

# Product Specification Sheet

## *Aesculin Blood Agar (modified)*

Intended Usage: A medium for the isolation and differentiation of bacteria involved in bovine mastitis.

For professional use only.

PB5023A	
Version: 03	Revision Date: March 2020

**Thermo Scientific™ Aesculin Blood Agar (modified)**

Form of Product	Poured plate
Storage	2 – 12°C, dark
Filling weight	17 g ± 5 %
Packaging	10 plates wrapped in film
pH	7.5 ± 0.2
Appearance	Flame red, opaque
Shelf life	8 weeks
Intended Usage	A medium for the isolation and differentiation of bacteria involved in bovine mastitis. For professional use only.
Technique	Depends on the different methods. For information see product information.

Typical formulation*	g/l
'Lab Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	7.5
Aesculin	1.0
Agar	16.0
Defibrinated Sheep Blood	70.0 ml

\*Adjusted as required to meet performance standards.

## Quality Control

1. Control for general characteristics, labelling and printing.
2. Contamination check  
≥ 72 h @ 20 – 25 °C, aerobic  
≥ 72 h @ 30 – 35 °C, aerobic
3. Microbiological control

Positive Controls	Growth
<b>Inoculum 50 – 120 colony forming units (cfu), quantitative, control medium TSA</b> <b>Incubation conditions: 18 – 24 h @ 36 ± 1°C, aerobic</b>	
<i>Enterococcus faecalis</i> ATCC®29212™	1 – 2 mm, grey shiny colonies. Aesculin positive.
Colony counts shall be ≥ 50% of the control medium TSA.	
<b>Inoculum 10<sup>3</sup> – 10<sup>4</sup> cfu, qualitative, control medium COL+SB</b> <b>Incubation conditions: 18 – 24 h @ 36 ± 1°C, aerobic</b>	
<i>Enterococcus faecium</i> ATCC®19434™	Good growth, dark grey colonies. Aesculin positive.
<i>Staphylococcus aureus</i> ATCC®6538™	Good growth, yellow shiny colonies with haemolysis.

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## Description

Mastitis in cattle causes large losses in dairy cattle farming. After fertility disorders, udder disorders are the second most important cause of death among dairy cattle. The bacteriological investigation of milk samples is the most important diagnostic measure in the detection of udder disorders.

Over 130 different microorganisms were isolated from milk samples in connection with mastitis among cattle<sup>1</sup>; however, a relatively narrow spectrum of bacteria number among the most frequent bacterial pathogens: *Staphylococcus aureus*, *Streptococcus agalactiae*, *S. dysgalactiae* and *S. uberis* as well as other streptococci and coliform bacteria. Due to the inclusion of blood, aesculin blood agar ensures the growth of staphylococci, streptococci and permits direct detection of enterococci and *S.uberis* by means of the aesculin cleaving. The growth of coliform bacteria, pseudomonads and yeasts is also possible on the non-selective agar. Due to the relatively high agar content, the medium exhibits a high gel strength so that all samples of a quarter milk can be spread onto a single plate. In comparison with coliform bacteria, streptococci as well as yeasts grow in smaller colonies than, for example, on Columbia agar with sheep blood.

## Technique

1. The samples are taken from the initial quarter milk. Clean teats, milk foremilk, evaluate and discard.
2. Disinfect teats with 70% alcohol swab.
3. Milk approx. 10-15ml of milk secretion into a sterile sample container.
4. Centrifuge samples at 3,000 rpm for 10 minutes. Heat the fat layer over an open flame and pour off with the skim milk.
5. Spread one loop of sediment on one quarter of an aesculin blood agar plate per milk sample and incubate for 24-48 hours at  $37 \pm 1^\circ\text{C}$ . If slow growing organisms are suspected, extend the incubation for up to 5 days.
6. Evaluate aesculin cleavage under UV light (366 nm).

## Characteristic Colony Morphology

Species	Colony Morphology
<i>Staphylococcus aureus</i>	Medium-sized (0.5-1 mm diameter), white-yellow, regular colonies with smooth, glossy surface. Possibly $\beta$ -haemolysis, aesculin-negative, coagulase-positive.
<i>Streptococcus agalactiae</i>	Small, unpigmented, smooth, round colonies with $\beta$ -haemolysis. Aesculin negative, Lancefield group B, CAMP test positive (should not be tested on this medium) *
<i>Streptococcus dysgalactiae</i>	Small, unpigmented, smooth, glossy colonies with slight $\alpha$ -haemolysis, aesculin-negative, Lancefield group C.
<i>Enterococcus faecalis</i> , <i>S. uberis</i>	Medium-sized, pigmented, smooth, round colonies with $\alpha$ -haemolysis, aesculin-positive.
Coliform bacteria e.g. <i>E. coli</i> , <i>Klebsiella</i> species, <i>Enterobacter</i> species, <i>Citrobacter</i> species.	Large, unpigmented, smooth, glossy, partially mucilaginous colonies, rarely with haemolysis.
Yeasts	Slower growth (often only after 48 hours), medium-sized, white, bulging colonies.

\*The CAMP test for *S. agalactiae* should not be performed on aesculin blood agar, rather on a blood-containing agar without aesculin (e.g. Columbia agar with Sheep Blood<sup>PLUS</sup>, Product code PB5039A).

## Literature

1. Quinn, P.J., Carter, M.E. Markey, B. und G.R. Carter (1994). "Clinical Veterinary Microbiology" Chapter 36: Mastitis; Mosby Yearbook Europe Ltd, London, UK.