

# The North German outbreak of E. coli O104:H4



The outbreak of E. coli O104:H4 from a source in northern Germany in May/June 2011 is likely to become the focus of microbiologists, food technologists, food safety professionals, epidemiologists and health professionals for many years to come.

The speed and ferocity of this expanding infection, its novelty and above all its impact on its victims and the people directly involved in their care will ensure that it is never forgotten.

As we go to press there are still a number of unanswered questions!

A patient infected by enterohaemorrhagic E. coli (EHEC) can take from 2-10 days (WHO) to start showing the symptoms of abdominal cramps and diarrhoea, but it usually takes 3-4 days.

The first EHEC patient in northern Germany is believed to have been hospitalised on Friday 13th May, which suggests that the infection of that patient took place around the 8th/9th May.

On the evening of the 13th May a party of government officials and Danish tourists enjoyed a meal in the Kartoffelkeller restaurant in Lübeck.

## Development of haemolytic uraemic syndrome

Meanwhile, the worsening infection led to some of the patients developing haemolytic uraemic syndrome (HUS) which occurs when the toxins from the infection cause a break down of the blood cells, the destruction of blood platelets and extreme kidney damage. The time taken to reach this stage will vary according to the person's age and initial state of health. The first patient with HUS was admitted to University Medical Center Hamburg-Eppendorf on the 18th May.

Next day the hospital had eight patients with HUS and the authorities were told. Within five more days north German hospitals had reported 373 confirmed cases of HUS, of which two had died.

The authorities had a number of puzzles to solve. Where had the infection come from? Epidemiology interviews were already being carried out. It is difficult enough for a healthy person to remember the details of the meals they have eaten six days previously, but interviewees said that the symptoms also inhibited the patients' ability to remember and communicate.

The next puzzle was why relatively few children and infants were infected and

relatively more adult women (then 70%)? Was this a consequence of domestic chores or of diet?

The interview data from the sick compared to the control interviews of a similar cross section of 'the well' showed a lower consumption of meat and dairy and a higher consumption of vegetables and salads in the infected people. This was no smoking gun, but perhaps it gave an area on which to focus.

The higher than average development of HUS and the speed of infection was another worry and there was no definite identification of the strain.

## Strain responsible identified

On May 24th Helge Karch, the director of the Robert Koch Institute's (RKI) EHEC consulting laboratory at the Münster University Hospital in western Germany announced that the strain responsible was E. coli O104:H4. A variant E. coli O104:H21 had been identified in 1994 in a small outbreak associated with milk in Helena, Montana, USA, which also involved a larger female proportion, and there was another case of a Korean woman in 2004.

The USA's CDC has also pointed to an E. coli O104:H4 strain similar to the current outbreak found in two patients in the Republic of Georgia in 2009.

The University Medical Centre Hamburg-Eppendorf and BGI-Shenzhen laboratory were later to produce the genetic sequence of the outbreak strain which they described as 'super toxic and highly infectious'.

They also found that it could resist up to eight antibiotics.

The epidemiological evidence stimulated the RKI to announce that they believed that tomatoes, lettuce or cucumbers were the most likely sources of the infection.

Testing of produce led local officials in Hamburg to say on the 26th May that they had isolated EHEC bacteria from four cucumbers, of which three were organic Spanish cucumbers on sale in a wholesale market in Hamburg. Scientists had speculated in the previous few days that manure used on an organic farm might have spread the bacteria to vegetables.

After the announcement, stores started taking Spanish cucumbers off the shelves and the German public was advised to avoid cucumbers, tomatoes and lettuces. This chain of consequences led to a collapse in the produce market.

With an average of 70,000 people entering and departing from Germany each day it was not long before the first cases began to appear outside the country.

On the 28th May the UK's Health Protection Agency said that three people in the UK who had recently been to Germany had fallen ill. By the first week in June there were 11 infected people in the UK and a total of over 100 patients were located in 11 European countries outside Germany and in the USA.

On the 31st May the first person outside Germany passed away in Sweden and the total number of infected people had now reached 1200, with 16 people having died.

The medical staff and hospitals in northern Germany were now trying to deal with an avalanche of cases with non urgent patients being sent home, and retired staff coming in to help. The intensive care beds and dialysis machines

Country	HUS	EHEC
Austria	1	3
Czech Republic	0	1
Denmark	8	12
France	0	2
Greece	0	1
Luxembourg	0	2
Netherlands	4	4
Norway	0	1
Poland	2	1
Spain	1	1
Sweden	17	30
Switzerland	0	5
United Kingdom	3	3
Canada	0	1
USA	3	2
Germany	773	2374

Deaths: Germany 34 - Sweden 1

**Table 1. WHO outbreak data: 14th June – 3255 cases.**



17 were overwhelmed. One patient was reported as being in Accident and Emergency of one hospital with 19 EHEC/HUS patients.

The German authorities believed they were getting close to finding the source. By the 1st of June, however, they admitted that the EHEC on the Spanish cucumbers did not match the outbreak strain. By then, however, Russia was banning the import of vegetables and produce from all EU countries.

Around the 2nd of June the Agriculture Ministry of Lower Saxony identified and closed a farm south of Hamburg which grows bean sprouts from adzuki, alfalfa, broccoli, peas, lentils and mung beans for eating raw in salads.

The imported seeds were sprouted and distributed from around the area of the outbreak.

The public were advised to stop eating the bean sprouts.

Two of the farm's employees had the infection.

Forty tests were carried out on water, ventilation and work surfaces. The first results, however, showed no *E. coli* O104:H4. Was it the wrong location? Had the normal production/cleaning processes flushed the bacteria out of the sys-

tem? On the 4th June the search reached the Kartoffelkeller restaurant in Lübeck where you may recall that on the 13th May some government officials and Danish tourists had dined. By now 18 of them had become ill, one was on kidney dialysis, one had died, and two others had HUS.

Four days later the German authorities stated that they believed the outbreak was past its peak with fewer cases being reported daily.

On the 10th of June the RKI announced that scientific evidence showed that the cause of the outbreak was contaminated bean sprouts from the farm.

Those who ate the sprouts were nine times more likely to become infected than those who did not. The epidemiology taskforce found that 26 restaurants and cafeterias that had received produce from the farm could be linked to O104:H4 patients.

The future for many HUS survivors will be difficult. Food suppliers must now learn from this outbreak and prevent an occurrence in the future.

14th June 2011

## A solution for detection

Until recently the O104 serogroup had not been identified as a potential risk and was not included in the European Food Safety Authority's (EFSA) scientific report regarding the monitoring and reporting of STEC/VTEC in food.

It is also outside the current USDA definition of STEC/VTEC of interest. As such, current rapid screening methods specific for the detection of the STEC/VTEC serogroups were not designed to detect O104.

However, as a member of the STEC/VTEC group, all pathogenic strains of *E. coli* including O104 will contain the *stx1* and/or *stx2* genes responsible for the production of a group of potent cytotoxins known as shiga toxins.

The Assurance GDS Pathogen Detection Platform from BioControl Systems is able to rapidly test and detect the specific O groups of STEC/VTEC identified by EFSA and USDA as well as the ability to detect any member of the STEC/VTEC group, including *E. coli* O104.

This method for detection of the top non-O157 STEC combines a proprietary immunomagnetic separation (IMS) procedure targeting the O26, O45, O103, O111, O121, and O145 O serogroups and molecular analysis targeting the *eae* and *stx1* and *stx2* genes to satisfy the criteria established by EFSA and the USDA for the detection of the top non-O157 STEC. The method accomplishes this with two sequential assays. Following a short 10 hour enrichment in mEHEC media and the IMS procedure to capture all six target O serogroups, the Assurance GDS Top STEC (*eae*) assay will detect the presence of the *eae* gene using highly specific primers and probes in just 75 minutes.

Positive samples can be immediately analysed with the Shiga Toxin Genes for Top STEC assay which detects and differentiates the *stx1* and *stx2* genes in just 75 minutes. Alternatively, the order can be reversed to test samples first with Shiga Toxin Genes for top STEC followed by analysis of positive enrichments with the Top STEC (*eae*). The STEC detection assays allow the option to forego the IMS sample preparation procedure, providing users with the flexibility to directly detect the key pathogenicity genes associated with STEC (*eae*, *stx1* and *stx2*) regardless of the organism's specific O-serogroup.

With the alternate protocol, after enrichment in mEHEC for 12-18 hours, depending on the food matrix, the Shiga Toxin Genes for Top STEC assay can be used to screen directly for the presence of *stx1* and *stx2* genes from any *E. coli* regardless of serotype.

The Shiga Toxin assay can be used as a stand-alone assay to detect potentially pathogenic strains that may not contain the *eae* gene, such as the recent *E. coli* O104 outbreak strain. It can also be used in conjunction with the Top STEC (*eae*) assay to satisfy the prevailing definition of pathogenic STEC as an organism containing both *eae* and either *stx1* or *stx2*.

As the food industry continues to tackle the challenges presented by this emerging group of organisms the knowledge and understanding of how to best address the public health risk of STEC/VTEC will undoubtedly evolve.

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## Rapid O104 testing

Biotecon Diagnostic's in-house laboratory has developed a rapid test protocol for EHEC, including the specific detection of the dangerous strain O104: H4.

The test enables the direct detection of human pathogenic EHEC strains within 24 hours. The results will identify EHEC bacteria in the food or enable the food without contamination to be quickly released for sale or for processing. "This makes it possible for us to specifically identify the dangerous strain of *E. coli* O104: H4 in the shortest possible time and help our customers to clear their ingredients or products," Dr Berghof-Jäger, company founder and CEO of Biotecon Diagnostics Ltd, told International Food Hygiene.

EHEC bacteria are not easy to differentiate between harmless *E. coli* using standard tests. Unfortunately specific test kits for *E. coli* O104: H4 are not currently available on the market.

The Biotecon Diagnostics test procedure carries out a real time PCR test that identifies all EHEC by amplification of the genes for Shiga toxins *stx1* and *stx2*, and the *eae* gene. Results that are presumptive positive for *E. coli* O104: H4 are put through a second PCR test which specifically targets the genes that are found in the *E. coli* O104:H4 strain. The German laboratory is accredited to the ISO 17025 standard and this service is available 24 hours.

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## Key technological tool

Pall Corporation's GeneDisc System is being used by Germany's national reference laboratory to expedite testing of food samples for the toxic strain (STEC) of the pathogen known as *E. coli* O104:H4.

"We are using this test kit for investigative screening of potential *E. coli* O104:H4 samples as well as for confirmation of presumptive positive samples," Dr Lothar Beutin of Germany's Federal Institute for Risk Assessment (BfR), told International Food Hygiene. "The GeneDisc system has proven highly effective in rapidly identifying the STEC and helping us manage this public health crisis."

The system is a rapid, simple and reliable testing solution for the detection of multiple food-borne contaminants. Based on real-time Polymerase Chain Reaction (qPCR) technology. It yields consistently repeatable results and virtually eliminates operator error. Presence or absence of multiple pathogens is indicated in as little as one hour after enrichment. The system enables producers to test simultaneously for the pathogenic *E. coli* O157 and four of the top six non-O157 STECs targeted by the USDA. The pathogenic *E. coli* O104:H4 test kit is the newest commercial assay in the GeneDisc product line.

The effectiveness of the GeneDisc platform in identifying STECs associated with haemorrhagic colitis and haemolytic uremic syndrome (HUS) – the two catastrophic illnesses presenting in the German *E. coli* outbreak – was reported in the International Journal of Food Microbiology in April 2010.

The study was conducted jointly by Health Canada, the French Food Safety Agency, and the BfR.

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# Infection to detection

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by **Tony Hines, Head of Food Security and Crisis Management, Leatherhead Food Research, UK.**

Imagine the scenario. You can eat either at a traditional restaurant, from a street vendor, or at an open air food stall. Two days later you are ill with frequent visits to the toilet. Another day later and you visit your doctor or the local hospital. Your family are becoming concerned. It is not like you to be unwell. Let's assume you are the first 'presenter' to your doctor or emergency room at the hospital. Samples will be taken and you will probably be admitted to hospital. The samples, will be sent to a laboratory and hopefully the strain of infection will be recognised quickly, within 48 hours. During this time your condition has worsened, talking and thinking are difficult and you can barely recognise your family, let alone remember what you ate 14 days ago or where you ate it. The laboratory has some results that show a rare strain of pathogenic food poisoning. The public health agency is advised.

Over the following days, more patients present to more doctors and more hospitals. A 'cluster' is identified and alarm bells ring. The street vendors have all packed up and gone away.

The agencies responsible for food safety start the detective work. They have to quickly find and close the source. Patients and families are interviewed. The answers are analysed and compared. Potential candidate locations, sources or products are identified. The public has to be warned, but is it the right conclusion? The death toll rises and the investigation continues. The spread of the infection continues: international travel alerts send travellers and tourists back home. The incident goes global.

Meanwhile, retail sales continue to fall and no simple explanation is forthcoming. Research institutes and government laboratories conduct widespread and detailed studies to try and confirm the root cause.

Traditional crisis management in the food industry where products or batches are quickly identified through supply chain management, traceability, risk assess-

ments and retail sales bear little resemblance to this topical example. Previously known cases from around the world will be investigated, production methods rigorously examined, water, irrigation and washing operations closely monitored. Questions will be asked on HACCP, production guidelines and best practice, and final sanitisation protocols.

The key question is still what caused the outbreak? Was it the seeds, the beans, the sprouts, the irrigation, the rinsing, the soil, the packaging, the environment, the storage, refrigeration, transport or the people? Was it negligence? Should an inspection or audit have identified the problem? Above all, was it preventable and predictable? Good food science, epidemiological detective work and microbiology may eventually give us the answer. However, the answer may never be known precisely. You have survived, but has your future health been damaged?

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## Enhanced E. coli test

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Neogen's test kit, Reveal 2.0 for E. coli O157:H7, provides a quick, accurate and easy method of detecting without compromising sensitivity or specificity. The AOAC process validated the accuracy of this E. coli O157:H7 system when testing food samples.

"The system cuts the wait time for results and eliminates the potential for cross-reactivity with E. coli O157 non-H7 or common cross-reactors like Citrobacter," Neogen's Ed Bradley told International Food Hygiene.

This system provides results in 12-20 hours, with only 15 minutes of test development time. It uses Neogen's new proprietary media which is specifically balanced for use with this end point test. The AOAC study found the test to be an effective procedure for the detection of O157:H7 in 65 and 375g samples of raw ground beef and beef trim. The testing system utilises a new antibody sourced from the USDA and incorporates USDA-specified sample sizes with reduced media volumes for more manageable samples.

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## The danger that lurks

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The recent German outbreak has reminded us all of the need for great vigilance in food hygiene, particularly for those with responsibility for production and delivery of safe food. Challenges can come from known pathogens and the sudden emergence of unknown strains. Food handling and processing environments and food contact surfaces are key to the spread of pathogens, so rigorous validated cleaning procedures are essential to prevent contamination.

To ensure cleaning continues to be effective, all cleaned surfaces must be regularly sampled and tested to ensure microbial levels are within acceptable limits and that dangerous pathogens are not present at all.

Products like Medical Wire's NRS II Transwabs can help to do this. These sampling swabs are premoistened with a neutralising solution. The neutralisers cancel the antimicrobial activities of disinfectors, allowing the microbes present to survive and be detected. NRS II Transwabs are supplied with a choice of accurate fill volumes to allow quantitative measurements of the environmental bioburden so that any changes or trends can be quickly identified. The collected samples can also be tested for particular pathogens.

Polywipes are premoistened blue sponge swabs that allow the sampling of larger areas, such as machines, conveyor belts, floors and walls. The sponges are premoistened with a phosphate buffer that also effectively neutralises trace antimicrobials, this time by rapid dilution. By regularly swabbing on defined areas and quantifying the microbial load, changes or trends can be monitored and responded to. Pathogens can also be detected if present.

Medical Wire also has some swab products for the detection of particular pathogens such as coliforms.

The Coliform Isolation Transwab will detect coliforms including *E. coli* on surfaces, and is an effective way of monitoring personal hygiene in food manufacturing premises. For example door handles can be swabbed. The swab is placed in the purple medium which is then incubated overnight (or up to 24 hours). Any coliforms present will multiply, causing the medium to turn yellow.

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## E. coli crisis in Europe

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Thermo Fisher Scientific has responded to the *E. coli* O104 crisis in Europe by increasing production and distribution of its Brilliance ESBL Agar plate, a chromogenic screening plate for the detection of Extended Spectrum  $\beta$ -Lactamase-producing (ESBL) organisms, such as the outbreak strain, within 24 hours.

Food microbiologists need to ensure that they can detect enterohaemorrhagic *E. coli* (EHEC) in foods, water and food processing environments in order to prevent, investigate or combat outbreaks of EHEC related food poisoning.

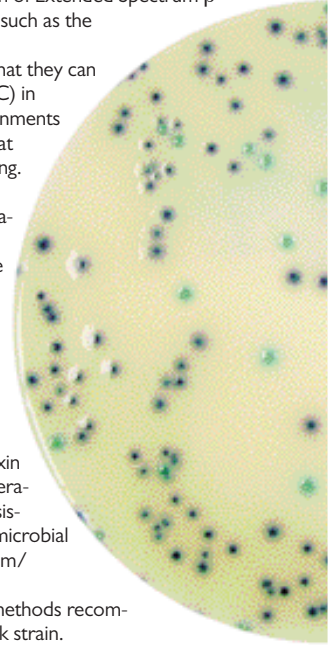
The current outbreak highlights the importance of rapid and reliable identification of EHEC, which cause bloody diarrhoea and haemolytic uraemic syndrome (HUS), for the assurance of food safety.

This large outbreak is unusual in several other ways. Historically, most outbreaks of HUS have been associated with *E. coli* O157, but the current outbreak strain belongs to the *E. coli* O104 serogroup.

The causative strain produces Shiga toxin 2 and shows high resistance to third generation cephalosporins (due to the ESBL resistance mechanism), as well as broad antimicrobial resistance to, among others, trimethoprim/sulphonamide and tetracycline.

Media to detect ESBL are among the methods recommended when screening for the outbreak strain.

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## High quality diagnostic tool

Pro-Lab Diagnostics offer a high quality tool to diagnose this life threatening disease. Prolisa EHEC EIA is available across the globe for the most rapid and accurate confirmation of toxic infection due to Enterohaemorrhagic *E. coli*.

Prolisa EHEC EIA is a microplate assay for the qualitative detection of Shiga Toxins I (ST-I) and II (ST-II) in faecal samples, and from either selective broth or agar enrichment cultures of faecal samples. It is intended for use as an aid in the diagnosis of Enterohaemorrhagic *Escherichia coli* (EHEC) infections.

EHEC can be acquired from food, water, person-to-person contact or human-to-animal contact. The organisms are typically resistant to the acidic environment of the stomach and colon where they attach to epithelial cells and produce potent cytotoxins called STs. The toxins enter the bloodstream and are distributed to endothelial cells containing toxin receptors in sensitive tissues, primarily the kidney, colon and central nervous system. The toxins act locally to damage tissue.

Microbiological tests can isolate ST-producing *E. coli* from stools, or detection of STs directly in stools or in enrichment cultures of stools (either on selective agar or in broth culture). Toxins can be detected in stools using immunoassays.

Polymerase chain reaction using toxin-specific primers may also be used to detect toxins indirectly.

Detection of EHEC infections improves patient care, reduces person-to-person transmission and prevents spread of foodborne outbreaks of disease by facilitating early reporting and trace back activities.

The Prolisa EHEC EIA test is performed by adding diluted samples to microplate wells on which rabbit polyclonal anti-ST-I & ST-II are bound. The wells are incubated at room temperature. A wash is performed to remove unbound material.

Monoclonal antibodies specifically recognising ST-I and ST-II are added to the wells. After washing, enzyme-conjugated rabbit anti-mouse IgG polyclonal antibody is added and incubated at room temperature. A reactive antibody-enzyme complex is formed if toxin is present. After washing to remove unbound conjugate, substrate is added and incubated for 10 minutes at room temperature. Colour develops in the presence of bound enzyme. Stop solution is added and the results are interpreted spectrophotometrically.

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## E. coli and bean sprouts

The International Sprout Growers Association (ISGA) describe a typical sprout growing system involving a bin, roughly 4ft wide x 5ft long x 4ft high.

The entire bottom of the bin is an open screen to allow the passage of water and air. Irrigation is supplied by an overhead spray bar that travels over the bean sprout bins. The volume of spray water is high so the suggestion is to apply a low volume of irrigation water through the crop in order to take the sample. This testing could enable the joint testing of irrigation water, the testing for seed contamination and the growing crop.

A study by Warriner et al at the University of Nottingham into *E. coli* and salmonella contamination of bean sprouts found that the contamination on the seeds 'becomes internalised within the subsequent sprouts and cannot be removed by post harvest biocidal washing'.

## Screening in 24 hours

The harmful *E. coli* O104 recently detected in Germany was screened using CHROMagar STEC chromogenic medium in under 24 hours incubation time.

Disease caused by Shiga toxin-producing *Escherichia coli* (STEC) ranges from self-limiting diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Serotype O157:H7, the most frequently implicated STEC causing haemorrhagic colitis and HUS, has been isolated from large foodborne outbreaks, as well as sporadic cases, in North America and abroad.

However, 60 STEC serotypes have been implicated in diarrhoeal disease, and several non-O157:H7 serotypes have been implicated as the cause of foodborne outbreaks and HUS in several countries suggest that non-O157:H7 STEC infections are as prevalent, or more so, than O157:H7 infection.

To address this challenge, CHROMagar has already been working on a new medium, designed for the detection of STEC (and not only O157). The result is the CHROMagar STEC, a highly selective medium, allowing the growth of a majority of STEC strains in mauve colony colour, while in general colouring in blue or inhibiting other bacteria.

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## Taking the safe route

Enterobacteriaceae of the species *Escherichia coli* (*E. coli*) are found in the normal gut flora of humans and mammals. However, when found in food and water they are an indication of faecal contamination. *E. coli* belonging to the group Enterohaemorrhagic *E. coli* (EHEC) are able to produce two cytotoxins, verotoxin 1 and 2. These toxins can produce clinical symptoms in humans from light gastroenteritis to severe haemorrhagic colitis. If this condition presents in immunocompromised groups, such as the elderly and young children, it can be life threatening or lead to severe post infection related conditions.

RayAI Ltd distribute a range of *E. coli* testing kits in a simple ELISA format in the UK on behalf of SafePath Laboratories LLC from California.

The range includes *E. coli* O157 antigen detection and a verotoxin assay developed to detect the toxins produced by the EHEC group including the current serovar *E. coli* O104 H21.

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## Accurate identification

The recent outbreak of *E. coli* has highlighted the need for efficient and accurate identification of *E. coli*. Microgen Bioproducts GNA-ID will identify all commonly encountered enterobacteriaceae, including *E. coli*, after 24 hours incubation from a primary isolation plate using a 12 substrate biochemical identification panel.

The Microgen GNA-ID is inoculated using a single colony which can be taken directly from a wide range of selective, non-selective and chromogenic agar plate media. There is no need to test colonies from a purity plate.

As with all the Microgen-ID systems, the easy to use strips of the identification system provides the reliability associated with conventional biochemical tests, plus the interpretation of results is provided with an easy to use computer database employing up-to-date taxonomy, with regular updates to the software available for customers registered as software users. The Microgen GNA-ID can also be combined with a second 12 substrate identification panel, the Microgen GNB-ID, to identify a wide range of oxidase-positive Gram-negative organisms making the Microgen GNA+B-ID system a flexible biochemical system for all Gram-negative organisms. They also manufacture an easy to use one step, latex agglutination test for the identification of *E. coli* O157.

This latex agglutination test confirms that *E. coli* strains are serogroup O157 therefore potentially EHEC. The Microgen *E. coli* O157 Latex provides results in under two minutes and can be used directly from a range of selective plate media and even with mixed cultures.

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# Outbreak timeline

**May 2nd**

Suspected start date *CDC*

**May 6th-8th**

Hamburg Harbour festival attracts 1.5 million visitors

**May 13th**

First EHEC patient hospitalised

Party of gov't officials and Danish tourists eat in the Lübeck restaurant *Independent*

**May 18th**

First HUS patient admitted to University Medical Center Hamburg-Eppendorf *Spiegel Online*

**May 19th**

UP to eight HUS patients EHEC infected at UMC. Head of department informs Robert Koch Institute (RKI) *Spiegel Online*

**May 20th**

276 HUS patients in German hospitals

**May 21st**

83 year old woman, hospitalised in 'mid-May', dies after a week. *The Independent*

**May 24th**

373 confirmed cases of HUS across Germany High proportion of women. 2 more deaths

RKI epidemiologist have patients report low dairy/meat intake and high raw veg intake compared with controls *Spiegel Online*

**May 24th**

Helge Karch of the RKI's EHEC laboratory at the Münster University Hospital says O104:H4 bacteria responsible for the outbreak. Advises that it contains additional DNA that heightens pathogenicity *Süddeutsche Zeitung*

**May 25th**

RKI believes tomatoes, lettuce or cucumbers are the most likely sources *Spiegel Online*

**May 26th**

German officials say three out of four Spanish cucumbers are contaminated. Public advised to avoid cucumbers, tomatoes and lettuces

The UK Health Protection Agency warns British travellers to Germany *HPA*

**May 29th**

300 infected – 40 HUS – 10 dead *Reuters*

**May 31st**

First death outside Germany in Sweden 15 deaths in Germany.

Spain claims country's produce wrongly blamed *Daily Mail*

**June 1st**

The German State Agriculture Secretary Robert Kloos admits Spanish cucumbers not source of O104 *Independent*

17 people dead 1,500+ ill across eight European countries, mainly in Northern Germany, or have travelled from Germany

**June 2nd**

1600 infected – 500 HUS – 18 dead

**June 2nd**

Russia bans imports of raw vegetables from EU

Lower Saxony identifies and closes farm which grows bean sprouts for eating uncooked in salads. Seeds imported from different countries

Public advised to stop eating bean sprouts

Two farm employees had the infection *BBC*

University Medical Center Hamburg-Eppendorf and BGI-Shenzhen produce DNA sequence of the E. coli strain. Preliminary analysis indicates it is 'super toxic' and highly infectious *ngsbioinformatics.com*

**June 4th**

Search looks at Kartoffelkeller restaurant in Lübeck where 18 people ate before becoming ill. One has died, two have HUS, another on kidney dialysis *Independent*

**June 4th**

BGI-Shenzhen and UMC Hamburg-Eppendorf advise of genes resistant to eight types of antibiotics *PRNewswire*

**June 5th**

Hospitals in north Germany struggle to cope and discharge patients with less serious illnesses *The Guardian*

1700 infected – 19 dead

**June 6th**

23 out of 40 tests at bean sprouts farm negative of EHEC bacteria. Seeds, water, ventilation and work surfaces targeted *FOCUS-Online*

**June 7th**

European Commission proposes to set aside €150 million (£134m \$220m) fund to compensate farmers *BBC*

**June 9th**

2909 infected – 760 HUS – 27 dead *WHO*

**June 10th**

RKI says epidemiological evidence concludes that the cause of the outbreak was contaminated bean sprouts from an organic farm in Lower Saxony

# The importance of traceability

by Donna Scholer, Chief Operating Officer, Microbiologics Inc.

Food processors know about the term traceability as it relates to the movement of product and steps within the production process; but, what about laboratory quality control (QC) traceability?

Have you ever wondered where your QC micro-organism strains originate from or what is your QC micro-organism strain's traceability? Food testing laboratories worldwide use QC micro-organisms to validate, verify, monitor and control the detection of foodborne pathogens and ensure product safety. Many industry standards and regulatory agencies require food manufacturers to monitor and prevent microbiological contamination. QC micro-organisms come from a variety of sources; but they are not all the same. An important factor that must be considered when choosing a supplier of reference materials is traceability. Traceability refers to the completeness of the information about every step in a process chain. It is the ability to verify the history, location, or application of an item by means of documented and recorded identification. For QC micro-organisms, traceability is critical. Without the provider's traceability, you could end up with micro-organisms that have been subjected to excessive subculturing, have been misidentified, contaminated or stored under conditions which could lead to mutation. If adequate traceability is not maintained, test results could be compromised, which may lead to serious consequences such as failure to detect pathogens in food samples.

The EU and the USA regulations require all food and feed producing companies to be able to trace their products and ingredients. It is just as important to know and understand the traceability of the quality control micro-organisms you are testing these products and ingredients against. How can you, after meticulously documenting the traceability of your materials throughout your product and process chain, neglect the traceability of the very materials you use to assure the world that your product is safe?

To support a claim of traceability you should look to the elements outlined by the National Institute of Standards and Technology:

- A clearly defined particular quantity that has been measured.
- A complete description of the measurement system or working standard used.
- A stated measurement result or value, with documented uncertainty.
- A complete specification of the stated reference at the time the measurement system or working standard was compared to it.
- An internal assurance program to establish the status of the measurement system or working standard at all times pertinent to the claim of traceability.
- An internal measurement assurance program to establish the status of the stated reference at the time that the measurement system or working standard was compared to it.

Today, several internationally recognised culture collections have partnered with commercial manufacturers to establish licensing programs that ensure the quality and integrity of the microbial ingredients contained in their products.

Manufacturers must meet certain quality requirements and undergo audits in order to obtain a license to commercially produce and sell micro-organism derivative products. Only a licensed commercial manufacturer can claim their microbial strains are traceable to an individual culture collection. A supplier should provide documentation or evidence that a QC micro-organism strain is traceable to its original source.

In addition to strain traceability, laboratories must also consider supply chain traceability for their micro-organisms. There are significant responsibilities that come with developing, handling, shipping, storing, using and disposing of biological materials in a way that preserves the highest possible standards for health, safety and the environment. Some manufacturers are now requiring laboratories to register their company information, including shipping address, in order to purchase biological material.

By registering every company that purchases their biological materials, a manufacturer is taking extra steps to ensure public health and safety by having complete documentation of the chain of custody from the manufacturer to the end-user.

"Our organisation is devoted to biological resource management and in its role in supporting public health, we are acutely attentive to the provenance and quality of the materials we provide," Dr Brian Pollok, ATCC President, told International Food Hygiene. "Whether in quality control testing, proficiency testing, or process validation, the source of the microbial strains used is vital in achieving reliable results. Quality control strains that carry the ATCC Licensed Derivative emblem are assured and traceable to a validated origin."

Laboratory technicians deserve to be certain that the materials they use are identified correctly and are traceable to a relevant source.

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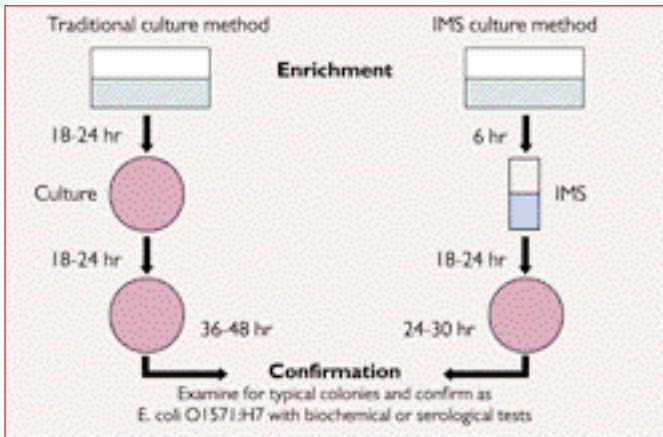


Fig. 1. *E. coli* O157:H7 traditional culture method versus IMS culture method.

## Faster detection

With the rapid isolation and identification of verocytotoxigenic strains of *E. coli* a high priority, Lab M's Captivate immunomagnetic separation (IMS) technique allows part of the conventional enrichment to be omitted, helping laboratories achieve faster results. Captivate uses antibody-coated microscopic paramagnetic particles for the specific immunomagnetic separation of micro-organisms. The beads have a magnetite core and a ceramic zirconium oxide coating and are designed for the immunomagnetic separation of target bacteria from enrichment cultures.

When incubated with a sample, the antibody-coated beads bind to cell surface antigens forming an antibody-antigen complex between the beads and the target molecules. Target cells are 'captured' and are then separated from background organisms and interfering materials using a magnetic concentrator. Non-specifically bound material is removed by washing and beads are then plated to selective media or subjected to other analyses.

Analogous to selective enrichment, IMS offers greater speed and can be a less damaging alternative to the use of antibiotics and other harsh reagents.

Fig. 1 shows how it can accelerate the isolation of *E. coli* O157:H7, for example, compared with standard culture methods.

IMS is highly effective in enhancing the isolation rates of target organisms, even from potentially problematic samples. Very fatty, particulate or viscous samples can interfere with bead recovery, but sample dilution normally overcomes this, reducing the effect of the matrix and allowing more efficient bead recovery.

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## Missing the important isolate?

The most recent outbreak of enterohaemorrhagic *E. coli* in Germany has once more generated the need for rapid and reliable diagnostic tests for that pathogen.

The currently used reference method, ISO 16654 can only detect the serotype O157 but not the German outbreak strain that is a O104 serotype as well as all the other non-O157 but enteropathogenic *E. coli* strains. The newly developed GeneGen-EHECplus Multiplex PCR assay can detect all the relevant genes (stx1, stx2, eae, rfb, iha, fliC<sub>H4</sub>) necessary for a rapid and reliable diagnosis of any enteropathogenic/enterotoxigenic *E. coli* isolate (EHEC, STEC, VTEC).

The concomitant differentiation of the current German outbreak strain is also possible using that test. Moreover, this assay can be used for any type of food and faecal samples. GeneGen-EHECplus is the ideal tool for a preventive screening in routine food testing laboratories to ensure optimum protection of consumers, to avoid future outbreaks as well as to monitor the actual outbreak in Germany. Furthermore, molecular testing should be more deeply implemented in the regular testing regime for those enteric pathogens.

Despite all that, a revision of ISO 16654 should also be discussed.

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