GUIDANCE FOR INTERPRETATION OF PCR ASSAYS FOR VEROCYTOTOXIGENIC

E COLI: FOR HEALTH PROTECTION TEAMS AND MICROBIOLOGISTS

1. Purpose of this document

The purpose of this document is to provide broad recommendations for clinical and public health action and risk assessment when PCR assays on faecal samples yield positive results for Verocytotoxigenic *E coli* (VTEC).

2. Introduction

Molecular diagnostic methods are becoming more widely available. PHE and NHS laboratories in England have developed in- house multiplex assays for detecting the major bacterial, parasitic and viral pathogens causing gastro-intestinal illness, and commercial systems are also available and in use in a small number of laboratories. In Scotland, diagnostic Virology laboratories routinely use molecular methodologies for detection of GI pathogens, and commercial platforms which detect bacterial and protozoal pathogens are currently being evaluated. Molecular detection of VTEC, including non- O157 serogroups, is currently only performed routinely in the Scottish *E coli* O157/VTEC Reference Laboratory.

The Scottish Government VTEC/ *E coli* O157 Action Plan for Scotland 2013- 2017 includes a recommendation on rapid microbiological confirmation of non- O157 VTEC infection. There is currently no convenient culture method available for non- O157 VTEC, and rapid screening by molecular methodologies is the best diagnostic option. In preparation for the introduction of PCR assays for GI pathogens including VTEC into diagnostic laboratory use, we have attempted to answer questions that may arise in relation to appropriate clinical and public health response to positive PCR results, while culture confirmation is in progress. We have also addressed issues relating to the processing of clearance samples.

3. VTEC: background

The term VTEC refers to all strains of *E coli* that produce verocytotoxin (VT) or possess VT genes. VTEC of serogroup O157 are the most common in the UK and are the only VTEC for which routine standard tests are performed in diagnostic laboratories. However the number of non- O157 VTEC detected at the SERL has risen steadily in recent years and these are now identified in approximately one quarter of all laboratory confirmed infections (REF: SERL Annual Report 2013/14).

4. Diagnostic laboratories: Investigation of acute diarrhoea

Faecal specimens should always be investigated from persons with acute diarrhoea (whether bloody or not) who:

- Are especially vulnerable;
- Have severe or protracted illness;
- May be part of an outbreak or household/extended family spread;
- Have recently returned from abroad;
- Have had a biologically plausible exposure.

In addition, if acute diarrhoea is bloody and there is no other explanation, VTEC infection should be suspected and faeces sent to the diagnostic laboratory for culture as quickly as possible.

Investigation for VTEC infection should be carried out on all diarrhoeal samples received by diagnostic Microbiology laboratories.

Microbiological tests currently carried out by diagnostic labs to identify *E coli* O157

Most diagnostic laboratories will carry out a morphological identification, a slide agglutination (or latex kit) test, and biochemical tests to identify the organism. When all of these three types of procedures have been conducted and are positive, their result may be referred to in laboratory terms as a **presumptive (locally confirmed) isolate** i.e. an isolate that satisfies all the following conditions:

- Typical colonial morphology on appropriate selective medium
- Positive O157 agglutination (latex kit or slide agglutination with antiserum)
- Biochemical identification of *E coli .*

Identification of a presumptive (locally-confirmed) isolate of VTEC O157 or other VTEC should be notified immediately **to the patient's responsible clinician and the local HPT.**

Presumptive cases require further laboratory testing or epidemiological assessment.

5. Current guidance on isolate and faecal sample referral (Health Protection Network, HPS http://www.documents.hps.scot.nhs.uk/about-hps/hpn/vtec.pdf)

Referral to SERL

Isolates

Diagnostic laboratories should immediately refer to the SERL **isolates** of:

- *E. coli* O157 for confirmation of identity, verocytotoxin gene detection and typing;
- *E. coli* with negative O157 agglutination tests if there is a strong clinical suspicion of severe VTEC infection (by arrangement with the SERL Consultant Microbiologist or Clinical Scientist).

Faecal samples

Diagnostic laboratories should immediately refer to SERL **faecal samples** from:

- Cases of suspected HUS/ TMA, or bloody diarrhoea in whom conventional laboratory testing has failed to yield a pathogen;
- All symptomatic contacts of cases of VTEC infection or any VTEC outbreak-associated case in whom conventional laboratory testing has failed to yield a pathogen;
- All asymptomatic contacts of cases of infection with sorbitol fermenting (SF) verocytotoxin gene positive *E coli* O157.

Serum samples

Diagnostic laboratories should immediately refer to SERL **serum samples** from likely cases of *E. coli* O157 infection who:

- Have severe clinical infection **and**
- Have either failed to provide a faecal sample or faeces is VTEC culture negative.

6. PCR testing in local diagnostic laboratories

Some local laboratories are now undertaking PCR testing for *E coli* O157 and non-O157 VTEC.

Gastro-intestinal multiplex PCR tests are designed to be used as preliminary screening tests. A positive result should always be confirmed by culture and referral of the isolate or, if culture is unsuccessful, referral of the faecal sample to the Reference Laboratory. It is therefore important that culture facilities are retained by diagnostic laboratories when molecular screening tests are introduced.

Clinical and public health information on the case should be used to make a judgement on the clinical significance of the result, pending culture confirmation. Advice can be obtained from the Consultant Microbiologist or Principal Scientist at the SERL.

If a validated PCR test is reported as positive for verocytotoxin genes or *E coli* O157, we recommend that it is used by front line workers to initiate a public health response and control strategy.

In certain circumstances e g HUS cases, it may be necessary to refer samples negative by local PCR to the SERL, as some VTEC strains are not detected by currently available commercial platforms. Please discuss with the SERL Consultant Microbiologist or scientific staff.

a. Use of PCR assays for primary clinical diagnosis

Laboratories using PCR methods for primary diagnosis of VTEC infection should report all positive results to the local HPT and must make it clear that the result was obtained by a PCR test. Where possible laboratories should attempt local confirmation of the result by culturing the sample for *E coli* O157 and if positive sending the isolate to the reference laboratory, as normal, for confirmation and typing. Faecal samples positive by PCR but negative on culture should be sent to the SERL for PCR confirmation and culture of VTEC including non- O157 strains (see Table 1)

In all cases it is important to assess the clinical significance of the positive PCR result by discussing the case with the patient's physician.

In the absence of a confirmatory culture or reference laboratory test, the significance of the multiplex PCR test result is assessed on a case by case basis depending on individual circumstances. The presence of the nucleic acid of the organism does not always equate to presence of live organisms in the faecal specimen. Treatment with antimicrobial agents may kill the organism (in case of bacterial / parasitic pathogens), however the DNA may still be detectable.

Advice can be obtained from the Consultant Microbiologist or Principal Scientist at the SERL

b. Current guidance for microbiological clearance (Health Protection Network, HPS http://www.documents.hps.scot.nhs.uk/about-hps/hpn/vtec.pdf)

Microbiological clearance for:

- NSF *E.coli* O157 with or without verotoxin genes is confirmed by conventional laboratory testing (culture) at the local diagnostic laboratory;
- SF *E.coli* O157 with or without verotoxin genes is confirmed by PCR for detection of *vtx*1, *vtx*2, and *rfb*O157 genes;
- Non-O157 VTEC is confirmed by PCR for detection of *vtx*1 and *vtx*2 genes.

In the case of SF and non-O157 VTEC, microbiological clearance must be confirmed by the SERL, which does not routinely culture the organism on clearance samples, but reports samples as positive or negative on the basis of interpretation of a PCR test carried out without enrichment. In exceptional circumstances e g prolonged PCR positivity, advice may be obtained from the SERL and it may be helpful to re- culture the sample to inform a risk assessment on the need for continued exclusion.

c. Use of local PCR tests for microbiological clearance when the isolate has not been cultured by the diagnostic laboratory

Discuss with the Consultant Microbiologist or Principal Scientist at the SERL (see Table 1)

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Guidance for the interpretation of PCR assays for Gastrointestinal Pathogens: Health Protection Agency, 2013

Table 1: Actions for diagnostic laboratory when a PCR carried out locally is positive

GI pathogen	Local confirmation following positive PCR	Reference Laboratory confirmation, typing etc	Clearance Specimens (when indicated)	Screening of asymptomatic contacts (where indicated)	HPT action on PCR positive specimens
Verotoxigenic *E coli* O157	Culture for *E coli* O157	Send *E coli* O157 isolate for confirmation and typing	Local culture for *E coli* O157 (except SF strains- discuss with the SERL)	Local culture for *E coli* O157 (except SF strains- discuss with the SERL)	Discuss with microbiologist
Verotoxigenic *E coli* Non- O157 serogroups	None	Send faecal specimen to the SERL	Discuss with the SERL	PCR and culture for non-O157 VTEC (at the SERL)	Discuss with microbiologist