PT19 on spent irrigation water

NRL Ireland

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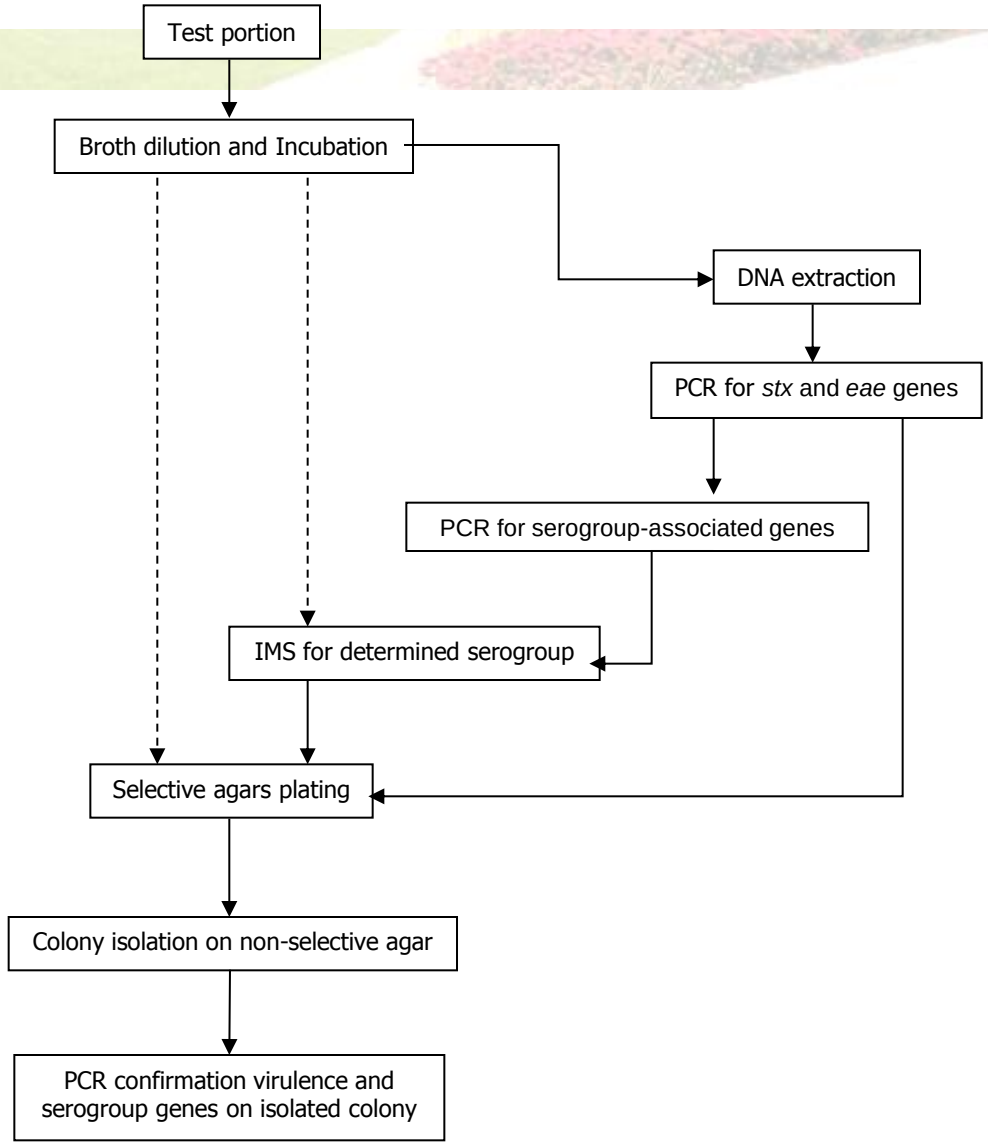
Methodology



Screening

- **Day 1**: Wed 5th
  - Centrifuge water samples 4500 for 30min at 4 degrees using refrigerated centrifuge.
  - Discard supernatant and add 10 x volume of pellet of BPW. Incubate 18-24h at 37°C.
- **Day 2:** Thurs 6th
  - Take 1 ml from each tube. Heat kill.
  - DNA extraction
  - PCR screen for stx 1 & 2 and eae
  - Serogroup PCRs: O157,O26, O103, O111, O145, O104, O45 & O121

Screening Results

Sample	vtx1	eae	O145	VIC
EURL PT 19 6817	No Ct	No Ct	No Ct	25.63-25.78
EURL PT 19 7553	26.92-27.80	28.44-29.60	29.41-29.44	25.29-26.10
EURL PT 19 8074	30.97-31.65	32.46-32.57	32.55-33.02	25.42-25.67

- 6817 negative for all targets in all PCR
- 7553 and 8074
  - *vtx*1 positive
  - *eae* positive
  - O145 positive
  - Negative for the rest of serogroups

Isolation Part 1: IMS

- **Day 3:** Tuesday 18th
  - Put up control for IMS of O145
- **Day 4:** Wednesday 19th
  - Centrifuge (500g for 1 min) 5ml aliquot of enriched culture
  - IMS with O145 (CAPTIVATE O145 CAP006-050 LAB M)
  - Selective agars:
    - Chromagar STEC (Oxoid)
    - TBX (Biokar)
    - mRBA (Biolog)

Isolation Part 1: IMS continued

- **Day 5:** Thursday 20th
  - Nice clean selective agar plates
  - Latex agglutination O145 (Pro-lab)
  - Many different morphology colonies checked
  - Only a weak reaction found in one colony
  - IMS isolation not too promising -> Direct plating attempted

Isolation Part 2: Direct Plating

- **Day 6:** Friday 21st
  - Centrifuge (500g for 1 min) 5ml aliquot of enriched culture
  - Direct plating
- **Day 7**: Monday 24th
  - A lot of background flora. Very difficult to detect isolated colonies.
  - Subbed onto a second set of Selective agar plates.
  - Change of type, e.g. mRBA -> STEC
- **Day 8**: Tuesday 25th
  - Latex agglutination on *second* subbed selective agar plates:
    - **7553: Several positive colonies identified from STEC agar plate**
    - **8074: One single small colony identified from STEC agar plate**
  - Positive colonies subbed onto the *third* set of STEC plates

Isolation Part 2: Direct Plating cont'd

- **Day 9**: Wednesday 26th
  - Third set of selective agars -> Five colonies selected -> NA plating & PCR water
  - PCR water: Heat kill and DNA extraction
- **Day 10**: Thursday 27th
  - PCR for stx1, stx2 & eae
  - Serogroup PCR
  - Indole confirmation on isolates from NA
  - Each colony confirmed for presence of vtx1, eae, O145 genes and indole positive reaction

Confirmation PCR results

Well Name	CY5 (stx2)	FAM (stx1)	ROX (eae)	VIC (IAC)	CY5 (O145)	Result
PT19 7553 1	No Ct	13.57	13.83	No Ct	17.02	stx1 & eae & O145
PT19 7553 1	No Ct	14	14.03	No Ct	16.7	stx1 & eae & O145
PT19 7553 2	No Ct	15.86	15.8	No Ct	18.51	stx1 & eae & O145
PT19 7553 2	No Ct	15.92	15.85	No Ct	18.38	stx1 & eae & O145
PT19 7553 3	No Ct	16.72	16.74	No Ct	19.4	stx1 & eae & O145
PT19 7553 3	No Ct	16.99	16.65	39.94	19.32	stx1 & eae & O145
PT19 7553 4	No Ct	15.99	15.69	No Ct	18.61	stx1 & eae & O145
PT19 7553 4	No Ct	16.06	15.76	No Ct	18.25	stx1 & eae & O145
PT19 7553 5	No Ct	13.94	13.64	No Ct	15.75	stx1 & eae & O145
PT19 7553 5	No Ct	14.05	13.76	No Ct	16.46	stx1 & eae & O145

Well Name	CY5 (stx2)	FAM (stx1)	ROX(eae)	VIC (IAC)	CY5 (O145)	Result
PT 19 8074 1	No Ct	16.66	16.65	39.95	16.56	stx1 & eae & O145
PT 19 8074 1	No Ct	16.59	16.59	No Ct	16.31	stx1 & eae & O145
PT 19 8074 2	No Ct	15.65	15.57	No Ct	15.4	stx1 & eae & O145
PT 19 8074 2	No Ct	15.53	15.47	No Ct	15.32	stx1 & eae & O145
PT 19 8074 3	No Ct	17.88	17.69	36.63	17.91	stx1 & eae & O145
PT 19 8074 3	No Ct	17.73	17.82	29.87	17.7	stx1 & eae & O145
PT 19 8074 4	No Ct	15.29	15.06	30.69	15.44	stx1 & eae & O145
PT 19 8074 4	No Ct	15.38	15.28	No Ct	15.43	stx1 & eae & O145
PT 19 8074 5	No Ct	15.43	15.37	No Ct	15.02	stx1 & eae & O145
PT 19 8074 5	No Ct	15.52	15.42	No Ct	14.89	stx1 & eae & O145

Learning

- Direct plating better than IMS
- Direct plating needs to be repeated on selective agars to obtain well isolated colonies
- Changing selective plates helps to obtain isolated colonies
- STEC agar > mRBA > TBX
- No pooled colonies strategy applied but it has been used in other occasions successfully. However ‘sweeps’ for PCR showed pointless unless there were isolated colonies
- Latex helpful but not many serogroups available

PT samples are “special”

- Routine method is *Hit and miss*
- Higher attempts to isolate than routine samples
  - Selective agar colonies ex IMS were negative for latex
  - Uncounted number of colonies examined ex direct plating

Thanks for your attention