



## Methyl Red Indicator

I007

### Intended use

Methyl Red Indicator is recommended in IMViC test for detection of acid production from glucose fermentation.

### Composition\*\*

#### Ingredients

Methyl Red	0.2 gm
Ethyl alcohol	60.0 ml
Distilled water	40.0 ml

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Add 1-2 drops of methyl red indicator (I007) to 5 ml of a 24 - 48 hours old culture of the organism under investigation, interpret the colour result immediately. Acid produced due to fermentation of glucose by bacterial culture gives red colouration to the medium. Negative reaction is indicated by development of yellow colour.

### Principle And Interpretation

The Methyl red test is a biochemical test to detect acid producing organisms from glucose through the mixed acid fermentation pathway. It is used as a part of IMViC tests, a set of four biochemical test used for the differentiation of many species of Enterobacteriaceae. Enterobacteriaceae may produce sufficient quantities of strong acid, that can be detected by methyl red indicator during the initial phases of incubation. Only organisms that can maintain this low pH after prolonged incubation (48-72 hours) and overcoming the pH buffering system of the medium can be called as methyl red positive. The development of stable red colour in surface of the medium indicates sufficient acid production to lower pH to 4.4 and constitutes positive test. While other organisms may produce lesser quantities of acid from test substrate, an intermediate orange colour between yellow and red may be developed. This indicate negative test.

### Type of specimen

Clinical samples, Water samples

### Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established 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. The MR test should not be read before 48 hours, because some organisms will not have produced enough products from the fermentation of glucose.
2. MR-negative organisms may also not have had sufficient time to convert those products and will appear MR positive.
3. If the methyl red test results are inconclusive (orange) after 48 hours, continue incubation of the broth for an additional three days and retest the broth culture.
4. MR-VP testing should be used in conjunction with other confirmatory tests to differentiate organisms among the Enterobacteriaceae.

## 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** : Pinkish red solution.
- **Clarity** : Clear solution without any particles.
- **Reaction** : Red at pH 4.4 and Yellow at pH 6.2 at 25 °C
- **Sensitivity** : A mixture of 0.1ml of the solution, 100ml of carbon dioxide free water and 0.05ml of 0.02M hydrochloric acid is red. Not more than 0.1ml of 0.02M sodium hydroxide is required to change the colour to yellow.

## Cultural Response

Organism	Growth	MR Test
Cultural Response	Cultural response is observed after 18-24 hours at 37°C in MR-VP medium (M070) Methyl Red test is performed by using Methyl red indicator.	
* <i>Klebsiella aerogenes</i> ATCC 13048 (WDCM 00175)	Luxuriant	Negative (Yellow colour observed)
<i>Escherichia coli</i> ATCC 25922 (WDCM 00013)	Luxuriant	Positive (Red colour observed)
<i>Klebsiella pneumoniae</i> ATCC13383 (WDCM 00097)	Luxuriant	Negative (Yellow colour observed)

(\*) 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. Color Atlas and Textbook of Diagnostic Microbiology, 4th edition, Elmer W. Koneman. Stephen D. Allen., William M. Janda., Paul C. Schreckenberger., Washington C. Winn.
2. Godkar B. P., 1996, Textbook of medical laboratory technology: 25(341)
3. Horobin R. W. and Kiernan J. A., 2002, 10th ed., CONN'S Biological Stains, A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine: 10(117-118)
4. INDIAN PHARMACOPOEIA 2018, VOL-I, 4.3(960).



Storage temperature



Do not use if package is damaged



In vitro diagnostic medical device



CE Marking



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