Tools for easy mastitis diagnostics in the field

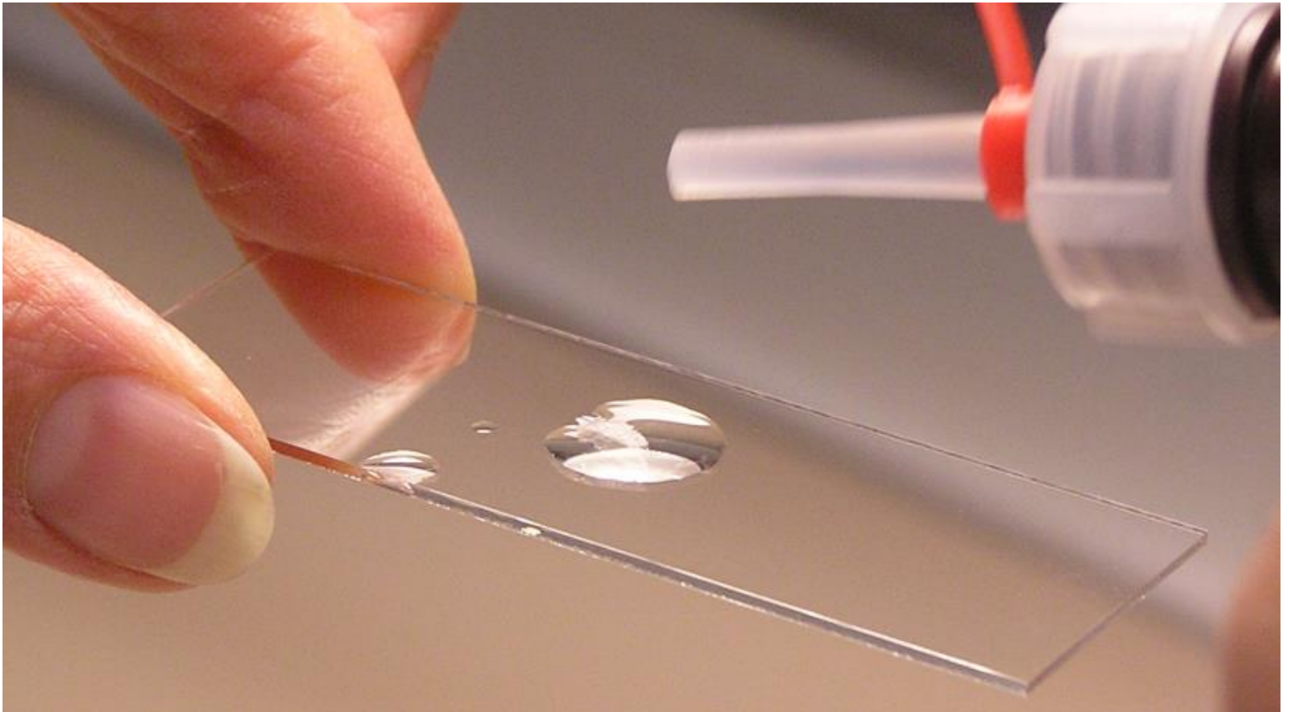

Photo: Susanne André

This booklet presents the most common diagnostic tools suitable for field laboratory conditions to facilitiate the diagnostic procedure of clinical mastitis.

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Potassium hydroxide-test (KOH-test)

The main use of this test is to quickly differentiate Gram-positive bacteria from Gram-negative bacteria.

Principles of analysis:

The lipopolysaccaride component in the cell wall of Gram-negative bacteria will be lysed when exposed to potassium hydroxide and release DNA-content which will appear gelatinous.

Safety aspects:

3-5 % potassium hydroxide (KOH) is a mild irritant, make sure to protect hands and eyes.

Reagent/material:

3-5 % potassium hydroxide (KOH)
Glass slide
Sterile bacteriological loop

Test procedure:

Mix material from one or more colonies with a small amount of 3-5 % KOH on a glass slide.

Preferably use colony material from non-selective medium (blood agar) to optimise the result.

Use the sterile bacteriological loop

to mix the solution in a circular motion.

Test for thread formation by elevating the loop a few millimetres above the glass slide.

Interpretation:

The test result should be checked immediately.

Positive result
- Thread formation, gel forming within 60 s = Gram-negative bacteria.

Negative result - The KOH-solution remains liquid = Gram-positive bacteria.

Sources of error:

Not enough colony material/too much KOH will give a false negative result.

Certain strains of streptococci and staphylococci might cause an increase in viscosity.

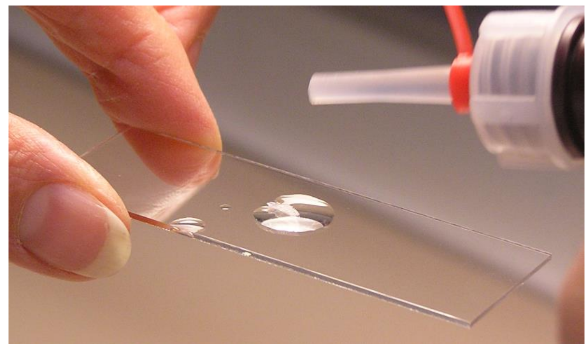
In addition, enterococci might cause a weak positive reaction.

Catalase test

This test is mainly used for the rapid differentiation of staphylococci (catalase-positive) and streptococci (catalase-negative).

Principles of analysis:

The test detects the production of catalase in bacteria. Catalase is an enzyme that splits hydrogen peroxide (H_2O_2) into oxygen and water.



Reagents/material:

Hydrogen peroxide 3% (H_2O_2)
Glass slide
Sterile bacteriological loop

Test procedure:

Using a sterile bacteriological loop, transfer one colony from a bacterial culture on to a glass slide (make sure to avoid contamination with blood agar since this might give a false positive result).

Add one drop of hydrogen peroxide.

Interpretation:

The test result must be checked immediately.

Positive test result: Gas formation, bubbles
Negative test result: Absence of gas formation

Please note that bacterial species other than staphylococci may be catalase-positive.

Sources of error:

Contamination/dirt on the glass slide may give a false positive result.

Contamination of the test reagents with blood or blood agar will give a false positive result since red blood cells contain catalase which splits hydrogene peroxide.

Enterococcal growth may be difficult to distinguish from staphylocccal growth, it may give a yellow colour change on mannitol salt agar and have a weak positive catalase-reaction.

Rapid agglutination test for staphylococci

The purpose of the test is to differentiate *Staphylococcus aureus* from other stapylococcal species.

Principles of analysis:

The tests consists of dyed latex particles covered in antibodies directed against surface proteins of *S. aureus*. When the particles come in to contact with bacteria, agglutination occurs.

Reagents/material:

Agglutination test

Saline solution, NaCl (0,86-0,90%)

Sterile bacteriological loop

Pipette

Test procedure:

CAUTION **–** Do not touch the circles on the reaction cards since this may cause cross-contamination. The quality of the reagents will deteriorate and give false results if they absorb moisture. Make sure to reseal the bags within 2 minutes.

Colony material from blood agar or mannitol salt agar may be used to perform the test.

Add one drop of saline solution (NaCl) in each circle within the elliptic area of the test and the control. Do not place the drop directly on to the dried latex particles.

Use a sterile bacteriological loop

to transfer colony material (one colony) and carefully mix this with the drop of NaCl. Make sure the solution is evenly mixed.

Mix the solution with the the dried latex particles in the control area until the solution is evenly spread out within the entire field.

Use a new sterile bacteriological loop to repeat the above procedure, this time on the test area.

Gently rock the card for a maximum of 20 s and check for agglutination.

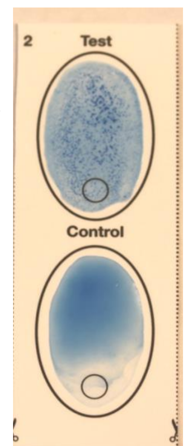
Interpretation:

Positive test **:**

Agglutination of the blue latex particles within 20s . The strain may be *S. aureus*.

*Negative test***:**

No agglutination, an even blue solution at the end of the 20 s.



Soruces of error:

Staphyloccal species other than *S. aureus* may display a positive test result.

Insufficient amount of colony material may cause a false negative result.

The tested material is not pure culture (other bacteria than staphylococci may have a positive reaction).

Penicillinase test with cefinase

The purpose of this test is to detect penicillinase (β-lactamase) producing abilities in staphylocicci. β-lactamase-producing staphylococci are resistant to penicillin.

Principles of analysis:

The production of the enzyme β-lactamase will hydrolyse the β-lactam ring and degradation of nitrocefin will follow which causes a colour change of the disc from yellow to red.

Reagents/material:

Method 1:
Cefinase disc impregnated with nitrocefin

Method 2:
Cefinase disc impregnated with nitrocefin
Physiological saline solution (NaCl)
Glass slides
Unused petri dishes/plastic bags
Sterile bacteriological loops

Procedure:

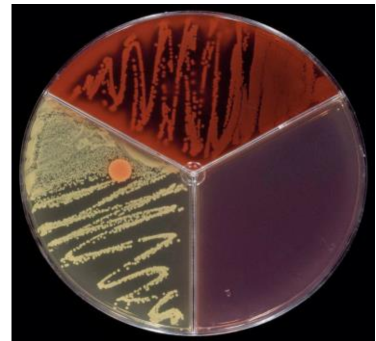
CAUTION! Always ensure the purity of the colonies subjected to testing.
Leave the cefinase cartridge out at room temperature before opening it. The cefinase disc cannot be placed on agar containing blood – this may cause a false positive reaction.

Avoid touching the cefinase discs with your bare hands.

Method 1: To be used with bacteria displaying growth on the mannitol salt agar combined with a yellow colour change:

Place the cefinase disc (using a needle or similar) directly on to the staphylococcal colonies on the mannitol salt agar.

Incubate the plate at +37°C for 15 - 30 minutes. Most positive staphylococci will show a reaction within 5 minutes, whereas some will take up to one hour before the reaction is seen. If left out at room temperature, there will be a delay to a positive reaction.



Method 2:

Place the cefinase disc on a glass slide or in the lid of the petri dish.

Moisten the cefinase disc using NaCl.

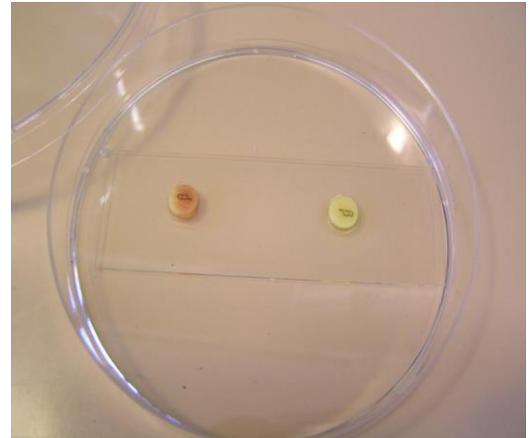
Transfer staphylococcal colonies to the cefinase disc using a sterile bacteriological loop. Test colonies growing on the blood agar as a first hand choice.

Place the slide in an empty petri dish or in a small plastic bag to retain moisture during incubation.

Incubate according to the above instructions.

Interpretation:

Positive result: A positive reaction will elicit a colour change of the disc from yellow to red in the area where the culture was applied. This indicates that the strain subjected to testing produces penicillinase. Even the smallest colour change to red is indicative of a positive result. Commonly, the disc changes colour only partially .



Negative result: No colour change, the disc remains yellow or orange. No pencillinase production.

Sources of error:

The cefinase disc should be yellow when it is unused (unused discs are yellow to orange in colour and may appear slightly patchy which can make the interpretation more difficult).

The cefinase discs are sensitive to transport and storage conditions. Please, adhere to the manufacturer's instructions for storage and pay attention to expiration dates.

Take care to ensure the purity of the culture subjected to testing.

This method of testing is limited to staphylococcal species only.

If left for 2> hours the red colour change will start to fade.

In this case, a new test should be conducted.

Lancefield grouping of streptococcal species

The test is used to classify streptococcal species according to Lancefield group (A,B, C, D, E, F or G). The main purpose is to differentiate *Streptococcus agalactiae* from other streptococcal species.

Principles of analysis:

Antibodies against streptococcal species belonging to the Lancefield groups A-G are bound to latex particles. When bacterial matter comes in to contact with the latex particles agglutination occurs.

Reagents/material:

Agglutination kit

Super Q water (autoclaved)

Sterile bacteriological loops

Pasteur pipette or similar

Agglutination card (”tilt-board”, comes in Streptex-kits)

Mixing sticks

Small sealable plastic tubes (for the extraction enzyme)

Extraction enzyme:

Dilute the enzyme using 11 ml Super Q water

Dispense 0,4 ml of extraction enzyme in a sealable plastic tube

Freeze the tubes at ≤- 18,0°C

Durable for a minimum of 6 months (according to the manufacturer's instruction), alternatively store at 2-8°C, (durability 3 months).

Procedure:

IMPORTANT! Shake the bottles of reagents thoroughly before use homogenise the solutions.

Leave the reagents out at room temperature before use.

Use a white sterile loop to mix colony material from the streptococci subjected to testing with 0,4 ml extraction enzyme (thawed).

Incubate at +37,0 ° C ±1,0° C for 10-60 minutes.

Using a pasteur pipette (or similar), place one drop of the bacterial suspension in each circle on the agglutination card.

Add one drop of each reagent to each drop of bacterial suspension already distributed on the card. Mix the reagents with the bacterial suspension using a mixing stick or a sterile loop (a separate stick for each field!)

Gently rock the agglutination card for a maximum of one minute.

Interpretation

Positive test: agglutination (white precipitate) is visible

Negative test: No agglutination

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Sources of error:

False results can be obtained if:

- The culture is not pure
- Bacteria other than streptococci is tested.