



PT 19 – irrigation water

NRL Dolný Kubín, Slovakia



Department of Food Hygiene

Department of Molecular-biological Analysis

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Delivery of samples

Treatment
(Initial processing of samples)

Preparing of the enrichment broth

RT-PCR screening
(DNA extraction, vt1/vt2/eae and serogroups detection)

samples with positive results

Streaking the enrichment broth on to solid media

Preparing the pools No. 1-5
(Isolation 1)

Preparing the pools No. 6-10
(Isolation 2)

PCR testing
(vt1/vt2/eae detection)

PCR testing
(vt1/vt2/eae detection)

negative results

positive results only in pool No. 6



Treatment



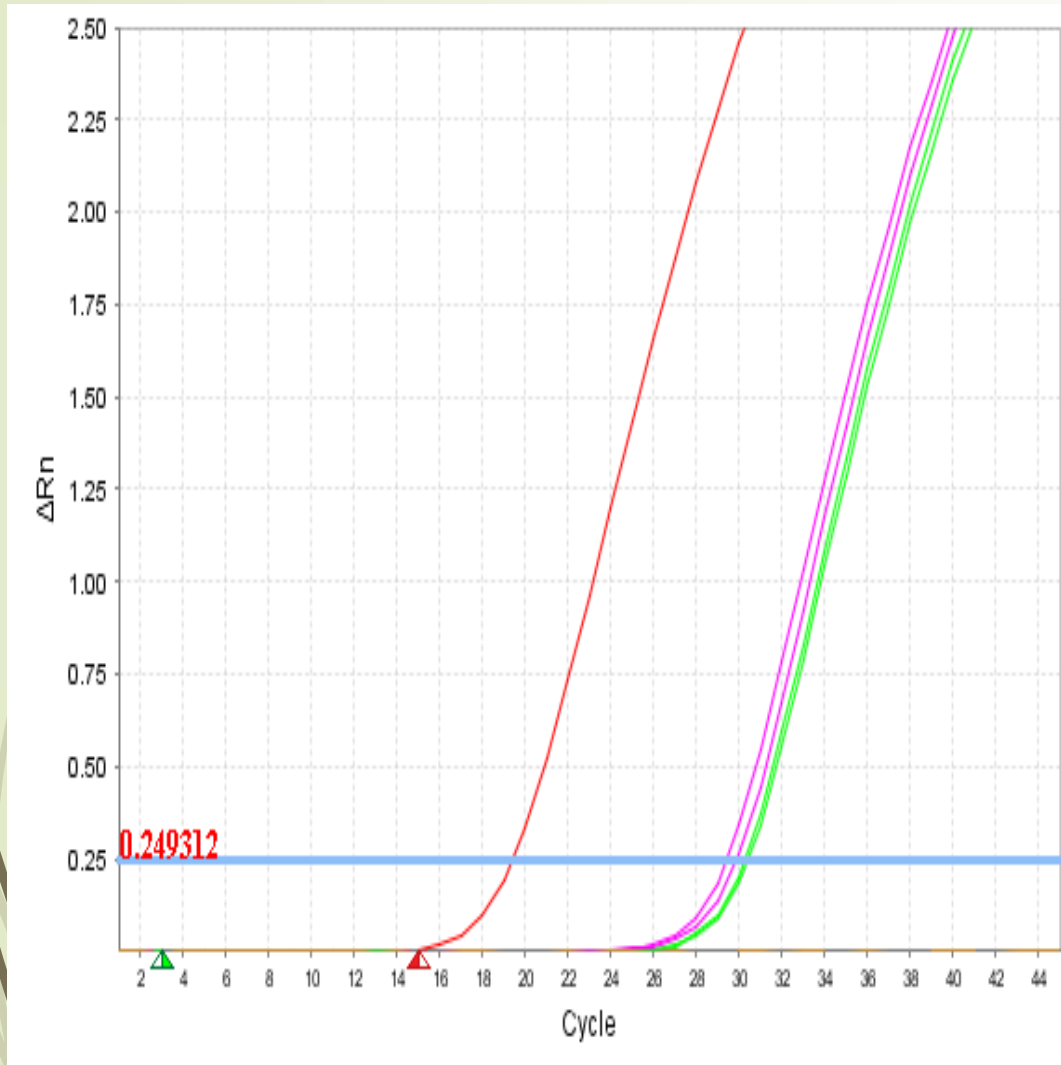
- ▶ Samples arrived 4.4. 2017 afternoon – stored refrigerated
- ▶ Analyzes started 5.4. 2017
- ▶ 1 sample centrifuged at PCR department - later as „negative one“
- ▶ 2 samples centrifuged at other department – both positive
- ▶ Each sample – divided into 4 tubes
- ▶ 30 min. at 4,500 X g at 4°C
- ▶ Both positive samples - 2/4 tubes opened and the content flow out
- ▶ We used only the sediment from the remaining 2 tubes (a half volume of enrichment broth)



RT-PCR screening

- ▶ DNA extraction from two enrichment broth subsamples per sample (A, B)
- ▶ Sample No. 2583 – negative
- ▶ Sample No. 6682, 6985 – positive for vt1+, vt2+, eae+
- ▶ Continue to isolate the strain : from the enrichment step in BPW
we innoculated 5 solid media :
 1. **TBX**
 2. **CT- SMAC**
 3. **Mc Conkey**
 4. **Mc Conkey + Rhamnose**
 5. **Mc Conkey + Sorbitol**

RT-PCR screening – amplification plot for vt1

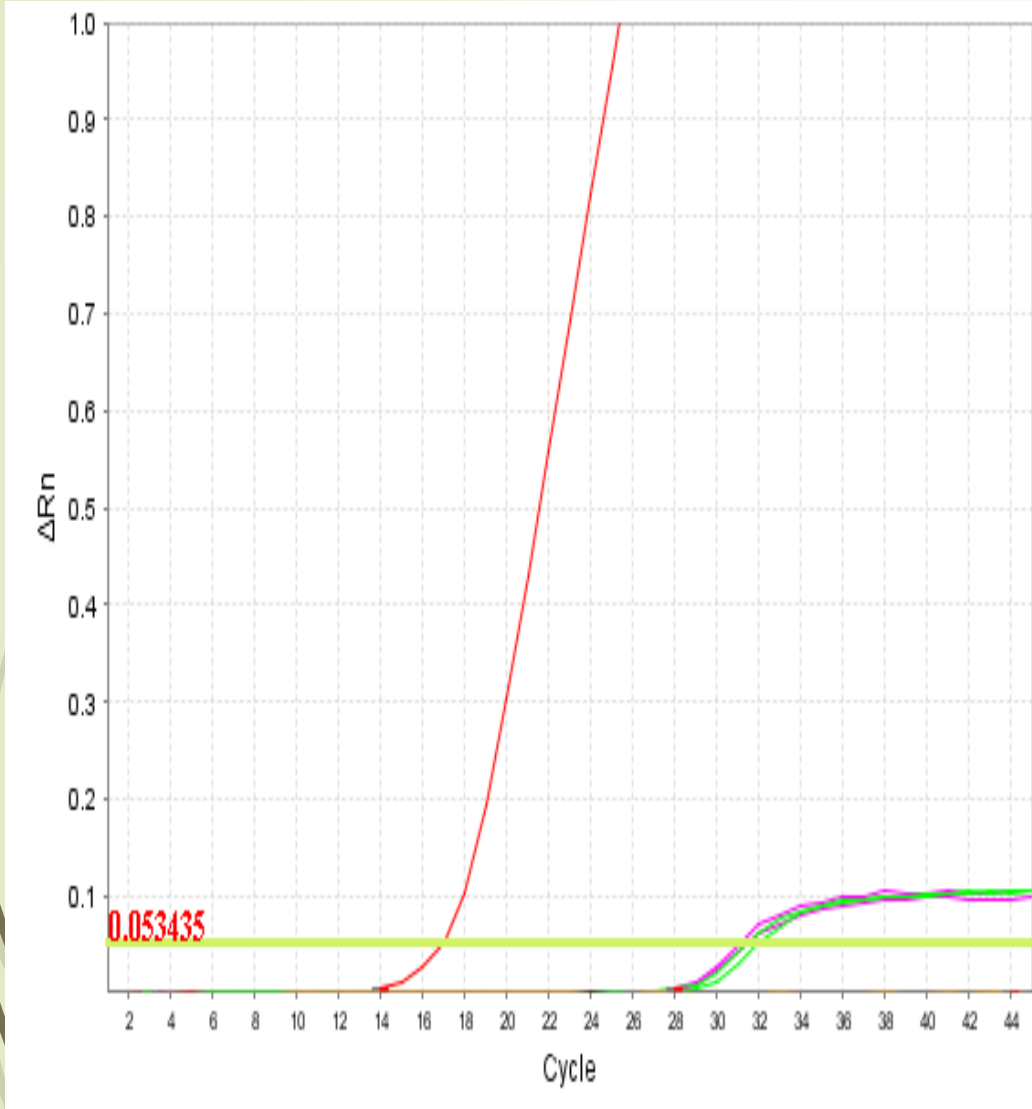


Legend:

- 2583 A, 2583 B
- 6682 A, 6682 B
- 6985 A, 6985 B
- positive control vt1
- non template control

Sample	Signal	Ct value	Results
2583A	not detected	>45	vt1-
2583 B	not detected	>45	vt1-
6682 A	detected	30.59	vt1+
6682 B	detected	30.44	vt1+
6985 A	detected	30.05	vt1+
6985 B	detected	29.61	vt1+

RT-PCR screening – amplification plot for vt2

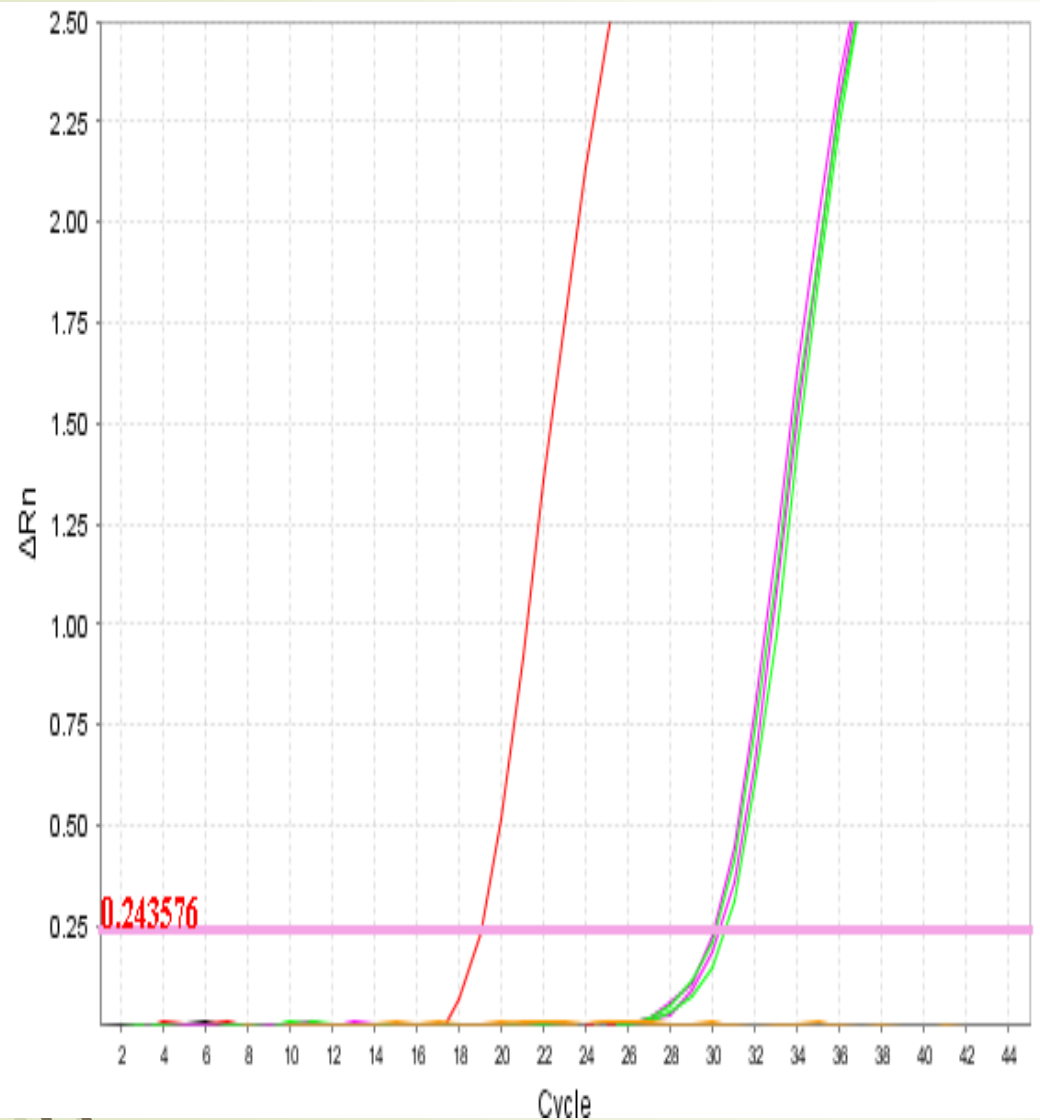


Legend:

- 2583 A, 2583 B
- 6682 A, 6682 B
- 6985 A, 6985 B
- positive control vt2
- non template control

Sample	Signal	Ct value	Results
2583A	not detected	>45	vt2-
2583 B	not detected	>45	vt2-
6682 A	detected	32.14	vt2+ !!!
6682 B	detected	31.58	vt2+ !!!
6985 A	detected	30.84	vt2+ !!!
6985 B	detected	3047	vt2+ !!!

RT-PCR screening – amplification plot for eae

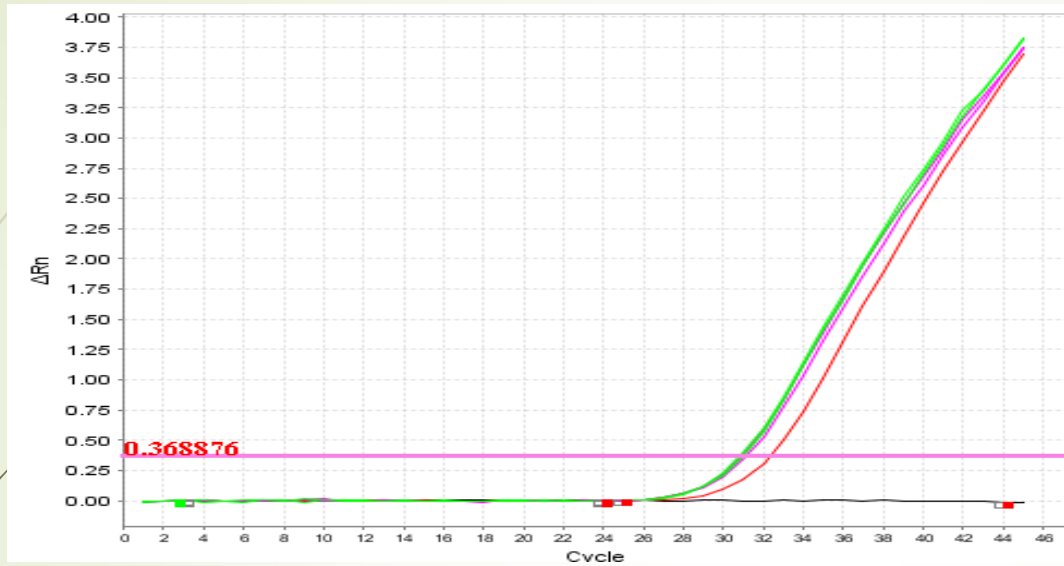


Legend:

- 2583 A, 2583 B
- 6682 A, 6682 B
- 6985 A, 6985 B
- positive control eae
- non template control

Sample	Signal	Ct value	Results
2583A	not detected	>45	eae-
2583 B	not detected	>45	eae-
6682 A	detected	30.56	eae+
6682 B	detected	31.00	eae+
6985 A	detected	30.84	eae+
6985 B	detected	30.47	eae+

RT-PCR screening – serogroups



amplification plot – serogroup O145

Legend:

- 6682 A, 6682 B
- 6985 A, 6985 B
- positive control
- non template control

	O26		O103		O111		O145		O157	
Sample	Signal	Ct value	Signal	Ct value	Signal	Ct value	Signal	Ct value	Signal	Ct value
6682 A	not detected	>45	not detected	>45	not detected	>45	detected	30.87	not detected	>45
6682 B	not detected	>45	not detected	>45	not detected	>45	detected	31.02	not detected	>45
6985 A	not detected	>45	not detected	>45	not detected	>45	detected	30.96	not detected	>45
6985 B	not detected	>45	not detected	>45	not detected	>45	detected	31.15	not detected	>45

Isolation 1

➤ 7.4.2017 - Similar for both positive samples :

Pool No 1. TBX - 10x white colonies

Pool No 2. CT- SMAC - 5x red and 5x pale colonies

Pool No 3. Mc Conkey - 10x pale

Pool No 4. Mc Conkey + Rhamnose - 5x red + 5x pale

Pool No 5. Mc Conkey + sobitol - 5x red + 5x pale

All 5 pools were PCR negative

Isolation 2

From the previous plates (avoid taking the same ones)

6682

Pool No 6. TBX - 4 x blue colonies



vt1+, vt2-!!!, eae+

Pool No 7. CT- SMAC - 10x colonies

Pool No 8. Mc Conkey - 10x

Pool No 9. Mc Conkey + Rhamnose - 10x

Pool No 10. Mc Conkey + sobitol - 10x

6985

Pool No 6. TBX - 5 x blue colonies



vt1+, vt2-!!!, eae+

Pool No 7. CT- SMAC - 10x colonies

Pool No 8. Mc Conkey - 10x

Pool No 9. Mc Conkey + Rhamnose - 10x

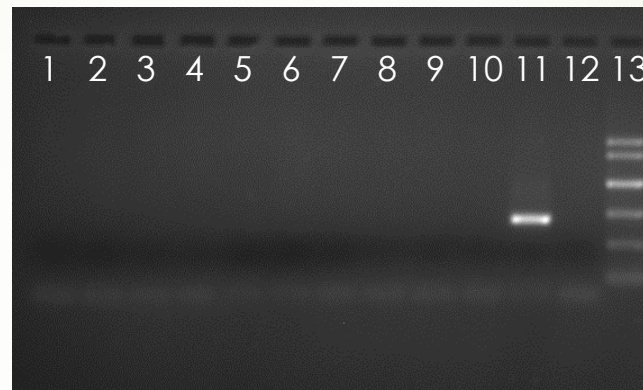
Pool No 10. Mc Conkey + sobitol - 10x

PCR testing (isolation 1/isolation 2)

- vt1, vt2, eae detection (Paton & Paton, 1998) with gadAB amplification (McDaniels *et al.*, 1996)
- confirmation of O145 serogroup in positive pools



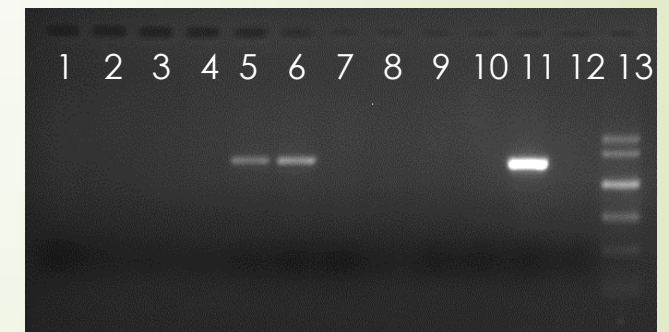
vt1 (180 bp)



vt2 (255 bp)



eae (384 bp)



gadAB (670 bp)

Lane No.1 – 5 = pool No. 6 – 10, sample 6682

Lane No. 6 – 10 = pool No. 6 – 10, sample 6985

Lane No.11 = positive control vt1/vt2/eae/gadAB

Lane No.12 = non template control

Lane No.13 = PCR DNA ladder 50, 150, 300, 500, 750, 1000 bp

Mc Daniels, A. E., Rice, E. W., Reyes, A. L., Johnson, C. H., Haugland, R. A., Stelma, G. N., (1996) Confirmational identification of *Escherichia coli*, a comparison of genotypic and phenotypic assays for glutamate decarboxylase and beta-D-glucuronidase. *Appl. Environ. Microbiol.*, **62**, 3350-3354.

Paton, J.C., Paton, A.W., (1998) Detection and characterisation of Shiga Toxigenic *Escherichia coli* by using Multiplex PCR Assays for *stx1*, *stx2*, *eaeA*, Enterohemorrhagic *E. coli* *hlyA*, *rfb* /O111/, and *rfb* /O157/. *Journal of Clinical Microbiology*, **36**/2/, 598-602.



Conclusion :

RT PCR screening :

- ▶ 2583 vtx1-, vtx2-
- ▶ 6682 O145, vtx1+, vtx2+, eae+
- ▶ 6985 O145, vtx1+, vtx2+, eae+

Isolated strain

O145, vtx1+, vtx2-, eae+

O145, vtx1+, vtx2-, eae+