IFU

Instructions for Use

HARDY DIAGNOSTICS
A Culture of Service™

BOVINE BLOOD MEDIA

Cat. no. A188	Bovine Blood Agar, 15x100mm Plate, 17ml	10 plates/bag
Cat. no. A189	Bovine Blood Agar with Esculin, 15x100mm Plate, 17ml	10 plates/bag
Cat. no. A143	Bovine Selective Strep Agar, 15x100mm Plate, 17ml	10 plates/bag
Cat. no. A157	Bovine Selective Staph Agar, 15x100mm Plate, 17ml	10 plates/bag
Cat. no. J129	Bovine Blood Agar with Esculin / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J312	Mastitis Triplate (Bovine Selective Strep Agar / MacConkey Agar / Blood Agar), 15x100mm Triplate, 7ml/section	10 plates/bag

INTENDED USE

Hardy Diagnostics Bovine Blood Media is recommended as a general purpose growth media for the cultivation, selective isolation, and differentiation of organisms responsible for mastitis in dairy populations.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Bovine mastitis, the inflammation of the mammary gland in dairy cattle, is mainly caused by infections from bacteria. Over 130 different microorganisms have been isolated from bovine mastitic milk with *Staphylococcus* and *Streptococcus* species being the most common etiologic agents.[6] *E. coli* and *Klebsiella* are commonly found in manure, bedding, and sometimes can be found in water supplies in the dairy environment. These organisms can cause acute mastitis, but high counts of *E. coli* and *Klebsiella* are more likely due to dirty teats or contamination of the milk by manure.

Bovine Blood Agar is designed to aid in the presumptive identification of *Staphylococcus* and *Streptococcus* species based on hemolysis patterns and the ability of certain mastitis-causing bacteria to hydrolyze esculin.[3-7] Bovine blood cells have been added to this media to facilitate the growth of various organisms, as well as for the observation of hemolytic reactions. The absence of reducing sugars and carbohydrates allows hemolysis to occur without hindrance. *Staphylococcus aureus* will appear round and shiny with golden-yellow colonies demonstrating a zone of beta-hemolysis while *Streptococcus* species will demonstrate alpha-, beta-, or non-hemolytic patterns and are often white to gray in color.[3-7]

Bovine Blood Agar with Esculin contains esculin (full strength) to differentiate group D streptococci from *Streptococcus agalactiae*, as *S. agalactiae* is not capable of esculin-hydrolysis. When esculin is hydrolyzed by organisms it forms dextrose and esculetin, which react with a compound in the media to produce a darkening or blackness around the colonies.[1,2]

Bovine Blood Agar with Esculin, Modified is similar to Bovine Blood Agar with Esculin, except with half-strength

esculin.

Bovine Selective Strep Agar is a selective medium based on the modified Edwards formulation. *Streptococcus agalactiae* normally produces narrow zones of beta-hemolysis or are non-hemolytic on regular blood agar plates. Bovine Selective Strep Agar contains beta toxin, which causes hemolytic and non-hemolytic strains of GBS to appear strongly beta-hemolytic, thus increasing the sensitivity of the detection method. This formulation (modified Edwards agar combined with sheep blood and beta toxin) is also known as TKT Agar. The medium also contains esculin to further differentiate the members of the genus *Streptococcus* (see description for Bovine Blood Agar with Esculin). Therefore on Bovine Selective Strep Agar: *S. agalactiae* , which is incapable of esculin hydrolysis, should appear strongly beta-hemolytic with no darkening of the media, while *S. uberis* should appear non-hemolytic with obvious darkening of the media. [3-7] Selective agents have been added to inhibit *Staphylococcus* species and gram-negative organisms.

Bovine Selective Staph Agar is a selective medium that will allow for the growth of *Staphylococcus* species, while inhibiting *Streptococcus* species and gram-negative organisms.

MacConkey Agar is a selective and differential medium for the isolation of gram-negative bacilli (including coliform organisms and enteric pathogens), on the basis of lactose fermentation. If the gram-negative organisms ferment lactose, the colonies will appear pink. Non-lactose fermenting organisms (i.e. *Pseudomonas* species) will also grow on MacConkey Agar but will produce a colorless or opaque colony. The bile salts in the medium inhibit the growth of gram-positive bacteria.

Mastitis Triplate, a three sectioned plate containing Bovine Selective Strep Agar (section I), MacConkey Agar (section II), and Blood Agar (section III), is designed to aid in the growth and presumptive identification of mastitis related organisms. The Blood Agar is used to grow all organisms as well as demonstrate hemolysis patterns. MacConkey Agar is used for the selective isolation and differentiation of coliforms, while Bovine Selective Strep Agar is used in the selective isolation and differentiation of *Streptococcus* species.[3-7]

Hardy Diagnostics Beta Toxin (Cat. no. Z306) is a simplified and modified version of the traditional CAMP procedure. Beta Toxin contains extracted *S. aureus* beta-hemolysin and is added to Bovine Blood Agar, Bovine Blood Agar with Esculin, or Bovine Blood Agar with Esculin, Modified to evaluate the CAMP reaction on primary isolation. The modified CAMP procedure has demonstrated a high degree of reliability in the identification of *S. agalactiae*.[4-7] In this method the inoculated plate is observed for CAMP reaction after overnight incubation. A positive CAMP reaction is noted by an enhanced zone of beta-hemolysis and presumptively identifies *S. agalactiae*.[3-7]

FORMULA

Ingredients per liter of deionized water:*

MacConkey Agar:	
Peptone	17.0gm
Lactose	10.0gm
Sodium Chloride	5.0gm
Proteose Peptone	3.0gm
Bile Salts	1.5gm
Neutral Red	30.0mg
Crystal Violet	1.0mg
Agar	13.5gm

Final pH 7.1 +/- 0.2 at 25ºC.

Bovine Blood Agar:

Tryptose	20.0gm
Sodium Chloride	5.0gm
Bovine Blood Cells, Washed	40.0ml
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

In addition to the above Bovine Blood Agar ingredients;

Bovine Blood Agar with Esculin contains:	
Esculin	1.0gm

Final pH 7.6 +/- 0.2 at 25ºC.

Bovine Blood Agar with Esculin, Modified contains:	
Esculin	0.5gm

Final pH 7.6 +/- 0.2 at 25ºC.

Bovine Selective Strep Agar contains:	
Selective Agents	0.015gm
Beta Toxin	20.0ml
Esculin	1.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

Bovine Selective Staph Agar contains:	
Selective Agents	0.015gm

Final pH 7.4 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Sample Collection: It is important that a milk sample be taken to ensure that the pathogens originated from the mammary gland and not from dust or fecal particles on the udder surface. To ensure the contaminant is from the milk, the teat surface and orifice should be wiped with seventy percent (70%) ethyl alcohol. It is also essential to obtain a sample before the cow has been treated with antimicrobial agents. Consult listed references for additional information on specimen collection. [4]

Method of use for Bovine Blood Media :

1. Allow the media to warm to room temperature before inoculating the surface of the medium with the milk samples.

2. Prepared media should be inoculated, incubated, and results recorded according to accepted procedures described in the reference texts. [1-7]

3. Observe Bovine Blood Agar for growth and hemolysis patterns. Additionally, observe Bovine Blood Agar with Esculin, Bovine Blood Agar with Esculin, Modified, and Bovine Selective Strep Agar for a darkening around the colonies.

4. Observe MacConkey Agar for lactose fermenting (pink) and non-lactose fermenting (clear) colonies.

Method of use for *S. agalactiae* and *S. dysgalactiae* :

1. Dip a sterile cotton swab into the Beta Toxin (Cat. no. Z306). Ensure that the swab is saturated with Beta Toxin.

2. Inoculate the dried surface of Bovine Blood Media (Cat. no. A188 or A189) with the saturated swab by swabbing a single line across the agar. See listed references for accepted application procedures for Beta Toxin.[5-7] If the surface of the media shows excess moisture (droplets on the surface of the media or on the petri plate lid), incubate the plates for 10 to 30 minutes with the lids slightly ajar prior to swabbing the media surface.

3. Repeat this single line swabbing procedure with a second swab to ensure an even distribution of Beta Toxin.

4. Allow media swabbed with Beta Toxin to dry prior to inoculating with the sample to be tested. Drying of Beta Toxin prepared plates can be accelerated by incubating the swabbed media prior to use.

5. Media prepared with Beta Toxin should be inoculated with the milk sample according to accepted procedures described in the listed reference texts.[5-7]

6. After 18-24 hours of incubation, observe plates for growth and enhanced beta-hemolysis.

INTERPRETATION OF RESULTS

All Bovine Blood Media should be examined for colonies with alpha-, beta-, and non-hemolytic patterns. Only Bovine

Blood with Esculin, Bovine Blood with Esculin, Modified, and Bovine Selective Strep Agar should be examined for esculin-hydrolysis as indicated by a darkening around the colonies.

Typical Reactions of Commonly Isolated Organisms on Mastitis Triplate and Mastitis Quadplate				
Organism	**Blood Section**	**MacConkey Section**	**Selective Strep Section**	**Selective Staph Section**
S. aureus	Growth	No growth*	No growth*	Growth, hemolytic
Environmental Staph	Growth	No growth*	No growth*	Growth, non-hemolytic
S. agalactiae	Growth	No growth*	Growth, enhanced beta-hemolysis	No growth*
Environmental Strep	Growth	No growth*	Growth, non-hemolytic; darkening with group D	No growth*
Coliforms	Growth	Growth, pink colonies	No growth*	No growth*
Non-coliforms	Growth	Growth, clear or colorless colonies	No growth*	No growth*

*Growth in this section does not affect interpretation; reactions given are typical of pure-isolates of these organisms. Sample may contain no organisms, one organism, or multiple organisms.

Consult listed references for the identification of colony morphology and further biochemical tests required for identification. (1,2,4,6)

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, Beta Toxin (Cat. no. Z306), other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	**Inoculation Method***	**Incubation**			**Results**
		Time	**Temperature**	**Atmosphere**	
Bovine Blood Agar (Cat. no. A188):					
Staphylococcus aureus ATCC® 25923	A	18-24hr	35°C	Aerobic	Growth; beta-hemolytic
Streptococcus agalactiae	A	18-24hr	35°C	Aerobic	Growth; beta-hemolytic

ATCC® 12386					
Streptococcus agalactiae ATCC® 13813	**	18-24hr	35°C	Aerobic	Growth; enhanced beta-hemolysis in the presence of beta toxin
Streptococcus uberis ATCC® 700407	A	18-24hr	35°C	Aerobic	Growth; non-hemolytic
Streptococcus dysgalactiae ATCC® 43078	**	18-24hr	35°C	Aerobic	Growth; no enhanced beta-hemolysis in the presence of beta toxin
Bovine Blood Agar with Esculin (Cat. no. A189, J129):					
Streptococcus uberis ATCC® 700407	A	18-24hr	35°C	Aerobic	Growth; non-hemolytic with darkening around colony
Staphylococcus aureus ATCC® 25923	A	18-24hr	35°C	Aerobic	Growth; beta-hemolytic with no darkening around colony
Streptococcus agalactiae ATCC® 12386	A	18-24hr	35°C	Aerobic	Growth; beta-hemolytic with no darkening around colony
Streptococcus agalactiae ATCC® 13813	**	18-24hr	35°C	Aerobic	Growth; enhanced beta-hemolysis in the presence of beta toxin
Streptococcus dysgalactiae ATCC® 43078	**	18-24hr	35°C	Aerobic	Growth; no enhanced beta-hemolysis in the presence of beta toxin
Bovine Selective Strep Agar (Cat. no. A143, J312):					
Streptococcus agalactiae ATCC® 13813	A	18-24hr	35°C	Aerobic	Growth; enhanced beta-hemolysis; no darkening around colony
Streptococcus uberis ATCC® 700407	A	18-24hr	35°C	Aerobic	Growth; non-hemolytic; darkening around colony
Streptococcus dysgalactiae ATCC® 43078	A	18-24hr	35°C	Aerobic	Growth; non-hemolytic; no darkening around colony
Staphylococcus aureus ATCC® 25923	B	18-24hr	35°C	Aerobic	Inhibited
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Inhibited
Bovine Selective Staph Agar (Cat. no. A157):					
Staphylococcus					

aureus ATCC® 25923	A	18-24hr	35°C	Aerobic	Growth; beta-hemolytic
Streptococcus agalactiae ATCC® 12386	B	18-24hr	35°C	Aerobic	Inhibited
Streptococcus uberis ATCC® 700407	B	18-24hr	35°C	Aerobic	Inhibited
Proteus mirabilis ATCC® 12453	B	24hr	35°C	Aerobic	Inhibited
Pseudomonas aeruginosa ATCC® 27853	B	24hr	35°C	Aerobic	Inhibited
MacConkey Agar (J129, J312):					
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Growth; colonies pink to red with bile salt precipitate surrounding the colonies
Proteus mirabilis ATCC® 12453	A	24hr	35°C	Aerobic	Growth; colonies colorless with no swarming
Salmonella enterica ATCC® 14028	A	24hr	35°C	Aerobic	Growth; colonies colorless
Enterococcus faecalis ATCC® 29212	B	24hr	35°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 6538	B	18-24hr	35°C	Aerobic	Inhibited
Blood Agar (J312):					
Streptococcus pyogenes ATCC® 19615	A	24hr	35°C	Aerobic	Growth; beta-hemolytic
Streptococcus pneumoniae ATCC® 6305	A	24hr	35°C	Aerobic	Growth; alpha-hemolytic
Staphylococcus aureus ATCC® 25923	A	24hr	35°C	Aerobic	Growth; white colonies
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Growth; off-white colonies
Enterococcus faecalis ATCC® 29212	A	24hr	35°C	Aerobic	Growth; non-hemolytic

* Refer to the document "Inoculation Procedures for Media QC" for more information.

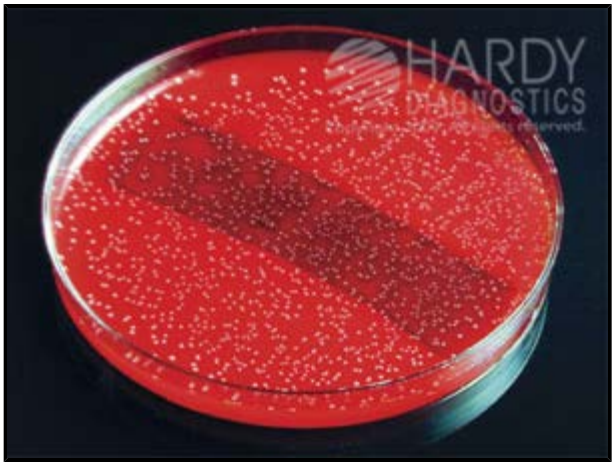
** Refer to the above Procedure section for a description of the recommended inoculation procedures.

USER QUALITY CONTROL

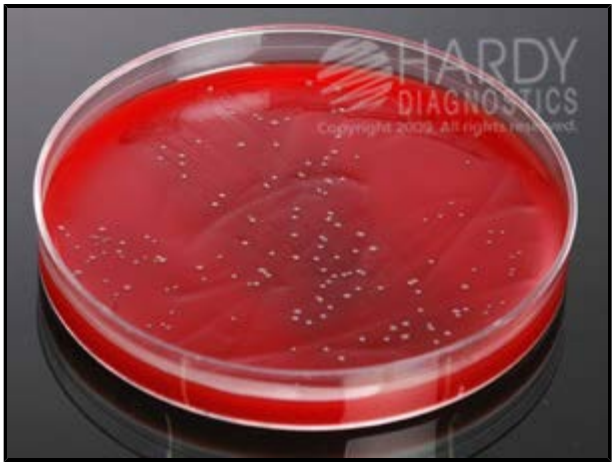
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

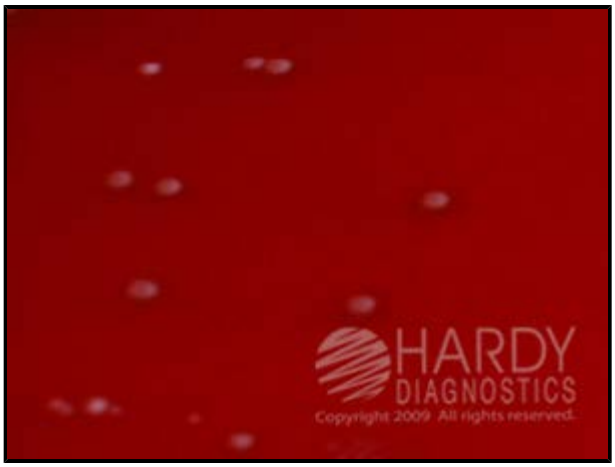
Bovine Blood Media should appear opaque, and cherry red in color.



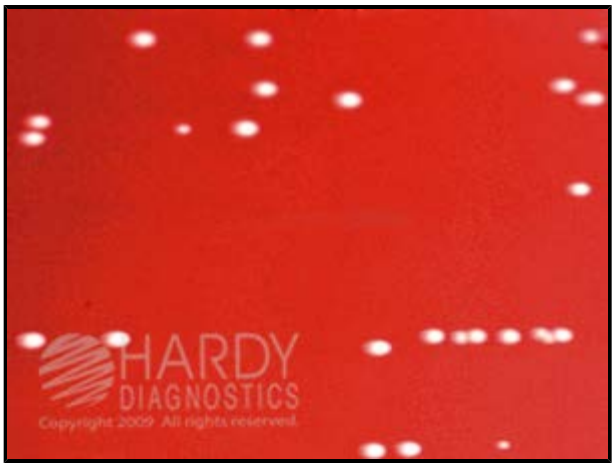
Streptococcus agalactiae (ATCC® 13813) colonies growing on Bovine Blood Agar (Cat. no. A188) showing enhanced beta-hemolysis in the presence of Beta Toxin (Cat. no. Z306). Incubated aerobically for 24 hours at 35ºC.



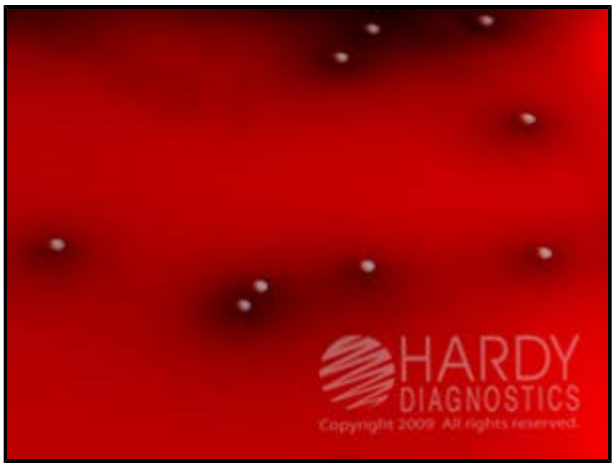
Streptococcus dysagalactiae (ATCC® 43078) colonies growing on Bovine Blood Agar (Cat. no. A188) in the presence of Beta Toxin (Cat. no. Z306) without enhanced beta-hemolysis. Incubated aerobically for 24 hours at 35ºC.



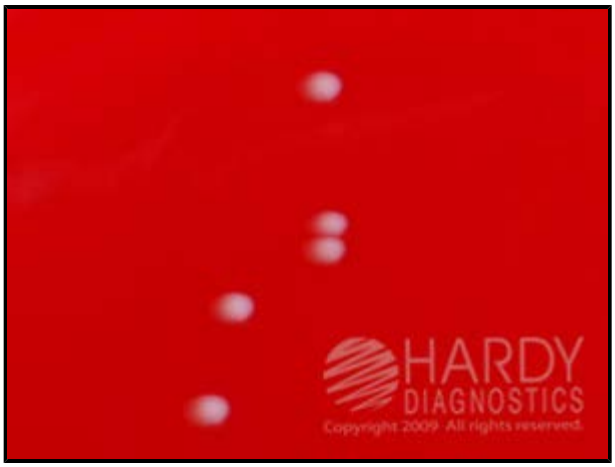
Streptococcus dysagalactiae (ATCC® 43078) colonies growing on Bovine Blood Agar (Cat. no. A188). Incubated aerobically for 24 hours at 35ºC.



Streptococcus uberis (ATCC® 700407) colonies growing on Bovine Blood Agar (Cat. no. A188). Incubated aerobically for 24 hours at 35ºC.

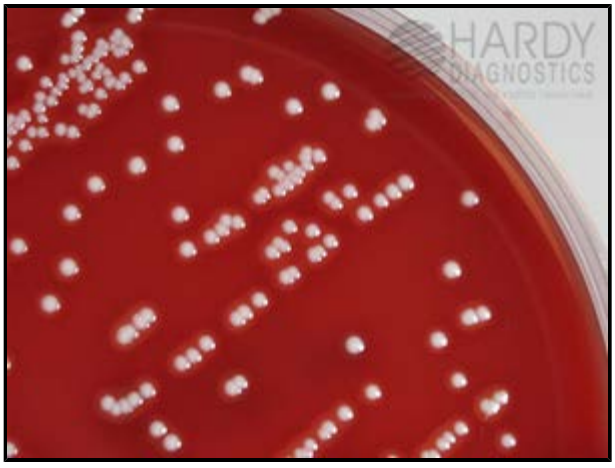


Streptococcus uberis (ATCC® 700407) colonies growing on Bovine Blood Agar with Esculin (Cat. no. A189) and Bovine Selective Strep Agar (Cat. no. A143). Incubated aerobically for 24 hours at 35ºC.

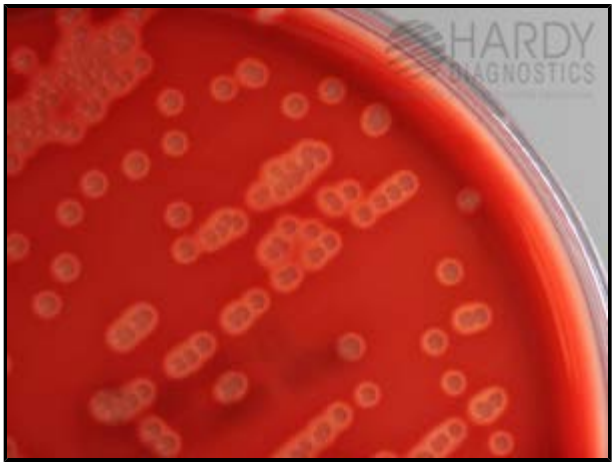


Streptococcus dysagalactiae (ATCC® 43078) colonies growing on Bovine Blood Agar with Esculin (Cat. no. A189) and Bovine Selective Strep Agar (Cat. no. A143). Incubated aerobically for 24 hours at 35ºC.

Streptococcus agalactiae (ATCC® 13813) colonies growing on Bovine Selective Strep Agar (Cat. no. A143) showing beta-hemolytic colonies. This strain is not hemolytic on a regular blood agar plate. Incubated aerobically for 24 hours at 35ºC.



Staphylococcus aureus (ATCC® 25923) colonies growing on Bovine Selective Staph Agar (Cat. no. A157). Incubated aerobically for 24 hours at 35ºC.



Showing beta-hemolysis from *Staphylococcus aureus* (ATCC® 25923) colonies growing on Bovine Selective

Staph Agar (Cat. no. A157). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

2. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology.* J.B. Lippincott Company, Philadelphia, PA

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4. Quinn, P.J., et al. 1994. *Clinical Veterinary Microbiology*. Wolfe Publishing, London, England.

5. National Mastitis Council. 1999. *Laboratory Handbook on Bovine Mastitis*. NMC Inc., Madison, WI.

6. Carter, G.R., et al. 1995. *Essentials of Veterinary Microbiology*, 5th ed. Williams & Wilkins, Philadelphia, PA.

7. A Practical Look at Contagious Mastitis, www.nmconline.org/contmast, 04/18/02.

IFU-10080[B]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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