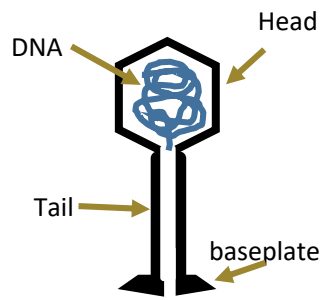


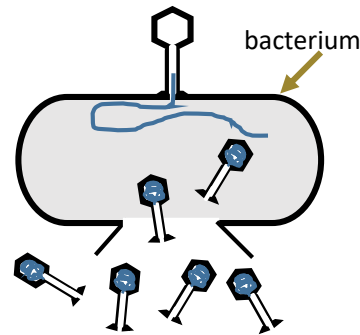
Phage tests for bovine TB

What is a phage?

A bacteriophage or phage is a virus which infects bacteria. Phages have variable life cycles, but generally they inject their DNA into the host bacterium, which is then used to make new phage organisms. In many cases the phage then ruptures the host, releasing new phages which in turn can infect more bacteria.



Simplified phage diagram



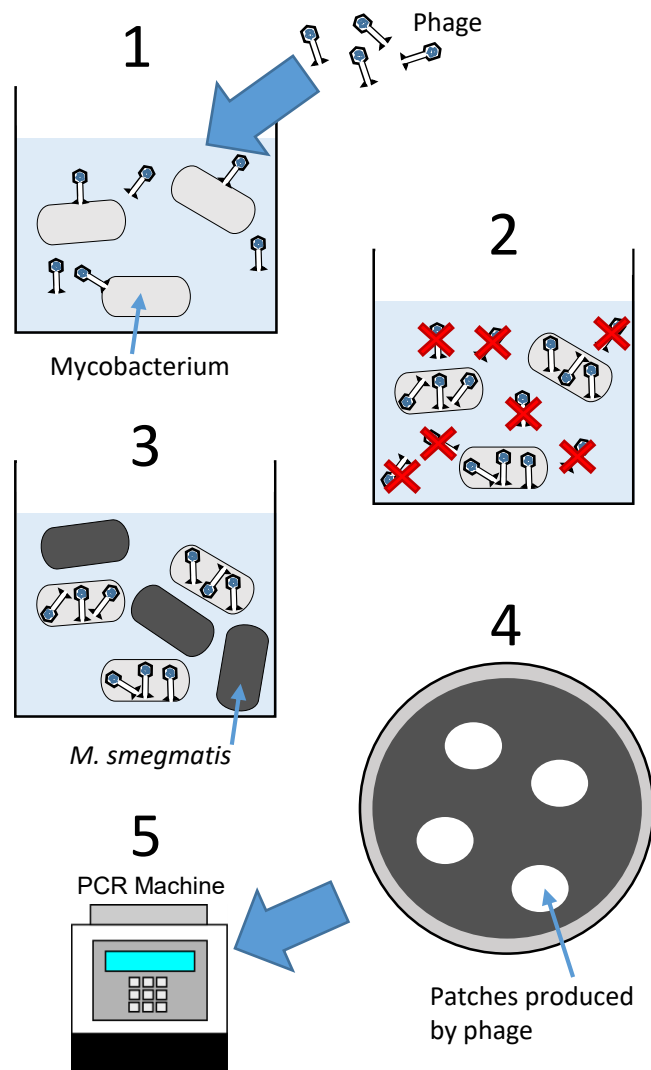
Example phage lifecycle

How can phage testing identify infected animals?

Phage-based methods of detection use phages capable of infecting the target bacterium to detect living bacterial cells. Phage-based methods have been developed to detect *Mycobacterium avium* subspecies *paratuberculosis* (which causes Johne's disease) in blood and milk [1,2,3]. Recent studies have also trialled phage-based methods for identifying *Mycobacterium bovis* (which causes bovine TB) in blood [4]. The process involves several steps (see below):

Process steps (based on Swift et al. 2016)

1. Mycobacteria are isolated from a blood sample and phage (which can only infect mycobacteria) are added.
2. Phages which have not infected mycobacteria are inactivated using chemicals.
3. A fast growing bacterium *M. smegmatis* is added to the solution.
4. The solution is incubated producing a surface covered by bacteria ('bacterial lawn'). If mycobacteria (such as *M. bovis*) were present in the original sample, then the phage from these mycobacteria will infect the surrounding *M. smegmatis*, producing patches in the bacterial lawn (called 'plaques'). If there were no mycobacteria in the original sample there will be no phage (as they were killed in step 2) and no patches in the bacterial lawn.
5. Samples are taken from the plaques and tested for the presence of *M. bovis* DNA using PCR techniques.



Patches produced by phage

How accurate are phage tests for bovine TB?

Phage testing of cattle blood will produce a positive result if the blood tested contains viable *M. bovis* (a condition called bacteraemia). There is little information available on how often bovine TB infection results in *M. bovis* in cattle blood [5]. However a recent study has compared the results of phage testing to results from the tuberculin skin test [4]. This study found a positive phage result in 66% of cattle which reacted to the skin test, and a positive result in 85% of those with visible lesions. In this study 45 animals from a herd with no history of TB all produced negative results using the phage testing method used. The skin test is not perfect and may leave some infected animals in the herd, but it is currently unclear how many of these animals would be identified using phage testing.

How long do phage tests take?

Current published methods for identifying *M. bovis* infection using phage testing take 48 hours to produce results [4]. However, phage testing times can be reduced substantially (to a few hours) by using analytic methods which test samples for evidence of phage activity (either natural products or by using fluorescent compounds), rather than incubating samples for longer periods [6]. As phage testing is an active area of research, changes to testing methods are likely to occur in the near future

Are phage-based tests currently available?

Phage based test for *M. bovis* have not been formally validated. The Actiphage™ test designed by Nottingham University and PBD Biotech can be used if permission is obtained via an APHA case vet, subject to certain conditions. Further research will be needed to determine the accuracy and reliability of these methods before they may be available for widespread use.



Where can I find more info?

Information on a range of tb related topics can be found on on www.TBhub.co.uk

1. Botsaris (2016) International Journal of Food Microbiology
2. Swift (2013) Journal of Microbiological Methods
3. Stanley (2007) Applied and Environmental Microbiology
4. Swift (2016) Virulence
5. Maggioli MF (2016) Virulence
6. Van der Merwe (2014) Analyst

This factsheet has been created as part of a Knowledge Exchange project in collaboration with the regional TB eradication groups. If you would like to know more about this or other TB related topics please contact a.robertson@exeter.ac.uk or visit www.tbknowledgeexchange.co.uk