

## Mueller-Hinton agar (MHA) and MHA supplemented with horse blood and NAD (MH-F) – Version 1.0

MHA is used for antimicrobial susceptibility testing of non-fastidious organisms. MHA supplemented with 5% defibrinated horse blood and 20 mg/L  $\beta$ -NAD is used for testing *Streptococcus* spp., *Haemophilus* spp. and some other fastidious organisms. Agar plates may be purchased ready-poured from commercial sources or prepared locally as follows:

Reagents	
1.	MHA powder from commercial source.
2.	Defibrinated horse blood.
3.	$\beta$ -Nicotinamide adenine dinucleotide ( $\beta$ -NAD). Prepare a stock solution by dissolving $\beta$ -NAD in sterile deionized water to a concentration of 20 mg/mL. Filter sterilize the solution. Stock solution may be stored at -20°C in aliquots and defrosted as required. Do not refreeze unused solution.

Preparation of agar plates	
1.	Prepare and autoclave MHA according to the manufacturer's instructions.
2.	Cool medium to below 50°C.
3.	For MH-F, aseptically add 50 mL defibrinated horse blood and 1 mL $\beta$ -NAD stock solution per litre medium. Mix well and dispense immediately.
4.	Dispense medium into sterile Petri dishes to give a level depth of 4 mm $\pm$ 0.5 mm (25 mL in 90 mm diameter Petri dish, 70 mL in 150 mm diameter Petri dish).
5.	Allow the agar to set before moving the plates.
6.	The surface of the agar should be dry before use. Whether plates require drying and the length of time needed to dry the surface of the agar depends on storage and drying conditions. Do not over-dry plates.

Storage of agar plates	
1.	Store plates in vented plastic boxes at 8-10°C. Alternatively, store plates at 4-8°C in sealed plastic bags.
2.	For plates prepared in-house, plate drying, storage conditions and shelf life should be determined as part of the laboratory quality assurance programme.
3.	Commercially prepared plates should be stored as recommended by the manufacturer and used within the labelled expiry date.

Quality control	
1	Use a surface pH electrode to check that the pH is within the range 7.2-7.4.