

Standardisation of VTEC Detection & Typing in the UK

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Public Health England

Definition of Service

SERL

- Confirm and type isolates of *E.coli* O157, and other VTEC as appropriate, in order to supply detailed information for the management of human infection throughout Scotland.
- Detection, isolation and typing of VTEC in high-risk faeces.

GBRU

- Confirm and type (phage type and MLVA) isolates of *E.coli* O157, and other VTEC as appropriate, in order to supply detailed information for surveillance and outbreak investigation
- Detection, isolation and typing of VTEC from highrisk & PCR positive faeces

Facts & Figures

SERL

- ALL frontline Scottish clinical laboratories submit samples to SERL - no charge.
- ~5.3 million population
- ~235 cases *E. coli* O157

GBRU

 ALL frontline English clinical laboratories submit samples to GBRU - no charge.

- ~53 million population
- ~900 cases *E. coli* O157 per year

Incidence Rates

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Current Methodology (1)

	SERL		GBRU	
RT-PCR: O157 isolate	~	One reaction: vtx_1 , vtx_2 , vtx_{2f} , rfb_{O157} , IAC	~	One reaction: vtx_1 , vtx_2 , rfb_{O157} , IAC
Faecal extraction	~	Enrich & Instagene™	~	 Enrich & Instagene[™] Direct Qiasymphony
RT-PCR: faeces	~	VTEC only One reaction: <i>vtx</i> ₁ , <i>vtx</i> ₂ , <i>vtx</i> _{2f} , <i>rfb</i> _{O157} , IAC	~	VTEC : vtx_1 , vtx_2 , rfb_{O157} , IAC and/or EPEC (+ vtx_{2f}), EIEC and EAEC on request
AST	~	Disk diffusion 15 antibiotics	×	No R-typing
Serotyping	~	Send to GBRU Real-time PCR: O26, O103, O111, O145, eae	~	Full range of phenotypic serotyping

Current Methodology (2)

	SERL		GBRU	
Phage typing	~	Ahmed <i>et al</i> , 1987	\checkmark	Ahmed <i>et al</i> , 1987
MLVA	~	All O157	\checkmark	All O157
PFGE	~	Non-O157	×	Service discontinued
NGS	×	Not yet in routine use	~	Non-O157 and national outbreaks

Standardisation is crucial

Standardisation of MLVA

- Identify and address standardisation issues between data produced at SERL and GBRU
- 67 isolates chosen to represent frequentlyoccurring UK phage types (21/28, 8, 2) together with representatives of less common types (14, 32, 34), which reflected, as far as possible, all of the tandem repeat copy numbers for each of the eight alleles in the MLVA scheme.

MLVA Loci

Reaction 1	O157-3	0157-34	O157-9	0157-25
	ECS ₂₇₁	ECS ₅₃₃₁	ECS ₃₄₉₀	ECS ₁₅₂₀
Reaction 2	0157-17	0157-19	O157-36	O157-37
	ECS ₅₄₂₆	ECS ₂₈₆₂	pO ₁₅₇₅₄	pO ₁₅₇₄₆

Hyytiä-Trees E, Smole SC, Fields PA, Swaminathan B, Ribot E. Foodborne Path Dis. 2006;3(1):118-31.

Nadon CA, Trees E, Ng LK, Møller Nielsen E, Reimer A, Maxwell N, Kubota KA, Gerner-Smidt P, the MLVA Harmonization Working Group. Euro Surveill. 2013;18(35):pii=20565.

Differences between MLVA protocols

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Allele Assignment

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Quality Assurance & Proficiency Testing

 Phage typing – each exchange a panel of 6 isolates per year

MLVA – use above panel for MLVA

Welcome the opportunity to standardise MLVA with our European colleagues

WGS

SERL

 Ion Torrent Project: 100x
 E. coli O157 from local region spanning a five year period.

GBRU

- Illumina GAII, MiSeq & HiSeq
- >800 genomes from UK
- Including ~100 Scottish human isolates and 100 Scottish cattle isolates

2014 – 2017: To determine the phylogenomics of human and cattle EHEC O157 and the strain genotypes associated with super-shedding and human disease.

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