PT 19 – irrigation water

NRL Dolný Kubín, Slovakia
ISO 13136:2012

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)

PCR testing (vt1/vt2/eae detection)

negative results

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

PCR testing (vt1/vt2/eae detection)

positive results only in pool No. 6
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 inoculated 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Signal</th>
<th>Ct value</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2583A</td>
<td>not detected</td>
<td>&gt;45</td>
<td>vt1-</td>
</tr>
<tr>
<td>2583 B</td>
<td>not detected</td>
<td>&gt;45</td>
<td>vt1-</td>
</tr>
<tr>
<td>6682 A</td>
<td>detected</td>
<td>30.59</td>
<td>vt1+</td>
</tr>
<tr>
<td>6682 B</td>
<td>detected</td>
<td>30.44</td>
<td>vt1+</td>
</tr>
<tr>
<td>6985 A</td>
<td>detected</td>
<td>30.05</td>
<td>vt1+</td>
</tr>
<tr>
<td>6985 B</td>
<td>detected</td>
<td>29.61</td>
<td>vt1+</td>
</tr>
</tbody>
</table>
RT-PCR screening – amplification plot for vt2

Legend:
- 2583 A, 2583 B
- 6682 A, 6682 B
- 6985 A, 6985 B
- Orange: positive control vt2
- Black: non template control

<table>
<thead>
<tr>
<th>Sample</th>
<th>Signal</th>
<th>Ct value</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2583 A</td>
<td>not detected</td>
<td>&gt;45</td>
<td>vt2-</td>
</tr>
<tr>
<td>2583 B</td>
<td>not detected</td>
<td>&gt;45</td>
<td>vt2-</td>
</tr>
<tr>
<td>6682 A</td>
<td>detected</td>
<td>32.14</td>
<td>vt2+ !!!</td>
</tr>
<tr>
<td>6682 B</td>
<td>detected</td>
<td>31.58</td>
<td>vt2+ !!!</td>
</tr>
<tr>
<td>6985 A</td>
<td>detected</td>
<td>30.84</td>
<td>vt2+ !!!</td>
</tr>
<tr>
<td>6985 B</td>
<td>detected</td>
<td>3047</td>
<td>vt2+ !!!</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Signal</th>
<th>Ct value</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2583A</td>
<td>not detected</td>
<td>&gt;45</td>
<td>eae-</td>
</tr>
<tr>
<td>2583 B</td>
<td>not detected</td>
<td>&gt;45</td>
<td>eae-</td>
</tr>
<tr>
<td>6682 A</td>
<td>detected</td>
<td>30.56</td>
<td>eae+</td>
</tr>
<tr>
<td>6682 B</td>
<td>detected</td>
<td>31.00</td>
<td>eae+</td>
</tr>
<tr>
<td>6985 A</td>
<td>detected</td>
<td>30.84</td>
<td>eae+</td>
</tr>
<tr>
<td>6985 B</td>
<td>detected</td>
<td>30.47</td>
<td>eae+</td>
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</table>
## RT-PCR screening – serogroups

<table>
<thead>
<tr>
<th>Sample</th>
<th>O26</th>
<th>O103</th>
<th>O111</th>
<th>O145</th>
<th>O157</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Signal</td>
<td>Ct value</td>
<td>Signal</td>
<td>Ct value</td>
<td>Signal</td>
</tr>
<tr>
<td>6682 A</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
</tr>
<tr>
<td>6682 B</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
</tr>
<tr>
<td>6985 A</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
</tr>
<tr>
<td>6985 B</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
<td>&gt;45</td>
<td>not detected</td>
</tr>
</tbody>
</table>

**Legend:**
- 6682 A, 6682 B
- 6985 A, 6985 B
- positive control
- non template control

amplification plot – serogroup O145
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 colonies
- **Pool No 4. Mc Conkey + Rhamnose** - 5x red + 5x pale colonies
- **Pool No 5. Mc Conkey + sobitol** - 5x red + 5x pale colonies

All 5 pools were PCR negative.
Isolation 2

From the previous plates (avoid taking the same ones)

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

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

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

- **vt1 (180 bp)**
- **vt2 (255 bp)**
- **ea (384 bp)**
- **gadAB (670 bp)**


Conclusion:

**RT PCR screening:**

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

**Isolated strain:**

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