# TCH AGAR (1 µg/ml and 5 µg/ml)

## INTENDED USE
Remel TCH Agars (1 µg and 5 µg) are solid media recommended for use in qualitative procedures for differentiation of *Mycobacterium bovis* from *Mycobacterium tuberculosis* and other nonchromogenic, slow-growing mycobacteria.

## SUMMARY AND EXPLANATION
Studies by Bonicke, using thiophen-2-carboxylic acid hydrazide (TCH), resulted in the use of this drug in tuberculosis diagnostic testing.[1] Vestal and Kubica also demonstrated *M. bovis* is predictably sensitive to low concentrations of TCH (1 µg/ml to 5 µg/ml), while most other slow-growing *Mycobacterium* spp., including *M. tuberculosis*, are resistant.[2,3]

## PRINCIPLE
TCH Agar base contains inorganic salts which are essential to the growth of mycobacteria, and glycerol, a source of carbon and energy. Casein hydrolysate serves as a growth stimulant for drug-resistant strains of *M. tuberculosis*. Sodium citrate, converted to citric acid, serves to hold inorganic cations in solution. Malachite-green dye is a selective agent which inhibits bacteria other than mycobacteria. OADC Enrichment contains oleic acid, which is required in the metabolism of mycobacteria, and albumin to protect against toxic substances that may be present in the medium. *M. bovis* is susceptible to low concentrations of TCH, 1 to 5 µg/ml. *M. tuberculosis* and most other slow-growing mycobacteria are usually resistant to the inhibiting action of this compound.

## REAGENTS (CLASSICAL FORMULAE)*
**Base medium:**

| | | | |
|---|---|---|---|
| Dipotassium Phosphate | 1.5 g | Ferric Ammonium Citrate | 0.04 g |
| Monopotassium Phosphate | 1.5 g | Malachite Green | 1.0 mg |
| Casein Hydrolysate | 1.0 g | Pyridoxine Hydrochloride | 1.0 mg |
| Ammonium Sulfate | 0.5 g | Biotin | 0.5 mg |
| Monosodium Glutamate | 0.5 g | •OADC Enrichment | 100.0 ml |
| Sodium Citrate | 0.4 g | Glycerol | 5.0 ml |
| Magnesium Sulfate | 0.05 g | Agar | 15.0 g |
| | | Demineralized Water | 900.0 ml |

pH 6.6 ± 0.2 @ 25°C

The following ingredient is available per liter of medium:
TCH Solution ..... 1 µg/ml or 5 µg/ml

**•OADC Enrichment:**

| | | | |
|---|---|---|---|
| Albumin Fraction V | 50.0 g | Oleic Acid | 0.5 g |
| Dextrose | 20.0 g | Catalase (Beef) | 0.04 g |
| Sodium Chloride | 8.5 g | Demineralized Water | 1000.0 ml |

*Adjusted as required to meet performance standards.

## PROCEDURE
1. Prepare a barely turbid suspension of the test isolate growing on a drug-free medium in sterile water or saline. Inoculate a positive control and negative control organism with each test.
2. Dilute suspension to $10^{-3}$ and $10^{-5}$ (1:1000 and 1:100,000) with sterile water or saline.
3. Inoculate TCH Agar (1 µg/ml and/or 5 µg/ml) and a control medium (Middlebrook 7H11) using a sterile pipette.
4. Add 3 drops (or 0.1 ml) of each dilution to TCH-containing media and to the control medium.
5. Incubate in 5-10% $CO_2$ at 33-37°C for 14-21 days.
6. Record the time when definite growth is observed on the media.

## INTERPRETATION OF THE TEST
Sensitive - Growth of test isolate on TCH medium is <1% of the growth on the control medium
Resistant - Growth of test isolate on TCH medium is ≥1% of the growth on the control medium
Control - Confluent growth on TCH medium and control medium (equivalent to approximately $10^4$ organisms)

## QUALITY CONTROL
All lot numbers of TCH Agar (1 µg and 5 µg) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms 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 | INCUBATION | RESULTS |
|---|---|---|
| *Mycobacterium bovis* ATCC® 19210 | $CO_2$, up to 21 days @ 33-37°C | Sensitive |
| *Mycobacterium tuberculosis* ATCC® 25177 | $CO_2$, up to 21 days @ 33-37°C | Resistant |

(Continued on back)

## LIMITATIONS

1. Some strains of *M. tuberculosis* and *M. bovis* have been found to be inhibited by 1 µg/ml of TCH.[4]
2. Studies have shown the susceptibility of an isolate to a concentration of 1 µg/ml TCH is highly reproducible. However, the isolate should also be tested with a concentration of 5 µg/ml TCH, to confirm results obtained with the lower concentration.[4,5]
3. Isoniazid-resistant strains of *M. bovis* may also be resistant to TCH.[3]

## BIBLIOGRAPHY


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5. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press, Washington, D.C.


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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