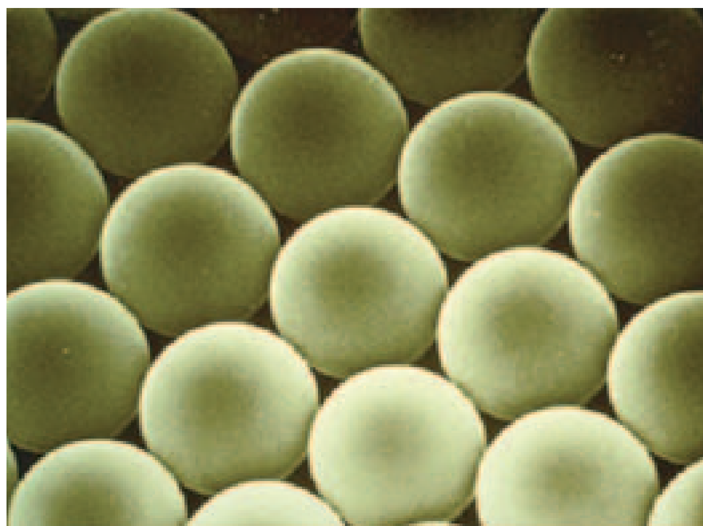
Is there a bug on board?

Phil Duncanson reviews the process of immunomagnetic separation

IMMUNOMAGNETIC separation (IMS) is the process of using small super-paramagnetic particles or beads coated with antibodies against surface antigens of cells. Utilising this technique, methods have been described for the efficient isolation of certain eukaryotic cells from fluids such as blood. Additionally, this technique has been shown to be suitable for the detection of prokaryotic organisms such as bacteria and viruses.

The technique of IMS is assisted in the fact that bacteria immunologically bound to magnetic beads usually remain viable and can continue to multiply if nutritional requirements are provided. The immuno-magnetically-isolated fraction can then be washed to remove



non-specifically attached organisms before being placed on suitable growth media. Both polyclonal and monoclonal antibodies have been employed in IMS. These antibodies can be linked to the beads either directly or indirectly, using beads pre-coated with anti-mouse or anti-rabbit antibodies. Several magnetic solid phases in particle form are commercially available for magnetic separation of biological organisms, organelles, or molecules. Common to all of these particles is that specific binding molecules can be attached to them. Most particles are super-paramagnetic; i.e. they are magnetic in a magnetic field but are non magnetic as soon as the magnetic field is removed. This is important because, once separated by a magnet, particles should attach to each other through intermagnetic force but then return directly back into suspension. Physical parameters, i.e. the shape and size of the particles, are also important. In order to perform identically in a suspension, with respect to sedimentation and kinetics of binding to other molecules, identical size and form of the particle are preferred.

The IMS technique has several advantages for micro-

biologists. When working with samples heavily contaminated with non-target organisms, IMS facilitates the purification of the target organism. Additionally, larger volumes of samples can be employed and captured target organisms can be concentrated to a volume suitable for analysis. Isolation of specific bacteria by the antigen-antibody reaction has generally been accomplished by inoculating the bead samples to cultivation broths or onto solid media selective for the target bacteria. Identification can then be accomplished by routine or conventional methods. However, increasingly IMS is being chosen as a precursor to a number of downstream detection methods. These methods whilst using very different technologies all serve to answer the question *'is there a bug on board?'*

There are an increasing number of methods downstream of the IMS process to confirm the presence of target microorganisms. We will examine some of the more common ones:

The first application of IMS technology to microbiological science was the separation of bacteria from other non-target organisms for delivery to liquid or solid culture media. Bacteria do not need to be detached from the beads, as attachment apparently has no effect on their growth. Both solid and liquid media have been used for cultivation of several bacterial species immunologically bound to magnetic beads, however, enumeration of CFU must take into account that each colony is not always the product of a single cell; several cells might be attached to a cluster of beads to initiate a single colony. Despite this, IMS has been shown to be a quantitative technique and enumeration of CFU correlates to the initial inoculum.

Both intact bacteria and their soluble antigenic determinants can be detected after magnetic extraction from the test sample, using a second antibody in a sandwich format. The application of this technology performed on an automated platform (BeadRetriever™, Dynal Biotech Ltd.) was recently applied to the rapid detection of a *Salmonella enteritidis* outbreak at a bakery (Duncanson *et al.*, 2003).

Bead Retriever automated IMS platform from Dynal Biotech Ltd

The ability of Polymerase Chain Reaction (PCR) to amplify specific DNA elements drastically reduces the need for the large quantities of test material. In theory, one copy of the target gene is sufficient for successful amplification. In many ways, the extreme sensitivity of PCR can be compared with cultivation of bacteria on non-selective media, when a single live bacterium can be detected upon initiation of a single colony. However, certain disadvantages limit the technique for diagnostic use. The sample volume traditionally used in PCR ranges from <1 to 20µl. For several microbiological applications, such as testing for *Salmonella* spp. in foods, requirements are often one cultivatable organism per 25g of sample. Reduction of the sample to <1 to 20µl restricts the test sensitivity to a theoretical minimum of 5,000 to 100,000 organisms per ml. An additional factor hindering the diagnostic use of PCR directly is the sensitivity of the *Taq* polymerase to inhibitor elements in the sample.

The use of IMS as a pre-PCR step appears to solve several of these problems. The bacteria in the sample are concentrated to a suitable volume of 10 to 100µl, and specific *Taq* polymerase inhibitors are simultaneously removed. Furthermore, there have been several reports of surveys examining the application of PCR to diagnostic microbiology that have concluded that IMS is essential to increase sensitivity and reduce inhibition. These have included the detection of *Listeria* from ham samples (Hudson *et al.*, 2001), *Salmonella* from alfalfa seed homogenates (Liao & Schollenberg, 2003), and MRSA from clinical samples (Francois *et al.*, 2003).

Dynabead with bacteria attached to the surface

While there have been many IMS methods and particles for various pathogens described in the literature, several are available commercially. For example, Dynabeads® anti-*E. coli* O157 for the physical selective enrichment and improvement in the sensitivity and specificity of the conventional culture method. Whilst Dynabeads® anti-*Cryptosporidium* kit is an integral part of US EPA Method 1622 and UK DETR Water Supply Regulation 1999 SI No. 1524 for the detection of *Cryptosporidium* from potable water samples.

It has been well-described in the literature that DNA amplification, nanotechnology and lab-on-a-chip diagnostics may all have a role to play in the future of diagnostic microbiology. Whilst much of the investment in research has focused on the end-detection techniques, little work has been focused on the sample preparation. If these novel techniques are to provide answers comparable to current result reporting such as presence/absence of a pathogen in 25g of food then efforts are needed to concentrate and deliver these pathogens to a test system. It is in this area that IMS excels.

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