APPENDIX II
SEROLOGICAL TESTING

Serological Testing

Serological testing is performed to identify *Salmonella*, *Shigella* and *E. coli* 0157. Testing is performed by a slide agglutination test using somatic (O) or flagella (H) antisera.

Materials:

1. Glass slides
2. Bacteriology loop
3. Appropriate *Salmonella*, *Shigella* or *E. coli* antisera. (Store refrigerated)
4. Sterile saline

Procedure:

1. With a China-marker, divide the slide into two.
2. Put a drop of saline in each section.
3. Using a loop, emulsify the organism in each drop of saline to give a homogenous, fairly dense suspension.
4. Add one drop of undiluted antiserum to one of the suspensions and mix. The other suspension serves as a control.
5. Rock the slide gently and observe for agglutination using indirect lighting over a dark background. Agglutination should be strong and clearly visible within one minute.
6. If there is agglutination in the saline control, the test is invalid.

*Salmonella* spp.

If there is little or no H₂S in the TSI tube and the isolate resembles Salmonella, test with the Vi antiserum first. The antisera are tested in the following order:

1. Polyvalent O (A – S)
2. Polyvalent H (phase 1 and 2)
3. Polyvalent H (phase 2) –
4. If polyvalent O is positive, then proceed with other O antisera.
5. If the organism does not react with the polyvalent O antiserum, and the isolate is still suspected to be Salmonella due to the biochemical reactions, send to PHL for identification.

_Shigella_ spp.

1. Serological confirmation of Shigella isolates is based only on O antigen testing.
2. If the isolate is biochemically a Shigella species, but fails to agglutinate with the grouping sera, test suspect colonies with the following antisera in the following order:
   a) _S. sonnei_
   b) _S. flexneri_
   c) _S. dysenteriae_
   d) _S. boydii_
3. Send to PHL for confirmation.

_E. coli_

1. _E. coli_ 0157 antiserum is used.
2. Known positive (LPTP 8608-3) and negative (ATCC 25299) must be tested with each batch of testing.