Investigating molecular diagnosis of tuberculosis using transrenal DNA. The nucleic acid extraction step.

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Background

Tuberculosis (TB) is one of the most prevalent infectious diseases in the world. It is caused by the bacterium Mycobacterium tuberculosis (Mtb) and results in >3 million deaths a year. While global awareness has improved, diagnostic methods remain very poor especially in the parts of the developing world where HIV co-exists, resulted in a TB epidemic.

The most important role for biotechnology in improving health in developing countries has been set in improving infectious disease diagnosis. But diagnostic methods for diseases like TB have remained limited with poor clinical utility partly because the most commonly used clinical sample, sputum, is highly heterogeneous and difficult to extract DNA from.

Recently detection of the M. tuberculosis DNA in the urine of patients with TB has been reported. However results have been variable and studies have not approached this systematically.

Before studying this phenomenon in more detail we are investigating optimal extraction methods for purifying DNA from urine. Furthermore we are interested in purifying the free transrenal DNA (tDNA) that passes through the kidneys from part of the circulating nucleic acids in plasma and serum (CMNPs).

Aims

Test different methods for purifying free DNA from urine for:

- Sizes of DNA recovered
- Removal of PCR inhibitors

Results, Experiment A: Recovery & Inhibition

-Purpose: to assess a number of extraction methods for purifying free DNA (3500 bp) from urine

Xenomics and PEG methods yield the most detectable target amplicon using Mtb16S competitor A.

However this “real time PCR” assessment will also be susceptible to any sample inhibition.

Inhibition assessment

Effect of extraction technique

Assessment of recovered 3500 bp molecule

Inhibition is not sample specific

Strategy

DNA spiked into urine:

10,000 copies 450 bp
100,000 copies 71 bp
qPCR reactions

Mtb 16S rDNA fragment

Average ratio = 0.16

Conclusion

- Xenomics extraction method is capable of purifying a range of different sized free DNA molecules from urine.
- We are currently assessing the PEG method for purification of smaller molecules.
- We will use the Xenomics method (along side the PEG method) to fully characterise the phenomenon of Mtb tDNA in patients with TB.

Results, Experiment B: Smaller molecules

-Purpose: to assess Xenomics extraction method ability to purify smaller DNA molecules

Xenomics extraction method extracts 71 bp and 450 bp molecules with similar efficiency.

Copy recovered DNA

Average ratio = 0.16

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