

## Instructions for Use

### Horse Blood Lysed (HBL)

**REF - HBL**

#### 1. Intended Use

Horse Blood Lysed (HBL) is aseptically collected, lysed horse blood used as an enrichment supplement for the preparation of culture media.

#### 2. Composition\*

<u>Ingredient</u>
Defibrinated Horse Blood    100%

\*Adjusted/supplemented as needed to meet performance requirements

#### 3. Summary and Explanation

Lysed horse blood is used for special purposes in culture media. It has been used for many years in *Corynebacterium diphtheriae* media where better growth was observed after lysis of horse blood. It is also documented that lysed blood stimulates the growth of *Haemophilus influenzae* due to the release of nicotinamide adenine dinucleotide from ruptured erythrocytes. In antibiotic susceptibility testing, lysed horse blood is added to the medium to improve the reactions with trimethoprim and sulphonamides. Most culture media, unless specially processed for susceptibility testing, contain amounts of thymidine which can antagonise the inhibitory effects of these antimicrobials. When horse blood is lysed, the erythrocytes release an enzyme thymidine phosphorylase which converts thymidine into the much less antagonistic compound thymine.

#### 4. Preparation Instructions

Dependent on final medium produced. Aseptically add the required Horse Blood Lysed volume to the sterilised base medium and allow to mix prior to final product dispensing.

#### 5. Physical Characteristics

	Appearance
Appearance and Colour	Dark red liquid

#### 6. Materials Provided

Horse Blood Lysed (HBL) 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
HBL025-P030-25	1 x 25ml in a Polycarbonate Universals
HBL1.2L-1LB-1200	1 x 1.2L in a 1L Media Bag
HBL100-N100-100	10 x 100ml in a 100ml Square PETG Bottles
HBL1L-1LB-1000	1 x 1L in a 1L Media Bag
HBL1L-N1L-1000	1 x 1000ml in a 1000ml Square PETG Bottles
HBL500-N500-500	1 x 500ml in a 500ml Square PETG Bottles

#### 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 and cool prior to adding the Horse Blood Lysed (HBL) to the medium and mix well before dispensing to the final container.

Organism	Incubation	Result (Specificity)
<i>E. coli</i> (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: Grey colonies
<i>S. pyogenes</i> (NCTC 12696)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies
<i>S. pneumoniae</i> (NCTC 12977)	37 ± 1°C in 5% CO <sub>2</sub> for 18-24 hours	Growth: Grey/green colonies

	Sterility Testing: Incubate at 37 ± 1°C aerobically for 72 hours and at 20 ± 5°C aerobically for 120 hours	
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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 Horse Blood Lysed (HBL) 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 Horse Blood Lysed (HBL) 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 aerobically for *Escherichia coli* (NCTC 12241) and *Streptococcus pyogenes* (NCTC 12696), and 37 ± 1°C in 5% CO<sub>2</sub> for *Streptococcus pneumoniae* (NCTC 12977). All samples were incubated 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 pyogenes* (NCTC 12696) and *Streptococcus pneumoniae* (NCTC 12977). Therefore, it can be concluded that Horse Blood Lysed (HBL), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

## 13. Limitations of the Media

As Horse Blood Lysed (HBL) is not to be used independently and is instead designed to be added to an existing medium, limitations will vary depending on the final medium produced.

## 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 Horse Blood Lysed (HBL) 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 Horse Blood Lysed (HBL) 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, Horse Blood Lysed (HBL) 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 16/04/2024 - New document created
- 002 24/04/2025 - Updated IVDR status and Section 9: Specificity

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



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IFU/HBL REV. 002

TABLE OF APPLICABLE SYMBOLS

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