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Grant awarded £6,000 (1 Year)

Development of a NASBA-based method for detection of viable *Mycobacterium paratuberculosis*

The objective of the proposed project will be to devise a rapid method which to detect living (viable) *Mycobacterium paratuberculosis* cells, as an aid to the study of this microorganism's role in Crohn's disease.

The approach for detection of viable cells will be based on detection of messenger RNA (mRNA). Messenger RNA is produced in living cells, and is rapidly degraded after death, or outside the cellular environment. Its detection is therefore regarded as being diagnostic of the viability of the organism.

The first stage of the project will involve development of a nucleic acid sequence based amplification (NASBA)-based method to detect viable *M. paratuberculosis*. NASBA is an alternative technique to the polymerase chain reaction (PCR); it uses nucleotide primers, which match gene sequences, to mediate amplification of mRNA from those genes. As a gene-based method, it can confirm the identity of the target organism, simultaneous to its detection. But unlike PCR and its variants, NASBA operates under conditions which should allow the unambiguous detection of viable cells. It does not employ temperatures at which genomic DNA opens up to allow binding of primers which mediate its amplification. Detection of DNA does not signify detection of viable cells, as it can survive cell death and is robust enough to persist extracellularly. Complete removal of DNA from RNA is very difficult if not impossible to achieve using current technologies, and amplification of background DNA can confuse a signal from amplification of mRNA, giving a false result.

The next stage of the project will verify that the NASBA-based method detects only viable *M. paratuberculosis*, by applying the method to untreated and heat killed cells.

The final stage of the project will determine the sensitivity of the method in terms of the number of cells which can be detected, optimising it if appropriate to allow the detection of low numbers of cells.

A method to rapidly detect living cells of *M. paratuberculosis* will greatly aid in the investigation of this organism's role in Crohn's disease; and if it is found that this role is significant, a NASBA-based detection method would be useful in several ways. Patients could be rapidly diagnosed, food and environmental samples screened if appropriate, and processes and measures to eliminate

the agent effectively evaluated. The proposed project is thus of relevance to NACC's aims of understanding and controlling Crohn's disease.