

# DEFIBRINATED HORSE & SHEEP BLOOD FOR READY-TO-USE CULTURE MEDIA



## Donor Animals

All donor animal bleeding procedures are licensed by the Home Office under the "Animals (Scientific Procedures) Act 1986". This license ensures that the welfare of the animals is paramount and the premises meet the high standard of a designated area. All animal technicians have external training and hold Personal Licenses issued by the Home Office to perform the strictly defined animal bleeding protocols.

All new donor animals (horses and sheep) go through strict veterinary supervised quarantine procedures before entering donor stock groups. Each donor animal is microchipped with a unique number for instant recognition and traceability, with all medical and movement records computer recorded and archived. Stringent veterinary supervision ensures all donor animals are kept in the best of health, whilst adhering to the most modern and up-to-date disease preventative stock management practices. State of the art farming practices are used to control pasture rotation and fodder production. High quality summer grazing using minimal chemical fertilisation allows donor animals a perfect natural grazing environment during the summer months. Purpose built animal housing with open concrete courtyards guarantee donor animals a stress free winter housing. Sheltered feeding passages filled with high quality fodder assists the delivery of consistent quality blood products throughout the most adverse of seasonal weather conditions.

Animal blood harvest intervals are strictly monitored according to Home Office Licence protocols. Using gamma irradiated blood collection bags, a defined volume of blood is harvested from the jugular vein and then mechanically defibrinated using specialised agitation methods.

Home Office Licensed

ISO 9001 / ISO 13485

CE Marking 98/79/EC

Over 40 years experience – proven track record

High quality donor blood products

Guaranteed Packed Cell Volume

This controlled defibrination process minimises any erythrocyte degradation regardless of animal species. Once this is complete, the blood bags are then taken from the farm to the production facility to be prepared for final blood batch pooling and dispensing. The PCV range for horse blood is 38-45% and for sheep blood it is 30-40%. The PCV is checked for each donor animal sample prior to blood batch pooling and also on the final pooled batches.

Horse and sheep blood are essential nutritional supplements for microbiological culture media; they also function as a diagnostic indicator in media and test systems for routine clinical diagnosis. In addition to the defibrinated blood products, E&O also offers lysed blood, citrated blood, filtered serum and plasma and Alsever's solution based products for various applications.

Donor animal blood performance, sterility and appearance testing are carried out in E&O's UKAS ISO 17025 accredited test laboratory and all production activities are ISO 9001/ ISO 13485 certified.

All finished animal blood products are shipped according to customer requirements with packaging and documentation to meet location legislation. Long distance shipments are packed with cool packs and released within 48 hours of animal harvest ensuring the freshest of products with the longest shelf-life expectation. Quality control documentation is not released until full testing has been completed.

Quality that you can trust with zero contamination

Shipped worldwide

Volumes to suit your batch manufacture

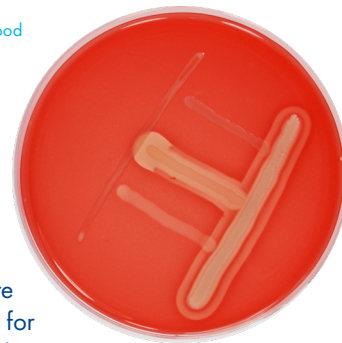
HORSE & SHEEP BLOOD  
SHELF-LIFE 56 DAYS



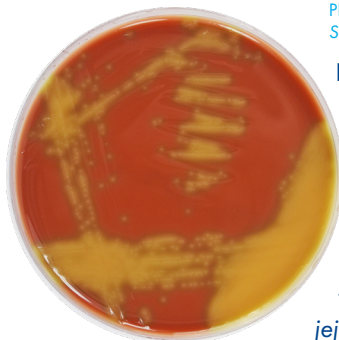
## Quality Control:

PP1747 Columbia agar with 5% Sheep Blood  
CAMP test for *L. monocytogenes*

Rigorous quality control testing is carried out on all defibrinated horse and sheep blood batch samples. Accurate representative aliquots from every production batch are aseptically added to a sterile molten Columbia Agar Base and poured into 90mm petri-dishes. After cooling and drying a defined proportion of these plates are then inoculated with specified reference organisms. After appropriate incubation, growth is examined and measured for productivity colonial morphology and haemolytic reactions. For sheep blood, the CAMP test is also performed according to the requirements of ISO 11290 for *L. monocytogenes*. All uninoculated batch sample poured plates are kept for sterility testing. These plates are then divided and incubated at  $37 \pm 1^\circ\text{C}$  for 3 days/  $20 \pm 5^\circ\text{C}$  for 14 days - each plate is examined daily for any contaminants. The appearance and colour of the blood is also recorded by measuring the pre- and post-incubation colour of the poured plates against a reference colour chart. Finally the PCV is checked through centrifugation to confirm that it falls within the defined acceptance criteria.



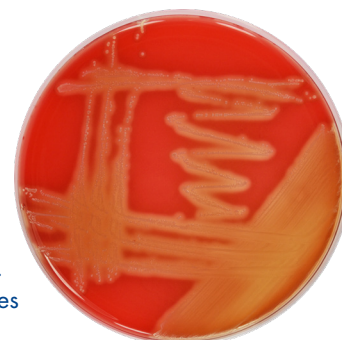
PP0100 Columbia Chocolate agar with 5% Horse Blood  
*S. pneumoniae* NCTC 12977



Horse blood is preferred to sheep blood for the manufacture of chocolate media products. In sheep blood, NAD (Factor V) is rapidly degraded due to a high level of NADase activity; horse erythrocytes lack measurable NADase activity. Therefore, the use of horse blood in chocolate media products allows for the optimal recovery of *Haemophilus* species as the nutritional qualities of the blood are better suited for this particular application. This property has been exploited for the fastidious medium required in the EUCAST disc diffusion method for antibiotic sensitivity testing. The MH-F medium consists of Mueller Hinton Agar supplemented with 5% defibrinated horse blood and NAD (20mg/L). This medium facilitates the testing of a wide range of fastidious bacterial species from *Haemophilus influenzae* to *Campylobacter jejuni*.

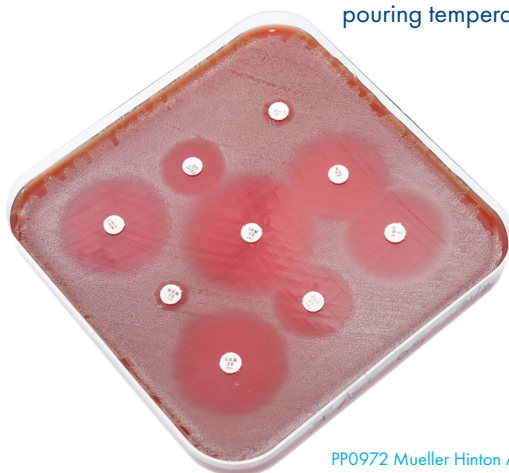
Lysed horse and sheep blood – defibrinated blood is lysed by the addition of a fixed concentration of saponin. The saponin disrupts the red blood cell membrane by complexing with the cholesterol present in the lipid bilayer. This leads to pore formation and rapid lysis of the red blood cells. This results in a nutritious supplement that can be used in media products where the growth of fastidious organisms is required e.g. *Neisseria gonorrhoeae*, *Campylobacter jejuni*, etc. The defibrinated blood products can also be used to prepare laked horse and sheep blood. Preparation of laked blood for anaerobic media preparation, MIC testing, etc, can be prepared according to ISO 20776-1:2006, where the blood is frozen and thawed five to seven times at  $-20^\circ\text{C}$  to lyse the red blood cells followed by centrifugation to clarify.

PP1747 Columbia agar with 5% Sheep Blood  
*S. pyogenes* NCTC 12696



Horse blood contains erythrocytes (red cells) that possess a slightly lower mean corpuscular volume (MCV) than sheep erythrocytes. Horse erythrocytes are also more flexible so they are more robust. This is an important factor as sheep erythrocytes are more delicate and the blood harvesting process can exert a damaging effect on the blood if the process is not subject to rigorous control. Horse erythrocytes separate quickly from the serum component and tend to readily pack at the bottom of the container during storage. Sheep erythrocytes, which have a more rounded shape, stay in suspension in the serum so sheep blood does not require as much agitation as horse blood prior to use.

We advise that all blood products are refrigerated between  $2-8^\circ\text{C}$  and added to culture media within the recommended shelf life (56 days). We also advise that the bottles/bags of defibrinated blood are slowly warmed up to room temperature ( $20-25^\circ\text{C}$ ) and gently shaken or rolled to re-suspend the erythrocytes prior to being added to the sterilised, molten agar. The temperature at which the blood is added to the molten agar should be no higher than  $45^\circ\text{C}$ . The ideal finished pouring temperature should be  $40-42^\circ\text{C}$ .



PP0972 Mueller Hinton Agar with 5% Horse Blood and NAD (MH-F) *S. pneumoniae* NCTC 12977

## Antimicrobial Susceptibility Testing

As described above, erythrocytes in horse blood lack NADase activity. This property has been exploited for the fastidious medium required for the EUCAST disc diffusion method for antibiotic sensitivity testing. The MH-F medium (PP0972) consists of Mueller Hinton Agar supplemented with 5% defibrinated horse blood and NAD (20mg/L). This medium facilitates the testing of a wide range of fastidious bacterial species from *Haemophilus influenzae*, *Streptococcus pneumoniae* to *Campylobacter jejuni* as per the requirements of the EUCAST standard method.