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# YEAST FERMENTATION BROTH w/ BROM THYMOL BLUE and DURHAM TUBE, w/ and w/o CARBOHYDRATES

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## INTENDED USE

Remel Yeast Fermentation Broths with Brom Thymol Blue (BTB) and Durham Tube (DT), when supplemented with carbohydrates, are liquid media recommended for use in qualitative procedures for the determination of yeast fermentation reactions.

## SUMMARY AND EXPLANATION

Carbohydrate fermentation tests are useful for yeast identification when other tests fail to identify an isolate. Wickerham developed a chemically defined broth medium to determine yeast carbohydrate assimilation reactions which occur in the presence of oxygen.<sup>1</sup> Fermentation reactions, on the other hand, occur in the absence of oxygen and are evidenced by gas formation. In separate studies, Ahearn and Haley used a modification of Wickerham's formula to determine the fermentation reactions of yeast isolates using a battery of carbohydrates.<sup>2,3</sup>

## PRINCIPLE

Peptone provides nitrogenous compounds and amino acids essential for the growth of yeasts. Yeast extract provides B-complex vitamins. The anaerobic utilization (fermentation) of a carbohydrate results in the formation of ethanol and carbon dioxide. A positive fermentation reaction is indicated by the presence of gas (bubbles) in the inverted Durham tube. The development of a yellow color in the fermentation test (acid pH) indicates the carbohydrate has been assimilated. The base medium employed to test fermentation reactions provides the vitamins necessary for the growth of yeasts.

## REAGENTS (CLASSICAL FORMULAE)\*

Base Control Medium:

|                    |       |                          |           |
|--------------------|-------|--------------------------|-----------|
| Peptone.....       | 7.5 g | Brom Thymol Blue.....    | 16.0 mg   |
| Yeast Extract..... | 4.5 g | Demineralized Water..... | 1000.0 ml |

pH 6.9 ± 0.2 @ 25°C

The following optional ingredients are available per liter of medium:

|                  |        |                 |        |
|------------------|--------|-----------------|--------|
| Cellobiose ..... | 10.0 g | Maltose .....   | 10.0 g |
| Dextrose.....    | 10.0 g | Raffinose ..... | 10.0 g |
| Galactose.....   | 10.0 g | Sucrose .....   | 10.0 g |
| Lactose.....     | 10.0 g | Trehalose.....  | 10.0 g |
|                  |        | Xylose.....     | 10.0 g |

\*Adjusted as required to meet performance standards.

## PROCEDURE

1. Prepare the inoculum by making a suspension of the test isolate in sterile demineralized water or saline equal to a No. 1 McFarland turbidity standard or equivalent (REF R20411).
2. Transfer 0.1 ml of the suspension to each Yeast Fermentation Broth w/ BTB and Carbohydrate being tested.
3. Simultaneously inoculate a tube of Yeast Fermentation Broth w/ BTB Base Control as a negative control.
4. Incubate the tubes aerobically with caps loosened at 25-30°C.
5. Examine at 7 days and hold up to 24 days before reporting as negative. Daily agitation during incubation has been reported to facilitate growth of yeast isolates.<sup>3</sup> Avoid getting bubbles in Durham tubes.

## INTERPRETATION OF THE TEST

Positive Test - Fermentation is indicated by accumulation of gas (CO<sub>2</sub>) in the Durham tube.

Negative Test - No gas production in the Durham tube

## QUALITY CONTROL

All lot numbers of Yeast Fermentation Broths w/ BTB and DT w/ and w/o Carbohydrates have been tested for performance and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. Control organisms should be selected that demonstrate a positive and negative reaction for each carbohydrate tested. If aberrant quality control results are noted, patient results should not be reported.

## LIMITATIONS

1. A heavy inoculum may cause a false-positive reaction due to endogenous carbohydrates present in the yeast cells.<sup>3</sup>
2. The brom thymol blue indicator will turn yellow due to carbohydrate assimilation. All fermented carbohydrates will be assimilated, but not all assimilated carbohydrates will be fermented.<sup>4</sup>
3. Assimilation or fermentation studies are never incubated at 35-37°C because disaccharides and similar carbohydrates can be broken down into their component sugars at this temperature.<sup>5</sup>

## BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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