

## **SSGN - SSGNC Mastitis Culture**



# **Quad Plate User's Manual**

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## Warnings and Precautions

- 1.** Sample collection is critical. If the milk sample becomes contaminated by contact with manure, dirty hands or poorly cleaned teats, the mastitis culture results will be invalid.
- 2.** Do not use milk from an animal being treated with antibiotics. Allow for proper flush time according to the instructions of the antibiotic manufacturer.
- 3.** Do not use milk collected from a flow meter. This milk will contain bacteria from the entire herd or string.
- 4.** The milk samples should be plated in a clean environment away from the animals.
- 5.** Never touch the colonies on the plate with your fingers. These colonies are live microorganisms and could be spread.
- 6.** After the SSGN or SSGNC plate is read, pour enough standard household bleach to flood the surface of the plate. Place the top and bottom in a zip lock bag and dispose away from the animals.
- 7.** If a SSGN or SSGNC plate shows no growth it should not be used again. Disinfect the plate as in step 6 and properly discard.

## Introduction

This user manual contains instructions for the SSGN (Staph, Strep and Gram Negative) plate and the SSGNC (Staph, Strep, and Gram Negative Chromogenic) plate. The bacterial growth medias in both plates are identical. The only difference is in Section 2 of the SSGNC where an enzyme substrate specific for *E. coli* has been added. This substrate is utilized by 95% to 97% of all *E. coli* isolates to strain the colonies blue or purple. An exception is *E. coli* O157:H7 which does not turn blue or purple, but will give the typical pink precipitate surrounding a pink colony on both plates.

The Eurofins dairy mastitis quad plates for Staph, Strep, and Gram Negatives (SSGN and SSGNC) are screening methods to aid the dairy producer to identify the prevalence of these bacteria in lactating animals. Follow up therapy or sanitation strategies should be implemented in consultation with your veterinarian, herdsman or dairy co-op field man. Proper use of the Eurofins mastitis quad plates will aid the dairy producer in taking corrective action sooner, optimizing milk quality, and aid in maintaining the health of the herd.

## Materials Required

1. A quad plate containing four differential and selective bacterial growth media.
2. A 10 micro-liter inoculating loop.
3. An alcohol wipe.
4. A sterile collection vial.
5. Regular bleach solution.
6. Zip-lock freezer bags.
7. An incubator set at 35 C (95 F).

# Whole Herd Milk Collection Technique

## Guidelines:

Collecting milk samples to determine the contagious mastitis status of each cow in your herd is essential if *Strep agalactiae* or *Staph aureus* is present. The best results will come if samples are collected at the normal milking time. We suggest one person milking and one person collecting.

Flow meters are not acceptable for collecting milk samples. All milk is run through the same line. Therefore, all samples being collected this way will be contaminated with the same type of bacteria.

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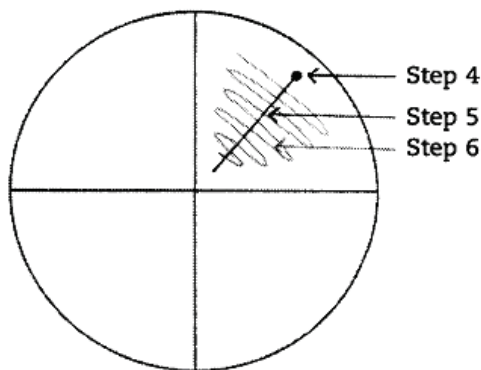
## Steps:

1. Identify the sterile collection vial with the cow ID number of use pre-numbered tubes. Do not put fingers, teats, or any objects inside the vial or cap.
2. Thoroughly wash and dry the cow teats for milking. Pay particular attention to the teat end.
3. Use one alcohol pad per cow and thoroughly sanitize the teat end. If the pad comes away dirty, use another until the pad remains clean. Begin with the teats on the far side so your arms do not redeposit bacteria on the teat ends.
4. Collect equal amounts of milk from all four teats. Begin with the teats closest to you as to not redeposit bacteria on the teat ends.
5. Squirt 2-3 strips of milk to flush out the teat canal, then squirt one strip into the collection container from each teat. Hold the container to the side so no manure drops into the vial as you strip out the teat. Only fill the vial 1/2 full.
6. Cap Vial and put into an ice bucket.
7. Wash and dry hands, then move to the next cow.
8. If you cannot inoculate the SSGN or SGNC plate within 15 minutes of collection, refrigerate, keep on ice, or freeze the sample.

# Procedure for Inoculation of Culture Plates

## Steps:

1. Label outside bottom of plate with cow name/number
2. Invert vial containing milk several times to ensure milk is adequately mixed.
3. Insert round end of the sterile loop into the milk filling the loop.
4. Touch loop to surface of first quadrant of agar.
5. Lightly drag droplet of milk down center of agar surface. Do not pierce the surface of the agar.
6. Lightly streak surface several times across initial streak to spread sample over as much surface of quadrant as possible
7. Repeat steps 3-6 for all three additional quadrants.
8. Replace lid and turn plate upside down.
9. Place in incubator set to 35C (95F) and examine at 24 and 48 hour intervals to identify reactions. Refer to interpretation sheet for identification.
10. Follow directions enclosed with kit for proper disposal.



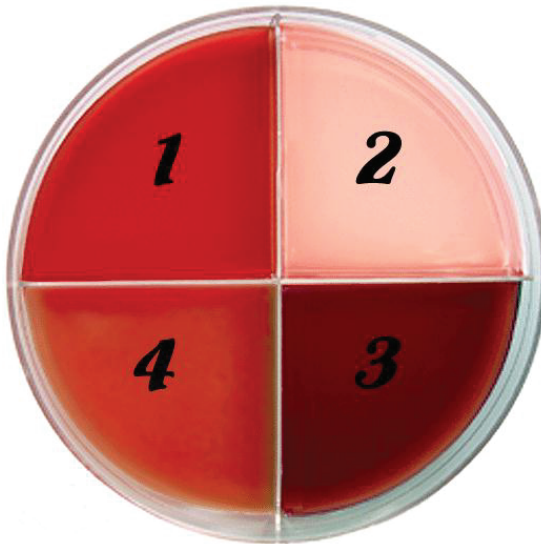
# Procedure Results

	Date	ID of Animal/ Quarter	Quad 1 Results	Quad 2 Results	Quad 3 Results	Quad 4 Results	ID of Organism
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

# SSGN-SSGNC Mastitis Quad Plate

Blood Ager

Gram Negative



Staph

Strep

Each section of the quad plate contains different bacterial nutrients capable of distinguishing distinct groups of bacterial.

**Section 1** Non-selective blood agar capable of growing most bacteria. It is used as growth control.

**Section 2** Specific for Gram negatives such as coliforms, E.coli, and Klebsiella.

**Section 3** Specific for Strep.

**Section 4** Specific for Staph

## SSGN-SSGNC Plate Interpretation Instructions

The following pictures illustrate typical biochemical reactions of the target microorganisms on the four sections of selective nutrient media. Because microorganisms grow at different rates, read the plate at 24 and 48 hours. If the plate does not show any typical reactions after 24 hours, continue incubation and read again at 40-48 hours. Do not read the plate if it is incubated for more than 50 hours. If the plate shows a typical reaction as described in the following summary after 24 hours or after the second reading at 40-48 hours, appropriate corrective action should be implemented. Discard the plate after one use even if no growth occurs.

The Colonies shown on these pages are pure laboratory cultures and are used to illustrate typical microbial reactions on the selective growth media. Actual mastitis raw milk samples could look quite different due to multiple types of bacteria growing in the sample, overgrowth, or poor sample collection.

If there is growth only in section 1 or no growth in any quadrant, clinical symptoms may be due to organisms not presented in this manual. Contact your veterinarian or a reference lab.

# Summary of SSGN Media Reactions

## Staph Aureus

Growth on section 1 and section 4.  
The colonies on section 4 will show hemolysis or clearing of the red color of the media surrounding the colony.  
Growth or no growth in section 2 and 3 does not affect this diagnosis.

**1. Growth on blood agar**

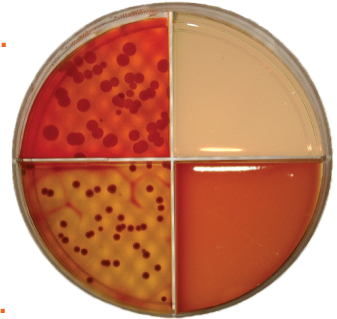
**4. Growth with enhanced hemolysis on Staph agar**

1.



4.

1.



4.

# Summary of SSGN Media Reactions

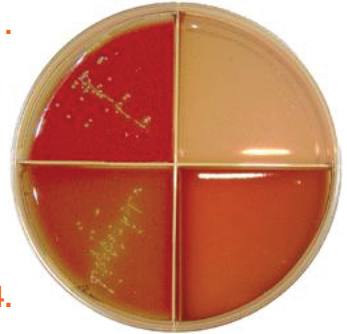
## Environmental Staph

Growth on section 1 and section 4.  
The colonies on section 4 will not show any hemolysis or clearing.  
Growth or no growth in sections 2 and 3 does not affect this diagnosis.

**1. Growth on blood agar**

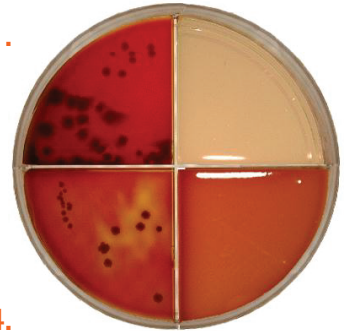
**4. Growth with no hemolysis on Staph Ager**

1.



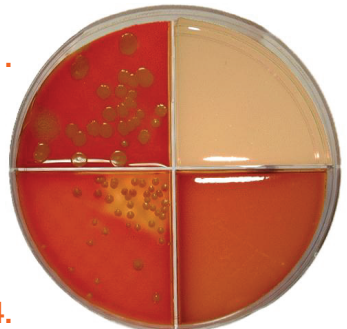
4.

1.



4.

1.



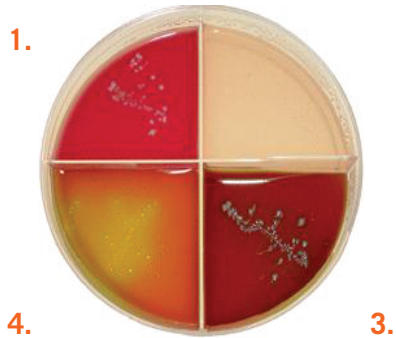
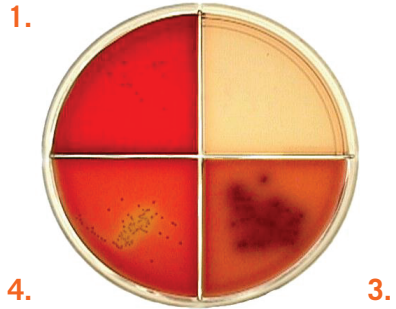
4.

# Summary of SSGN Media Reactions

## Environmental Strep

Growth on sections 1, 3, and 4. The colonies on section 3 may have brownish color but no hemolysis.

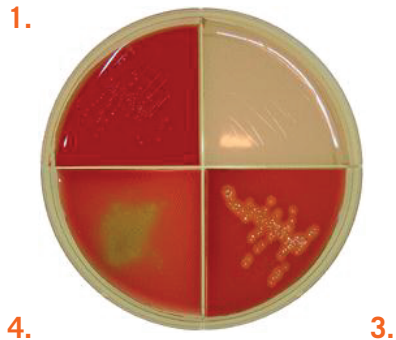
- 1. Growth on blood agar
- 3. Growth on strep agar can be with or without darkening or brownish color
- 4. Growth on Staph agar



## Strep Agalactiae

Growth on section 1, 3, and 4. The colonies on section 3 will show hemolysis or clearing surrounding the colonies. Growth or no growth in section 2 does not affect this diagnosis.

- 1. Growth on blood agar
- 3. Growth with enhanced hemolysis on the Strep agar
- 4. Growth on Staph agar



# Summary of SSGN Media Reactions

## Coliforms/E.coli SSGN

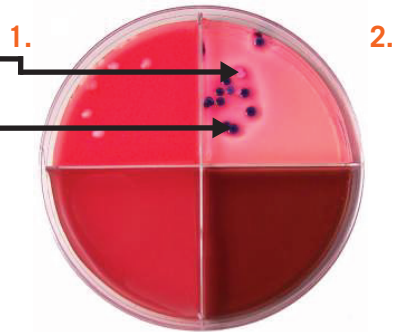
Growth on Sections 1 and 2 is indicative of Gram Negative organisms on both the SSGN and SSGNC plates. Coliforms give pink colonies on both plates. E. coli colonies on the SSGNC plate will be blue or purple while E. coli colonies on the SSGN plate will be pink.

1. **Pink colonies indicate coliforms.**
2. **Additional pink precipitate indicates E.coli**



## Coliform/E.coli SSGNC

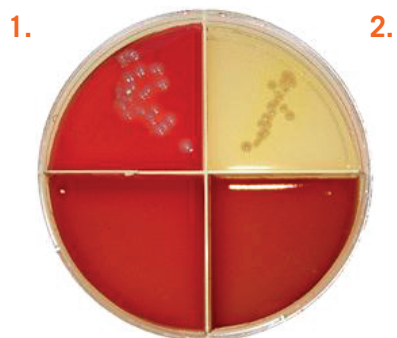
1. **Growth on blood agar**
2. **Pink = Coliforms**
- Blue/Purple Colonies = E. coli**



## Non-Coliforms SSGN and SSGNC

Growth on section 1 and 2 is indicative of Gram Negative organisms. Non-coliform organisms give white colonies on both plates.

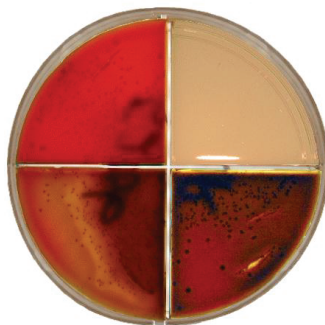
1. **Growth on blood agar**
2. **Gram Negatives (White Colonies)**



# Summary of SSGN Media Reactions

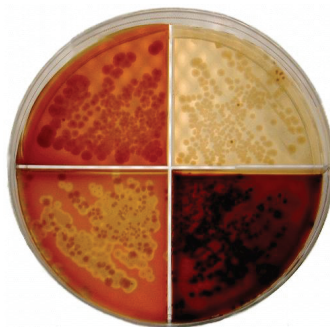
## Overgrowth

Needs to be re-sampled



## Mixture

Coliform/Non-Coliform/Staph/Strep  
Needs to be re-sampled



## Other

If there is growth only in section 1 or no growth in any quadrant, clinical symptoms may be due to organisms not presented in this manual. Contact your veterinarian or a reference lab.

# Procedure Results

	Date	ID of Animal/ Quarter	Quad 1 Results	Quad 2 Results	Quad 3 Results	Quad 4 Results	ID of Organism
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							



# Eurofins Mastitis Media Plates

Item ID	Item Description
222200	<p><b>Mastitis SSGN Quad Plate:</b> Staph, Strep, Gram Negative all in one.</p> <p><b>Section 1:</b> Non-selective blood agar capable of growth most bacteria. It is used as a growth control.</p> <p><b>Section 2:</b> Is specific for Gram Negatives such as Coliforms, E. coli, and Klebsilla.</p> <p><b>Section 3:</b> Is specific for Streptococcus</p> <p><b>Section 4:</b> Is specific for Staphylococcus</p>
222210	<p><b>Mastitis SSGNC Quad Plate:</b> Staph, Strep, Gram Negative all in one. Same as item 222200 except that E. coli colonies on the SSGNC plate will be blue or purple while E. coli colonies on the SSGN plate will be pink with a pink precipitate surrounding the colony. An exception on the SSGNC plate is E. coli 0157:H7 which does not turn blue or purple, but will give the typical pink precipitate surrounding a pink colony.</p>
223000	<p><b>Strep ID Tri Plate:</b> For differentiation and presumptive identification of streptococci species (Enterococcus Faecalis, Streptococcus Bovis, Streptococcus Agalactia, Streptococcus Dysgalactia, Streptococcus Uberis)</p>
222900	<p><b>Mycoplasma Plate:</b> For cultivation and selective isolation of mycoplasma</p>
222100	<p><b>Milk Strep/Staph Bi Plate II:</b> Staphylococcus media for isolation of Staph organisms including Staph Aureus. Streptococcus media for isolation of Strep organism including Strep Agalactia.</p>
222000	<p><b>Milk General Bi Plate I:</b> Non selective Blood Agar for Total Count and Hemolytic Reactions / seletive Gram Negative Agar.</p>
222400	<p><b>Sensitivity Agar Plate I:</b> Agar media for sensitivity testing using the Kirby Bauer method.</p>
222500	<p><b>Sensitivity (Fastidious) Agar Plate with Blood:</b> Agar media with 5% blood added for growth of the more fastidious bacteria.</p>
222600	<p><b>MacConkey Agar Plate:</b> For isolation and differentiation of Gram Negative Bacilli.</p>
222700	<p><b>Strep Media Plate (TKT-FC):</b> Meets National Mastitis Council recommendations.</p>
222800	<p><b>Staph Media Plate (KLMB):</b> Meets National Mastitis Council Recommendations</p>
222810	<p><b>Staph Agar Quad Plate (KLMB):</b> Cultures 4 samples at a time ( One udder by quadrant or four cows), meets National Mastitis Council recommendations</p>
222300	<p><b>Blood Agar Plate:</b> Tryptic Soy Agar with 5% sheep blood.</p>

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