© Article originally published in the Autumn 2014 edition of **waterline** - The Journal of the Water Management Society

Immunomagnetic Separation (IMS) for the detection of Legionella

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Immunomagnetic separation (IMS) has justified its relevance and reliability over many years and is a fully proven and accepted technique; particularly when capturing cells from difficult samples.

There are a wide range of techniques for the detection of microorganisms and the majority of laboratories still use traditional plate culture for the growth and isolation of microorganisms including *Legionella*. However, with traditional plate culture and confirmation of *Legionella* taking from 5-14 days there have been a number of rapid assays developed to reduce the time period for reporting of microbiological results.

One such rapid assay, immunomagnetic separation (IMS) has largely been driven by food safety where there has been widespread adoption of the IMS process in assessing contamination during manufacturing.

This brief paper discusses how the IMS process is being applied in the rapid detection of *Legionella* for risk management.

Applications

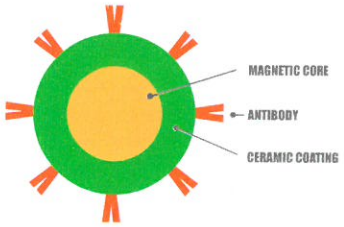
IMS is an established technique that can reliably isolate specific cell types from a liquid or culture for the purposes of early detection, characterisation and enumeration. This is best described as a specific sorting process for the concentration, isolation and purification of microorganisms. By avoiding the need for the standard enrichment process necessary with some culture techniques, IMS can save a considerable amount of time. The technique is widely used to enhance isolation rates, to create concentrated samples to improve the performance of other processes such as PCR or as an integral part of capture processes for automated isolation systems.

Deployment of IMS has been particularly widespread in the rapid detection of *Escherichia coli O157:H7* [1]. The process has proven equally successful in the process of detection in the difficult to culture obligate intracellular pathogen *Cryptosporidium parvum*. [2]

Methodology

The process utilises microscopic magnetic particles which are coated with antibodies specific to the target microorganism. These beads are manufactured to different sizes using a high speed blending process and comprise a magnetic core within a ceramic zirconium oxide coating.

The beads are mixed with a sample suspension thought to contain the target microorganisms to allow the antibodies to bind to the appropriate cell surface antigens. Critically, these bonds do not form successfully to dead cells so IMS does not appear to suffer from false positives when a sample is comprised of dead organisms.

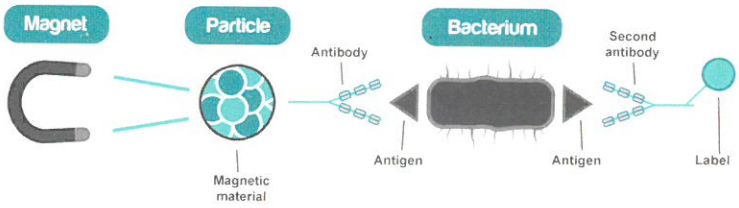


A strong magnetic separator is then deployed to attract the beads with attached 'captured' cells into a concentrated sample which can then be washed to remove all other non-targeted cells present in the original sample. This enables IMS to be highly specific in complex samples where there could be interference from other microorganisms. For example, *Pseudomonas* sp. is known to create interference by suppressing the growth of *Legionella* sp. in traditional culture techniques.

Measurement

IMS enumeration is achieved by application of a second stage colour marker-based antibody to the captured cells. The higher concentration of target cells present in the sample the higher the number of colour markers that will be retained. The colour response will therefore be directly proportional to the number of cells; sufficient to allow a semi-quantitative measurement by the naked eye, above the detection limit. Some advanced IMS processes have developed quantitative colorimetry, where an algorithm provides an accurate cell count corresponding to the colorimetric reading of a sample.

Once IMS has produced a concentrated sample, the exact identity (species or serogroup) of the microorganism may be further identified using '*polymerase chain reaction*' (PCR) if required. *Or culture can be used if a physical isolate is required as in public health investigations e.g. IMS for 0157 still requires a culture step*. The combination of IMS-PCR has been previously documented [3] in the rapid detection of *Salmonella* in food samples.



Application of IMS for the detection of Legionella

Legionella was not one of the first candidates for the protocol as it is comparatively easy to isolate from water samples. It was not until a combined IMS/Colorimetry process was developed that the rapid detection capability of IMS could be harnessed for accurate and rapid detection *and* enumeration of *Legionella* within a water sample. This patented process has been validated internationally by the AOAC [4] to be within the scope of the ISO 11731 reference method.

Now IMS is able to perform as an ideal control mechanism (in support of traditional laboratory testing), enabling management of a water system in 'real time'; to both initiate remedial action and validate that it has been successful. New IMS field test kits for routine sampling enable facility managers to save valuable time over sending samples off to laboratories for culture or PCR so remediation is both timely and creates minimal disruption.

Benefits of IMS in detection of *Legionella*

- IMS provides accurate results <40 cfu/L within 1 hour.
- IMS can detect all *Legionella pneumophila* sero groups and 92% of all species.
- IMS will only detect *Legionella* cells and is not subject to interference by *Pseudomonas*.
- IMS will not detect dead *Legionella* cells i.e. after remediation by heat/chemicals.
- IMS is ideal for real-time management of *Legionella* risk.
- To save cost, only positive IMS samples require further speciation by PCR.

1. Rapid detection of *Escherichia coli* O157:H7 by immunomagnetic separation and real-time PCR. Zhu Fu, Snezna Rogelj, Thomas L. Kieft, International Journal of Food Microbiology, Vol 99, Issue 1, March 2005 pp47-57

2. Source: An immunomagnetic separation–real-time PCR method for quantification of *Cryptosporidium parvum* in water samples. Melanie Fontaine, Emmanuelle Guillot Journal of Microbiological methods, Volume 54, Issue 1, July 2003 pp 29-36

3. Detection of Salmonella in food samples by the combination of immunomagnetic separation and PCR assay. 27th September 2000. G Jeníková, J Pazlarová, K Demnerová Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czech Republic. Internatl microbiol (2000) 3:225–229

4. Journal of AOAC International, Volume 95, Number 5, September/October 2012, pp. 1440-1451(12). Rodriguez Albalat et al AOAC Certificate No 111101

This article has been edited by Dr Jimmy Walker and Dr Samuel Collins (Public Health England). The views expressed in this publication are those of the authors and not those of the editors, PHE or any other Government Agency.