



## METHYL RED

### INTENDED USE

Remel Methyl Red reagent is recommended for use in qualitative procedures to test the ability of an organism to produce and maintain stable end products from glucose fermentation.

### SUMMARY AND EXPLANATION

The methyl red test was first described in 1915 by Clark and Lubs who used it to aid in the differentiation of the coliform aerogenes group of enteric bacteria.<sup>1</sup>

### PRINCIPLE

The methyl red test is based on the use of an indicator, methyl red, to determine the pH of the medium following glucose fermentation.<sup>2</sup> All members of the *Enterobacteriaceae* are glucose-fermenters which result in the production of acidic metabolic byproducts. After initial incubation all will give a positive methyl red reaction if tested.<sup>3</sup> However, after further incubation (2-5 days), methyl red-positive organisms continue to produce more acids and overpower the phosphate buffer, resulting in a low pH and a red color development. Methyl red-negative organisms further metabolize the fermentation products by decarboxylation, producing acetylmethylcarbinol (acetoin), which results in a neutral pH and no red color.

### REAGENTS (CLASSICAL FORMULA)\*

Methyl Red ..... 0.2 g  
Ethyl Alcohol (CAS 64-17-5) ..... 600.0 ml  
Demineralized Water (CAS 7732-18-5) ..... 400.0 ml

\*Adjusted as required to meet performance standards.

### PRECAUTIONS

**WARNING! FLAMMABLE**, keep away from heat, sparks and flame! Avoid breathing vapor and eye/skin contact.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Take precautions against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

### STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Protect product from light.

### PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

### SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.<sup>4</sup>

### MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Pipette, (7) Test tubes, (8) MR-VP Broth (R061430).

### PROCEDURE

- Using a light inoculum from a pure, 18-24 hour culture inoculate MR-VP Broth.
- Incubate in ambient air at 35-37°C for a minimum of 48 hours. (Prolonged incubation up to 5 days may be required.)

- Following incubation, aseptically transfer 1 ml of the medium to a separate test tube to perform the Methyl Red (MR) test.
- Add 1-2 drops of Methyl Red reagent to the aliquot of MR-VP Broth.
- Observe for an immediate red color development on the surface of the medium.

### INTERPRETATION

Positive Test - Red color development on surface of medium

Negative Test - Yellow color on surface of medium

### QUALITY CONTROL

All lot numbers of Methyl Red have been tested using the following quality control organisms and have been found to be acceptable. Testing of a positive and negative control should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

#### CONTROL

*Escherichia coli*  
ATCC® 25922

#### INCUBATION

Ambient, 48h @  
35-37°C

#### RESULTS

Positive

*Klebsiella pneumoniae*  
ATCC® 27736

Ambient, 48h @  
35-37°C

Negative

### LIMITATIONS

- Avoid testing an extremely turbid broth-inoculum mixture. Bacterial growth is inhibited if the inoculum exceeds the maximum cell concentration of about 10<sup>9</sup> viable cells/ml.<sup>5</sup>
- The following variables should be standardized to obtain optimal and reproducible results: (a) the inoculum density, (b) the total volume of broth, and (c) the size of the test tube used. An orange color reaction often occurs when too large a volume of broth is used.<sup>5</sup>

### BIBLIOGRAPHY

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### PACKAGING

REF 21236, Methyl Red ..... 25 ml/Btl

### Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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CAS (Chemical Abstracts Service Registry No.)

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