



***"In vitro"* evaluation of urine preservatives to assess DNA stability for the diagnosis of tuberculosis**

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Justification of study

- **Timely and accurate diagnosis is a critical obstacle to TB control.**
- **Implementation of alternative methods to sputum microscopy would be a tremendous advantage. (e.g. Real Time qPCR)**
- **There is growing evidence that short DNA fragments arising from human or bacterial cells, dying throughout the body, pass through the renal barrier and appear in urine as transrenal DNA (Tr-DNA). (Umansky S, et al 2006 Expert Rev Mol Diagn 6:153-63)**
- **Molecular diagnosis of Tuberculosis using urine is becoming increasingly utilized**
- **The storage of urine prior to nucleic acid analysis is a paradox**
- **Total nucleic acid in urine has previously been reported to degrade when stored over a long period of time. (Cannas A, et al 2009 PLoS ONE 4(9))**
- **Previous investigations recommend different storage strategies (Cannas A, et al 2009 PLoS ONE 4(9))**
- **Routine diagnostic trDNA testing will require banking of samples for repeat testing (Green C, et al 2009 Lancet Infect Dis 9:505-11)**
- **In the current study we propose to assess the stability of TBtrDNA in prior to molecular analysis and improve detection sensitivity of MTB assay**



Objectives

- To "*in vitro*" evaluate the use of urine preservatives (EDTA, K₂EDTA, Sodium azide and Guanidine isothiocyanate) to assess DNA stability
- Optimising the TB TrDNA assay to improve detection sensitivity by using:
 - Higher volume of urine to be extracted (1 or 9 ml to 15 ml)
 - MTB micro assay (IS1081, 39bp amplicon)



Methods (1)

1. Preparation of urine sample with preservative

- 20 urine specimens from health adults (10 males + 10 females) from adults in Mbeya, Tanzania
- Kept on ice prior to the addition of the preservative

2. Spiking of urine sample

- Sonicated genomic MTB DNA (10 μ l, 10^4 copies) into 250 μ l of PCR grade water.
- Add 10 μ l of genomic MTB DNA solution to each 20 ml of urine sample.

3. Addition of preservative

Addition of preservative in the urine: Final concentration for:

Sodium azide (SA)	10 mM
Guanidine isothiocyanate (GTC)	4 mM
EDTA	40 mM
K2EDTA	10 mM



Methods (2)

3. Extraction of DNA

- i. Day 0 (base line) (Immediately extraction)
- ii. Incubation of urines at 4C
- Days: 1, 3, and 7 extractions

4. Measuring the DNA content

- i. Each sample was first assessed for inhibition by SPUD assay. (Nolan T, et al 2006 : Analytical Biochemistry 351; 308–310)
- ii. Use of ALU target: Human DNA
- iii. Use of IS1081 (39 bp) target: *M. tuberculosis* DNA

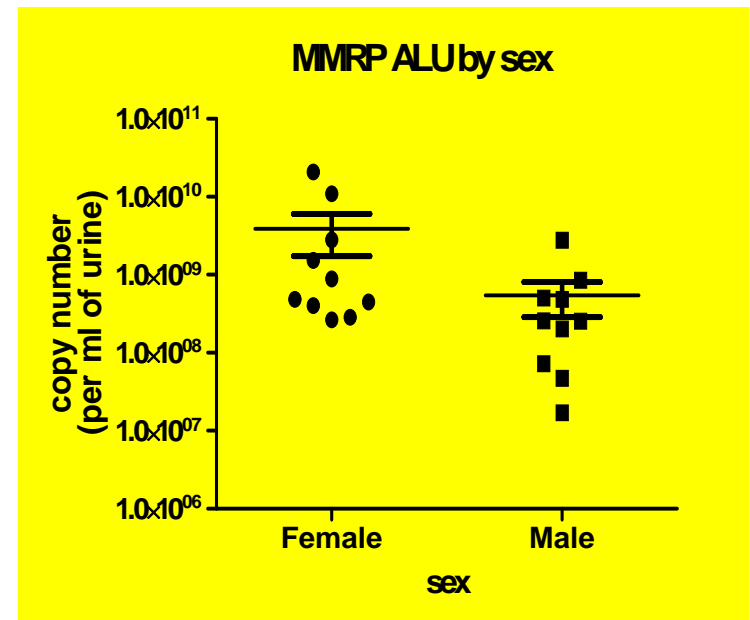
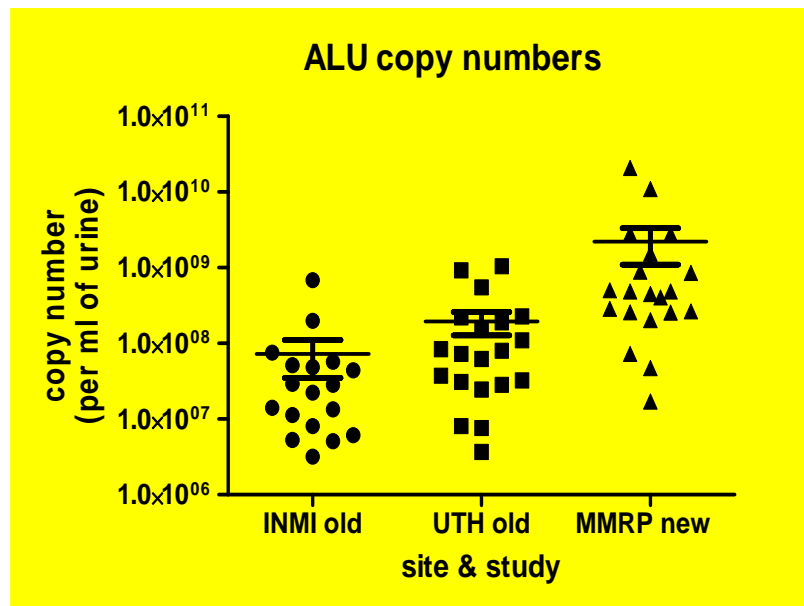
5. Analysis of data

- The data were analysed by quantitative real time PCR. (Rotor gene 6000, corbett) and Graph pad prism.



Results (1)

- The amount of human DNA in extracted urine samples were slightly greater than that previous reported from similar studies in Italy and Zambia at baseline
- Also less human DNA content was observed in urine of males at base line, 3.2×10^9 copies/ml against 2.5×10^{10} in females.





Discussion & Conclusions

- Here we found that, the stability of DNA in urine from healthy adults in Mbeya was good regardless of urine preservative employed except there was an order of magnitude change in the cell-free MTB DNA after 7 days storage with SA at 4 C.
- For low hazard, cost and ease of use, EDTA is adequate to store urine at 4°C for a week in Mbeya.



Future perspectives

- **The diagnostic potential of Tr-DNA based tests**
- ✓ **Detection of other infectious disease agents HIV, Malaria**
- ✓ **Broad public health screening; safe, non-invasive platform**
- ✓ **Therapeutic monitoring**
- ✓ **Tumor diagnostics**
- ✓ **Prenatal detection**
- ✓ **Transplantation monitoring**
- ✓ **Detecting in vivo cell death**



THANKS FOR THE MYCOBACTERIA AND YOU ALL

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