Middlebrook 7H10 Agar

Moderately selective medium for the isolation and cultivation of mycobacteria.

INTENDED PURPOSE

Solid medium used for the selective isolation of *Mycobacterium* spp., especially *Mycobacterium tuberculosis*, from clinical specimens as well as for the cultivation of pure cultures of mycobacteria. This medium is intended as an aid in the diagnosis, requiring additional tests to complete the diagnostic results.

DESCRIPTION

There are many culture media that have been devised for the cultivation of mycobacteria over the years. The early ones were egg-based formulations which included Lowenstein-Jensen Medium and Petragnani Medium. Later, Dubos and Middlebrook developed various formulations containing oleic acid and albumin, which protected *Mycobacterium* from toxic agents, helping for the growth of tubercle bacilli. Subsequently, Middlebrook and Cohn improved the formulation to develop 7H10 medium, which allowed faster and more luxuriant growth of *Mycobacterium* species.

Middlebrook 7H10 Agar (with OADC supplement and glycerol) is used for isolation, cultivation, and sensitivity testing of *M. tuberculosis*. It has been reported that the 7H10 medium tends to grow fewer contaminants than the egg-based media commonly used for the cultivation of mycobacteria.

TYPICAL FORMULA* (Per Litre of Purified Water)

Basal medium	
L-Glutamic Acid	0.5 g
Sodium Citrate	0.4 g
Pyridoxine Hydrochloride	0.001 g
Biotin	0.0005 g
Ferric Ammonium Citrate	0.04 g
Ammonium Sulfate	0.5 g
Disodium Phosphate	1.5 g
Monopotassium Phosphate	1.5 g
Magnesium Sulfate	0.025 g
Malachite Green	0.00025 g
Agar	15.0 g
Calcium Chloride	0.0005 ml
Zinc Sulfate	0.001 g
Copper Sulfate	0.001 g
Final pH 6.6 ± 0.2 at 25°C	

OADC supplement	
Oleic Acid	0.06 ml
Catalase	0.003 g
Bovine Albumin (Fraction V)	5.0 g
Glucose	2.0 g
Sodium Chloride	0.85 g

OADC: Oleic acid, Albumin, Dextrose, Catalase.

Glycerol supplement	
Glycerol	5.0 ml

*Adjusted and/or supplemented as required to meet performance specifications.

Complete medium

Base + Supplements

Middlebrook 7H10 Agar Base + **Middlebrook 7H10 (OADC) Supplement** + **Glycerol**

Supplementation of the agar base is required to obtain mycobacterial growth.

METHOD PRINCIPLE

Glutamic acid, sodium citrate, pyridoxine, biotin and ammonium sulfate supply growth factors. Ferric ammonium citrate, magnesium sulfate, calcium chloride, zinc sulfate and copper sulfate are sources of trace ions. Phosphates help maintaining the pH of the medium. Malachite green is the selective agent inhibiting the contaminant microbial flora. Malachite green serves as pH indicator as well. Agar is the solidifying agent. Glycerol and glucose are energy sources. Sodium chloride maintains the osmotic equilibrium. Albumin protects the tubercle bacilli against toxic agents. Catalase destroys toxic peroxides that may be present in the medium. Oleic acid is a fatty acid utilized in the mechanism of mycobacteria.

PREPARATION

Dehydrated medium.

Suspend 19.5 g of powder in 900 ml of distilled or deionized water containing 5 ml of Glycerol supplement. Heat to boil until completely dissolved. Autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and aseptically add 100 ml Middlebrook 7H10 supplement. Mix well and pour into sterile final containers (this medium is prepared in slant tubes or plates).

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Autoclave, sterile Petri plates, test tubes, inoculating loops, swabs, incubator, quality control organisms.

SPECIMENS

Specimens submitted for mycobacterial culture fall into two categories: specimens normally contaminated with resident flora, and specimens from normally sterile sites. Contaminated specimens require a decontamination step before culture to reduce the likelihood of overgrowth by organisms other than mycobacteria. Specimens should be obtained before antimicrobial therapy (where possible) and promptly delivered to the laboratory for examination.

Refer to specific guidelines for more detailed information.

TEST PROCEDURE

Inoculate processed specimen onto the medium. Process clinical specimens as soon as possible. Concentration of samples (for example centrifugation) may be required. Depending on the specimen type and method used, it may be necessary to go through one or more of the following stages before culture: homogenization, digestion/ decontamination, and neutralization.
Keep plates and tubes shielded from light and incubate at 35 ± 2°C for up to 8 weeks in aerobic atmosphere enriched with 5-10% carbon dioxide.

For more detailed information, consult appropriate guidance.

INTERPRETING RESULTS

Examine weekly for growth, pigment production and colony morphology. Carry out identification tests according to established laboratory procedures.

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store slant tubes and prepared plates at 2-8 °C.

SHELF LIFE

Ready-to-use plates: 6 months.
Medium in tubes: 1 year.
Dehydrated medium: 4 years.
Supplements: 2 years.

QUALITY CONTROL

Appearance of OADC Supplement: Amber liquid, limpid or slightly opalescent.

Appearance of Glycerol Supplement: Dense colourless substance of oily appearance.

Appearance of Dehydrated Medium: Free-flowing, homogeneous. Light beige with green tint.

Appearance of Prepared Medium: Slightly opalescent, light yellowish green.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Specification
Mycobacterium intracellulare	ATCC® 13950	10^3-10^4 CFU	up to 21 d/ 35 ± 2°C/ 5-10% CO_2	Good growth
Mycobacterium scrofulaceum	ATCC® 19981			
Escherichia coli	ATCC® 25922	10^4-10^6 CFU		Partial to complete inhibition
Staphylococcus aureus	ATCC® 25923			
Streptococcus pyogenes	ATCC® 19615			

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of Middlebrook 7H10 Agar was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Negative culture results do not rule out active infection of mycobacteria.

Some factors of unsuccessful cultures are:

- The specimen was not representative of the infectious material, e.g. saliva instead of sputum.
- The mycobacteria were destroyed during digestion and decontamination of the specimen.
- Gross contamination interfered with the growth of mycobacteria.
- Proper aerobic conditions and increased CO_2 tension were not provided during incubation.

Since this medium is only partially selective, bacteria other than mycobacteria may grow if specimens are not appropriately pretreated for decontamination.

WARNING AND PRECAUTIONS

1) **For *in vitro* diagnostic use (IVD).**
2) **For laboratory professional use only.**
3) Operators must be trained and have certain experience. Please read the instructions carefully before using the product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
4) Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.
5) Do not use if the product or packaging appears to be damaged.
6) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
7) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
8) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
9) Do not mix reagents of different batches. Please use the product within the validity period.
10) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
11) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
12) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
13) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below.

Product	Format	Packaging	Ref.
Middlebrook 7H10 Agar*	Plate 90 mm	20 plates	10453
	Slant tube	10 x 8.5 ml	30368
Middlebrook 7H10 Agar Base	Dehydrated media	500 g	611022
Middlebrook 7H10 (OADC) supplement	Bottle	4 x 50 ml	81035
Glycerol supplement	Bottle	4 x 50 ml	80021 •

* Complete medium

• Not CE-IVD marked