

Article for *International Food Hygiene*

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**Detecting the Source of a Food Poisoning Outbreak
What Automating Microbial Quality Control Offers
By**

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Introduction

With just three full-time staff and around 3,000 food and water samples to process each year, the UKAS accredited Microbiology Department at the Western General Hospital, Edinburgh, needs to utilise the most efficient and effective methods available to meet increasing demands. Food samples tested include raw and cooked meats from local butchers and food manufacturers plus processed foods sent in by the environmental health department. These are commonly examined for aerobic bacteria such as *Salmonella*, *E.coli* 0157, *Bacillus cereus* and *Listeria monocytogenes* as well as anaerobes, for example *Campylobacter*.

The majority of these samples are negative with around 99.9 per cent being pathogen free. However, the comprehensive range of samples and number of different bacteria to test for, means that to continue protecting the health of the people of Edinburgh, West and East Lothian the Microbiology Department has to keep assessing and incorporating new methods and systems to provide rapid and accurate answers.

Culturing Bacteria

The Microbiology Department initially carries out a viable count of the bacteria in each sample it receives. To do this, staff make food samples into liquids, dilute and spiral plate them onto plate count agar using a WASP Spiral Plater (Don Whitely, Shipley, West Yorkshire, UK). Plates are incubated for an average of 24 hours at 37°C.

Spiral plating is more commonly utilised in the Department instead of serial dilutions because it can be used with highly contaminated samples that contain up to four million c.f.u (colony forming units) per gram of food. Also in a comparison of the two methods staff found that spiral plating reduces the time to prepare plates by up to 50

per cent. It also reduces the costs of media, requires less incubator space and produces less hazardous waste.

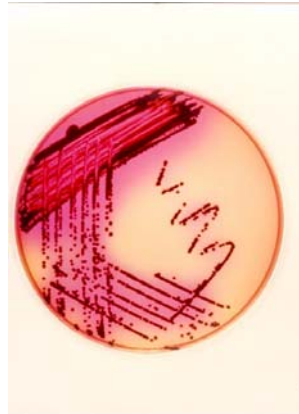
If the particular food retailer requires it, or if there is a reason to suspect that a sample contains high levels of a bacteria such as *E.coli*, then the samples are placed into selective broth and incubated for an average of 48 hours to culture specific organisms. The broth is then streaked out onto one of a range of selective media for example TBX (Tryptone Bile X-glucuronide) medium (Oxoid, Basingstoke, UK) to detect *E.coli* (Figure 1).

Figure 1: TBX agar showing *E.coli* as blue/green colonies (courtesy of Oxoid)



The Department uses selective media such as Baird Parker Agar for *Staphylococcus aureus*, violet red bile glucose agar for coliforms, XLD (Xylose Lysine Desoxycholate) medium (Oxoid) for Salmonella, (Figure 2), Oxford media for Listeria and blood free charcoal agar for Campylobacter. This method of culturing bacteria, as a basic identification has been used successfully for many years in the Microbiology Department. Although selective media are constantly being improved, the time for culturing bacteria remains at an average of 48 hours. Therefore, this is currently a rate-limiting step, where time saving is not possible at this stage of the detection process.

Figure 2: XLD media showing Salmonella colonies as red with black centres (courtesy of Oxoid)



Sample Enumeration.

To count colony numbers on spiral plates, the Department uses a ProtoCOL automated colony counter (Synbiosis, Cambridge, UK). The system (Figure 3) combines a camera integrated with software, which analyses and automatically allows for variations between plates and samples. The software is flexible enough to compensate for debris, bubbles, touching colonies, different sized colonies, different coloured media and variations in agar thickness.

ProtoCOL analyses plates, in accordance with published methods and automatically transfers the results to computer, thus preventing transcription errors. It can also present the results in a GLP compliant report format.

Figure 3: ProtoCOL automated colony counter from Synbiosis



The Department chose to automate this part of the detection process because it offers the biggest increases in sample throughput. Each plate is read and analysed in a couple of seconds, something which manually used to take around 15 minutes, so staff can now read 100 plates in the time it used to take to read one.

Case Study of a Salmonella Outbreak

The time saving methods of spiral plating and automated colony counting implemented in the Department of Microbiology not only prove useful for routine work but are invaluable in an emergency situation such as assisting in tracing the source of a food poisoning outbreak.

A good example of this occurred last year when the Food Standards Agency (FSA) declared a "national outbreak" of food poisoning in Scotland after 36 people presented with symptoms of nausea, vomiting and fever after eating contaminated food. Eight people were taken to hospital, but none was seriously ill. All 36 affected tested positive for *Salmonella enteritidis* using serological identification of culture isolated from stool samples.

In the majority of people, *Salmonella enteritidis* does not cause serious illness but it still has a one per cent fatality rate effecting mainly the elderly, infants, and those who are immuno-compromised. Therefore, for these people at risk, it was imperative that the FSA quickly identified the source of the *Salmonella enteritidis* infection.

Pinpointing the Source

To prevent further cases, the FSA set up an outbreak control team, which included the Western General Hospital's Microbiology Department. Its initial investigation showed that a number of those affected had eaten at three Chinese restaurants.

The FSA gathered over 50 samples including spices, meat and egg fried rice from the three restaurants, which presented the most cases of Salmonella poisoning. These were sent to the Microbiology Department for testing.

Initially staff spiral plated the samples onto plate count agar and incubated these for 24 hours at 37°C. Simultaneously, they placed food samples into peptone water and incubated for 48 hours at 37°C, after which time the peptone water was streaked out onto XLD agar. The ProtoCOL system was used to count the spiral plates and produced total viable count results for all of the samples in just over 15 minutes, a task which would have taken 25 hours manually. Food samples that contained between 10⁴-10⁵ viable bacteria per gram of food were noted as potential suspects. The XLD plates were then used as the confirmatory test of which samples contained Salmonella. The Salmonellas were taken from XLD plates and subjected to additional

investigation, including biochemical and antisera tests to confirm which was *Salmonella enteritidis*. All of this work eventually narrowed down the source of contamination to chicken in a sauce supplied to restaurants by a local retailer. The chicken was withdrawn from sale, the retailer was placed under investigation and there were no further cases of this type of *Salmonella enteritidis* reported.

Conclusions

The results of a recent FSA commissioned survey showed that nine per cent of 800 fresh, frozen, domestic and imported chickens tested in Scotland are infected with Salmonella. From this fact alone, it is clear that the Western General Hospital's Microbiology Department must be able to cope with an increase in sample throughput caused by a food poisoning outbreak since there is the potential for one to occur at anytime.

Using time saving methods such as spiral plating in conjunction with a ProtoCOL automated colony counter the Microbiology Department can easily handle routine quality control of food rapidly. Automated colony counting means that the Department has the capacity to not only handle extra routine samples but also has the time to carry out the more specialist tests necessary to locate the source of an outbreak.

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