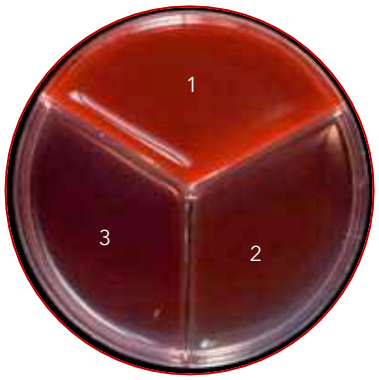
SWEDISH VETERINARY AGENCY

SELMA® and SELMA PLUS®

Selective growth media for diagnostics of clinical mastitis developed by SVA

SELMA is a three-partitioned and SELMA PLUS a four-partitioned agar plate for identifying bacteria in milk samples from clinical mastitis. The plates contain three and four different selective media, respectively, thus greatly facilitating safe and rapid bacteriological diagnostics.



SELMA

SELMA PLUS

Agar fields

1) Bovine blood agar with esculin where all aerobic bacteria grow
2) MacConkey agar where only gram-negative bacteria grow
3) Mannitol salt agar where staphylococci and enterococci grow. When yellow colonies grow on the mannitol salt agar this field can also be used to test staphylococci for penicillinase production.
4) PGUA agar for identification of *Escherichia coli*.

Note that fields 3 and 4 on SELMA PLUS are reversed in this figure.

IMPORTANT INFORMATION TO USERS OF SELMA PLATES

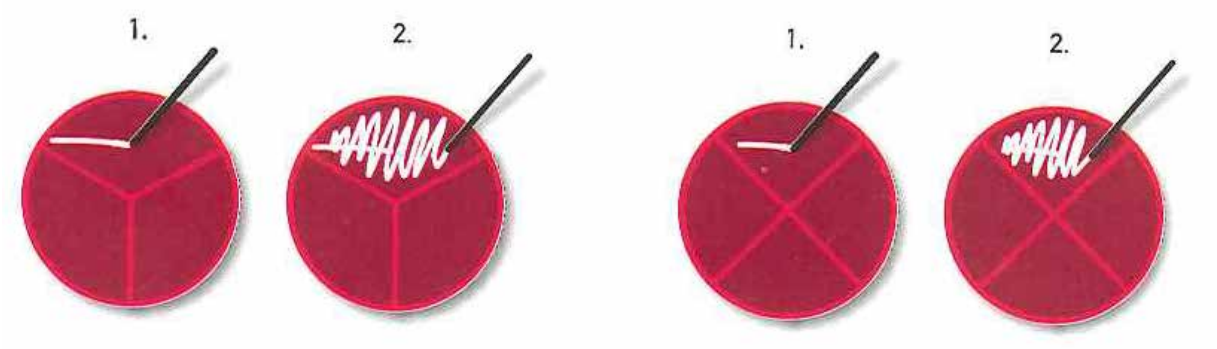
SELMA and SELMA PLUS are specifically developed for culturing of milk samples!

Every batch of SELMA and SELMA PLUS is produced according to QC/QA standards by the Department of Vaccines and Laboratory Products, and is tested for optimal properties for culturing and typing of mastitis pathogens. Acute clinical mastitis is in most cases caused by a single bacterial species which grows in pure culture. When culturing samples from cases of subclinical mastitis, mucous membranes, skin and wounds the sample will often contain a mixed culture of different bacterial species. In these cases it is, therefore, not appropriate to use the SELMA plates as the small size of the agar fields makes it difficult to discern single colonies, which is important for correct evaluation of bacterial growth. For culturing of other samples than milk we therefore recommend our bovine blood agar plates without partitions.

INSTRUCTIONS FOR CULTURING

If condensation water coats the plastic lid gently remove it. Shake the test tube. Dip a 10µl plastic loop (blue) in the milk once for each field and spread on the plate as indicated in the pictures below starting with the bovine blood agar (field 1).

For correct diagnostics culture only one milk sample per plate.



As soon as possible after inoculation, the plate is incubated with the lid down at 37°C for 18–48 hours. The first reading is done after 18–24 hours. If there is no growth, the plate should be incubated for another period of 24 hours and re-examined to check for presence of more slowly growing bacteria.

READING

Inspect the plate in both reflecting light (small colonies appear) and translucent light (haemolysis appear). No growth or sparse growth of contaminants on the blood agar (field 1) indicates that the milk contains such a small amount of bacteria that it is not meaningful to proceed with further bacteriological testing.

DIFFERENTIATION OF BACTERIA

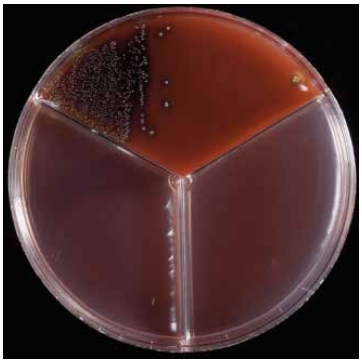
Udder pathogens grow as follows (S=SELMA, S+=SELMA PLUS):

Almost all aerobic bacteria grow on blood agar (field 1). Streptococci grow only on blood agar (field 1). Staphylococci and enterococci grow on blood agar (field 1) and mannitol salt agar (field 3 on S and field 4 on S+). Gram-negative bacteria grow on blood agar (field 1), MacConkey agar (field 2) and PGUA agar (field 3 on S+).

Mannitol salt agar differentiates *Staphylococcus aureus* from coagulase-negative staphylococci.

MacConkey agar identifies Gram-negative bacteria.

PGUA agar differentiates *Escherichia coli* from *Klebsiella* spp.



Streptococci

Staphylococci

Gram-negative bacteria

GRAM-POSITIVE BACTERIA

Staphylococci

Staphylococcus aureus (*S. aureus*) and coagulase-negative staphylococci (CNS) grow on blood agar (field 1) and mannitol salt agar (field 3 on S and field 4 on S+). A characteristic of *S. aureus* colonies is that they often are surrounded by double zones of haemolysis on blood agar, which is not observed for CNS. Surrounding the *S. aureus* colony is a clear zone and outside this is a diffuse zone. The mannitol salt agar field almost always changes to yellow around *S. aureus* colonies, while the agar color most often is unchanged around CNS colonies. Both *S. aureus* and CNS should be tested for penicillinase production according to the method explained below.

Penicillinase test

Staphylococci can easily be tested for penicillinase production. When the mannitol salt agar (field 3 on S resp field 4 on S+) around the colonies has turned yellow (field 3 on S resp. field 4 on S+) a Cefinase paper disc (CEF-F) can be placed on such colonies.

NOTE!

The test should only be performed when only one type of colonies is present. The test cannot be performed on bovine blood agar (field 1) or on colonies of bacteria other than staphylococci! Several other bacterial species than staphylococci can cause yellow coloration of the mannitol salt agar.

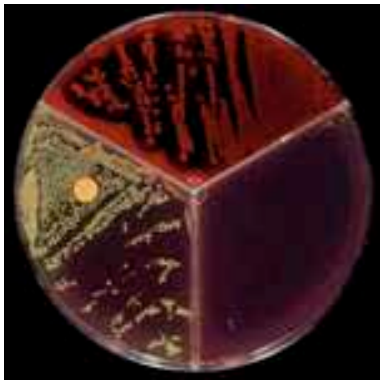
Incubate the plate at 37°C for 60 minutes. A pink to intense red coloring of the disc means that the isolate produces penicillinase (Pc:ase +). The color change is best detected when observing the bottom of the plate. Sometimes the color change only appears as small red spots where the colonies touch the disc.

When growth on mannitol salt agar (field 3 on S and field 4 on S+) is detected without yellow coloration the test should be carried out on a glass slide.

Place the Cefinase paper disc on a glass slide. Moisten the disc with sterile water or sterile saline solution. Transfer colony material from the blood agar (field 1) to the disc with a loop. Incubate at 37°C and check for red coloration within 15 to maximum 30 minutes. It is important that the disc is kept moist throughout the whole incubation period by keeping the slide in an empty petri dish.

Staphylococcus aureus
Pc:ase +

Staphylococcus aureus
Pc:ase + (underneath)



Staphylococcus aureus
Pc:ase - (underneath)

coagulase-negative staphylococci
(mannitol positive)

coagulase-negative staphylococci
(mannitol negative)

Stafylococcus pseudintermedius
(mannitol negative)

Streptococci

Most commonly, all streptococci grow into visible colonies on blood agar (field 1) within 18-24 hours. β-hemolytic streptococci, for example *Streptococcus agalactiae*, often grow with a clear hemolytic zone around the colonies. α-hemolytic streptococci, for example *Streptococcus dysgalactiae*, grow with a greenish hemolytic zone around the colonies. *Streptococcus uberis* can also give a greenish coloration, and cannot be

differentiated from other streptococci. Enterococci sometimes grow on mannitol salt agar (field 3 on S and field 4 on S+) as small colonies and color the medium yellow.

Streptococcus dysgalactiae

Streptococcus agalactiae
(with fluoroscope)

Streptococcus uberis

Enterococcus spp.

Trueperella pyogenes
(with fluoroscope)

Trueperella pyogenes

After incubation for 18-24 hours the colonies of *Trueperella pyogenes* can be observed on blood agar (field 1) as barely visible pin-pricks in reflective light. In translucent light a weak diffuse hemolysis may be visible. The colonies appear more distinct with a small hemolytic zone after incubation for another period of 24 hours.

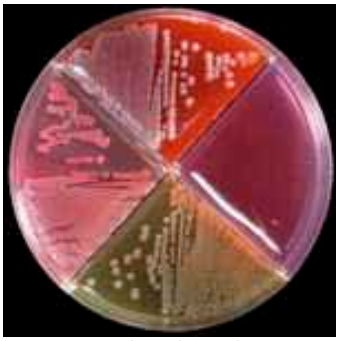
GRAM-NEGATIVE BACTERIA

Coliform bacteria often grow as small colonies on blood agar (field 1) and MacConkey agar (field 2) already within 4 to 6 hours of incubation. Only colonies of *Escherichia coli* (*E. coli*) and *Klebsiella* spp. are colored red-violet on MacConkey agar. Hemolysing *E. coli* has a clear hemolytic zone around the colonies on blood agar. *Klebsiella* colonies are often more smeary than *E. coli* colonies and may form threads when lifting colony material with a loop.

E. coli and *Klebsiella* spp. are easily differentiated using the SELMA PLUS plate. *E. coli* colors the PGUA agar (field 3 on S+) yellow-green. At abundant growth of *E. coli* the whole PGUA agar field is yellow-green. Otherwise only the area around the colonies is colored. Growth of *Klebsiella* spp. will not change the color on the PGUA agar.

Pseudomonas spp. grow as grey/green shimmering colonies with a typical smell of caramel on blood agar (field 1), MacConkey agar (field 2) and PGUA agar (field 3 on S+).

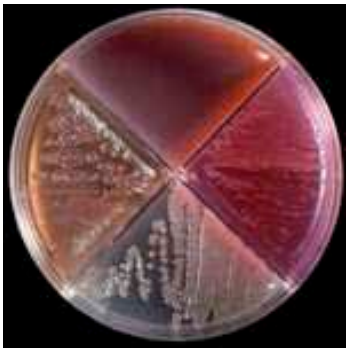
Proteus mirabilis often swarms on blood agar (field 1) and grows as single colonies on the other fields without color change.



Escherichia coli

Klebsiella pneumoniae

Pseudomonas spp.



Proteus mirabilis

Enterobacter cloaca

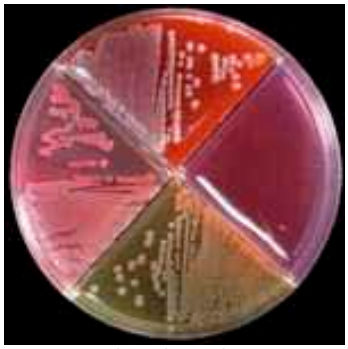
OTHER BACTERIA

Several other bacteria may sometimes cause mastitis, for example *Bacillus* spp. and species of *Enterobacteriacae*. Colonies of Bacillus are relatively large with irregular edges, often with hemolysis, and may sometimes also grow on mannitol salt agar (field 3 on S and field 4 on S+).

Yeast often grow on blood agar (field 1) but sometimes also on mannitol salt agar (field 3 on S and field 4 on S+) after 48 hours of incubation.

Corynebacterium bovis (usually considered non-pathogenic) forms white, dry, non-hemolysing colonies on blood agar (field 1) after incubation for 48 hours.

DIFFERENTIATION OF *E. COLI* AND *KLEBSIELLA*



Escherichia coli

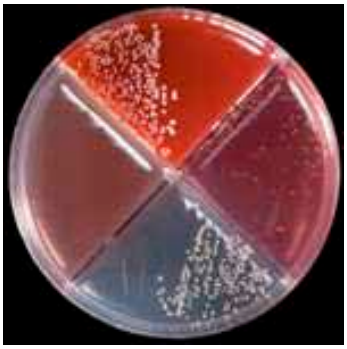
Klebsiella spp.

Escherichia coli

Klebsiella spp.

Pseudomonas spp.

Pseudomonas spp.



Yeast

Bacillus cereus

EVALUATION SCHEME

Species	Bovine blood agar with eskulin field 1	MacConkey agar field 2	Mannitol salt agar field 3 on SELMA, field 4 on SELMA PLUS	PGUA agar field 3 on SELMA PLUS
Coagulase negative staphylococci	+ possibly haemolysis		+	
Staphylococcus aureus	+ single or double haemolysis		+ yellow color	
Staphylococcus pseudintermedius	+ single or double haemolysis		+	
ß-hemolytic streptococci	+ clear haemolysis			
Other streptococci	+ possibly green haemolysis			
Enterococci	+ possibly black colonies		weak growth, yellow color	
Trueperella pyogenes	+ haemolysis			
Escherichia coli	+ possibly haemolysis	+ reddish purple colonies		+ yellow-green color
Klebsiella spp	+	+ reddish purple colonies		+ no color change
Pseudomonas spp	+ possibly haemolysis	+		+
Proteus spp	+ swarms	+ individual colonies		+ individual colonies
Yeast	+	(+)	(+)	(+)
Enterobacter cloacae	+	+		+
Bacillus spp	+ possibly haemolysis		(+)	

+ = bacterial growth
(+) = not always bacterial growth

VERIFICATION OF DIAGNOSIS

In case of an uncertain diagnosis the plate or the milk sample should be sent to a veterinary bacteriological laboratory for verification. Often it is also very important that the diagnosis is complemented with an antimicrobial resistance test of the isolated bacterium.

SVA is an accredited laboratory since 1998 and carries out resistance testing.

STORAGE AND SHELF LIVE

SELMA and SELMA PLUS have a guaranteed shelf life of 2 months from the production date if stored refrigerated and with the lid down. Cefinase has a shelf life of 1 year if stored refrigerated.

DESTRUCTION

Used plates should be destroyed by burning.

CONTACT

For more information contact substrat@sva.se

Phone: +46(0)18674167

For order order@sva.se

Adress: Swedish Veterinary Agency, SVA

SE-751 89 Uppsala, Sweden

SWEDISH VETERINARY AGENCY

SVAKOM41.12.eng