ImmunoCapture performed in microplates: comparison with IMS method

IMC developed by PhD student: D. LAROSE
Framed by: Dr. Patrice ARBAULT & Pr. Jean GUZZO

Tool actually tested in French NRL
Introduction: STEC isolation from food samples

2 standards for STEC detection in food

ISO 16654:2001 (for O157 only):
- Enrichment
- IMS
- Streaking

ISO TS 13136:2012:
- Detection of virulence genes
- Detection of serogroup genes
- Confirmation by isolation of the strain (facilitated by IMS)

⚠️ Limits of IMS with magnetic beads:

- Availability of magnetic beads for certain serogroups (O45, O121, O104)
- Practicality of magnetic beads (intensive washing step when a lot of samples are analyzed)
- Rate of confirmation of positive PCR samples (5 to 15%) (Bosilevac & Koohmaraie, 2011)
Introduction: A new approach to immunocapture

- Development of a new method for capture and enrichment of STEC: IMC
  - Use of a microplate (96 wells) for immunocapture of STEC

- Advantages of IMC:
  - Can be available for several serogroups (O26; O45; O103; O111; O121; O145; O157) – Immunocapture of several serogroups in the same well (up to 3);
  - Practicality and speed of the washing step by using a multichannel pipette;
    - Subculture in any broth directly in the well;
    - Streaking onto plate directly from the well.
**Introduction:** Flowchart of the IMC protocol

**Principle:**

- 100µL of enrichment broth in a well
- Incubation 1 hour at room temperature
- Washing step
- Enrichment 3 to 5 hours at 37°C
- Streaking on selective or non-selective medium
- Possibility to isolate STEC (if the sample was heavily contaminated)

- Or

- 1mL of enrichment broth
- Concentration 10X (by centrifugation)
- 100µL of concentrated enrichment broth in a well
Example of IMC for O157
Characterization of O157 antibodies

Specificity test of the *E. coli* O157 antibodies versus other serogroups (ELISA indirect : coating of LPS)

- High signal is obtained for *E. coli* O157 antigen
- No signal is observed with other serogroups
Characterization of O157 antibodies

Specificity test of the *E. coli* O157 antibodies versus other bacterial species (ELISA sandwich: coating of primary antibodies)

- An important signal is observed with *E. coli* O157 and *Salmonella* Urbana (the same O antigen)
- No signal observed for other species
- *anti-O157 antibodies are specific to the O157 antigen.*
Characterization of O157 antibodies

Sensitivity test of the *E. coli* O157 antibodies

- The maximum signal is obtained with 5 µg/mL of antibodies
- For higher concentrations, the signal is leveling off
Comparing magnetic beads vs microplate for immunocapture of *E. coli* O157

- Artificial contamination *after* the enrichment step:

  25g ground beef + 225mL BPW

  **Enrichment 18 hours at 37°C**

  Artificial contamination at $10^5$ CFU/mL with *E. coli* O157

  - IMS (magnetic beads: Captivate™ O157)
  - IMC (Nexidia microplate O157)

  Numeration of *E. coli* O157 in tube (beads) or in well (microplate) on CT-SMAC
Comparison of magnetic beads with microplate for immunocapture of *E. coli* O157

Concentration of *E. coli* O157 captured by magnetic beads or in the microplate well

- Concentration of *E. coli* O157 in suspension is higher with the IMC approach than with the magnetic beads approach
Importance of the enrichment step in the well after immunocapture

Enrichment of a ground beef → Artificial contamination with E. coli O157 (10^5 CFU/mL) → IMC → Enrichment in the well: 0; 3 or 5 hours

Concentration of E. coli O157 in the well

- Concentration of E. coli O157 in the well increased by more than 2 log with 5 hours of enrichment
Artificial contamination before the enrichment step:

- 25g ground beef + 225mL BPW + about 10 *E. coli* O157 cells

Enrichment at 37°C during 18 hours

- IMS (magnetic beads: Captivate™ O157)
- IMC (Nexidia microplate O157)

Counting of *E. coli* O157 in tube (beads) or in well (microplate) on CT-SMAC
Comparison of magnetic beads with microplate for immunocapture of *E. coli* O157

Concentration of *E. coli* O157 captured by magnetic beads or in the microplate well

- Concentration of *E. coli* O157 in suspension is higher with the IMC approach than with the magnetic beads approach.
Natural contamination (samples from the French NRL)

- Samples in which the presence of *E. coli* O157 was confirmed by IMS (Dynabeads® anti *E. coli* O157) and isolation on CT-SMAC or chromID™ O157:H7) were analyzed with microplate O157:

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Frozen enrichment broths
   ↓
IMC O157
   ↓
Streaking on CT-SMAC
   ↓
Confirmation with latex beads
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Is IMC able to confirm presence of *E. coli* O157 in samples confirmed by magnetic beads?
Results:

Washing of the wells is faster and less tedious than beads.

Good quality of isolation after IMC.
Comparison magnetic beads vs microplate for immunocapture of *E. coli* O157

**Results:**

- **38 samples** formerly found positive tested by IMC

  Observation of characteristic colonies (on CT-SMAC) for **33 samples** after IMC

  Confirmation of the presence of *E. coli* O157 with latex beads in **33 samples** (agglutination test performed on sorbitol negative colonies)

- For the 5 samples missed by IMC but formerly found positive by IMS, analysis are going to be repeated

- Conservation of samples at -20°C ⇒ may affect the viability/physiology of bacteria and consequently the capacity of microplate to confirm the presence of *E. coli* O157 in a few samples?
Conclusions and prospects

- **Further tests** of IMC are underway for O157 and other serogroups (138 samples from the NRL)
- **Advantages of IMC**
  - Easiness of use and fast when use with a multichannel pipette (high number of samples simultaneously)
  - Available for different serogroups (O26; O45; O103; O111; O121; O145; O157)
  - Possibility to search different serogroups in the same plate and/or in the same well (well coated with up to three types of antibodies)

- **IMC: a new way for immunocapture of STEC**
  - Could be used instead of magnetic beads
    - for detection of STEC in food
    - for confirmation of PCR-positive results
  - Could be used before PCR (to facilitate the detection of a serogroup by PCR)

- **IMC developed by Nexidia**
IMC as a confirmation protocol

Tests are underway for other serogroups: a lot of samples from NRL will be analyzed.

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Positive sample with IMS</th>
<th>Negative sample (not confirmed by IMS)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>O26</td>
<td>35</td>
<td>188</td>
<td>223</td>
</tr>
<tr>
<td>O145</td>
<td>10</td>
<td>95</td>
<td>105</td>
</tr>
<tr>
<td>O45</td>
<td>0</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>O121</td>
<td>0</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>O104</td>
<td>0</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>O103</td>
<td>45</td>
<td>93</td>
<td>138</td>
</tr>
</tbody>
</table>
Introduction: IMC as a confirmation tool

- Recommended media:
  - Isolation of *E. coli* O26: **CT-RMAC and RMAC**
  - Isolation of other serogroups: **CHROMagar STEC** and a medium for differentiation of *E. coli* β-glucuronidase positive (for example TBX)