Application
The Shigella antisera from SSI Diagnostica are intended for serotyping of Shigella strains by slide agglutination.

Description
The polyclonal Shigella O antisera against S. dysenteriae, S. flexneri and S. boydii are raised in rabbits. The antisera against S. sonnei are monoclonal antibodies (obtained by immunising mice and fusion of the spleen cells with a mouse myeloma cell line). The antisera are supplied in 3 ml bottles (sodium azide as preservation).

Cross-reactions have been removed by absorption.

Principle
When a bacterial culture is mixed with a specific antiserum directed against bacterial surface components, the cells are bound together through antigen-antibody bonds to form aggregates (agglutination). This is visible to the naked eye as clumps in the suspension.

Limitations
The latex reagent is intended for serotyping of pure cultures of capsulated pneumococci.

Materials Required but not Provided
• Selective or non-selective agar medium (eg, beef extract agar)
• Inoculating loop or toothpick
• Glass slides
• Physiological saline, pH 7.4
• Incubator 35-37 °C

Procedure
General
Physiological saline is used as a negative control and must be negative. If the negative control is positive (agglutinates), the strain is auto-agglutinating, i.e. O rough.

1. The Shigella strain is grown at 35-37 °C over night on a non-selective or selective agar medium.
2. Apply a small drop of antiserum (approximately 20 µL) on the glass slide.
3. Transfer culture from several colonies to the drop of antiserum and mix well. The amount of culture should be sufficient to give a distinct milky turbidity.
4. Tilt the slide gently for 5-10 seconds.
5. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).
6. A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogeneous milky turbidity. A late or weak agglutination should be considered negative.

Problems with agglutination
If a Shigella culture agglutinates poorly or if it does not agglutinate at all suspend it in saline and boil it in a waterbath for 15-30 minutes. Cool down the suspension and test it in saline to determine if it is rough. If the suspension is not rough use it to test for agglutination in antiserum.

Storage and Shelf Life
Store at 2-8 °C in a dark place. Expiry date is printed on the package. Turbidity due to lipoprotein precipitation is sometimes seen after prolonged storage. Precipitation and/or contamination can be removed by centrifugation (10,000 g) followed by sterile filtration (0.22 µM).

Information and ordering
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