responding to ULAM results in approaching investigating these patients. As the WHO guidelines have been changed to include asymptomatic patients with low CD4 counts and all symptomatic patients, future research should assess if patients that are being admitted with HIV are having adequate ULAM testing performed according to these guidelines.

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**Appendix 1: Summary Table of Cumulative Investigations**

| **ULAM Positive n = 144** | Positive n (%) | Negative n (%) | Not done n (%) |
|---|---|---|---|
| Sputum GeneXPert | 30 (20.8%) | 45 (31.2%) | 68 (47.2%) |
| Sputum Auramine | 6 (0.04%) | 48 (33.3%) | 90 (62.5%) |
| Sputum Culture | 33 (22.9%) | 32 (22.2%) | 78 (54.1%) |
| Blood TB culture | 34 (23.6%) | 61 (42.3%) | 49 (34.0%) |
| Urine TB culture | 14 (0.09%) | 40 (27.2%) | 90 (62.5%) |

In ULAM positive group 6.25% of patients had no investigations done

| **ULAM Negative n = 180** | Positive n (%) | Negative n (%) | Not done n (%) |
|---|---|---|---|
| Sputum GeneXpert | 18 (10%) | 90 (50%) | 71 (39.4%) |
| Sputum Auramine | 4 (2.2%) | 77 (42.7%) | 99 (55%) |
| Sputum Culture | 17 (9.4%) | 60 (33.3%) | 99 (55%) |
| Blood TB culture | 10 (5.5%) | 80 (44.4%) | 88 (48.8%) |
| Urine TB culture | 3 (1.66%) | 36 (20%) | 138 (76.6%) |

In ULAM negative group 16.5% of patients had no investigations done

## Appendix 2:

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

**Room G50- Old Main Building**
**Groote Schuur Hospital**
**Observatory 7925**
**Telephone** [021] 406 6492
**Email:** hrec-submissions@uct.ac.za
**Website**: www.health.uct.ac.za/fhs/research/humanethics/forms

23 July 2021

**HREC REF: 449/2021**

**Dr D Maughan**
Division of Internal Medicine
Email: deborah.maughan@uct.ac.za
Student: erweechristie@gmail.com

Dear Dr Maughan

**PROJECT TITLE: RETROSPECTIVE REVIEW OF USE OF URINARY LIPOARABINOMANNAN (LAM) IN THE DIAGNOSIS OF DISSEMINATED TUBERCULOSIS (TB) IN MEDICAL INPATIENTS AT A DISTRICT HOSPITAL IN CAPE TOWN -MMED CANDIDATE-DR CHRISTEE ERWEE**

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

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

**This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.**

**Approval is granted for one year until the 30 July 2022.**

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)

***The HREC acknowledge that the student: Dr Christie Erwee will also be involved in this study.***

**Please quote the HREC REF 449/2021 in all your correspondence.**

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

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

HREC/REF449/2021sa

Yours sincerely

**PROFESSOR M BLOCKMAN**
**CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE**
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007
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 Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. 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/REF449/2021sa

## Appendix 3: Instructions to authors (South African Medical Journal)

### Research

*Guideline word limit: 4 000 words*

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

*Structured abstract*

- This should be 250-400 words, with the following recommended headings:
    - **Background:** why the study is being done and how it relates to other published work.
    - **Objectives:** what the study intends to find out

    - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
    - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
    - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

*Main article*

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.

- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

*Results*

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

*Discussion*

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

*Conclusions*

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

**Tables**

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ ‖ then ** †† ‡‡ etc.

**References**

**NB:** *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.

- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
    - On the Crossref homepage, paste the article title into the ‘Metadata search’ box.
    - Look for the correct, matching article in the list of results.
    - Click Actions > Cite
    - Alongside 'url =' copy the URL between { }.
    - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

**Appendix 4:**

## SOP FOR THE USE OF URINE LAM STRIPS AT MITCHELLS PLAIN HOSPITAL

### Purpose

Lipoarabinomannan (LAM) is an antigen embedded in the cell wall of bacteria in *Mycobacterium tuberculosis* complex. Free LAM antigen is excreted by the kidneys in HIV-infected patients with advanced immunosuppression and disseminated tuberculosis (TB). A rapid point of care immunochromatographic lateral flow assay has been developed which can detect the presence of LAM in urine (Alere Determine™ TB LAM Ag [Urine LAM]), providing a qualitative result within minutes.

The urine LAM test has an excellent specificity for TB (over 98%), meaning that there are very few false positives. The sensitivity is around 40% for inpatients with advanced HIV, regardless of symptoms, but the yield drops substantially for those with CD4 counts above 150. Therefore, urine LAM has an important role as a rapid rule-in test for TB in HIV-infected patients with CD4 counts or stage 3/4 disease presenting to hospital. Note that TB is not excluded in patients with a negative urine LAM. The other limitation of the test is the inability of the test to provide susceptibility data for TB drugs.

The use of urine LAM can reduce investigations (such as abdominal ultrasound), length of stay, and time to starting TB treatment in the sickest patients, and should therefore be widely used in the correct clinical setting.

### Indications

- All HIV-infected inpatients with CD4 counts < 150 or stage 3/4 disease with suspected TB at any site and negative initial investigations
- HIV-infected inpatients with CD4 counts < 150 or stage 3/4 disease presenting with severe adverse effects after recently starting TB drugs (within 2 weeks) in whom the indication for TB treatment is in doubt

Do not perform urine LAM tests in symptomatic patients with a recent positive microbiological or molecular test for TB.

### Procedures

1. A limited supply of urine LAM strips will be kept in the Consultants' offices, and their use will be monitored and audited with the use of a register
2. When taking a urine LAM strip from the consultant room place a patient sticker and record date in the register
3. Use the test within 2 hours of opening the protective foil
4. Apply 60 µL of neat urine onto the the sample pad using the Microsafe capillary tube provided
5. Interpretation:
    a. A control bar is incorporated into the strip to ensure quality control and test validity – this should always change colour to purple/gray
    b. Invalid test = control bar does not change colour within 35 minutes
    c. Negative test = colour change in control bar only

   d. Positive test = **any** colour change in both control bar and test bar
6. A test can be interpreted as negative after a minimum of 25 minutes. Any colour change in both the test bar and control bar within 35 minutes indicates a positive result. Strips should not be read > 35 minutes after application of the urine sample
7. Used urine LAM strips, pipettes and urine samples must be discarded in appropriate medical waste containers
8. The results of the test must be CLEARLY DOCUMENTED in the patient's medical records, TB referral letters, and discharge summary
9. All patients with previous TB should have additional testing to determine drug susceptibility, even if the urine LAM is positive