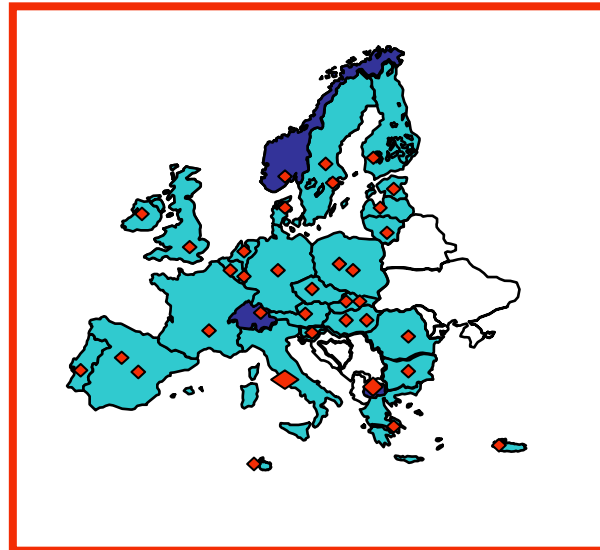


3rd inter-laboratory study on the detection of VTEC in carcass swabs - 2009

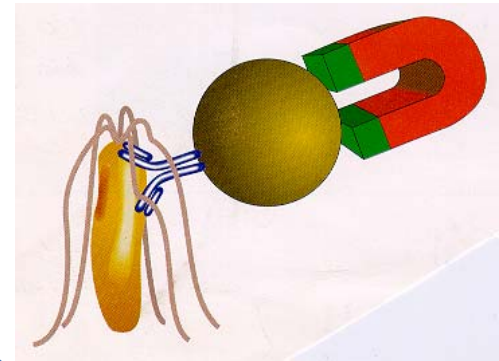
Gaia Scavia, Stefano Morabito



Aims of the study

- **Detection of the “Top 5” VTEC pathogenic serogroups (O157, O26, O103, O111, O145) in bovine carcass swabs**
 - VTEC O157 using the modified ISO 16654 procedure intended for food
 - All the “Top 5” using the Real-time PCR based ISO technical specification

2009 Ring test - Request



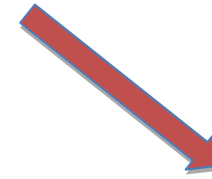
Mandatory Section

**Detection of VTEC O157 by
a modified ISO16654:2001**



2009 Ring test - Request

Facultative Section
Detection of "top 5"
VTEC serogroups by the
Real-time PCR ISO TS



- O157
- O26
- O103
- O111
- O145

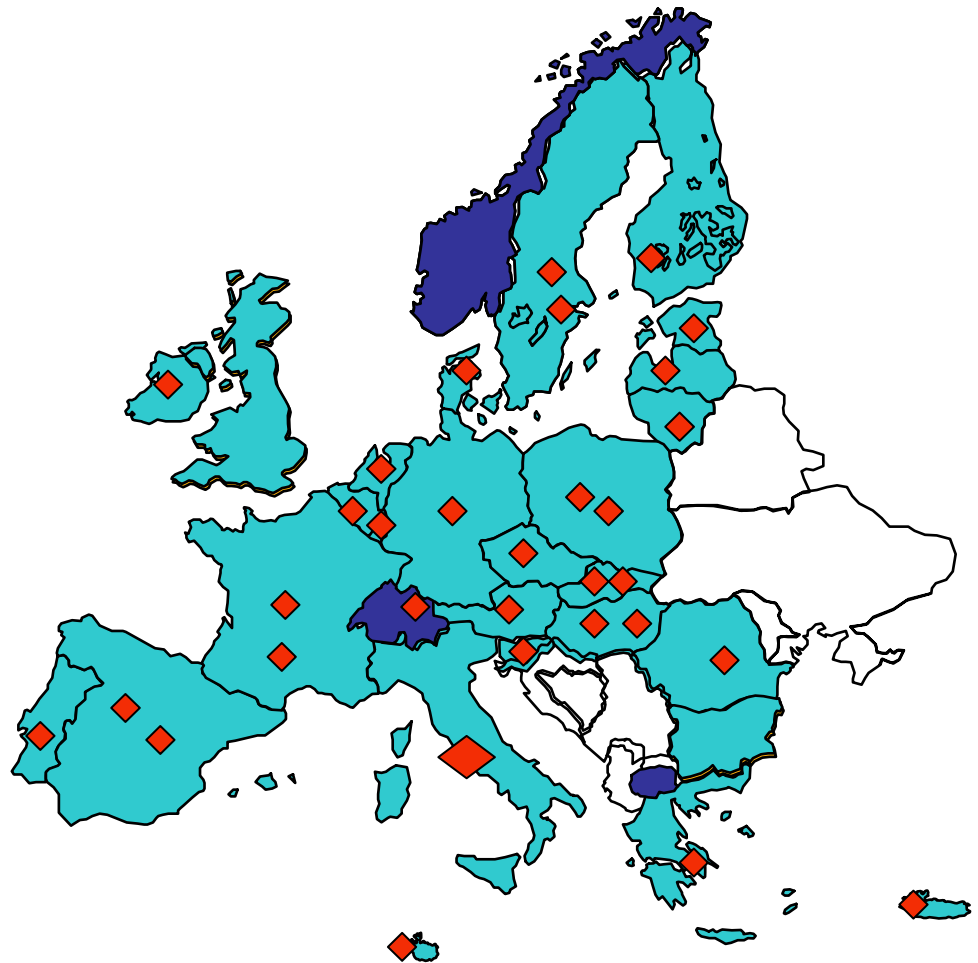
- *vtx1*
- *vtx2*
- *eae*



30 NRLs participating in the study

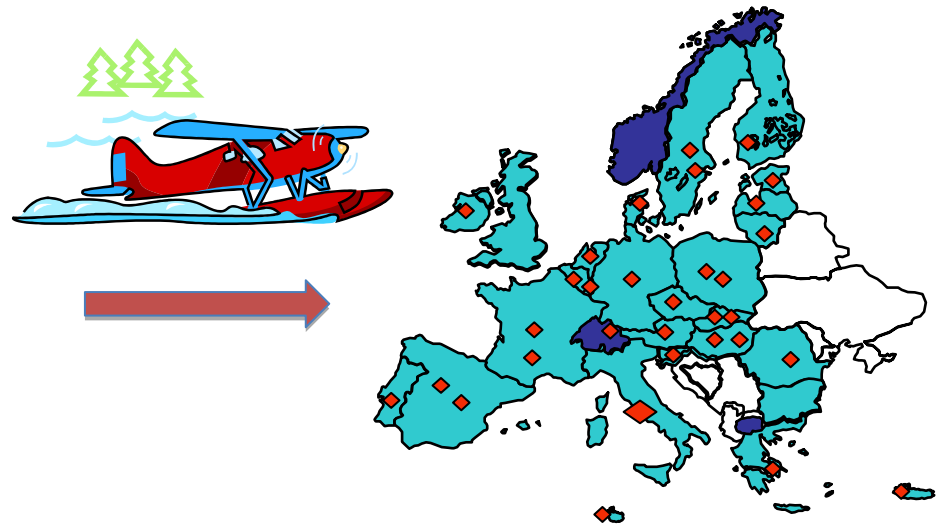
29 NRLs from
25 EU countries

+ the NRL of
Switzerland



30 NRLs participating in the study Shipment of samples

Refrigerated boxes



- ✓ 1 Lab received the samples after 10 days
- ✓ Range of temperature at delivery 1 – 12 °C
- ✓ 4 Labs received the samples at temperature > 8 °C

2009 Ring test - Samples

Sponges moisted with 10 ml of the following:

Sample/Contaminant	Sample A	Sample B	Sample C	Sample D	Sample E
VTEC O157 <i>vtx1, vtx2, eae</i>	2 CFU/ml	2X10 ³ CFU/ml	20 CFU/ml	none	none
VTEC O26 <i>vtx1, eae</i>	none	40 CFU/ml	4X10 ³ CFU/ml	40 CFU/ml	none
<i>E. coli</i>	10 ² CFU/ml	10 ² CFU/ml	10 ² CFU/ml	10 ² CFU/ml	10 ² CFU/ml
<i>K. pneumoniae</i>	2X10 ² CFU/ml	2X10 ² CFU/ml	2X10 ² CFU/ml	2X10 ² CFU/ml	2X10 ² CFU/ml
<i>S. faecalis</i>	5X10 ² CFU/ml	5X10 ² CFU/ml	5X10 ² CFU/ml	5X10 ² CFU/ml	5X10 ² CFU/ml

Materials and Methods

Analysis of the results

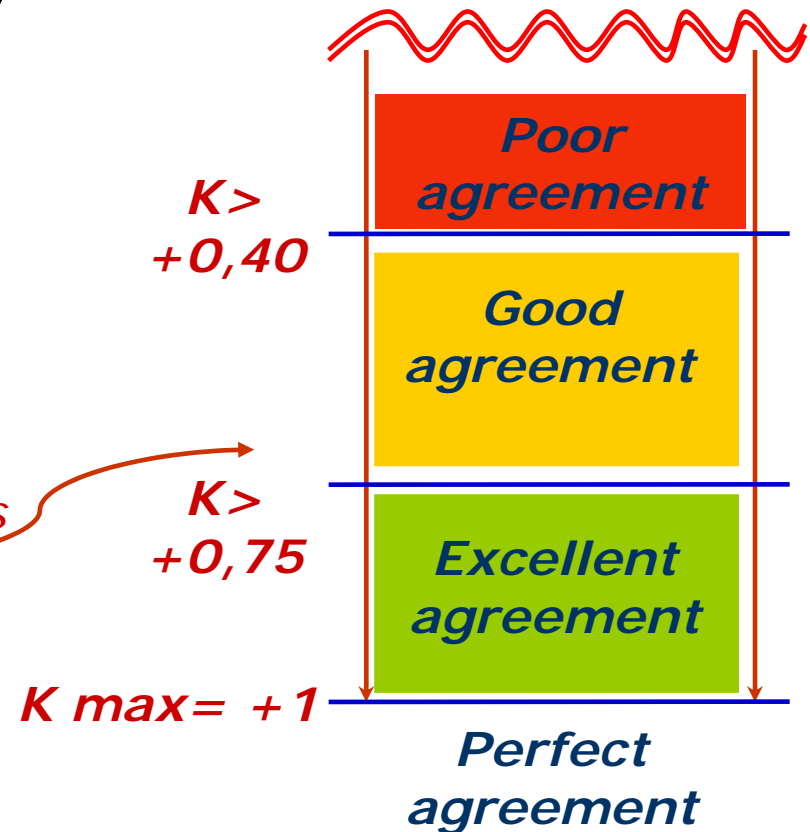
- **Agreement** of results with the true values (Gold Standard): **K test** (95% C.I.)

- **Kappa test**. Cohen's kappa is a measure of association between two measurements of the same item.

- K test estimates the level of agreement **beyond chance**

- How to interpret K value:

Fleiss J.L. (Statistical methods for rates and proportions, 1981)



- **Sensibility and Specificity**

Ring test 2009 - Results

Mandatory Section - Detection of VTEC O157 by a modified ISO16654:2001

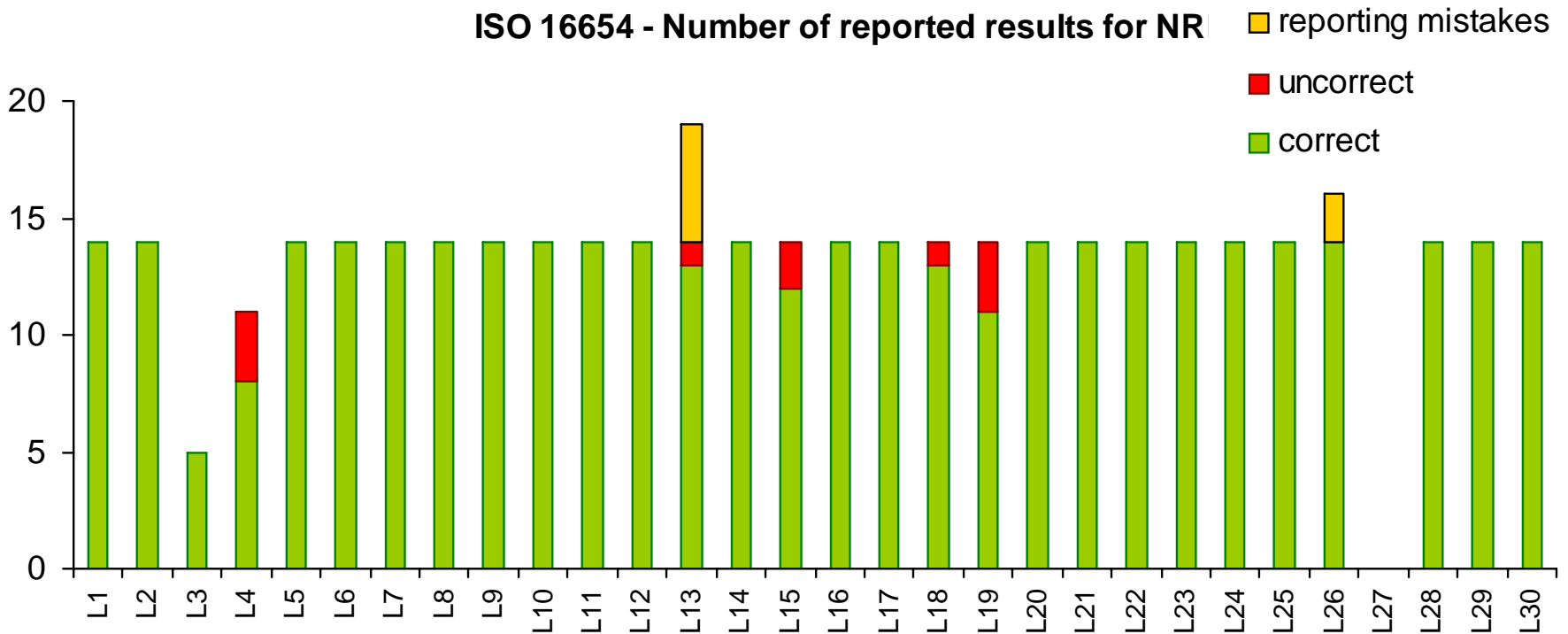
29 NRLs

Analytical results per NRL

Test	Sample lab	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L28	L29	L30		
isolation of O157	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	C	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Mandatory Section - General performances

ISO 16654 - Number of reported results for NR



ISO 16654 – Results: performances of the NRLs

ISO 16654 - isolation of O157 strains

Lab	K value	Se	Sp
L1	1,00	1,00	1,00
L2	1,00	1,00	1,00
L3	1,00	1,00	1,00
L4	0,62	0,67	1,00
L5	1,00	1,00	1,00
L6	1,00	1,00	1,00
L7	1,00	1,00	1,00
L8	1,00	1,00	1,00
L9	1,00	1,00	1,00
L10	1,00	1,00	1,00
L11	1,00	1,00	1,00
L12	1,00	1,00	1,00
L13	0,55	1,00	0,50
L14	1,00	1,00	1,00
L15	1,00	1,00	1,00
L16	1,00	1,00	1,00
L17	1,00	1,00	1,00
L18	1,00	1,00	1,00
L19	1,00	1,00	1,00
L20	1,00	1,00	1,00
L21	1,00	1,00	1,00
L22	1,00	1,00	1,00
L23	1,00	1,00	1,00
L24	1,00	1,00	1,00
L25	1,00	1,00	1,00
L26	1,00	1,00	1,00
L28	1,00	1,00	1,00
L29	1,00	1,00	1,00
L30	1,00	1,00	1,00

overall	0,97	0,99	0,98
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ISO 16654 - genotyping

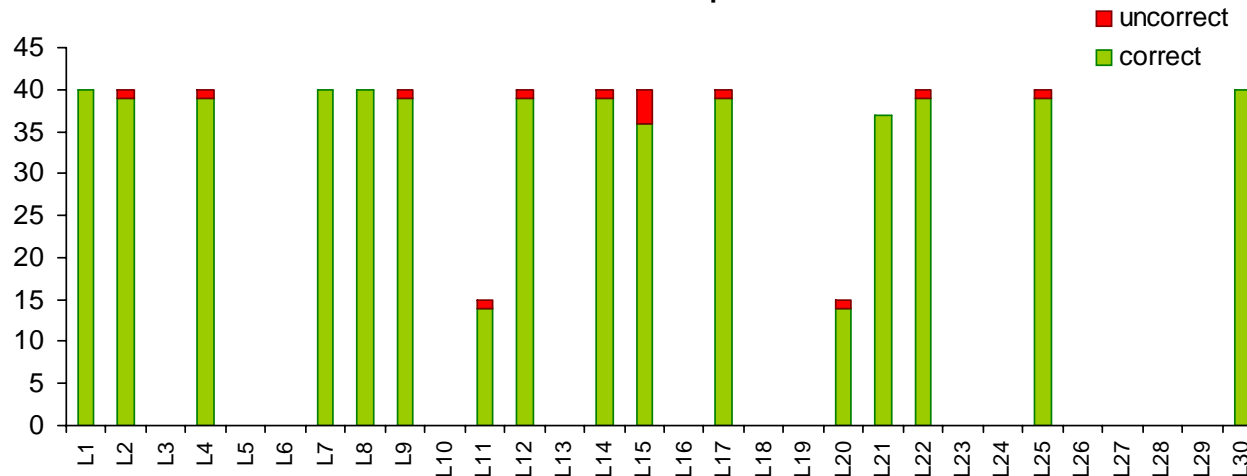
Lab	K value	Se	Sp
L1	1,00	1,00	na
L2	1,00	1,00	na
L3		na	na
L4	0,62	1,00	na
L5	1,00	1,00	na
L6	1,00	1,00	na
L7	1,00	1,00	na
L8	1,00	1,00	na
L9	1,00	1,00	na
L10	1,00	1,00	na
L11	1,00	1,00	na
L12	1,00	1,00	na
L13	1,00	1,00	na
L14	1,00	1,00	na
L15	0,67	0,67	na
L16	1,00	1,00	na
L17	1,00	1,00	na
L18	0,87	0,89	na
L19	0,67	0,67	na
L20	1,00	1,00	na
L21	1,00	1,00	na
L22	1,00	1,00	na
L23	1,00	1,00	na
L24	1,00	1,00	na
L25	1,00	1,00	na
L26	1,00	1,00	na
L28	1,00	1,00	na
L29	1,00	1,00	na
L30	1,00	1,00	na

overall	0,95	0,97	na
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Ring test 2009 - Results

Facultative Section - Real Time PCR Detection of Top 5 serogroups 16 NRLs

Real Time - Number of reported results for NRLs



serogroup **vtx1** **vtx2** **eae**
correct results **N_Labs** **%** **N_Labs** **%** **N_Labs** **%** **N_Labs** **%**

5	13	93%	15	94%	5	31%	15	94%
4	-	-	-	-	11	69%	1	10%
3	-	-	1	7%	-	-	-	-
2	1*	7%	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
0	-	-	-	-	-	-	-	-

* O157 not performed

Ring test 2009 - Facultative Section Results 16 NRLs

Analytical results (serogroups) per NRL

Test	sample lab	L1	L2	L4	L7	L8	L9	L12	L14	L15	L17	L21	L22	L25	L30
O157	A	+	+	+	+	+	+	+	+	+	+		+	+	+
	B	+	+	+	+	+	+	+	+	+	+		+	+	+
	C	+	+	+	+	+	+	+	+	+	+		+	+	+
	D	-	-	-	-	-	-	-	-	-	-		-	-	-
	E	-	-	-	-	-	-	-	-	-	-		-	-	-
O26	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D	+	+	+	+	+	+	+	+	+	+	+	+	+	+

14/14 NRLs who did the assay detected correctly the presence of serogroup-associated genes

O145	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Ring test 2009 - Facultative Section Results 16 NRLs

Real Time PCR Detection of virulence genes

Analytical results per NRL

Test	sample	L1	L2	L4	L7	L8	L9	L11	L12	L14	L15	L17	L20	L21	L22	L25	L30
vtx1	A	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
	B	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

- ✓ 15/16 (94%) detected correctly the presence of *vtx1*
- ✓ 5/16 (31%) detected correctly the presence of *vtx2*
- ✓ 15/16 (94%) detected correctly the presence of *eae*

2009 Ring test - Samples

Sponges moisted with 10 ml of the following:

Sample/Contaminant	Sample A	Sample B	Sample C	Sample D	Sample E
VTEC O157 <i>vtx1, vtx2, eae</i>	2 CFU/ml	2X10 ³ CFU/ml	20 CFU/ml	none	none
VTEC O26 <i>vtx1, eae</i>	none	40 CFU/ml	4X10 ³ CFU/ml	40 CFU/ml	none
<i>E. coli</i>	10 ² CFU/ml	10 ² CFU/ml	10 ² CFU/ml	10 ² CFU/ml	10 ² CFU/ml
<i>K. pneumoniae</i>	2X10 ² CFU/ml	2X10 ² CFU/ml	2X10 ² CFU/ml	2X10 ² CFU/ml	2X10 ² CFU/ml
<i>S. faecalis</i>	5X10 ² CFU/ml	5X10 ² CFU/ml	5X10 ² CFU/ml	5X10 ² CFU/ml	5X10 ² CFU/ml

Facultative Section - Performance of the NRLs

Real Time PCR Detection of Top 5 VTEC serogroups

Real Time - serogroup identification

Lab	K value	Se	Sp
L1	1,00	1,00	1,00
L2	1,00	1,00	1,00
L4	1,00	1,00	1,00
L7	1,00	1,00	1,00
L8	1,00	1,00	1,00
L9	1,00	1,00	1,00
L12	1,00	1,00	1,00
L14	1,00	1,00	1,00
L15	1,00	1,00	1,00
L17	1,00	1,00	1,00
L21	0,70	1,00	1,00
L22	1,00	1,00	1,00
L25	1,00	1,00	1,00
L30	1,00	1,00	1,00

overall	0,98	1,00	1,00
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Real Time - genotyping

Lab	K value	Se	Sp
L1	1,00	1,00	1,00
L2	0,84	0,91	1,00
L4	0,84	0,91	1,00
L7	1,00	1,00	1,00
L8	1,00	1,00	1,00
L9	0,84	0,91	1,00
L11	0,84	0,91	1,00
L12	0,84	0,91	1,00
L14	0,84	0,91	1,00
L15	0,41	0,73	0,75
L17	0,84	0,91	1,00
L20	0,84	0,91	1,00
L21	1,00	1,00	1,00
L22	0,84	0,91	1,00
L25	0,84	0,91	1,00
L30	1,00	1,00	1,00

overall	0,86	0,93	0,98
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Isolation of O26 after performing Real-time PCR (14 NRLs)

Test	sample lab	L1	L2	L4	L7	L8	L9	L12	L14	L15	L17	L21	L22	L25	L30
isolation of O26	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	+	+	+	+	+	-	+	+	+	-	-	-	-	-
	C	+	+	+	+	+	+	+	+	+	-	-	-	-	-
	D	+	+	+	+	+	+	+	+	+	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O26 VTX1	A	+	+	+	+	+	-	+	+	-	-	-	-	-	-
	B	+	+	+	+	+	-	+	+	-	-	-	-	-	-
	C	+	+	+	+	+	-	+	+	-	-	+	+	+	+
	D	+	+	+	+	+	-	+	+	-	-	+	+	+	+
	E	+	+	+	+	+	-	+	+	-	-	+	+	+	+
O26VTX2	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O26 eae	A	+	+	+	+	+	-	+	+	-	-	-	-	-	-
	B	+	+	+	+	+	-	+	+	-	-	-	-	-	-
	C	+	+	+	+	+	-	+	+	-	-	-	-	-	-
	D	+	+	+	+	+	-	+	+	-	-	-	-	-	-
	E	+	+	+	+	+	-	+	+	-	-	-	-	-	-

13 /14 NRLs isolated O26 from the 3 samples

1 /14 NRLs missed O26 from sample B

1/14 NRLs reported the isolation of O111 from 2 samples

Test	sample	L1	L2	L4	L7	L8	L9	L12	L14	L15
isolation of O111	A	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-
	D	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	+	+
O111 eae	A	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-
	D	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	+	+

Isolation of O26 without performing Real-time PCR (5 NRLs)

Test	sample lab	L11	L13	L20	L24	L29
isolation of O26	A	-	-	-	-	-
	B	+	-	+	+	+
	C	+	+	+	+	+
	D	+	+	+	+	+
	E	-	-	-	-	-
O26 VTX1	A	-	-	-	-	-
	B	+	-	+	+	+
	C	+	+	+	+	+
	D	+	+	+	+	+
	E	-	-	-	-	-
O26VTX2	A	-	-	-	-	-
	B	-	-	-	-	-
	C	-	-	-	-	-
	D	-	-	-	-	-
	E	-	-	-	-	-
O26 eae	A	-	-	-	-	-
	B	+	-	+	+	+
	C	+	+	+	+	+
	D	+	+	+	+	+
	E	-	-	-	-	-

3 / 5 NRLs isolated O26 from the 3 samples

2 / 5 NRLs missed O26 from sample B

Isolation of O26 (other methods): performances of the NRLs

isolation of O26 (other methods): serogroup identification

Lab	K value	Se	Sp
L11	0,62	0,67	1,00
L13	0,62	0,67	1,00
L20	1,00	1,00	1,00
L24	1,00	1,00	1,00
L28	1,00	1,00	1,00
overall	0,84	0,87	1,00

isolation of O26 (other methods): genotyping

Lab	K value	Se	Sp
L11	0,68	1,00	1,00
L13			
L20	1,00	1,00	1,00
L24	1,00	1,00	1,00
L28	1,00	1,00	1,00
overall	0,92	1,00	1,00

Detection of VTEC O157

Comparison between modified ISO 16654 and the Real-time PCR TS

13 NRLs performed both methods

Modified ISO 16654

12 / 13 isolated correctly O157

1 missed the identification in sample C

Real-time PCR TS

13 / 13 detected correctly the presence O157

2009 Ring test – Concluding remarks: Strengths

NETWORK DIMENSION AND PERFORMANCES

- ✓ 28 NRL from 25 MS participated to the Ring test
- ✓ 1 NRL from 1 non-MS participated to the Ring test

DIFFUSION OF THE SKILL ON THE FUTURE STANDARD FOR VTEC IN FOOD

- ✓ The network demonstrated to properly handle the methods for both
O157 and non-O157 VTEC

2009 Ring test – Concluding remarks: Unmatched requests

- ✓ **1 Lab** did not receive the samples (after 10 days)
- ✓ **1/29 Labs** did not genotype the isolated VTEC O157 strain
- ✓ **3/16 Labs** performed an incomplete Real Time method (1 Lab did not include O157 PCR, 2 Labs did not genotype the isolated strain)
- ✓ **5 Labs** attempted the isolation of non-O157 without performing the Real Time PCR screening

2009 Ring test – Concluding remarks: Weakness

NUMBER OF LABS CURRENTLY PERFORMING REAL TIME PCR

- ✓ 16 out of 29 NRL accepted to participate in the facultative part of the ring test

DIFFICULTIES IN MATCHING THE REQUESTS AND REPORTING THE RESULTS CORRECTLY

- ✓ Partially overlapping procedures to be applied to the same samples
- ✓ Bad explanation of the requests ?
- ✓ Report forms to be improved ?

2009 Ring test – Concluding remarks: Troubleshooting

NUMBER OF LABS CURRENTLY PERFORMING REAL TIME PCR

CRL will continue to provide knowledge, procedures and training to the NRLs

DIFFICULTIES IN MATCHING THE REQUESTS AND REPORTING THE RESULTS CORRECTLY

- ✓ Detailed guidelines will be provided explaining the requests
- ✓ Next Ring test will be presumably performed on a single method (provided as a SOP and not in form of guideline)
- ✓ We are currently working on a format to upload the results via website

Thanks to all the NRLs for their collaboration

