

NNN Modified Medium (Twin Pack)

Intended Use:

Recommended for cultivation of Leishmaniae and Trypanosomes

Composition**

Ingredients	g / L
Part A	-
HM extract #	3.000
Peptone Sodium	5.000
chloride	8.000
Agar	15.000
Final pH (at 25°C)	7.3 ± 0.2
Part B	-
Sodium chloride	8.000
Potassium chloride	0.200
Calcium chloride	0.200
Potassium dihydrogen phosphate	0.300
Dextrose	2.500
(Glucose) Final pH	$7.0\pm~0.2$
(at 25°C)	

**Formula adjusted, standardized to suit performance parameters # Equivalent to Meat extract

Directions

Part A: Suspend 31 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 10% of sterile defibrinated rabbit or human blood after inactivation at 56°C for 30mins. Mix well and dispense in 5 ml amounts in test 25 ml flasks. Allow tubed media cool in tubes or amounts in to slanted position. Part B : Suspend 11.2 grams of Part B in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add approximately 2 ml in tubes or 10-15 ml in flasks over solidified Part A medium.

Principle And Interpretation

The protozoan family *Trypanosomatidae* includes members from the genera *Leishmania* and *Trypanosoma*, which are flagellates that inhabit the blood and tissues of humans. NNN Medium was developed by Novy, McNeal (1) and modified by Nicolle (2). NNN Modified Medium is a modification of the original medium and consists of two phases, blood agar (Part A) and Lockes solution (Part B) (3). This modified medium is commonly used for diagnostic work (4,5). This medium consists of a blood agar base and an overlay medium. The blood agar base is a highly nutritious medium that supports the growth of fastidious organisms like *Leishmania* and *Trypanosoma*. The specimens are inoculated into the liquid phase of the diphasic medium and incubated. This favours the development of organisms in the insect vector. The amastigotes transform to promastigotes in about 24 hours (4).

Type of specimen

Clinical samples - Tissue samples, lesion aspirates, etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional 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.

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Limitations

- 1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
- 2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate
- the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement. 3. Further serological and biochemical testing is required for complete identification.

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

Part A : Cream to tan homogeneous free flowing powder Part B: White to cream homogeneous free flowing powder **Gelling** Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared medium

Basal medium :Light amber clear to slightly opalescent gel. After addition of sterile defibrinated rabbit or human blood : Red coloured opaque gel Part B : Colourless clear liquid

Reaction

Reaction of 3.1% w/v aqueous solution (Part A) at 25°C. pH : 7.3±0.2 Reaction of 1.12% w/v aqueous solution (Part B) at 25°C. pH : 7.0±0.2

Cultural Response

Cultural characteristics observed after an incubation at 21-26°C for 48-72 hours, with added sterile defibinated rabbit or human blood.

Organism	Growth
Leishmania donovani	luxuriant
Trepanosoma cruzi	luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

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 (6,7).

Reference

- 1. Novy F. G. and McNeal W. J., 1904, J. Inf. Diseases B, 1:1.
- 2. Nicolle A (1908) Comptes rendus de l Academie des Sciences (Paris) 146:842.
- 3. Cruickshank R., Duguid J. P., Marmion B. P., Swain R. H. A. (Eds) 1975, Medical Microbiology, 12th Edition, Vol. II, Churchill Livingstone.

4. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.

5. Taylor A. R., Baker J. R., (Eds.), 1978, Methods of Cultivating Parasites in vitro, Academic Press, London, pp 55-88

6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.



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In vitro diagnostic

IVD



]_30°C Storage temperature

> Do not use if package is damaged

Disclaimer :

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