Barritt Reagent A (for VP test)

R029

Intended use

Barritt Reagent A (for VP test) is used in Voges-Proskauer test for detection of acetoin production by bacterial culture.

Composition**

Ingredients

a-Naphthol (1-Naphthol)	5.0 gm
Absolute ethanol	100.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Grow test culture in MR-VP Medium (M070).
2. Add 0.6 ml of Reagent A and 0.2 ml (2 drops) of Reagent B (R030) for 10 ml medium.
3. Shake tubes gently for 30 seconds to 1 minute to expose the medium to atmospheric oxygen in order to oxidize the acetoin (acetylmethylcarbinol) so as to obtain a colour reaction.
4. Allow tube to stand at least 10 to 15 minutes.

Principle And Interpretation

VP test is helpful in identifying members of the family Enterobacteriaceae. Initially all enterics will give a positive MR reaction if tested. However, after further incubation, required by the test procedure (2-5 days), MR - positive organisms continue to produce acids, resulting in a low pH (acidic) that overcomes the phosphate buffering system and maintain an acidic environment in the medium (pH 4.2 or less). MR negative organisms further metabolize the initial fermentation products by decarboxylation to produce neutral acetyl methylcarbinol (acetoin), which results in decreased acidity in the medium and raises the pH towards neutrality (pH 6.0 or above). In the presence of atmospheric oxygen and alkali, the neutral end products, acetoin and 2, 3-butanediol, are oxidized to diacetyl, which react with creatine to produce a red colour.

Type of specimen

The specimen is any isolated colony on primary or subculture plates.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
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. Increased exposure of organisms to atmospheric oxygen in the microtechnique decrease the incubation period.
2. False-positive VP results may occur if VP tests are read beyond one hour following the addition of reagents. A copper like color may develop, resulting in a potential false positive interpretation.
4. Shaking the tubes enhance VP reaction.
3. With prolonged incubation, some VP positive organisms can produce acid condition in the medium, yielding weak positive reaction or false negative VP reaction.
5. Do not add more than 2 drops of KOH per 2ml of medium. Excess amount of KOH can give a week positive reaction, which may be masked by the formation of copper like color because of the reaction of KOH with alphanaphthol alone.
6. Reagents must be added in specified order. A reversal of order may result in the weak positive or false negative VP results.

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 reddish brown coloured solution.
- **Clarity :** Clear without any precipitate.

Cultural Response

Organism	Growth	VP Test
Cultural Responce	24-48 hours old cultures grown in MR-VP Medium (M070). reagent (Part A) (R029) and 0.2 ml of Barritt Reagent (Part B) (R030) in Biochemical identification was carried out by adding 0.6 ml of Barritt R	
**Klebsiella aerogenes* ATCC 13048 (WDCM 00175)	Luxuriant	Positive (Cherry red colour formation)
Escherichia coli ATCC 25922 (WDCM 00013)	Luxuriant	Negative (No red colour formation)
Klebsiella pneumoniae ATCC13383 (WDCM 00097)	Luxuriant	Positive (Cherry red colour formation)

(*) formerly known as *Enterobacter aerogenes*

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.
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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. Color Atlas and Textbook of Diagnostic Microbiology,4th edition ,Elmer W. Koneman. Stephen D.Allen., William M. Janda., Paul C. Schreckenberger.,Washington C.Winn.

Storage temperature

Do not use if package is damaged

In vitro diagnostic medical device

CE Marking

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