Development and Evaluation of Latex Agglutination Tests, Immunomagnetic Separation Beads (IMS), and ELISAs for Non-O157 Shiga Toxin-Producing E. coli (STEC)

Rubio F.1, Glaze T.1, Medina M.2, Fratamico P.2, Fan, X.3

Abraxis LLC, Warminster, PA1, USA: USDA-ARS, Wyndmoor, PA.2

Objectives

- Development of Latex Agglutination Tests, Immunomagnetic Separation Beads (IMS), and ELISAs for the detection of E. coli non-O157 STEC.

Introduction

Escherichia coli (E. coli) are common bacteria and part of the normal flora of the gastrointestinal tract. However, some E. coli strains, including the Shiga toxin-producing E. coli (STEC) are able to produce a toxin, known as Shiga toxin, and can lead to serious human illnesses from mild diarrhea to life-threatening conditions, such as hemolytic uremic syndrome (HUS). A recent report from the Centers for Disease Control and Prevention (CDC) showed that non-O157 STEC infections are more common than illnesses caused by E. coli O157:H7. A review of records of non-O157 STEC isolates forwarded by state public health labs to the CDC reference laboratory between 1983-2002 showed that six serogroups: O26, O45, O103, O111, O121, and O145, of the 61 serogroups identified accounted for 71% of the isolates recovered in the USA.

In 1994, the USDA-Food Safety and Inspection Service (FSIS) declared E. coli O157:H7 an adulterant in beef products, and on September 2011 E. coli serogroups O26, O45, O103, O111, O121, and O145 (top 6 non-O157 serogroups) were declared as adulterants on raw, non-processed beef products or components in the same manner as E. coli O157:H7. The USDA-FSIS performs a verification sampling program to test for these pathogens in samples collected from federally inspected establishments and retail stores. The testing protocol utilized and mandated by the USDA-FSIS is described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter SB.02 “Detection and isolation of non-O157 Shiga-Toxin Producing Escherichia coli Strains (STEC) from Meat Products”.

Most diagnostic methods for E. coli STEC have been designed to detect only serogroup O157 in food and water. With the recent classification by USDA-FSIS of serotypes O26, O45, O103, O111, O121, and O145 as adulterants in beef products, there is a need to develop methodology that would enable the detection of those STEC serogroups, and especially methods that involve the use of such latex agglutination and immunomagnetic beads described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter SB.02, as well as ELISAs.

Methods

- Affinity purified antibodies (Abraxis LLC) prepared against O26, O45, O103, O111, O121, and O145 were covalently coupled to red latex particles according to the procedure described by Medina et al.
- Affinity purified antibodies (Abraxis LLC) prepared against O26, O45, O103, O111, O121, and O145 were coupled to 3 micron super paramagnetic particles using procedures similar to those described by Fratamico et al. with some modifications.
- Sandwich type ELISA formats were developed using Costar 96 well microtiter plates.
- E. coli cells used in latex agglutination assays were prepared as follows: O26, O45, O103, O111, O121, and O145 were grown in TSA broth at 37°C, centrifuged at 10,000 rpm for 15 min, resuspended in PBS, and then fixed in PBS before resuspending in PBS. Cells were then heat inactivated and treated with formalin to a final concentration of 0.5%.
- Ground beef was inoculated with ca. 10^-2 CFU/g. Enrichment was at 42°C for 18 h in mTSA with 8 mg/L novobiocin (as described in the FSIS MLG SB.02). IMS was performed on 1 ml of the enrichment using 20 µl of beads, following the Invitrogen protocol (room temperature rotation for 10 min and 3 washes in PBST). This was followed by plating the beads onto mRBA (as described in MLG SB.02), and then picking three presumptive positive colonies and testing by the PCR for stx and eae genes and for serogroup-specific genes.
- IMS protocol and real-time PCR confirmation assays were performed as described in Fratamico et al.
- Invitrogen IMS beads, EPEC/VTEC O26, O103, O111, O145.

Results and Discussion

- Latex agglutination test performed on the top 6 non-O157 target and non-target serogroups grown on TSA agar plates. Results indicate positive results (agglutination) for target and negative for non-target serogroups. Figure 1.
- When comparing Abraxis and Invitrogen IMS beads, not much difference in terms of appearance of colonies on the plates were seen. However, it was easier to pick the correct colonies using the Abraxis beads. Colonies could not be identified from plates processed with Invitrogen’s IMS beads (O26, O103, O111, and O145). Results are presented in Table 1.
- Very sensitive ELISA was obtained for serotype O103 using a streptavidin-biotin sandwich antibody ELISA approach (Figure 2). 100 cells could be detected in a 50 µl sample.

Table 1. Number of colonies confirmed by real-time PCR assays out of total presumptive colonies picked from mRBA

<table>
<thead>
<tr>
<th>STEC serogroup</th>
<th>Abraxis</th>
<th>Dynabeads</th>
</tr>
</thead>
<tbody>
<tr>
<td>O26</td>
<td>2/3</td>
<td>2/3</td>
</tr>
<tr>
<td>O45</td>
<td>2/3</td>
<td>2/3</td>
</tr>
<tr>
<td>O103</td>
<td>1/3</td>
<td>0/3</td>
</tr>
<tr>
<td>O111</td>
<td>1/3</td>
<td>0/3</td>
</tr>
<tr>
<td>O121</td>
<td>2/3</td>
<td>2/3</td>
</tr>
<tr>
<td>O145</td>
<td>3/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

IMB beads for serogroups O45 and O121 were prepared as described previously (Fratamico et al., 2011) using streptavidin-coated Dynabeads that were bound with biotinylated polyclonal antibodies against E. coli serogroups O45 and O121.

Conclusion

- Simple and rapid latex agglutination assays were developed for the detection of non-O157 Shiga-toxin producing E. coli belonging to serogroups O26, O45, O103, O111, O121, and O145. Antibodies for the target serogroup are specific.
- Developed IMS beads performed better than Invitrogen’s.
- Very sensitive ELISA (O103) with the detection level of 100 cells is possible using a streptavidin-biotin sandwich antibody ELISA approach. Future work will involve the development of ELISAs to detect the O26, O45, O111, O121, and O145 serotypes.

References


USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter SB.02 “Detection and isolation of non-O157 Shiga-Toxin Producing Escherichia coli Strains (STEC) from Meat Products.”