PYR Agar

M1489

PYR Agar is used for the isolation and identification of *Streptococcus pyogenes* .

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from	500.000
Peptic digest of animal tissue	20.000
Dextrose	2.000
Sodium chloride	2.000
Disodium phosphate	0.400
Sodium carbonate	2.500
Chromogenic mixture	0.100
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 52 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

PYR hydrolysis is a presumptive test for both group A and group D enterococcal streptococci (1). The PYR test determines the activity of enzyme L-pyrrolidonyl arylamidase (PYR) produced by *Streptococcus pyogenes* but not by other b-haemolytic *streptococci* (2). Free b-napthylamide is then detected by addition of the diazo dye complex, N ,N-dimethylaminocinnamaldehyde. Development of a red colour is indicative of PYR hydrolysis (3). PYR test is a highly sensitive test, which replaces bacitracin and salt tolerance (growth in 6.5% NaCl) tests (1). PYR Agar is recommended for detection and presumptive identification of *S. pyogenes* based on PYR hydrolysis (4).

Todd Hewitt Broth Base (M313) acts as the basal medium to which the agar and substrate for PYR enzyme are added (3).

Beef heart infusion and peptic digest of animal tissue provide nitrogenous nutrients. Dextrose is the carbohydrate serving as an energy source. Disodium phosphate serves as buffering agent and sodium chloride maintains osmotic balance. Chromogenic mixture provides substrate for PYR enzyme. After an incubation at 35-37°C for 18-24 hours, add 1 drop of PYR reagent (R043) directly to suspected surface growth on plate. Observe for colour change after 2 minutes. The chromogenic mixture is hydrolysed by *S. pyogenes* to L-pyrrolidone and b-naphthylamine. The PYR reagent reacts with b-naphthylamine to form a red coloured Schiffs Base indicating a positive reaction.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.2% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH
7.60-8.00

Cultural Response
M1489: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	PYR (on addition of PYR reagent, R044)
Cultural Response			
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	positive, red colouration
Enterococcus faecalis ATCC 29212	50-100	luxuriant	positive, red colouration
Escherichia coli ATCC 25922	50-100	luxuriant	negative
Streptococcus agalactiae ATCC 12386	50-100	luxuriant	negative

Storage and Shelf Life

Store dehydrated medium and prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Facklam R. R., Thacker L. G, Fox B., Eriquez L., 1982, J. Clin. Microbiol., 15 (6), a, 987-990.
2. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Edition, Lippinocott Williams and Wilkins, N.Y. 407-410.
3. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company
4. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

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