Sulphanilic Acid, 0.8% R015

Intended use

Sulphanilic Acid, 0.8% is analytical reagent used along with α- Naphthylamine solution (R009) for determination of nitrate reduction.

Composition**

Ingredients	**-**
Sulphanilic acid	8.0 g
30% Acetic acid	1,000.0 ml
Final pH (at 25°C)	1.6 ± 0.1

**Formula adjusted, standardized to suit performance parameters

Directions

1. Inoculate growth from an 18-24 hours pure culture into Nitrate Broth.
2. Incubate at 35°C for 12 to 24 hours. Very rarely prolonged incubation upto 5 days may be required.
3. Add 0.5 ml a-naphthylamine (R009) along with 0.5 ml sulphanilic acid (R015).

Principle And Interpretation

The α- Naphthylamine solution and Sulphanilic acid is used to determine nitrate reduction by members of Enterobacteriaceae. The reduction of nitrates (NO_3) leads to the formation of nitrites (NO_2) and may progress to the liberation of nitrogen gas. The nitrate reductase producing organisms reduce nitrate to nitrite which reacts with sulphanilic acid to form a diazonium salt. This salt reacts with α- naphthylamine to form a red coloured, water soluble azo dye which results in the visualization of pink-red colour. A distinct red colour formation within 1-2 minutes indicates reduction of nitrate to nitrite.

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 nitrate reduction test may be used as an aid in the identification of bacteria. Additional biochemical testing using pure culture is recommended for complete identification.
2. Nitrate Broth and Nitrate Reagents A and B are not recommended for use in determining nitrate utilization by Mycobacterium spp.
3. Due to the possible presence of nitrite in the culture media, a low nitrite media such as Nitrate Agar or Nitrate Broth should be used for the nitrate reduction test.
4. A negative zinc reduction (no color change) test, in combination with a negative nitrite reaction, is presumptive indication that the nitrate was reduced beyond the nitrite stage. Although a very common end product of nitrite reduction is nitrogen gas, other end products may be formed. Additional testing may be required to determine the final end products of the reaction.
5. To avoid false-negative nitrite reduction reactions, negative nitrite reactions must be verified by the addition of zinc dust to the medium.
6. Excess zinc dust has been reported to cause false-positive nitrite reduction reactions due to complete reduction of previously unreduced nitrate to ammonia.

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 :** Colourless to light yellow solution with characteristic odour.
- **Clarity :** Clear with no insoluble particles.
- **Reaction :** Reaction of the solution at 25°C pH :1.6 ± 0.1
- **Cultural Response:** Add 0.5 ml. of 0.8% Sulphanilic Acid (R015) and 0.5 ml. α- Naphthylamine Solution (R009) into 18-24 hours old cultures in Nitrate Broth.

Organism	Growth	Nitrate Reduction
Acinetobacter calcoaceticus ATCC 43498	Luxuriant	Negative (No colour change)
Klebsiella aerogenes ATCC 13048 (WDCM 00175)	Luxuriant	Positive (Developme nt of distinct red colour)
Escherichia coli ATCC 25922 (WDCM 00013)	Luxuriant	Positive (Developme nt of distinct red colour)
Salmonella enterica subsp. *enterica* Typhimurium ATCC 14028 (WDCM 00031)	Luxuriant	Positive (Developme nt of distinct red colour)

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. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition. Vol. 2.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015)
3. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
4. 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.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
7. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
8. MacFaddin J.,1980, Biochemical tests for identification of medical bateria.
9. Godkar B. P., 1996, Textbook of medical laboratory technology:25(340)

Storage temperature

Do not use if package is damaged

In vitro diagnostic medical device

CE Marking

HiMedia Laboratories Pvt Limited
C-40,21/Y, MIDC, Wagle Ind Area,
Thane(W)–400604,Maharashtra,India

CEpartner4U,ESDOORNLAAN 13,3951
DB MAARN,The Netherlands,
www.cepartner4u.eu

Revision : 03/2023

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications.The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified.Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.