Instructions for Use

REF - DHB **Defibrinated Horse Blood**

1. Intended Use

Defibrinated Horse Blood (DHB) is aseptically collected, defibrinated horse blood with a packed cell volume (PCV) of 38% to 45%. Defibrinated Horse Blood (DHB) is an enrichment supplement for the preparation of culture media typically used to isolate clinically significant pathogens including *Staphylococcus* and *Streptococcus* species.

2. Composition*

Ingredient	
Defibrinated Horse Blood	100%

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Horse blood is collected aseptically by venepuncture and immediately mechanically defibrinated in such a way as to maximise fibrinogen removal and minimise erythrocyte rupture. Since it is not possible to sterilise blood by heat treatment, filtration or chemical means, strict sterility testing is conducted to ensure that the blood is free from contamination. Defibrinated horse blood is added to an agar base at 5% to 10% v/v to improve growth of fastidious organisms and/or to confirm the presence and type of haemolysis. Haemolytic reactions seen with horse blood agars may differ to those seen with equivalent sheep blood agars so careful attention to the specific test methodology and to the test results is advisable.

4. Preparation Instructions

Dependent on final medium produced. Aseptically add the required defibrinated horse blood volume to the sterilised base medium and allow to mix prior to final product dispensing.

5. Physical Characteristics

	Appearance
Appearance and Colour	Cherry red liquid

6. Materials Provided

Defibrinated Horse Blood (DHB) can be provided in various formats listed in the table below. Each container is labelled with (abbreviated) product name, product code, lot number and expiry date.

Product Code	**Product format**
DHB100-N100-100	100ml in a Square PETG Bottle
DHB006-P030-6	6ml in a Polycarbonate Universal
DHB050-N100-50	50ml in a Square PETG Bottle
DHB025-P030-25	25ml in a Polycarbonate Universal
DHB1L-1LB-1000	1L in a Media Bag
DHB500-N500-500	500ml in a Square PETG Bottle

7. Materials Needed but not Provided

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

8. Specimens

Blood containing agars can be used to isolate several different pathogenic bacteria from a wide variety of clinical specimens. Dependent on final medium produced. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Dependent on final medium produced. Follow supplier's instructions on preparing the dehydrated culture medium, sterilise & cool prior to adding the Defibrinated Horse Blood (DHB) to the medium and mix well before dispensing to the final container.

10. Quality Control

Organism	Incubation	Result (Specificity)
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: Grey colonies
S. agalactiae (ATCC 12386)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies with β-haemolysis
S. pyogenes (NCTC 12696)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies with β-haemolysis
S. pneumoniae (NCTC 12977)	37 ± 1°C in 5-10% CO_2 for 18-24 hours	Growth: Grey/green colonies with α-haemolysis
	Sterility Testing: Incubate aerobically at 37 ± 1°C for 72 hours and at 20 ± 5°C for 120 hours	

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

12. Performance

To fully verify Defibrinated Horse Blood (DHB) performance, Samples from the batch of freshly collected blood are tested immediately and after 7 days storage at 15-25°C during which the blood is inspected daily for colour variation and clotting. At 0 and 7 days, a 5% blood agar is prepared using the Defibrinated Horse Blood (DHB) and an appropriate agar base. Plates are tested for sterility and microbial productivity (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 in the appropriate atmosphere for 18-24 hours. All samples grew and showed good recovery and the correct morphology of the required test organisms: *Escherichia coli* (NCTC 12241), *Streptococcus agalactiae* (ATCC 12386), *Streptococcus pyogenes* (NCTC 12696) and *Streptococcus pneumoniae* (NCTC 12977). Therefore, it can be concluded that Defibrinated Horse Blood (DHB), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Dependent on final medium produced.
- Blood from different animal species can produce different haemolytic patterns. For example, *Enterococcus faecalis* (NCTC 12697 / ATCC 29212) will appear beta-haemolytic on culture media containing horse blood but non-haemolytic on sheep blood.

14. 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 Defibrinated Horse Blood (DHB) Safety Data Sheet.

Do not use if blood is not specified colour, has been stored inappropriately or the packaging has been damaged. Any unused blood should be disposed of according to local guidelines.

15. Storage conditions and Shelf life

Store Defibrinated Horse Blood (DHB) in the original container with the lid tightly closed at between 2 and 8°C in low humidity conditions away from direct light. Kept under these conditions, Defibrinated Horse Blood (DHB) may be used up to date of expiry shown on the product label.

Dispose of in accordance with local and national authority requirements.

Version History*

001 09/01/2023 - New document created
002 24/04/2025 - Updated IVDR status

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

EC REP
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TABLE OF APPLICABLE SYMBOLS

REF / o REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place