Pulling Power! Magnetic Separation Supports Specific *E. coli* Isolation in Foodstuffs

Lisa Green, Product Manager, Lab M Ltd

In 2011 a serious outbreak of illness in Europe was attributed to the consumption of fresh sprouted seeds contaminated with *Escherichia coli* O104:H4, a rare serotype of *E. coli*. The outbreak caused more than 3500 cases of disease, including over 750 incidents of haemolytic uraemic syndrome (HUS) and a number of fatalities. With calls for greater vigilance, the spotlight has since been thrown on the testing procedures to be applied to such foodstuffs. These are made more challenging by the biochemical ambiguity of the causative organism, whose appearance on traditional culture media is like that of other *E. coli* species. Here, the already proven technique of immunomagnetic separation has been put forward as the only procedure that makes specific isolation of *E. coli* O104 possible in a way that results in a viable microbiological culture.

So what is immunomagnetic separation and why is it so useful?

Immunomagnetic separation is a sophisticated sorting process that can be used for the concentration, isolation and/or purification of microorganisms. It contributes to the development of rapid methods by allowing omission of some of the time-consuming steps in the standard microbiological enrichment processes that are necessary when using conventional culture techniques alone.

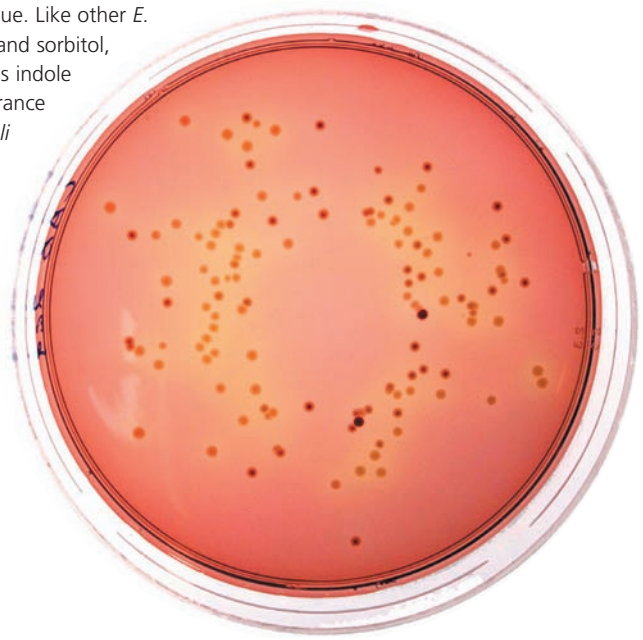
The principles of the technique are straightforward. Antibody-coated, microscopic paramagnetic particles are used to target specific microorganisms in a culture. When mixed with a sample suspension the antibody-coated beads bind to cell surface antigens forming an antibody-antigen complex between bead and target molecules, effectively 'capturing' the target cell. A magnetic concentrator is then used to effect the separation from background organisms and any interfering materials. After washing to remove any non-specifically bound material, the beads can be plated on to appropriate selective media or subjected to further analyses. The key to success lies not only in the utility of the immunomagnetic separation system itself, but more importantly in selecting a highly specific and stable antibody that can be bound to the surface.

Meeting the *E. coli* O104 challenge

E. coli are categorised by their O-specific lipopolysaccharide cell wall antigens, hence the naming system applied. Some *E. coli* harbour genes that code for Shiga toxin, which in certain circumstances can cause serious illness. Known as Shiga toxin-producing *E. coli* (STEC), these are also called verotoxigenic *E. coli* (VTEC).

While infection with Shiga toxin-producing *Escherichia coli* (STEC) generally does not have serious consequences for healthy adults, these organisms can cause diarrhoea and haemolytic uraemic syndrome (HUS) in the very young, the very old or those who are immunocompromised. The STEC serotype most often associated with outbreaks tends to be *E. coli* O157, while *E. coli* O104 (STEC O104) is generally quite rare in humans. However, the *E. coli* O104:H4 strain responsible for the 2011 outbreak described above has been described as exceptionally virulent, and most unusually for STEC, affected otherwise healthy adults and older children, rather than the more vulnerable sectors of the population more often associated with serious consequences of *E. coli* infection.

One of the challenges associated with *E. coli* O104 is that it is not biochemically unique. Like other *E. coli* species it ferments lactose and sorbitol, produces β-glucuronidase and is indole positive. It has the same appearance as non-O157 serotypes of *E. coli* on Sorbitol MacConkey Agar (SMAC) and Cefixime Tellurite supplemented SMAC (CT-SMAC), and appears like any other *E. coli* species on MacConkey Agar and Tryptone Bile Glucuronide Agar (TBGA).



Escherichia coli *O26 on Lab M Rhamnose MacConkey Agar*

Now, the development of a commercially available and highly specific immunomagnetic separation product (Captivate™ O104, Lab M, Heywood, UK) is starting to enable greater access to specific concentration and isolation of *E. coli* O104, including the *E. coli* O104:H4 serotype. Antibodies to the O104 serogroup have been attached to super paramagnetic particles via a covalent coupling process and can be used to work with organisms from food, animal feed, beverages, pharmaceutical or environmental samples.

E. coli *colonies on Harlequin™ SMAC-BCIG Agar: Translucent colonies* – E. coli *O157 (sorbitol negative, β-glucuronide negative), Pink/red colonies – sorbitol positive, β-glucuronide positive* E. coli*, Blue/Green colonies – sorbitol negative, β-glucuronide positive* E.coli.

A sample of the initial enrichment culture is mixed with the antibody-coated particles, mixed at room temperature and then placed in the magnetic separator. After washing and resuspension, the particles can be transferred to plating media, with confirmation of the identification of any resulting colonies made by serological or biochemical methods.

Looking ahead

The seriousness of the *E. coli* O104:H4 outbreak has prompted a greater focus on an organism with previously little history as a pathogen, and on its detection, isolation and identification in fresh foods. Immunomagnetic separation, a technique that is widely used to speed up the isolation of other food borne organisms, is proving to have a particularly valuable role here, providing a means of specific isolation and detection while maintaining a viable culture.

Captivate™ O104