

To: Technical Group
Cc: Executive Committee

BRIEFING PAPER

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THE RELEVANCE OF GENERIC *E. COLI* TO FOOD SAFETY

Escherichia coli (*E. coli*) is a bacterium that is commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless and some provide many health benefits to the host; for example, they prevent colonization of the gut by harmful pathogens.

However, there are small groups of *E. coli*, referred to as pathogenic *E. coli*, that can cause severe disease in humans, which are well known to be transmitted by food and/or water and have been implicated in major food borne outbreaks worldwide e.g. radish sprouts in Japan, Sprouted seeds in Germany, raw / undercooked beef products UK and USA.

Like generic *E. coli*, toxin-producing Shigatoxigenic *Escherichia coli* (STEC) are Gram negative, rod-shaped bacteria, but are characterised by the production of Shiga toxins (Stx). The infective dose of the STEC *E. coli* O157:H7 is estimated to be very low, in the range of 10 to 100 cells and patients whose illness progresses to haemolytic uremic syndrome (HUS) have a high mortality rate (3-5%).

STEC is destroyed by thorough cooking of foods i.e. all parts reach a temperature of 70 °C for 2 minutes. Most available information on STEC relates to serotype O157:H7, since it is easily differentiated biochemically from other *E. coli* strains. The reservoir of this pathogen appears to be mainly cattle. In addition, other ruminants such as sheep, goats, deer are considered significant reservoirs, while other mammals (such as pigs, horses, rabbits, dogs, and cats) and birds (such as chickens and turkeys) have been found infected.

Therefore *E. coli* O157:H7 is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Faecal contamination of water and other foods, as well as cross-contamination during food preparation (with beef and other meat products, contaminated surfaces and kitchen utensils), will also lead to infection. Examples of foods implicated in outbreaks of *E. coli* O157:H7 include undercooked beefburgers, cheese made from raw milk. unpasteurised fruit juices.

There are 200+ STEC serotypes, many of which have not been implicated in human illness. *E. coli* O157:H7 is the most important STEC serotype in relation to public health; however, other non O157 serotypes have frequently been involved in sporadic cases and outbreaks e.g. O104:H4 in sprouted seeds in Germany. Pathogenic *E. coli* are generally grouped based on their virulence properties or factors that they carry. Some groups can share similar virulence traits. For instance, the production of intimin protein, which allows the pathogen to attach to intestinal cells. Also, many of the virulence genes carried by these pathogenic *E. coli* groups reside on mobile genetic elements and can be transferred. As an example, the *E. coli* strain of serotype O104:H4 that caused a large outbreak in Germany, in 2011, produced Shiga toxin, but, genetically, the strain was known to only cause persistent diarrhoea in underdeveloped countries, and not implicated in major foodborne incidents. Hence, the O104:H4 strain that caused this outbreak appeared to be a strain that had acquired the ability to produce Shiga toxin.

In the United States a group often referred to as the “big 6” (O111, O26, O121, O103, O145, and O45) accounts for the majority of the non-O157:H7 serotypes isolated from clinical infections and, therefore, is a focus of concern. The ISO TS method 13136:2012 is for the detection of 5 serotypes: O157, O111, O103, O145 and O26.

Currently, it is difficult to determine which serotypes of *E.coli* are STEC and equally challenging to predict the emergence of strains that can acquire the genes for Shiga toxin production or other virulence factors and so cause human illness.

STEC are characterized by:

- production of Stx, including Stx1 and/or Stx2. Stx1, almost identical to the toxin produced by *Shigella dysenteriae* Type I. There are many subtypes of both toxins, however, Stx2 appear to be implicated in human illness and is most often associated with severe sequelae, such as **HUS**, which is characterised by acute renal failure, and
- the ability to produce intimin, a protein that enables bacterial attachment to epithelial **cells**. There are also several other virulence factors, including enterohaemolysin, but the role of these factors in pathogenesis remains undetermined.

Fresh Produce

Sources of STEC and transmission

An increasing number of outbreaks are associated with the consumption of fruits and vegetables (including sprouts, spinach, lettuce, coleslaw, and salad) whereby contamination may be due to contact with faeces from domestic or wild animals at some stage during cultivation or handling. STEC has also been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments.

Prevention

The prevention of infection requires control measures at all stages of the food chain, from agricultural production on the farm to processing, manufacturing and preparation of foods in both commercial establishments and household kitchens.

STEC cannot be eliminated from food unless it is cooked to 70°C for 2 mins or equivalent. Therefore, any fruit and vegetables that are ready to eat (RTE) must be protected from potential sources of STEC at all stages of the food chain, from cultivation to manufacturing and preparation of foods in both commercial establishments and household kitchens.

STEC is rare and the most prevalent source is known to be cattle, however, other ruminants such as sheep, goats and deer are considered significant reservoirs. Cross contamination of RTE produce with faeces from these animals must be prevented. The approach for this is documented in CFA 'Microbiological guidance for produce suppliers to chilled food manufacturers' second edition and includes guidance on hazard, risk assessment, traceability and growing controls (seed production, field history, farmyard manure, animals and birds, water, hygiene, harvesting and handling, transport and temperature control, storage and post-harvest handling).

Monitoring food safety

As with any food safety control, validation, verification and monitoring, is required. However for most RTE chilled foods microbiological testing cannot be a guarantee of food safety, due to the volume being produced (the number of samples required to generate statistically valid data is unrealistic), short shelf life (products cannot be quarantined and results would be available when the product is on the market or even after the product has been consumed) and the many external factors that can affect produce safety i.e. country of origin, temperature, rainfall, wild animal / bird activity, water sources for irrigation etc.

Pathogens are also rare and therefore indicator organisms, which are much more widespread, are routinely used to proactively identify any increasing trends and potential increased risk. For fresh produce, the indicator organism used is generic *E.coli*, which is a process hygiene indicator and has no direct food safety implications.

To support this, EC 2073/2005 (MCR) also includes generic *E.coli* within the process hygiene criteria for pre-cut fruit and vegetables and unpasteurised fruit and vegetable juices. The ISO analytical method for this organism (ISO 16649-1 or 2) is well established, routinely used and accredited by most laboratories.

Generic *E.coli* must not be confused with STEC, which is an *E.coli* with the ability to produce Shiga toxin. STEC must not be confused with *E.coli* O157:H7 which would only be an STEC if it contained stx gene and produced shiga toxin.

It is common to isolate generic *E.coli* from fresh fruit and vegetables due to their unavoidable contact with environmental factors such as soil, water, animals etc. Monitoring the levels of *E.coli* over time (trending) can proactively identify potential increased risk, allowing for further investigation to take place which includes ensuring all controls are in place and contact with known sources of STEC (cattle, wild animals, faecal contaminated water etc) has not occurred. The level of generic *E.coli* in isolation cannot guarantee food safety or indicate a food safety risk, it is an indicator of potential change which requires investigation.

Generic *E.coli* testing can be used at all stages e.g. growing, irrigation, harvesting, chilling, storage and post-harvest to monitor levels in soil, irrigation water as well as the produce itself.

Testing for *E.coli* O157 is covered by ISO 16654:2001, however, this method will not detect Stx 1 or 2 or any other virulence factor. Therefore the isolation of *E.coli* O157 does not indicate a food safety issue either, as without Stx, *E.coli* O157 will not cause illness.

It should be noted that laboratories will challenge their methods with a non toxin producing strain of O157, which has also been implicated in reported incidents e.g. mushrooms.

See Appendix 1 for further information on method issues.

Consumers and households

Preventive measures for *E. coli* O157:H7 infection are similar to those recommended for other foodborne diseases. Basic good food hygiene practices, can prevent the transmission of pathogens responsible for many foodborne diseases, as well as protect against foodborne diseases caused by STEC.

References

1. Bad Bug Book - Foodborne Pathogenic Microorganisms and Natural Toxins - Second Edition p67:
<https://www.fda.gov/media/83271/download>
2. <https://www.who.int/news-room/fact-sheets/detail/e-coli>

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STEC Methodology based on TS 13136 2012

Explanation of method and concerns

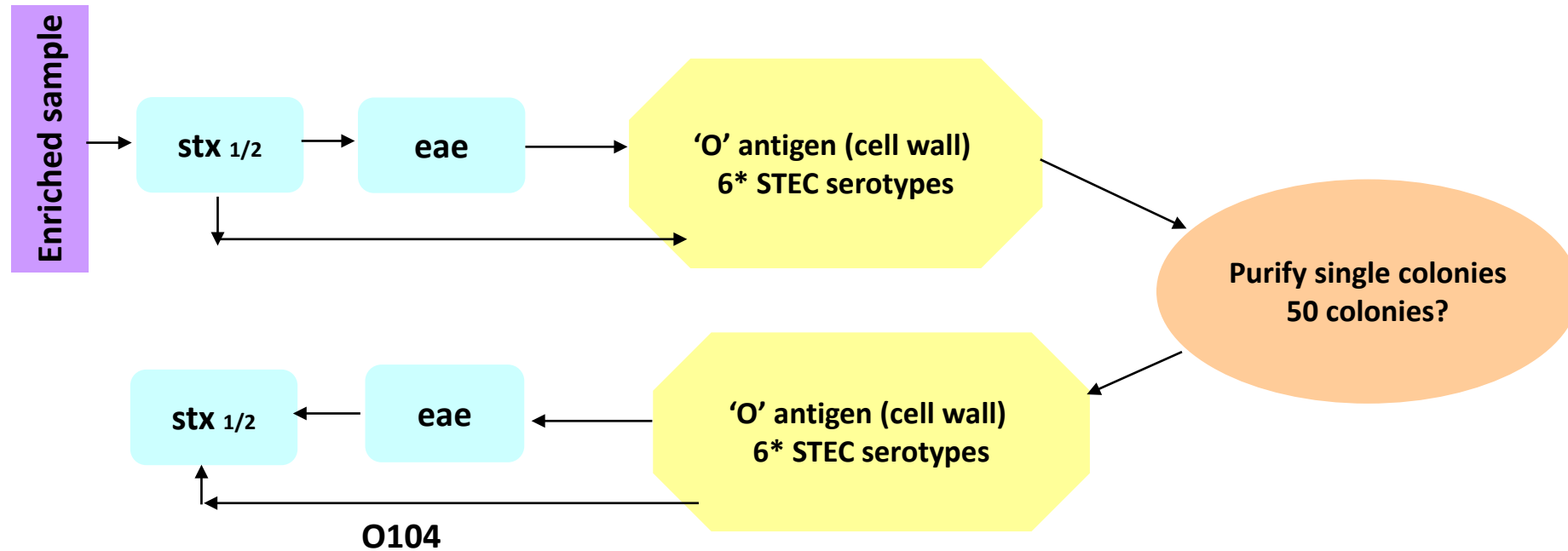
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15/12/22

Concerns surrounding STEC Testing

- Definition of pathogenic E.coli (stx + eae + serotype?)
- **Definition of** food groups 1 (STEC not eliminated) / 2 (STEC eliminated)
- Stage of method considered to be presumptive
- Actions to be taken if presumptive result reported for group 1 & 2
- Stage of method considered to be confirmed
- Actions to be taken if confirmed result reported for group 1 & 2

ISO/TS 13136:2012(en) Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

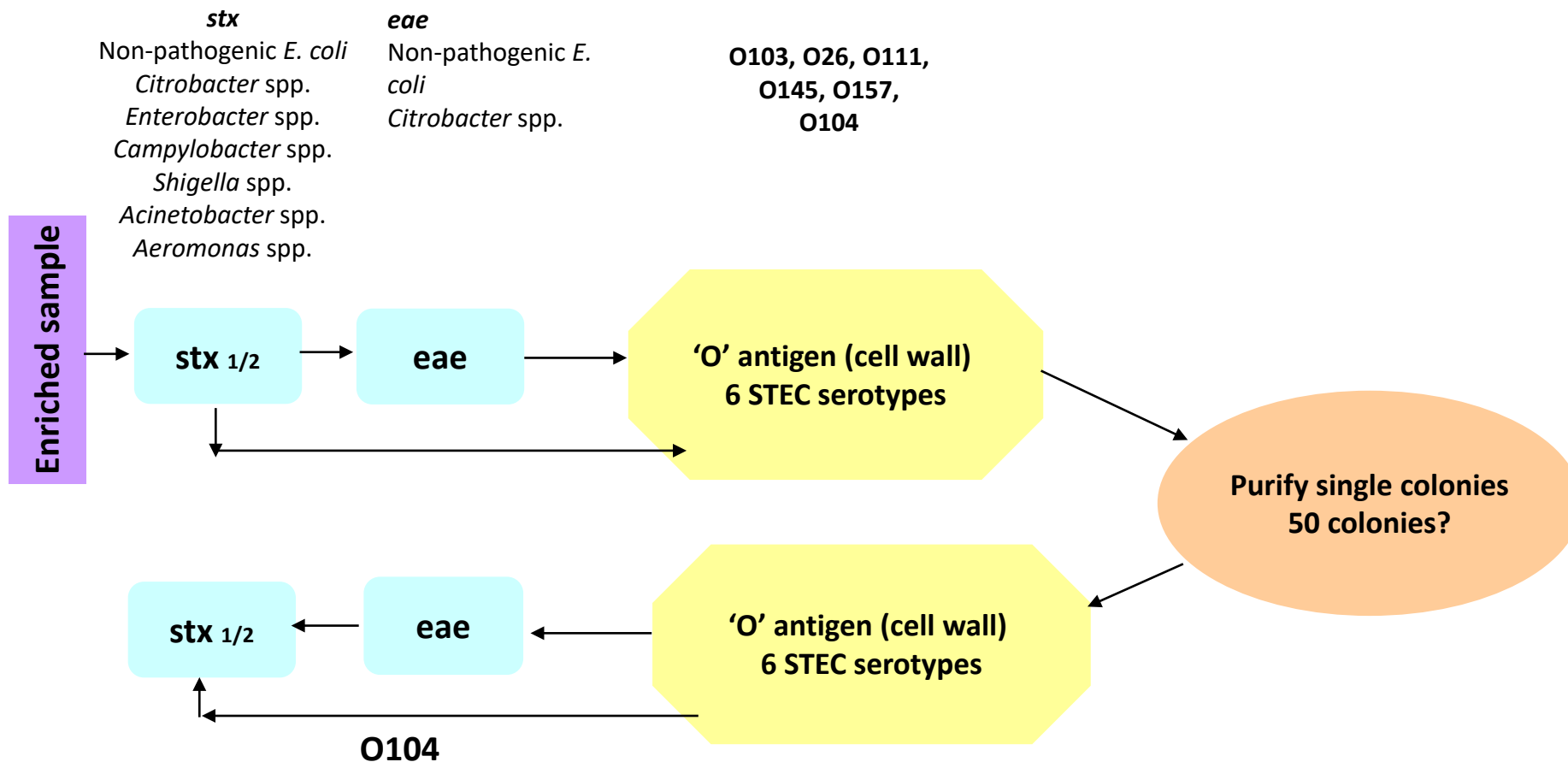


* O157, O111, O26, O103, O145 and O104

- **stx 1/2 + eae + O antigen = pSTEC (except for O104 (stx + O antigen))**
- Antigenic target = O (somatic) antigens - Typically an immunoassay-based detection
- Genetic targets = *stx*, *eae* - Typically a PCR-based detection

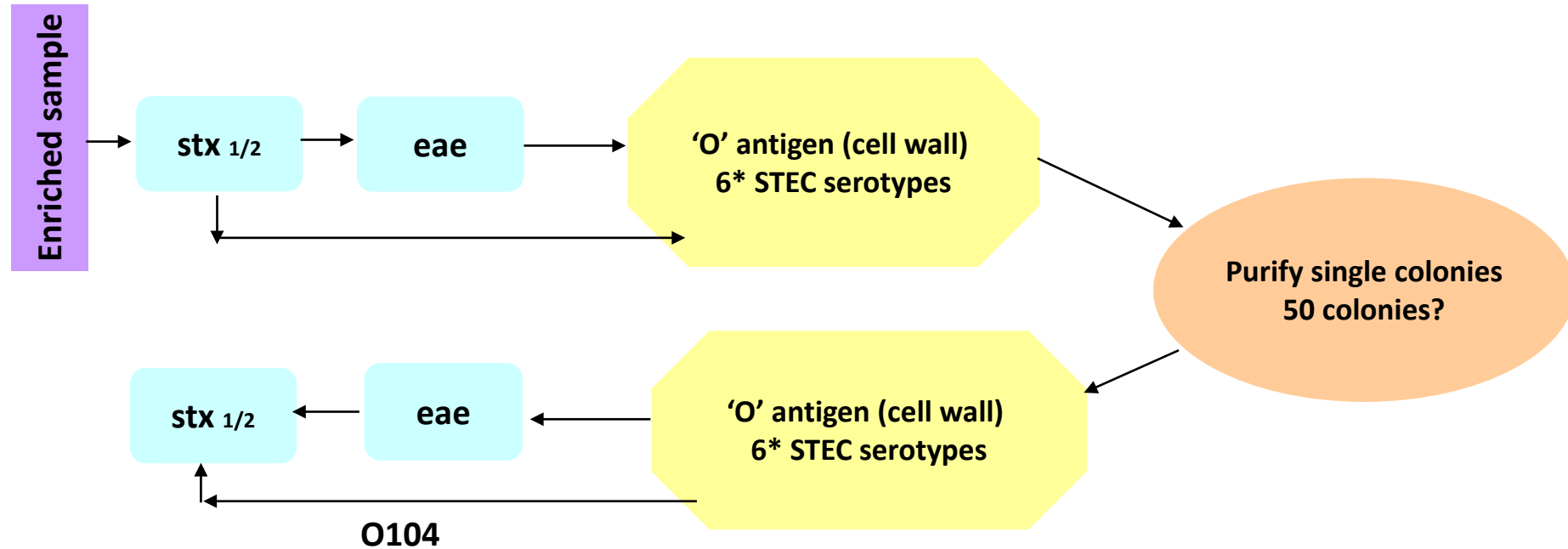
NOTE: Multiple bacteria can contribute individual genetic targets

Potential Issues



UK WORKING POLICY ON DETECTION OF STEC IN FOOD BY OFFICIAL CONTROLS AND FOOD BUSINESS OPERATOR SAMPLING AND TESTING

PRESUMPTIVE – Detection of Stx 1/2



CONFIRMED – detection of Stx ½ and eae IN one of the 6 serotypes

* O157, O111, O26, O103, O145 and O104

It is not possible to assess public health risks associated with the detection of stx gene(s) if their presence has not been confirmed in an isolated E. coli strain. The European Food Safety Authority (EFSA) opinion on 'VTEC-seropathotype and scientific criteria regarding pathogenicity assessment' 5 indicated that the detection of Shiga toxins alone, or of genes encoding for such toxins, is not a sound scientific basis for assessing the disease risk to the consumer. According to the opinion, the isolation of an STEC strain is needed to confirm the presence of stx gene(s) in addition to relevant virulence encoding genes in the same live cell rather than as free DNA or free stx phages in the enrichment culture.

HOWEVER:

FSA considers that the presence of STEC in food is confirmed when one or more of the stx genes are detected in an isolated E. coli strain.

The text is taken from the draft document

**‘UK WORKING POLICY ON DETECTION OF STEC
IN FOOD BY OFFICIAL CONTROLS AND FOOD
BUSINESS OPERATOR SAMPLING AND
TESTING’ 2016**

This provides some examples of the concerns
regarding testing food products for STEC
E.coli.

1. the definitions given for a presumptive
test result AND
2. The actions required to be taken by the
LA / FBO in the event of a presumptive
result.

**TABLE 1: SUMMARY OF ACTION REQUIRED IN RESPONSE TO UNSATISFACTORY TEST
RESULTS FROM BOTH OFFICIAL CONTROLS AND FBO SAMPLING AND TESTING**

	Presumptive test result	Confirmed test result
Definition	Detection of one or more <i>stx</i> gene(s) is considered a presumptive positive result if their presence has not been confirmed in an isolated <i>E. coli</i> strain	Presence of STEC is confirmed when one or more <i>stx</i> gene(s) are detected in an isolated <i>E.coli</i> strain.
Food Profile 1	Action required by LA/FBO	Action required by LA/FBO
Ready-to-eat (RTE) foods; Foods to be consumed with a mild heat treatment unlikely to remove the STEC risk And Food for which an FBO is not able to provide guarantees that a treatment that will remove the STEC risk will be applied	<p>The Competent Authority should be notified through incident reporting procedures.</p> <p>FBOs should prepare to take immediate action in readiness for a confirmed test result:</p> <ul style="list-style-type: none"> • Retain affected batch(s) still within FBO control; • Collate traceability information; • Notify onward supply chain and request retention of affected product still within their control; <p>If presumptive results are not confirmed, held product can be released on to the market with no further action.</p> <p>There may be instances where a withdrawal or recall may be required by the competent authorities on the basis of detection of <i>stx</i> genes alone, if, for example, there is epidemiological or other information that links the food to cases of illness.</p>	<p>The Competent Authority should be notified through incident reporting procedures.</p> <p>The affected batch(s) of food must be withdrawn from the market in accordance with Regulation (EC) 178/2002. Information on onward supply of the product will be required to determine whether a product recall from end users/ consumers is required.</p> <p>Investigations should be initiated by the FBO to identify and eliminate the source of STEC contamination and any other batches or products affected.</p> <p>The FBO should review the HACCP-based food safety management system, to ensure that STEC is identified as a specific hazard and that the risk from STEC in food is minimised.</p>

In Conclusion

- Positive E.coli O157 result alone may not cause illness
- The isolation of **an** stx gene **must** be **confirmed IN an E.coli strain**, usually with a gene for **attachment to enable** the isolate to cause serious illness
- Agree with the confirmed stage of the method
- FSA decision to call isolation of stx alone as presumptive is **extremely** misleading
- Action required in the event of a presumptive result is inappropriate and **impractical for** industry
- Food safety can only be controlled by routinely testing for indicator organisms e.g. generic E.coli and monitoring trends to proactively prompt investigation.
- HACCP & PRPs are paramount, supported by environmental and product monitoring.