Nessler's Reagent

R010

Nessler's Reagent is used to detect production of ammonia and ammonia salts.

Composition**

Ingredients	
Mercuric chloride	10.0 gm
Potassium iodide	7.0 gm
Sodium hydroxide	16.0 gm
Water (ammonia free)	100.0 ml
Final pH (at 25°C)	13.2±0.05

**Formula adjusted, standardized to suit performance parameters

Directions

Emulsify a 24 hours old culture of organism to be tested for urease test in 0.5 ml substrate in a test tube containing 2% urea. Place the tube in a water bath at 37°C for 3 hours. Remove the tube and add 0.1 ml of Nessler's reagent and similar amount to the negative control and blank tubes. Read the results after 3 - 5 minutes after adding the Nessler's Reagent. Both negative and control tubes must be absolutely colourless. When isolated colonies are to be examined, the volume of substrate is reduced to 0.3 ml and only one drop of Nessler's reagent is added.

For detecting NH3 production in L-arginine breakdown : Remove a loopful from a 4 day L-arginine culture and place into 0.5 ml of ammonia free distilled water. Add 1 drop of Nessler's reagent. Run the same check on the control.

Principle And Interpretation

Bacteria, particularly those growing naturally in an environment exposed to urine may decompose urea by means of the enzyme urease. The occurrence of this enzyme can be tested by growing the organism in the presence of urea and testing for alkali (NH3) production by means of a suitable pH indicator. An alternative method is to test for the production of ammonia from urea by means of Nessler's reagent (1) and/or to detect NH3 production due to L-arginine breakdown (2, 3).

Quality Control

Appearance
Pale yellow coloured solution.

Clarity
Clear with no insoluble particles.Note : On storage of the reagent, precipitate may develop. This will not affect the performance criteria of thereagent.

Reaction
Reaction of the solution at 25°C.

pH
13.05-13.25

Test
Emulsify a 24 hour old culture of organism to be tested for urease test, in 0.5 ml substrate containing 2% urea.Place the tube in a waterbath at 37°C for 3 hours. Remove tube and add 0.1 ml of Nessler's reagent.Read the results after 3-5 minutes.

Results
A positive reaction for presence of ammonia is a colour ranging from a pale yellow to a dark brown precipitate.

Storage and Shelf Life

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

Reference

1) Mackie and MacCartney,1989,Practical Medical Microbiology, Collee J.G.,Duguid J.p.,Fraser A.G.and Marmion B.p. (Eds.),13th ed., Churchill Livingstone, edinburgh.
2) Kauffmann F. and Moller U., 1955, Acta Pathol. Microbial. Scand.,36:173
3) MacFaddin J.,1980,Biochemical Tests for identification of Medical Bacteria,2nd ed. Williams and Wilkins, Baltimore.

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