



Nessler's Reagent

R010

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

1. 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.
2. Place the tube in a water bath at 37°C for 3 hours.
3. Remove the tube and add 0.1 ml of Nessler's reagent and similar amount to the negative control and blank tubes.
4. Read the results after 3-5 minutes after adding the Nessler's Reagent. Both negative and control tubes must be absolutely colourless.
5. 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.
6. 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.
7. 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 and/or to detect NH₃ production due to L-arginine breakdown.

Type of specimen

Used as biochemical reagent in diagnosis

Specimen Collection and Handling

1. For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
2. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
3. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The reaction time of ammonia with Nessler's reagent also affects the accurate quantification of NH_3 .
2. The result of the Nessler's method becomes inaccurate if there are interfering substances such as Cl_2 , Cl^- , hardness causing compounds (e.g., Mg^{2+}), and Fe^{2+} in target samples.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

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 the reagent.

- **Reaction** : Reaction of the solution at 25°C. pH : 13.2 ± 0.05
- **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 water bath 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 between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

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. Microbiol. Scand., 36:173
3. MacFaddin J., 1980, Biochemical Tests for identification of Medical Bacteria, 2nd ed. Williams and Wilkins, Baltimore.
4. Gaby, W. L., and L. Free. 1958. Differential diagnosis of pseudomonas-like microorganisms in the clinical laboratory.

- J. Bacteriol. 76:442–444.
5. Gaby, W. L., and C. Hadley. 1957. Practical laboratory test for the identification of *Pseudomonas aeruginosa*. J. Bacteriol. 74:356–358.
6. MacFaddin, J. 1972. Biochemical tests for the identification of medical bacteria. Williams and Wilkins Company, Baltimore, MD.
7. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
9. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
10. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.



Storage temperature



Do not use if package is damaged



In vitro diagnostic medical device



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