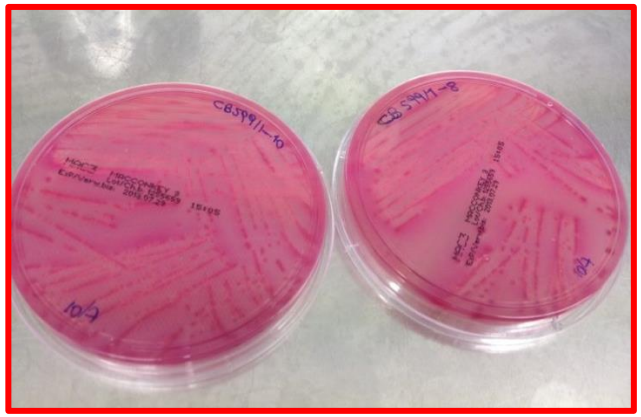
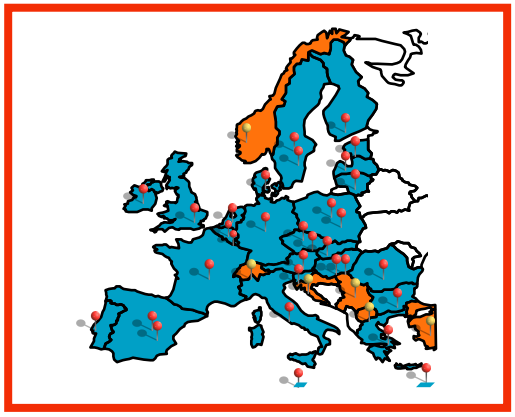
PT26

Identification and typing of Shiga toxin-producing *E. coli* (STEC)





The objectives of the study were:

- The detection of the main STEC virulence genes (*eae* and *stx* genes)
- The identification of a range of relevant STEC serogroups
- The subtyping of Shiga Toxins (Stx)-coding genes
- The identification of clusters of isolates based on genomic analysis (PFGE or WGS)

PT26: Design of the study (I)

1. Identification of the shiga toxin-producing *E. coli* main virulence genes by PCR amplification:

   ***stx1* type**, ***stx2* type** and the **intimin-coding *eae* gene**

2. Identification of **13 target O serogroups**:

   O26, O103, O111, O145, O157 ("top 5")
   O45 and **O121** (considered as adulterants in beef in the USA)
   O104 (relevant after the 2011 outbreak)
   O55, **O91**, **O113**, **O128**, **O146** (prevalent in human infections in Europe according to the ECDC data)

3. Subtyping of *stx* genes:

   stx1a, ***stx1c*** and ***stx1d***
   from ***stx2a* to *stx2g***

PT26: Design of the study (II)

Seven *E. coli* strains to be typed

Strain	Serotype	ST	Target virulence genes (*stx* subtypes)		
			stx1	***stx2***	***eae***
1	O121:H19	655	-	*stx2a*	+
2	O121:H19	655	-	*stx2a*	+
3	O128ab:H2	25	*stx1c*	*stx2b*	-
4	O91:H14	33	*stx1a*	*stx2b*	-
5	O55:H7	335	-	*stx2a*	+
6	O121:H19	655	-	*stx2a*	+
7	O145:H28	137	*stx1a*	-	+

Virulence genes detected by WGS-based virulotyping

Strain	Virulence genes
1	*cba, cma, eae, efa1, ehxA, espA, espB, espF, espI, espJ, espP, lpfA, nleA, nleB, nleC, stx2a, tir, toxB*
2	*cba, cma, eae, efa1, ehxA, espA, espB, espF, espI, espJ, espP, lpfA, nleA, nleB, nleC, stx2a, tir, toxB*
3	*ehxA, espI, iha, ireA, lpfA, mchB, mchC, mchF, stx1c, stx2b, subA*
4	*ehxA, espI, iha, lpfA, mchB, mchC, mchF, mcmA, senB, stx1a, stx2b, subA, tia*
5	*astA, efa1, espA, espB, espJ, etpD, nleA, nleB, nleC, stx2a, tir*
6	*efa1, ehxA, espA, espB, espI, espJ, espP, lpfA, nleA, nleB, nleC, stx2a, tir, toxB*
7	*celB, cif, efa1, ehxA, espA, espB, espJ, iha, nleA, nleB, nleC, stx1a, tir*

PT26: Participants

40 NRLs representing
27 EU MS

+ the NRLs of

Argentina

Chile

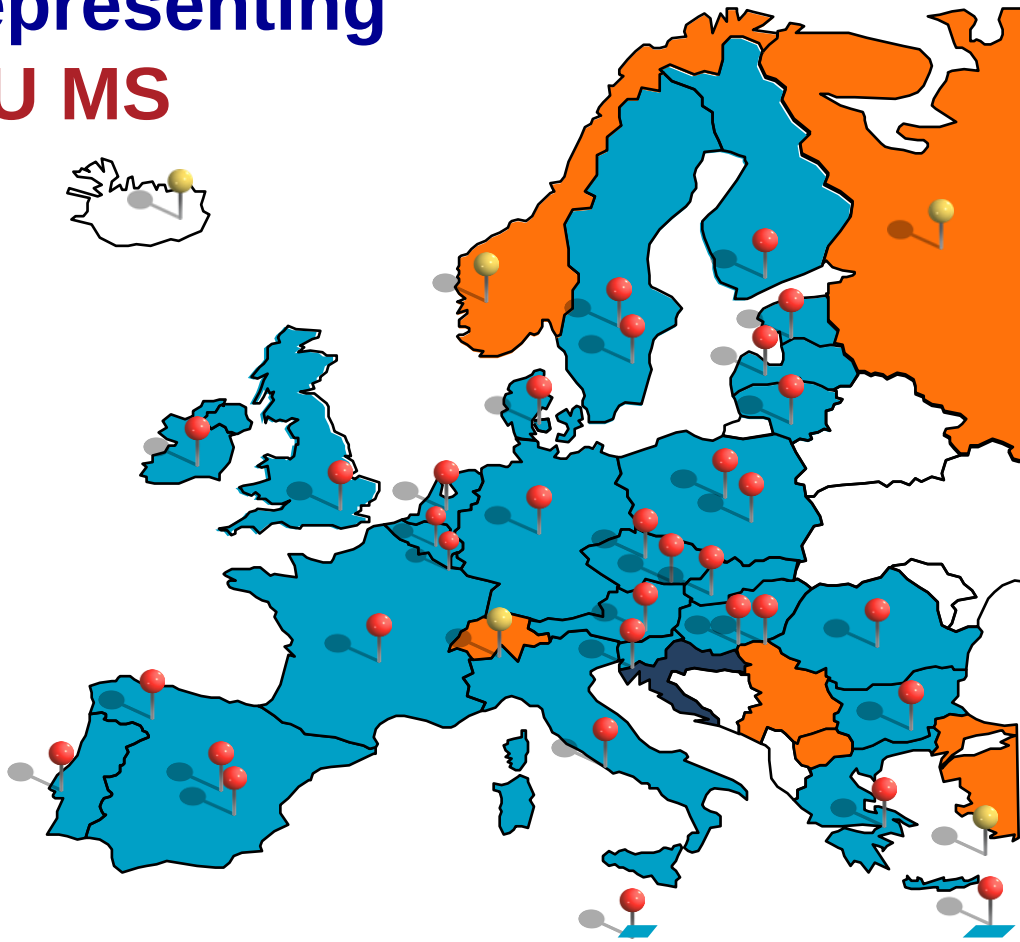
Iceland

Norway

Russia

Switzerland

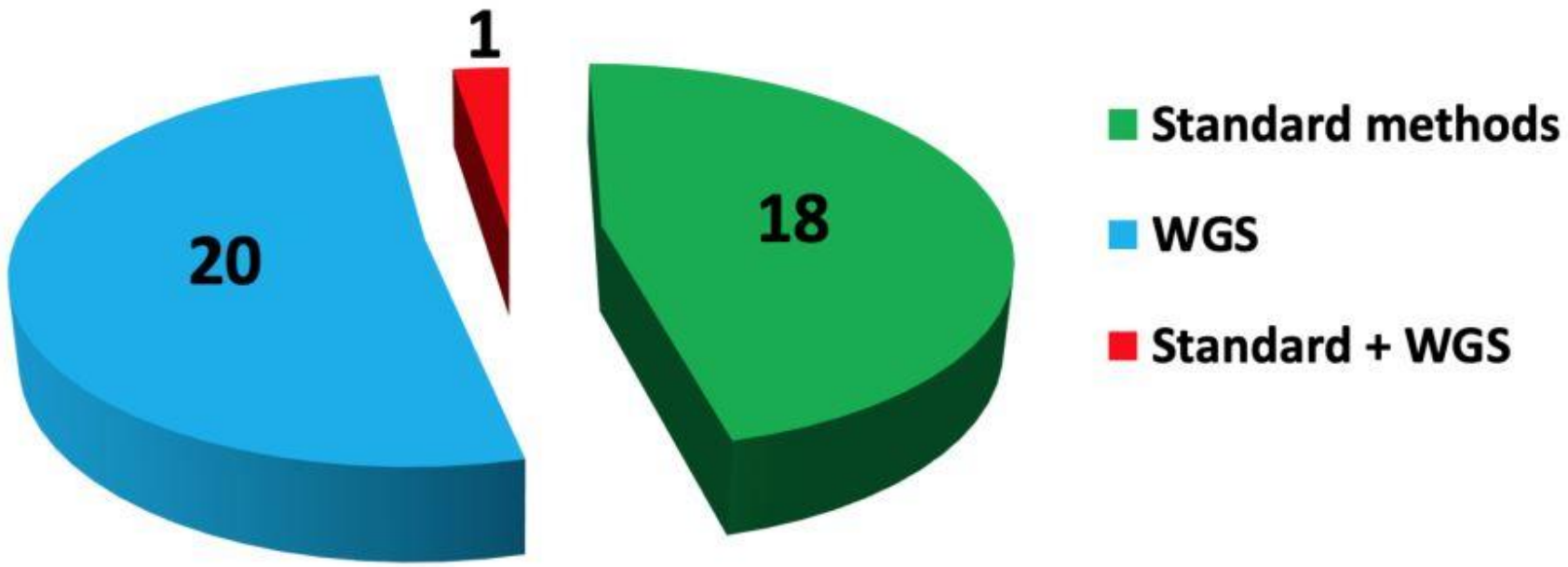
Uruguay



PT26: Samples

- ✓ 7 test strains as cultures in soft-agar
- ✓ Upon request, the needed control strains have been provided
- ✓ Test Samples were prepared on the 5th October 2019
- ✓ 8th October 2019, the homogeneity test was performed on a set of 5 randomly selected samples
- ✓ Samples labelled with randomly generated numerical codes shipped on the 14th October 2019
- ✓ Results submitted on-line via the web site from 39 NRLs

Number of laboratories reporting results/methods



Penalty Points for the identification of STEC virulence genes and serogroups

- **4 penalty points** to each incorrect or missing result concerning the identification of the *stx* genes
- **2 penalty points** to each incorrect or missing result concerning the identification of *eae* gene
- **2 penalty points** to each incorrect result concerning the identification of serogroups
- **1 penalty point** when the results of the serogroup identification were not uploaded ("null" field) or reported as "Not Done"
- **1 penalty point** to each incorrect result concerning the identification of the *stx* genes subtypes (not considered for the assessment of the laboratories' proficiency)

A threshold of 4 penalty points was set in order to identify the under-performant laboratories

PT26 – Results: Detection of virulence genes (I)

STANDARD TYPING METHOD

NRL	Detection of virulence genes in:																				
	Strain 1			Strain 2			Strain 3			Strain 4			Strain 5			Strain 6			Strain 7		
	stx1	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*
True value	-	+	+	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	-	+
L136																					
L187																					
L258																					
L295																					
L337																					
L355																					
L375																					
L413																					
L417																					
L443												+									
L543																					
L546																					
L556								-			-										
L676																					
L693																					
L775																					
L893																					
L925																					
L986																					

PT26 – Results: Detection of virulence genes (II)

WGS

NRL	Detection of virulence genes in:																				
	Strain 1			Strain 2			Strain 3			Strain 4			Strain 5			Strain 6			Strain 7		
	stx1	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*	*stx1*	*stx2*	*eae*
True value	-	+	+	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	-	+
L175																					
L203																					
L229															-						
L286																					
L376																					
L424																					
L513																					
L519																					
L537																					
L543																					
L597																					
L734																					
L737																					
L791																					
L810																					
L825		-			-										-		-	-			
L840																					
L843																					
L912																					
L967																					
L969																					

PT26 – Results: Identification of the serogroups (I)

NRL	Serogroup identification in Strain:						
	1	2	3	4	5	6	7
True value	O121	O121	O128	O91	O55	O121	O145
L136							
L187			O NT				
L258							
L295							
L337				O146			
L355	ONT					ONT	
L375		O103					
L413							
L417			ONT	ONT			
L443							
L543							
L546							
L556			ONT	ONT			
L676							
L693							
L775							
L893							
L925							
L986				ONT			

STANDARD METHODS

PT26 – Results: Identification of the serogroups (I)

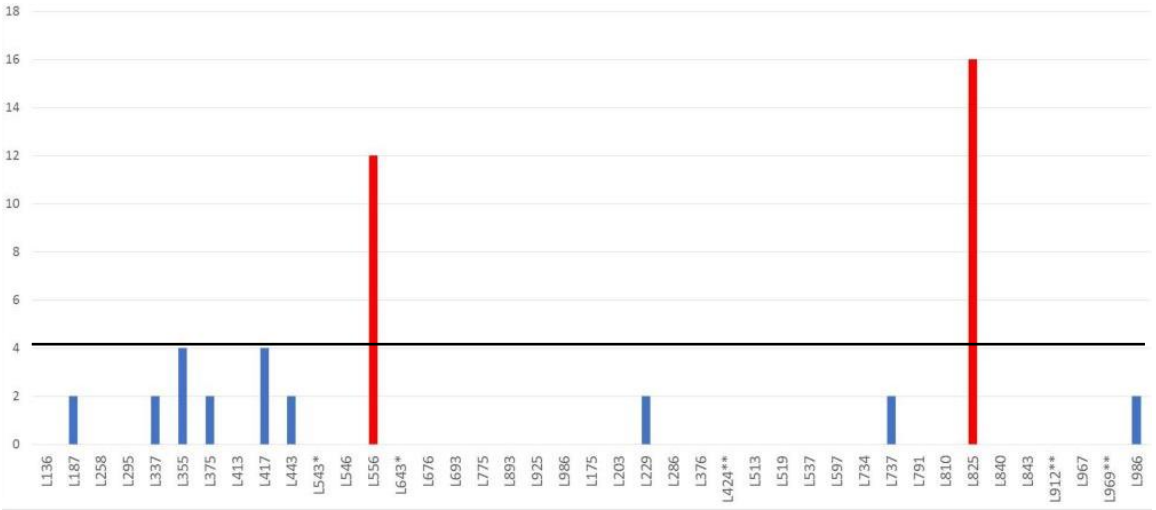
NRL	Serogroup / Serotype identification in Strain:													
	1		2		3		4		5		6		7	
True value	O121:H19		O121:H19		O128ab:H2		O91:H14		O55:H7		O121:H19		O145:H28	
L175														
L203														
L229														
L286														
L376														
L424														
L513														
L519														
L537														
L543														
L597														
L734														
L737					ONT									
L791														
L810														
L825														
L840														
L843														
L912														
L967														
L969														

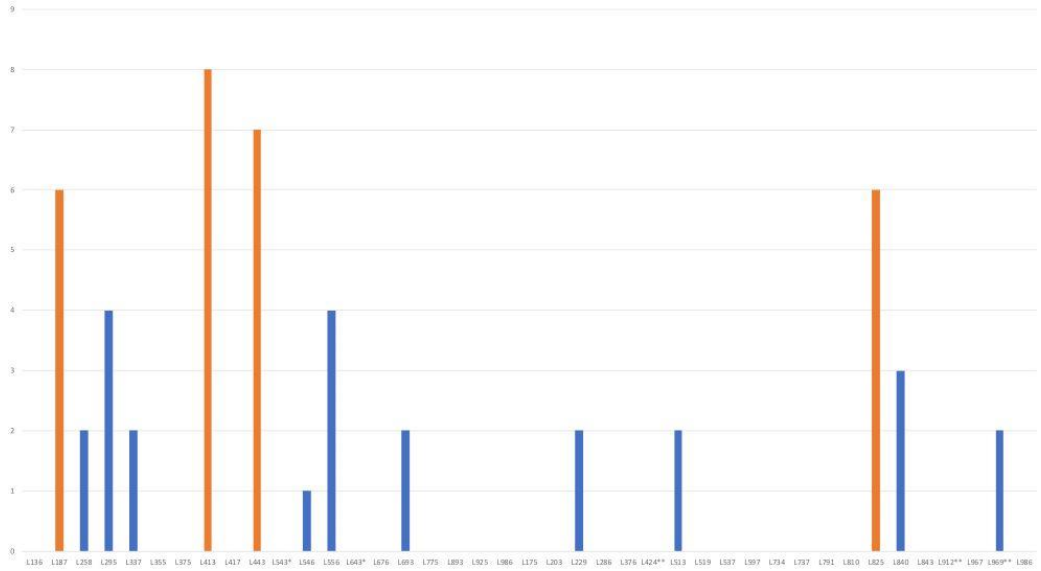
IDENTIFIED BY WGS

Evaluation of the laboratories' performance

Identification of *stx*, *eae* genes, top-13 serogroups

The red bars indicate the NRLs whose performance was considered as not satisfactory





Evaluation of results for detection of the *stx* genes subtypes by NRL

The orange bars indicate the laboratories accumulating a number of penalties over the threshold of four

PT26: Concluding Remarks

- ✓ 40 NRLs representing the 27 EU countries, as well as Argentina, Chile, Iceland, Norway, Russia, Switzerland and Uruguay taking part in the study
- ✓ Almost half of the laboratories **performed WGS with excellent performance in both the characterization and subtyping of the STEC isolates**
- ✓ Two laboratories underperformed in the characterization of STEC strains (detection of *stx*, *eae* and serogroups); some areas of improvement have been identified and will be managed
- ✓ Most of the laboratories submitted results for the *stx* subtyping, indicating that this assay is becoming widely adopted among the network of NRLs for *E. coli*, with a good performance on average. Apart from known criticalities of the typing method (e.g. discrimination between *stx2a* and *stx2c* genes in the PCR assay) the **network responded well to the stx subtyping exercise** (particularly through **WGS**)

Cluster Analysis

Voluntary exercise: performing cluster analysis on the 7 test strains

Methods:

- PFGE

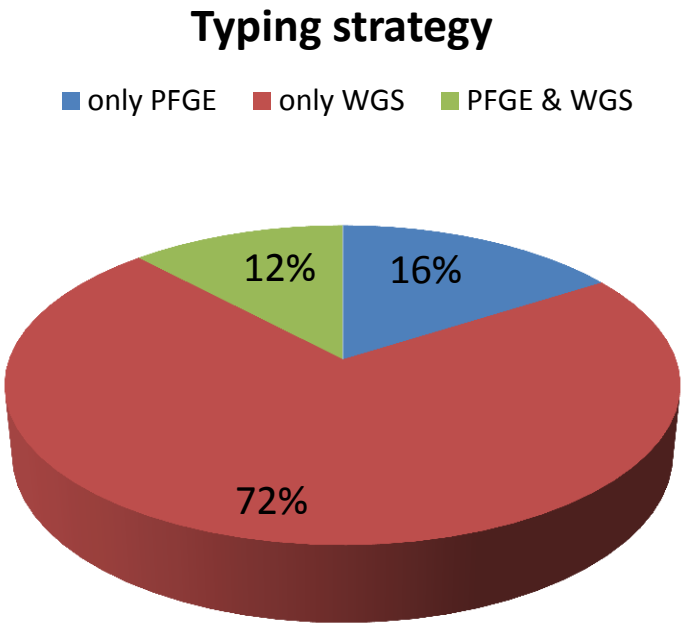
Provide the number of **total bands observed** *per* strain and the **number bands shared between each test strain and one of the isolates of the panel chosen as reference** by the laboratory.

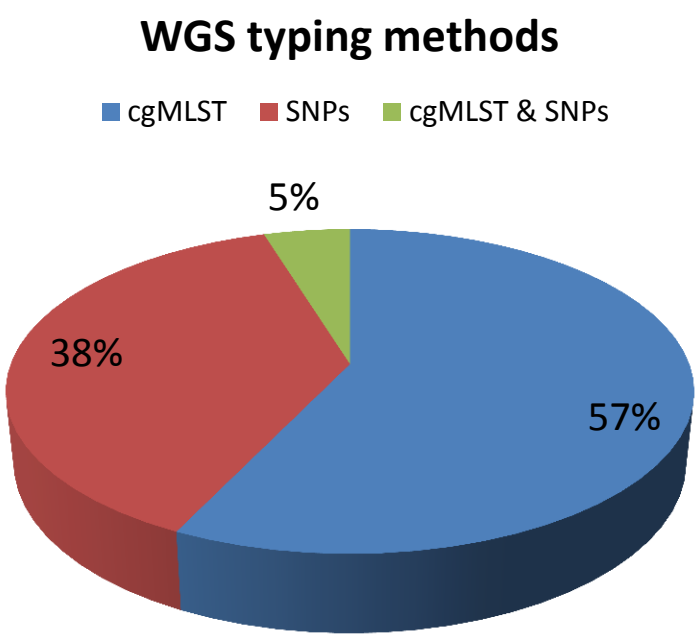
- WGS-based: SNPs/wg/cgMLST

Submit the **number of SNPs or allelic differences** observed between each strain and one test strain selected as reference

No gel image, .xml, .fastq or .fasta files submitted
Interpretation of the cluster performed by the NRLs

25 NRLs partecipated in this exercise





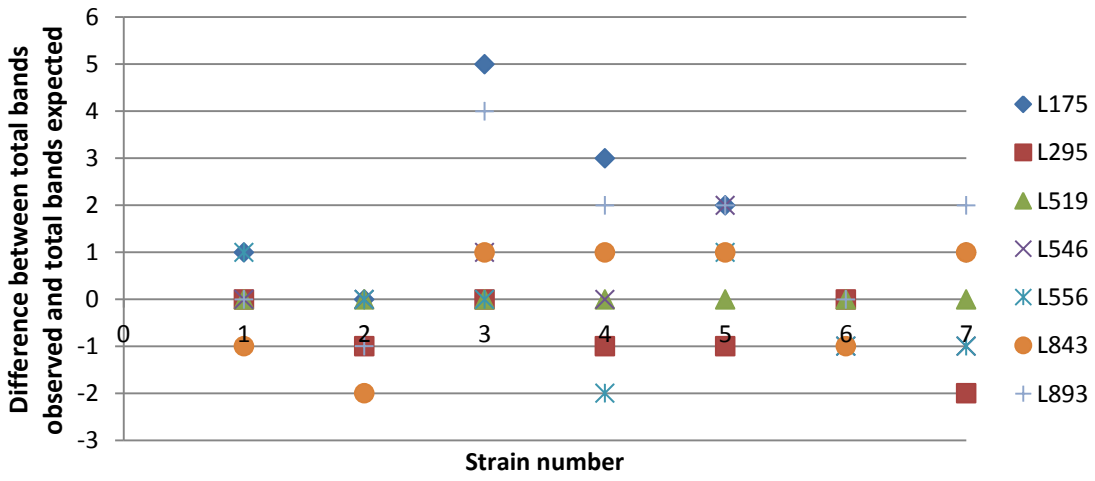
7/25 NRLs performed PFGE

21/25 NRLs performed WGS-based typing

PFGE-based typing results

NRL	Strain number							Different bands between strains 1 and 2
	1	2	3	4	5	6	7	
N. of expected bands	**18**	**19**	**15**	**18**	**14**	**19**	**16**	**≤2**
L175	19	19	20	21	16	19	17	0
L295	18	18	15	17	13	19	14	0
L519	18	19	15	18	14	19	16	2
L546	18	19	16	18	16	18	15	2
L556	19	19	15	16	15	18	15	0
L843	17	17	16	19	15	18	17	0
L893	18	18	19	20	16	19	18	0

Distribution of bands assigned in PFGE



WGS-based typing results

Strategy	Tool details	NRL	Strain number						
			1	2	3	4	5	6	7
		Clustering strains	Yes	Yes	No	No	No	No	No
Alleles-based, cgMLST	Enterobase 09-12-2019	L175							
	chewBBACA	L286							
	SeqSphere+, Enterobase 2513 loci	L424							
	SeqSphere+, Enterobase 2513 loci	L513							
	chewBBACA, INNUENDO 2360 loci	L519							
	SeqSphere+, Enterobase 2531 loci	L537							
	in house, SeqSphere, 1734 loci	L597							
	chewBBACA, INNUENDO 2360 loci	L676							
	SeqSphere+	L734							
	chewBBACA	L737							
	chewBBACA, INNUENDO 2360 loci	L791_1							
	SeqSphere+ v.1 scheme	L791_2							
	CGE cgMLST Finder v1.1, Enterobase scheme 2513 loci	L840_1							
	Enterobase	L912							
SNPs	CGE NDtree	L229							
	in house	L258							
	CGE CSI Phylogeny 1.4	L376							
	CGE CSI Phylogeny 1.4	L413							
	CGE CSI Phylogeny 1.4	L810							
	CGE CSI Phylogeny 1.4	L840_2							
	FDA SNP Pipeline	L843							
	Snippy 4.4.5	L967							
	in house	L969							

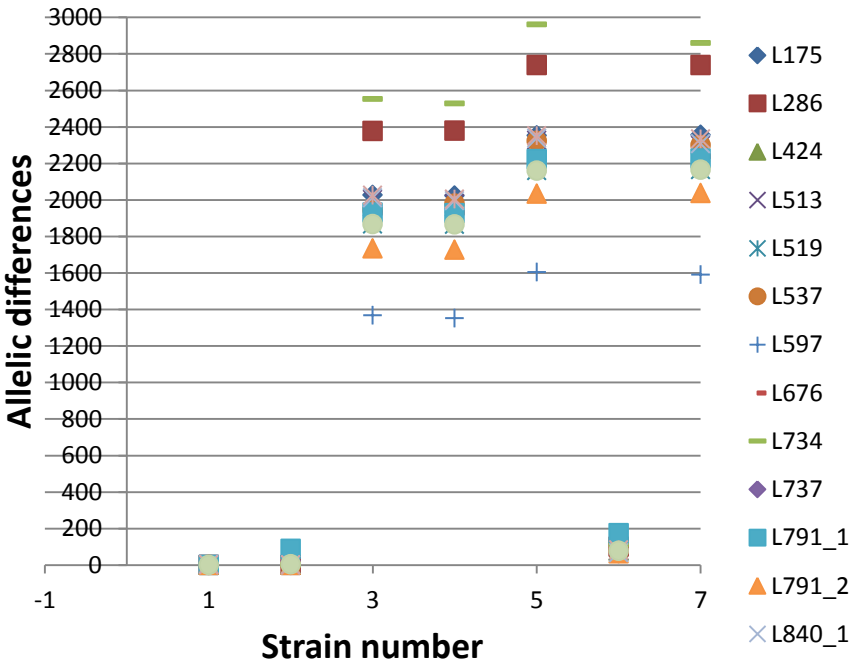
Wrong reporting, correct results

1 NRL

Strain 6: same serogroup of clustering strains

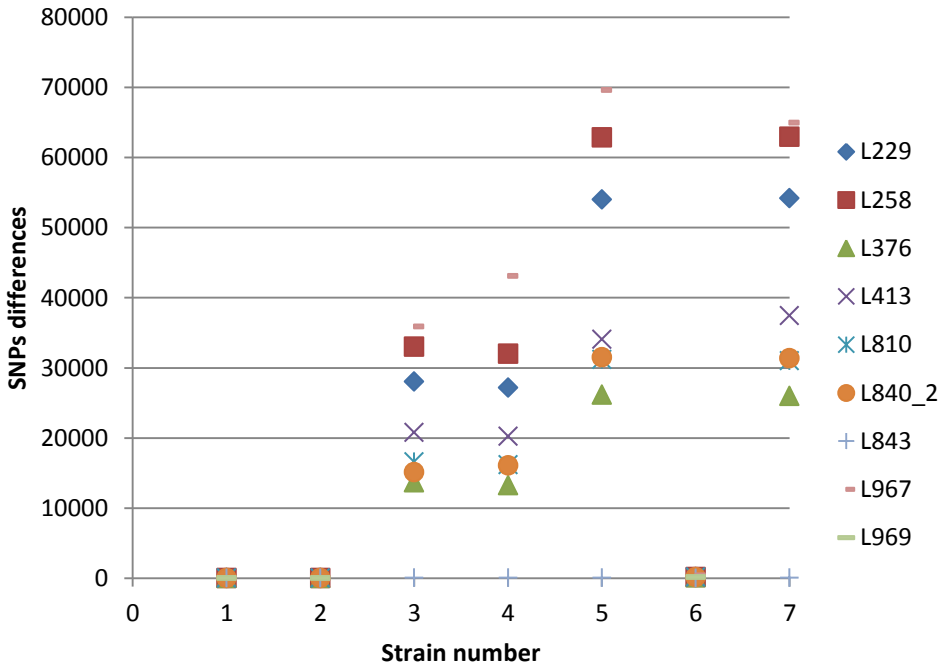
Cluster Analysis - WGS

Distribution of allelic differences from the strain chosen as cluster reference



Strain 6: mean 86 AD from 1-2

Distribution of SNPs distances from the strain chosen as cluster reference



Strain 6: mean 148 SNPs from 1-2

PT26 Cluster Analysis - Conclusions

- More than 60 % (25/40) of the laboratories participating in PT26 performed the voluntary cluster analysis exercise.
- The results showed that the participating laboratories that used PFGE were all able to identify the two related strains.
- Wide variability displayed by PFGE with respect to the number of bands detected in the different strains.
- The majority of laboratories that used WGS performed well regardless the method used (cgMLST or SNP analysis).
- Need to fine-tune some of the algorithms used to call the alleles or the SNPs, which did not work uniformly in all the laboratories.
- Need to define a threshold to call the clusters using the SNPs analysis.

Next PT rounds: PT27

27th inter-laboratory study on the detection of STEC in vegetables

3 artificially contaminated vegetable samples

25 g of basil will be tested for the presence of STEC according to the analytical method ISO TS 13136:2012

Shipment: **9 November 2020**

Deadline: **25 November 2020**

Novel reporting platform

Next PT rounds: PT28

28th inter-laboratory study on the detection and typing of pathogenic *E. coli*

The objectives of this study are:

1. The detection of the main STEC virulence genes (eae and stx genes).
2. The identification of a range of relevant STEC serogroups (at least the 13 serogroups indicated in the EURL-VTEC_Method_003).
3. The subtyping of Shiga Toxins (Stx)-coding genes.
4. Cluster analysis of the test strains – **Only WGS-based** - FACULTATIVE EXERCISE

8 STEC strains

Shipment: **9 November 2020**

Deadline: **31 December 2020**

Novel reporting platform