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Conventional methods for isolation and Identification of Streptococcus pyogenes

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ABSTRACT

The protocol outlines the steps for isolating and identifying Group A streptococci (Streptococcus pyogenes) from growth on primary culture media.

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Culture and identification of *Streptococcus pyogenes*

1 *S. pyogenes* isolates that were presumptively identified were collected from the different hospitals and outpatient centres that formed part of the study. Isolates were inoculated to 5% sheep blood agar plates that were incubated overnight at 35ºC.

2 Upon receipt, a beta-haemolytic colony was streaked onto a new 5% blood agar (HiMedia Laboratories Limited, Mumbai, In.) plate to obtain isolated colonies and incubated at 35-37ºC for 24 hours in an anerobic jar.

2.1 A bacitracin antibiotic disc was placed into the inoculum of each isolate. Any zone of inhibition to the antibiotic after incubation was considered to be an indication of drug susceptibility.

2.2 For quality control purposes, *S. pyogenes* ATCC 19615 (positive control), *S.agalactiae* ATCC 12386 (negative control), *Enterococcus faecalis* ATCC 29212 (negative control), and an uninoculated blood agar plate were incubated alongside the test isolates.

3 Following incubation, the pyrrolidonylarylamidase (PYR) test was performed on all beta-haemolytic isolates based on the manufacturer's instructions.

3.1 A PYR disc (HiMedia Laboratories Limited, Mumbai, In.) was placed on a glass slide on a petri dish and rehydrated with one drop of sterile deionized water.

3.2 Using a sterile loop, 2-3 colonies were added to the suspension on the glass slide and the mixture incubated at room temperature for 2 minutes.

3.3 One drop of the PYR chromogenic solution was placed on the disc and observations recorded after 3 minutes. The formation of a red colour was considered a positive result

4 Beta-haemolytic colonies from the test samples and controls were also exposed to the Voges-Proskauer (VP) Test (BD, New Jersey, USA).

4.1 A colony was subcultured to tubes containing 4 mL of trypticase soy broth (HiMedia Laboratories Limited, Mumbai, In.) and incubated at 35-37ºC for 24 hours.

4.2 Following incubation, 0.6 mL of alpha-naphthol solution and 0.2 mL of potassium hydroxide solution were added to the TSB tubes and mixed by vortexing for 10 sections.

4.3 Observations were recorded within 5 minutes. QC observations were made using *E. faecalis* ATCC 29212 (positive) and *S. pyogenes* ATCC 19615. Tubes that showed a yellow-brown colour were considered VP negative and indicative of *S. pyogenes*.

5 Confirmatory testing was performed using the Lancefield classification method with a Strep Grouping kit (Thermo Scientific Remel PathoDX, Remel Europe LTD, Kent, UK) following the manufacturer's instructions.