Instructions for Use

REF - PP0972 **Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F)**

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use. Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F) is used for antimicrobial susceptibility testing by the disk diffusion method as described by Bauer-Kirby [1].

2. Composition*

Ingredient	g/L
Beef extract	2.0
Acid digest of casein	17.5
Starch	1.5
Agar	17.0
Defibrinated horse blood	50ml
NAD	0.02

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

First described by Mueller and Hinton [2], Mueller Hinton Agar has been approved as the definitive medium for this purpose by European Committee on Antimicrobial Susceptibility Testing (EUCAST) [3] and Clinical and Laboratory Standards Institute (CLSI) [4]. Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F) is supplemented and is suggested by EUCAST for fastidious organisms, such as *Streptococcus pneumoniae*, *Streptococcus* groups A, B, C and G, Viridans group streptococci, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Listeria monocytogenes*, *Pasteurella multocida*, *Campylobacter jejuni* and *coli*, *Corynebacterium* spp., *Aerococcus sanguinicola* and *urinae* and *Kingella kingae* [3].

4. Principle

Beef extract and acid digest of casein supplies the required nitrogen, carbon, and vitamins in the medium. Starch absorbs any toxic metabolites produced. The medium contains low levels of thymidine and thymine and controlled levels of calcium and magnesium ions. Nicotinamide adenine dinucleotide (NAD) is added to make the medium suitable for use with more fastidious organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae*.

5. Physical Characteristics

	Medium
Appearance and Colour	Cherry red firm gel
pH	7.3 ± 0.1

6. Materials Provided

PP0972 Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F) is supplied in packs of 10 x 90mm P090 Petri dishes (24-26ml fill) (product code: PP0972-P090) or packs of 5 x 120mm PLSQ square dishes (55-57ml fill) (product code: PP0972-PLSQ); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., Antibiotic discs, sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

For testing individual isolated colonies for antimicrobial susceptibility testing by the disk diffusion method as described by Bauer-Kirby [1]. Use a sterile loop or cotton swab to pick colonies from an overnight culture on non-selective media. If possible, use several morphologically similar colonies to avoid selecting an atypical variant.

Suspend in saline and mix to an even turbidity. Adjust the density of the suspension to 0.5 McFarland by adding saline or more bacteria. Preferably use a photometric device to measure the turbidity. (Exception: *Streptococcus pneumoniae* is suspended to 0.5 McFarland from a blood plate such as E&O PP0120 Columbia Agar with 5% Horse Blood, but to 1.0 McFarland from a chocolate plate such as E&O PP0100 Chocolate Agar with 5% Horse Blood). [3]

Optimally, use the inoculum suspension within 15 minutes of preparation and always within 60 minutes.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

For full methods, see EUCAST [3] and CLSI [4].

The surface of the agar should be dry and room temperature before use.

Dip a sterile cotton swab into the inoculum suspension. For Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube to avoid over-inoculation. For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.

Spread the inoculum evenly over the entire surface by swabbing in three directions or by using a plate rotator. For Gram-positive bacteria, take particular care to ensure that there are no gaps between streaks. When inoculating several agar plates with the same inoculum, dip the cotton swab into the suspension for each agar plate.

Apply disks within 15 min of inoculation. Disks must be in close and have even contact with the agar surface. The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application at 35 ± 2°C in 5% CO_2 for between 16-24 hours dependant on organism species under test.

Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30cm from the eye. Measure zone diameters to the nearest millimetre with a calibrated ruler or calibrated callipers. If an automated zone reader is used, it must be calibrated to manual reading.

10. Quality Control

Organism	Incubation	Result (Specificity)
H. influenzae (NCTC 12975)	37 ± 1°C in 5% CO_2 for 18-24 hours	Growth: Opaque/Grey colonies
S. pneumoniae (NCTC 12977)	37 ± 1°C in 5% CO_2 for 18-24 hours	Growth: Grey/green colonies with α-haemolysis

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify PP0972 Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F) performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu and incubated at 37 ± 1°C for 18-24 hours. All samples showed expected recovery and morphology of the required test organisms: *Haemophilus influenzae* (NCTC 12975) and *Streptococcus pneumoniae* (NCTC 12977). Each lot of dehydrated Mueller Hinton Agar (E&O KM0183) has been tested according to ISO 16782:2016 [5]/CLSI and meets quality control criteria as stated in CLSI M100. Therefore, it can be concluded that PP0972 Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Stacking plates in the incubator may affect results due to uneven heating. The efficiency of incubators varies, but for most incubators, a maximum of five plates per stack is appropriate.
- A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. The growth should be evenly distributed over the agar surface to achieve uniformly circular (non-jagged) inhibition zones. If individual colonies can be seen, the inoculum is too light, and the test must be repeated.
- Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F) should not be used for testing trimethoprim-sulfamethoxazole, HTM should be used instead [4].

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP0972 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured, out of date or if cracks in the agar medium are present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Hudzicki J (2009) Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiol.
2. Mueller, H. and Hinton, J. (1941) A protein-free medium for primary isolation of the Gonococcus and Meningococcus. Proc. Soc. Expt. Biol. Med., 48, 330-333.

3. EUCAST (2025) Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 13.0
4. CLSI (2026) Performance Standards for Antimicrobial Susceptibility Testing. 36th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute
5. International Organization for Standardization (2016) 16782:2016 Clinical laboratory testing - Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing. Geneva, ISO.

Version History*

001 08/03/2023 - New document created
002 21/05/2026 - Updated references. Added limitation regarding trimethoprim-sulfamethoxazole.

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com
EC REP Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place