

EDWARDS AESCULIN MEDIUM

Dehydrated culture medium

1 - INTENDED USE

A selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci associated to bovine mastitis.

2- COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	10.00 g
Peptocomplex	10.00 g
Sodium chloride	5.00 g
Aesculin	1.00 g
Thallos sulphate	0.33 g
Agar	15.00 g
Crystal violet	1.30 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Bovine mastitis is an inflammatory response of the udder tissue in the mammary gland due to physical trauma or microorganism infections.¹ It is considered the most common disease leading to economic loss in dairy industries due to reduced yield and poor quality of milk.^{1,2} Many bacterial species have been identified as causative agents for bovine mastitis and among them, streptococci and in particular *Streptococcus agalactiae* play a key role. *S. agalactiae* is a Gram-positive pathogen known to cause bovine mastitis before it was appreciated as pathogenic in humans.³ It can be transmitted via milking machine and through oro-faecal route, particularly through contaminated drinking water.¹

In 1933, Edwards⁴ used an esculin blood agar containing crystal violet and sodium azide to isolate mastitis streptococci. A similar medium containing thallos acetate was used by McKenzie⁵ to isolate the causative agent of mastitis.

In Edwards Aesculin Medium crystal violet and thallium sulphate are the selective agents that suppress the growth of a wide variety of Gram-negative and Gram-positive bacteria with the exception of streptococci. Essential factors for microbial growth are provided by beef extract and peptocomplex. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Aesculin differentiates esculin-positive organisms (group D streptococci growing with black colonies) from esculin-negative organisms (*S. agalactiae* growing with blue to colourless colonies). Supplementation with blood provides additional growth factors and differentiates streptococci on the base of haemolytic pattern.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.3 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and aseptically add 5-7% of sterile defibrinated sheep blood. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-violet, fine, homogeneous, free-flowing powder
Solution appearance	violet, limpid
Prepared plates appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Edwards Aesculin Medium	Dehydrated medium	4014312	500 g (12.1 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, defibrinated sheep blood, ancillary culture media and reagents.

8 - SPECIMENS

Milk samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

9 - TEST PROCEDURE

Inoculate the centrifuged deposits of milk samples and streak with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Incubate at 35-37°C for 24-48 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.

S. agalactiae grows with blue to colourless, typically beta haemolytic, colonies.

Group D streptococci grows with black to brown (aesculinase positive) non-haemolytic colonies.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.



CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>S.agalactiae</i> ATCC 13813	35-37°C / 18-24h / A	growth, blue to colourless β-haemolytic colonies
<i>E.faecalis</i> ATCC 29212	35-37°C / 18-24h / A	growth, black to brown non-haemolytic colonies
<i>S.aureus</i> ATCC 25923	35-37°C / 18-24h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Edwards Aesculin Medium is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.

The productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *S. agalactiae* ATCC 13813, *S. agalactiae* ATCC 12386, *S. agalactiae* CB STR6.2. After incubation at 37°C for 24 hours the target strains exhibit good growth with blue to colourless colonies. CB STR6.2 and ATCC 12386 strains exhibit β haemolysis while ATCC 13813 strain is non haemolytic.

The specificity is evaluated with *E. faecalis* ATCC 19433. After incubation the strain grows with black to brown non haemolytic colonies.

The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922. The growth of the non-target strain is totally inhibited after incubation at 37°C for 24 hours

Note: CB: Biolife Microbial Collection

13 - LIMITATIONS OF THE METHOD

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Edwards Aesculin medium contains thallos sulphate and is classified as hazardous. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C / +30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).










16 - REFERENCES

1. Wei Nee Cheng, Sung Gu Han. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. *As-Austral J Anim Sci.* 2020 Nov; 33(11): 1699–1713.
2. Gomes F, Henriques M. Control of bovine mastitis: old and recent therapeutic approaches. *Curr Microbiol.* 2016;72:377–82.
3. Morven S, Edwards, Carol J. Baker, in *Infectious Diseases of the Fetus and Newborn (Seventh Edition)*, 2011.
4. Edwards SJ. *J. Comp. Pathol. Therap.* 1933; 46:211-217.
5. McKenzie DA. *Vet. Rec.* 1941; 53: 473-480.





TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

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

