

E. coli: a Jekyll and Hyde bacterium

E. coli is omni-present in the human gut and generally causes no clinically significant symptoms. However certain serotypes, in particular the formidable O157: H7 variant can be potentially lethal, particularly in immunocompromised subjects. Prevention of the onward spread of infection requires rapid isolation of infected cases, and thus rapid, specific diagnostic tests. Classical culture-based diagnostic methods are no longer suitable for recent variants. This article describes the performance of a new rapid diagnostic test developed to meet this current diagnostic challenge.

by Dr Frank Apostel & Helmut Leidinger

The bacterium *Escherichia coli* is found in the gut of humans and many other animals. Although most *E. coli* are harmless, some can cause disease. For example, enterotoxigenic strains produce a toxin in the gut. This typically results in diarrhoea, but such strains have the potential to give rise to more serious diseases.

One important group of bacteria capable of causing severe symptoms is the so-called Enterohaemorrhagic *E. coli* (EHEC). Infections by such bacteria can cause severe, often bloody, diarrhoea

accompanied by abdominal cramps. Usually (but not always), otherwise healthy adults recover completely within a week from infections by pathogenic strains of *E. coli* such as EHEC. However, in particularly vulnerable people, especially young children and the elderly, the illness can progress to the so-called Haemolytic Uraemic Syndrome (HUS), which is a serious condition that can lead to serious kidney damage and even death. The severe haemorrhagic conditions predominantly affect children up to five years of age; of the infected children 3-5% die of renal failure. Many of the

children who survive suffer from the sequelae of the EHEC infection for the rest of their life, with symptoms such as hypertension and impaired renal function.

One of the most important factors behind the pathogenicity of EHEC is that the bacteria produce toxins that are quite similar to the shigatoxins of *Shigella dysenteriae*. It is for this reason that such *E. coli* strains are called Shiga toxin-producing *E. coli* (STEC). A synonymous term for Shigatoxin-producing *E. coli* is Verotoxin-producing *E. coli* because of their toxicity to verocells.

Although the most important STEC serotype is O157, there are many other STEC serogroups that can cause disease; these are summarised as so-called "non-O157 STEC". Of these, the *E. coli* serogroups O26, O111, and O103 are those that, at least in the Western world, are the most pathological.

Routes of transmission

STEC pathogens are transmitted through the consumption of contaminated food products derived from not only domestic farm animals



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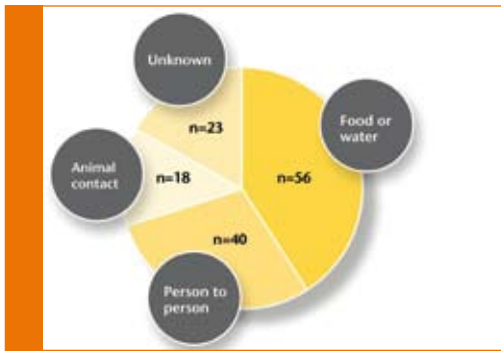


Figure 1. The routes of transmission of VTEC O157 *E. coli* in outbreaks recorded in England and Wales between 1995 and 2003. The data shown are typical of those found in other geographical areas. The principal route of contamination is via the consumption of contaminated food or water, closely followed by either person-to-person contact or contact with infected animals.

such as goats, sheep, pigs, but contaminated food products (e.g. venison) from wild animals can also be a source of contamination. Of these many potential contamination sources, the main route of bacterial transmission to man is through the consumption of contaminated beef products, i.e. meat and milk. However direct contact with infected people or animals and swimming in water contaminated with faecal matter are also other potential routes of infection. Figure 1 shows the transmission routes in 137 outbreaks of Verotoxin-producing (VTEC) O157 infection in England and Wales between 1995 and 2003. The data shown in Figure 1 are broadly similar to those found in other studies.

Even though as described above, there are many other Shigatoxin-forming serovars, the O157 serotype is still the most frequently detected, both in sporadic infections and in particular in epidemic situations. In HUS patients, this serotype has been identified as being the causative agent of the illness in by far the majority of cases. The importance and potential danger of the O157 serotype is illustrated by the requirement by many European health authorities for the immediate isolation of paediatric STEC patients and separation from their siblings. This is considered as being one of the single

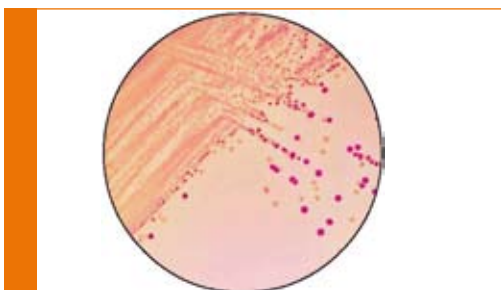


Figure 2. *E. coli* growing on MacConkey II Agar with Sorbitol.

most important measures for the prevention of any further spread of infection. However, the practical implementation and ultimate success of such isolation procedures is critically dependent on the existence of a method that can rapidly identify both Shigatoxins and the O157 serotype. Unfortunately, such a test is not part of current laboratory practice.

Laboratory analysis

The classic O157:H7 serotype (for many years the name used principally in the United States and in many other countries as a synonym for EHEC or STEC O157), has undergone genetic variations which mean that previously acceptable diagnostic methods are no longer sufficient. Other new diagnostic methods (preferably rapid tests) must be made available. For example, the culture on sorbitol-MacConkey agar (SMAC) [Figure 2] is no longer sufficient for reliable diagnosis. Whereas using this method the classic variant O157:H7 could be easily distinguished from other enterobacteria through its inability to metabolise sorbitol, new variants of this serovar have emerged which can not be distinguished on SMAC. The new variants are not only characterised by the ability to metabolise sorbitol, but also by significant changes in the many virulence factors and the absence of flagella.

Rapid diagnostics

To meet these new diagnostic challenges, the new RIDAQUICK Verotoxin/O157 Combi rapid test has been developed to satisfy the need to promptly identify both Shigatoxins and the O157 serotype. Using the new test system, the O157 antigen can be specifically identified within 20 minutes, while at the same time all human pathological Shiga toxin variants can also be identified. Only one stool-enrichment culture is needed, using mitomycin C-containing mTSB broth. Mitomycin C induces the formation of toxins from particular Shigatoxin producing *E. coli* variants (STEC), facilitating subsequent immunological detection. It is now possible to identify within just one day, the cause of a diarrhoeal illness mediated by Shigatoxin and/or O157 variants, and hence to be able to initiate appropriate measures for treatment and control.

Test evaluation

The new RIDAQUICK Verotoxin/O157 was evaluated on 159 samples, These included 51 EHEC reference strains, which represent a broad spectrum of 38 different serotypes, all known Stx subtypes (except for Stx2g produced by animal strains only), and six Stx-low producers. In addition, 96 stools, eight isolates from these stools and four animal isolates were submitted from routine clinical microbiological and consulting labs. All were investigated for the presence of Stx and O157 by the new

test. The results were compared to data derived from the genotypic detection, by PCR, of Stx encoding genes (stx) and of the *sfpA* gene specific for a subset of EHEC O157. To verify Stx production the Verocell cytotoxicity assay was additionally used.

Of the samples tested, 90.6% were correctly diagnosed for Stx production and/or serogroup O157 using the new test system. The serogroup O157 was correctly detected in 97.5% and Stx production was correctly determined in 91.8% of 146 samples compared with PCR detection and the cytotoxicity assay respectively. Sensitivity, specificity, positive and negative predictive values for Stx/O157 detections were 85.0/90.9%, 98.7/98.5%, 98.6/90.9%, and 86.7/98.5%, respectively. False negative results of Stx detection were mainly due to well known low producer strains in six isolates, to low amounts of EHEC in the stool enrichment broth (n=4) and to the presence of rare Stx subtypes (Stx2d, Stx2e) as sole Stxs (n=2). The low EHEC concentration also explained two false negative O157 results. In two further samples, false positive O157 results were detected.



Figure 3. The new RIDAQUICK Verotoxin / O157 Combi rapid test.

Summary

The new RIDAQUICK Verotoxin/O157 is a highly specific method to determine the presence of Stx and the serogroup O157 in stool enrichment cultures within 16 hours. Ongoing studies are investigating the test performance in comparison to Stx-targeting ELISA.

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