Cutting sample preparation time in Salmonella testing

Tytti Miettinen reports on an alternative approach to Salmonella testing.

almonella is a genus of gram-negative bacilli and a name guaranteed to remove most people's appetite. Ingestion of live Salmonella in food causes Salmonellosis, which is characterised by fever, abdominal pain, diarrhoea, nausea and vomiting.

According to the European Food Safety Authority, in 2004 there were 192,703 reported cases of human Salmonellosis in European Community member states, plus Norway. Although in many European countries incidence levels appear to be stable, or even falling, worldwide, Salmonella food poisoning is a growing problem.

Transmission of food-borne Salmonella typically occurs when the organisms are introduced to food preparation areas. Inadequate storage temperatures, inadequate cooking, and cross-contamination of cooked food are typical means by which the contaminating bacteria are allowed to multiply in food.

Comprehensive testing needed

Because of its low infective dose, many countries require the total absence of Salmonella in food products. The



Fig. 1. The MAGDA Salmonella 24-hour plating assay involves enhanced enrichment lasting just $5^{1/2}$ hours, followed by immunomagnetic capture instead of a selective enrichment. Detection is done by familiar and approved plating.

food industry is thus required to implement a large-scale testing programme based on thoroughly reliable Salmonella tests.

The traditional microbiological method for the determination of Salmonella spp. in foods is described as a standard method in ISO 6579. A pre-enrichment incubation typically lasting some 20 hours is followed by a selective enrichment lasting another 24 hours. Samples of the resulting broth are then plated out onto specified agar types and incubated. The whole process can last as long as five days.

The time requirement for this kind of test creates logistical difficulties for the food industry, increasing the residence time of the foodstuff at the production facility, adding costs and reducing shelf-life. Faster tests are essential to allow food products to be released in a more timely fashion.

A 24-hour plating assay

To meet the need for faster results, new 'rapid tests' have emerged, invariably based on alternative, time-saving detection methods and a higher degree of automation. While such tests, relying on ELISA, PCR and other technologies, offer a number of benefits, they are still characterised by long sample preparation times.

With its new MAGDA Salmonella test, Finland-based Raisio Diagnostics has adopted an alternative approach, retaining plating as an inexpensive and proven detection method, while introducing two main innovations that drastically reduce the length of the sample preparation process. There is a single, enhanced enrichment lasting just five-and-a-half hours, followed by a very practical immunomagnetic capture that removes the need for a selective enrichment. The PickPen device (Bio-Nobile) allows collection of bead-Salmonella complexes from 10ml samples of enrichment culture. Rather than immobilising magnetic particles and then removing the surrounding liquid, PickPen is used to lift the particles directly out of the liquid. Plating on agar plates of the user's choice is performed directly using the PickPen device, which has a flexible silicon rubber tip for efficient plating performance. The complete test leading to confirmation of positive colonies can be performed in 24 hours.

Specificity and sensitivity

In tests performed by Raisio Diagnostics the MAGDA protocol was run on a number of different Salmonella strains (n=30) as well as other bacterial strains (n=30). All of the Salmonella strains but none of the other bacterial strains were detected.



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Six food matrices (raw milk, raw fish, poultry, pastries, raw eggs and ready-cooked meal) were artificially contaminated with 4 different levels of Salmonella (0, 1, 3, and 10 cells/25g of sample) and tested using both MAGDA Salmonella and the ISO 6579:2002 reference method. The limit of detection of the MAGDA protocol was around 1 CFU/25g.

The MAGDA Salmonella test complements Raisio Diagnostics' rapid ELISA-based tests, TRANSIA Plate

Salmonella and TRANSIA Card Salmonella. Which test methodology users find most suitable will depend on their throughput requirement and on how much importance they attach to the evidence of a plate result. \blacklozenge

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