

Detecting Bovine Mastitis Contamination in the milk chain

Introducing

K091



MastiTest™

MastiTest ABST Test Kit



HiMediaLaboratories™

HiMedia Laboratories Pvt. Limited

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For life is precious

K091 MastiTest™ MastiTest ABST Test Kit

This ready to use kit is intended to ascertain antibiotic sensitivity as well as resistance in cases of Bacterial Mastitis in dairy animals.

What is Mastitis?

Mastitis (Mast: breast; itis :inflammation) is an inflammatory reaction of the udder tissue in bovine animals. It can be caused by microbial organisms or physical injury. It occurs when the white blood cells (leucocytes) increase in number in response to bacterial infection of teat canal, which are then released into the mammary glands. It is of major concern as the infected milk can enter the food supply chain and can pose a major risk to public health.

Early diagnosis and treatment can be economically beneficial to the common dairymen. MastiTest ABST Test Kit is developed so as to ascertain antibiotic sensitivity as well as resistance in case of bacterial mastitis.

Causative agents

Bacteria causing mastitis include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus bovis*, *Streptococcus disgalactiae*, *Brucella melintensis*, *Corynebacterium bovis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Pasteurella* species, *Proteus* species.

Mode of transmission

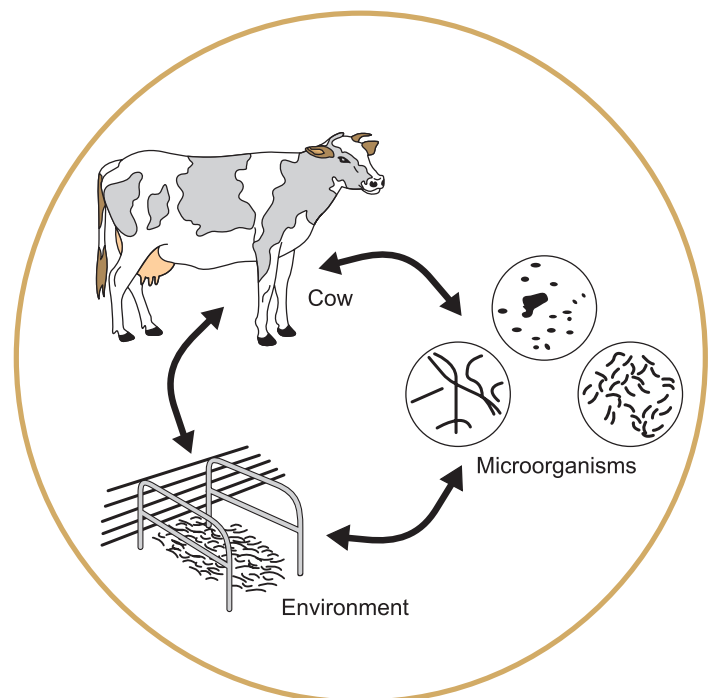
It is spread during the process of milking, either by machine or by hand, and by flies. It is also transferred from cow to cow by calf feeding from multiple cows.

Detection

Traditional methods involved collection of milk sample, detection of causative agents followed by antibiotic susceptibility, which is quite tedious, time consuming and requires a skilled person. This is overcome by the introduction of the most simple, rapid and cost effective MastiTest ABST Test Kit K091 which can be used even by a semi-skilled person.

Application

1. Veterinary Clinics
2. Veterinary Institute
3. Dairies
4. Research Institute for Veterinary
5. Semi-skilled Dairymen



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Types of Mastitis:

Mastitis can be basically classified into two major groups.

1. Contagious mastitis

It is caused by the bacteria on the skin of the teat and inside the udder. This can be transmitted from one cow to another during the milking process.

It can be further divided into

Clinical mastitis

1. Inflammation of the udder and clotted, bloody milk.
2. Abnormal secretions, swollen udder, may have fever.
3. Rapid pulse, loss of appetite dehydration and depression.
4. If gangrene develops, death can result.

Sub-clinical mastitis

1. Somatic cell count of the milk is increased.
2. Bacteriological culturing detects the presence of bacteria in milk
3. For a single clinical case, there are about 15-40 sub-clinical cases

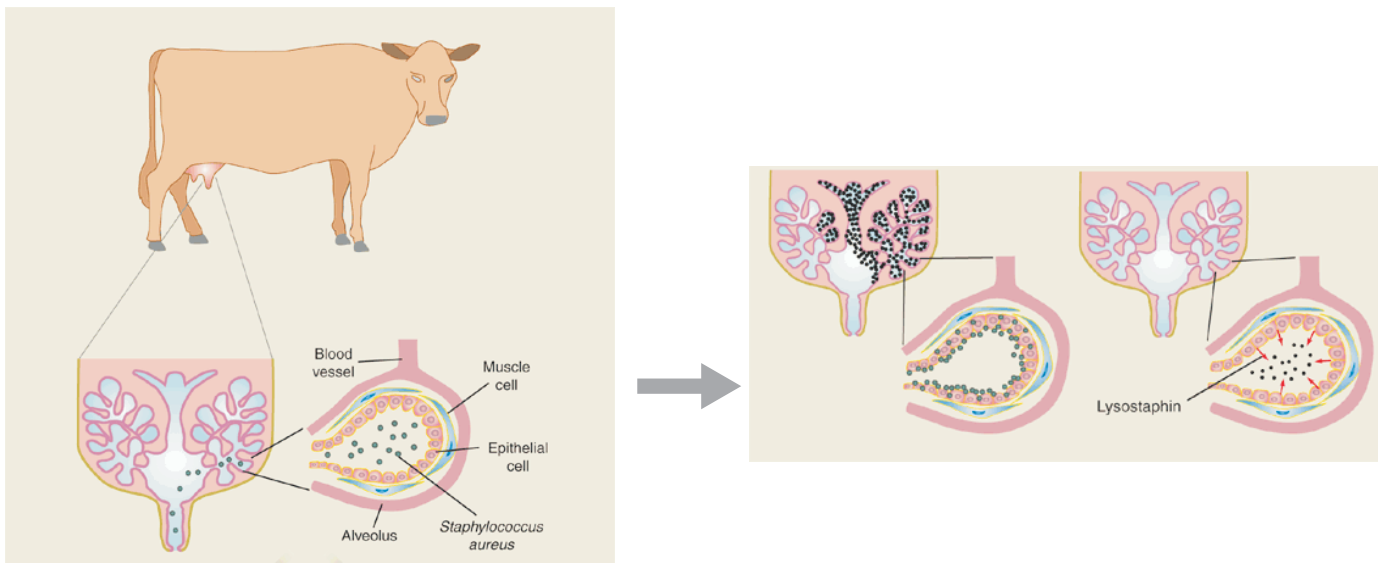
2. Environmental mastitis

It is mainly caused by organisms from a contaminated environment which are normally found in faeces, bedding materials and feed.

Economic Loss

The impact of mastitis on milk production, milk quality and the economic consequences are of major concern to the dairymen and community. It can result in significant annual loss as a result of discarded milk, extra labour and consequently a certain percentage of deaths.

1. Mastitis causes heavy loss in milk production due to contaminated milk affecting the dairy industry.
2. Subsequently the quality of related milk products is also hampered.
3. It alters milk composition and this contaminated milk can pose a major health hazard to public health.
4. Treatment cost is also elevated.



Development of the disease :

Bacterial mastitis is initiated after the bacteria pass through the teat duct and enter the cisternal area during the milking process. After the milking process the teat canal remains dilated for 1-2 hours while the canal of the damaged teat remains open permanently thus permitting the entry of the environmental organisms or from the injured skin of the animal.

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Salient features

1. Diagnosis and Treatment of Bacterial Mastitis made easier, faster & cheaper
2. This ready to use kit is intended to ascertain antibiotic sensitivity as well as resistance in cases of Bacterial Mastitis in dairy animals.
3. The Kit is easy to carry and due to its simplicity it can be used in field, veterinary hospitals or in farm premises by a semi-skilled person even in absence of basic facility of a clinical laboratory.
4. The results can be read explicitly within 18 hours as it involves only colour differentiation between the control and test vials.
5. Rapid and cost effective way of choosing the drug of choice for treatment as compared to the conventional methods that require 48 – 72 hours for the same.

Each Kit Contain

1. One sterile Sample collection vial with 10 ml marking containing diluent and glass beads (S-SAM).
2. One control vial (C-CTRL).
3. Eight test vials containing following antibiotic discs.
 - a. Ampicillin/Cloxacillin AX 128/128 mcg (T1-AX).
 - b. Amoxicillin/Cloxacillin ACX 128/128 mcg (T2-ACX).
 - c. Gentamicin GEN 128 mcg (T3-GEN).
 - d. Enrofloxacin EX 8 mcg (T4-EX).
 - e. Ciprofloxacin CIP 8 mcg (T5-CIP).
 - f. Tetracycline TE 128 mcg (T6-TE).
 - g. Chloramphenicol C 8 mcg (T7-C).
 - h. Streptomycin/Penicillin SPN 128/128 mcg (T8-SPN).
4. One sterile 5 ml syringe (SYN-5ml).
5. One sterile 18G needle (NEE-18G).
6. Two steriSwab™ (CO089).

Principle

Traditional methods include milk sampling and then culturing which is time consuming. This is overcome by the use of MastiTest ABST Test Kit. This kit is designed so as to detect mastitis along with simultaneous detection of its sensitivity and resistance against the most commonly used antibiotics in dairy animals. The test is easy, simple and fast. It is based on the inoculation of milk sample into the control vial and the antibiotic vials, and observing the visual colour change from the original blue colour. Change of colour is due to the presence of positive organism.

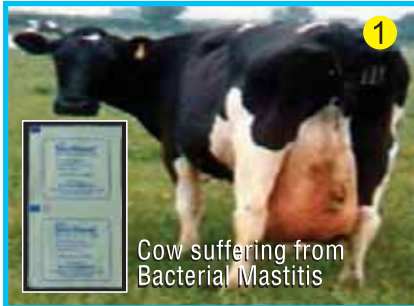
Direction

1. Wash your hands and wear gloves before collection of Milk samples.
2. Wash the udder of Bovine animals with sufficient water to remove dirt. Dry with a clean tissue / towel.

3. Disinfect the infected udder of the bovine animals using SteriSwab™ (Co089).
4. Remove 3 to 4 streams of milk from the quarter being sampled to avoid contamination.
5. Collect the milk sample from affected quarter/quarters aseptically in sterile closed container and label it.
6. Carry the sample, preferably to a dry clean area where the test is being performed.
7. Flip off the cap of the sample vial (S-SAM), control vial (C-CTRL) and the test vials (T1, T2, T3,.....T8).
8. Open the sterile 5 ml syringe (SYN-5ml).
9. Remove the needle from the syringe by rotating clockwise. Preserve this needle which has to be used as a venting needle.
10. Insert this needle in the rubber bunk of the sample vial (for venting).
11. Take care so that the venting needle is not in contact with the test material
12. Remove sterile 18G needle (NEE-18G) from pack and fix to the syringe (SYN-5ml) by rotating it anticlockwise.
13. Open the milk container carefully so as to avoid aerial contamination (give minimum exposure).
14. Collect 5 ml of milk sample from the milk container with the help of 5ml syringe (SYN-5ml).
15. Inject the 5 ml milk sample into the sample vial (S-SAM) containing diluent and glass beads. Rinse it by pumping the piston once or twice.
16. Mix the milk sample with the diluent of sample vial.
17. After proper mixing, draw 5 ml (**Test sample**) from the sample vial without any air gap in syringe (SYN-5ml).
18. Remove the venting needle from the sample vial, insert it in the slot of the control vial and then inject 1ml **test sample** into it. (Take care, so that the venting needle and 18G needle (NEE-18G) does not touch the material in the control vial (C-CTRL).
19. Likewise transfer 1 ml of **test sample** into the test vials T1, T2, T3 and T4, taking care to insert the venting needle in each of the test vial prior to injection of the **test sample**.
20. Now again draw 4 ml of the test sample from the sample vial using the same syringe and inject 1 ml each into the test vials T5, T6, T7 and T8.
21. Once all the test vials and control vial are injected with the test sample shake the vials to reconstitute the content of vials and incubate at room temperature (25-30°C) or in incubator (35-37°C, can give faster results).
22. After every 30 minutes, observe for change of colour without disturbing the vials upto a period of **16-24** hours.
23. Discard all the vials appropriately after noting the results.

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Disinfect the udder of the bovine animals using Steriswab (CO089)



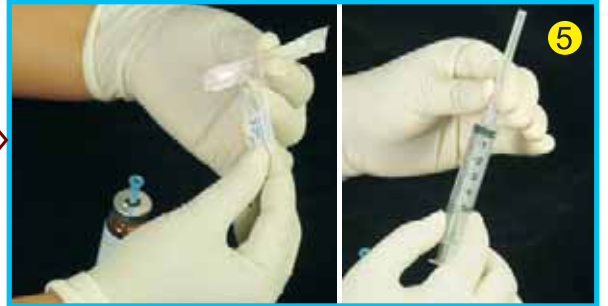
Collect the milk sample in sterile closed container



Flip off the cap of the sample vial (S-SAM), control vial (C-CTRL) and the tests (T1, T2, T3... T8).



Open the sterile 5 ml syringe (SYN-5ml) remove the needle from the syringe by rotating clockwise, preserve this needle which has to be used as a venting needle. Insert this needle in the rubber bunk of sample vial (for venting) Take care so that the venting needle is not in contact with the test material



Remove sterile 18G needle (NEE-18G) from pack and Fix to the syringe (SYN-5ml) by rotating it anticlockwise.



Collect 5 ml of milk sample from the milk container with the help of 5ml syringe (SYN-5ml).



Inject the 5 ml milk sample into the sample vial (S-SAM) containing diluent and glass beads. Mix the milk sample with the diluent of sample vial. Rinse it by pumping the piston once or twice.



After proper mixing, draw 5 ml of test Sample from the sample vial, without any air gap in syringe (SYN-5 ml) remove the venting needle from sample vial, insert it in the slot of the control vial. and then inject 1 ml test sample in to it. Take care so that the venting needle and 18 G needle (NEE-18G) does not touch the material in the control vial.



Like wise transfer 1 ml of **test sample** into test vials T1, T2, T3, T4 taking care to insert the venting needle in each of test vial prior to injection of the test sample.



Now again draw 4 ml of the test sample from the sample vial using the same syringe and inject 1 ml each into the test vials T5, T6, T7 and T8. Once all the test vials and control vials are injected with the test sample shake the vials to mix the contents and incubate at room temperature (25-30°C) or in an incubator (35-37°C, if available) for 16-24 hours.

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Result interpretation:

Note the results in the table below.

Vials	C-CTRL	T1-AX	T1-ACX	T1-GEN	T1-EX	T1-CIP	T1-TE	T1-C	T1-SPN
Observed colour after incubation									
Interpretation									

Interpret as follows:

1. The colour change from blue to colourless in control vial is an indication of luxuriant growth of the culture.
2. The colour change in antibiotic vial from blue to colourless is an indication of resistance towards the corresponding antibiotic.
3. The colour change in antibiotic vial from blue to light blue is an indication of intermediate susceptibility towards the corresponding antibiotic.
4. No colour change in the antibiotic vial is an indication of sensitivity towards the corresponding antibiotic.
5. Minimum change of colour in the corresponding antibiotic vial will be the antibiotic of choice for treatment.



1. Resistant 2. Intermediate 3. Sensitive

Results observed after inoculation of contaminated milk sample from an infected cow and incubation at room temperature for 16 to 24 hrs.



1. One control vial (C-CTRL) - Positive control
2. Ampicillin/Cloxacillin AX 128/128 mcg (T1-AX) - Resistant
3. Amoxycillin/Cloxacillin ACX 128/128 mcg (T2-ACX) - Resistant
4. Gentamicin GEN 128 mcg (T3-GEN) - Sensitive
5. Enrofloxacin EX 8 mcg (T4-EX) - Sensitive
6. Ciprofloxacin CIP 8 mcg (T5-CIP) - Intermediate
7. Tetracycline TE 128 mcg (T6-TE) - Sensitive
8. Chloramphenicol C 8 mcg (T7-C) - Intermediate
9. Streptomycin/Penicillin SPN 128/128 mcg (T8-SPN) - Intermediate

- Note :**
- Organisms other than bacteria may change the colour of control vials.
 - Milk samples obtained from animals already under treatment may take more time to give the result.
 - If the colour of the control vials does not change within 72 hours confirms organism other than bacteria or other slow growing organisms may be involved in the disease.
 - Failure to follow aseptic methods can give false results.

Storage

Store at 2-8 °C, away from direct light.

“Know- how from Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum”



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