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Research Article

Milk examination

Milk production potential is a function of the number of mammary epithelial cells in the gland, as well as the secretory activity of those cells [1,2]. Therefore, improved lactation performance can be achieved under conditions that enhance mammary cell proliferation (or decrease apoptosis), biochemical and structural differentiation of mammary epithelium, and synthesis and secretion of milk components [1].

Milk sample can be collected first wash the udder and the teats thoroughly and dry with a paper towel. Swab the teats with 70 per cent alcohol. The first couple of squirts should be discarded unless used for a field test and, about 10 ml milk squirted into a sterile 30 ml Macartney bottle or sterile 30 ml plastic universal container held nearly horizontally. Avoid touching the mouth of the bottle with the teat and code the samples numerically. The samples must be submitted chilled using either crushed ice or cooling bricks in an insulated container².

Milks must be refrigerated if transport is slightly delayed, or frozen if transport is delayed more than 2-3 days. Clinical mastitis episodes associated with Gram-negative bacteria frequently cause a profound leukopenia, neutropenia, lymphopenia and monocytopenia as a result of the endotoxemia and an increased packed cell volume². After sample is collected, the sample can be examined by agglutination test for mastitis and brucellosis.

Milk ring test

The milk ring test is a type of agglutination test that detects brucella antibodies in milk samples and is recommended as a screening test for bovine brucellosis by OIE [3]. It consists of *Brucella abortus* cells stained with hematoxylin, which are added to a sample of 1 to 2 ml of milk and incubated at 37°C for one hour³. If antibodies for *Brucella abortus* are present in the milk

the antigen agglutinate with the cells, and float to the surface where it forms a sharp blue or purple colored cream layer. The milk column underneath will remain white. The milk ring test (MRT) and ELISAs are available for detecting antibodies to *Brucella* infection in milk and the MRT test is cheap, simple and requires no specialised equipment to perform. It detects anti *Brucella* IgM and IgA bound to milk fat globules. When tests for detecting *Brucella* antibodies in milk and serum are considered, the principal methods for detecting infected herds and for diagnosing brucellosis in individual animals are the serological tests which are mainly used for diagnosis of brucellosis [4].

MRT antigen is prepared from concentrated, killed *B. abortus* strain 99 or 1119-3 cell suspension, grown as described previously. It is centrifuged at, for example, 23,000g for 10 minutes at 4°C, followed by resuspension in haematoxylin-staining solution⁵. The milk ring test indicates the presence of agglutinins (antibodies) in the milk, by addition of stained bacteria to the milk sample followed by incubation for 30-60 minutes. The agglutinins present in milk of cow or sheep will clump the *Brucella* stained agglutinin antigen complex rises the fat globules. This causes the cream layers at the top to bottom deplectocoloured [5]

Procedure MRT

The milk ring test should be performed by the following procedure [6-9]:

- Collect milk from an animal, sample is identified by date of collection and refrigerated
- Gentle mix the milk in sample tube to ensure distribution of cream
- Place 1 ml of milk in a plastic agglutination tube to give a column of milk about 2 cm high
- Add 1 drop of 0 milk ring test antigen or *B. abortus* suspension by pipette into test tube.
- Gentle mix thoroughly the contents within 1 minutes of adding antigens and avoiding frothing and incubation the mixture at 37 °C for 39-40 minutes and then examine

- The interpretation should be observed as: Positive test result: cream layer is deeply coloured and beneath cream layer is white, Negative test result: cream layer is white, milk beneath is white coloured as indicated at figure 1.

Mastitis test

Mastitis is a disease of many mammalian species. At least, 137 infectious causes of bovine mastitis are known to date and in large animals the commonest pathogens are *staphylococcus aureus*, *streptococcus agalactiae*, other *streptococcus* species and *Coliform* as reported by [10]. *Staphylococcus* sp. is the main aetiological agents of clinical and subclinical mastitis in cows while, *S. aureus* and *Escherichia coli* are most commonly isolated pathogen from the clinical mastitis, coagulase negative *Staphylococci* (CNS) are the most frequently isolated pathogens from the subclinical cows mastitis [11,12].

The major bacteria can be split into two categories, those that are cow associated (or contagious), and those which are environmental in origin [12]. The cow-associated bacteria are *Staph. Aureus* and *Strep. Agalactiae* while the main environmental bacteria are *Strep. Uberis*, *Strep. Dysgalactiae* and coliforms [13]. The most important major pathogens involved in bovine mastitis worldwide are *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Escherichia coli* and *Klebsiella* spp [13]. Mastitis can be tested by somatic cell count and california mastitis test.

California mastitis test

The California mastitis test (CMT) is a simple cow-side indicator of the somatic cell count of milk. It operates by disrupting the cell membrane of any cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel [14] (Figure 2).

Procedure of California Mastitis Test [15]:

- Collection of milk samples from each quarter of udders in the CMT paddle. The paddle has four shallow cups marked in different to identify the individual quarter from which milk sample was obtain.
- Take about 2 ml or 1 teaspoon of milk from each quarter
- Add an equal amount of CMT solution to each cup in the paddle

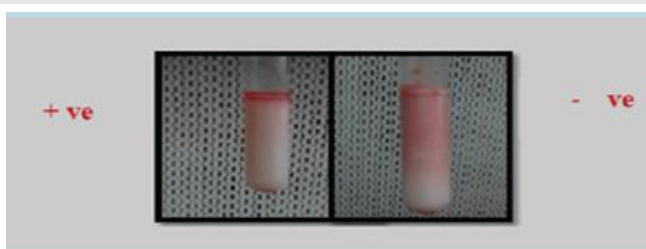


Figure 1: Milk ring test to detect the presence of brucella spp in milk sample [7].

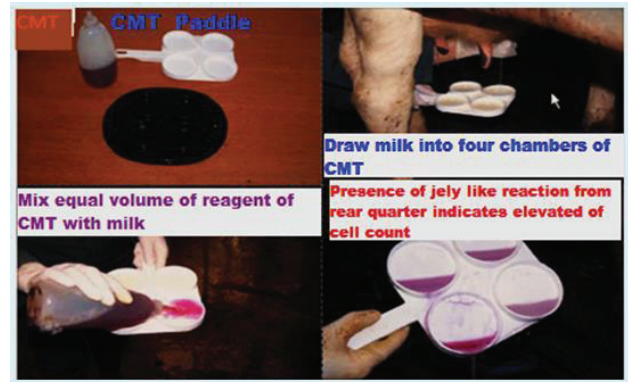


Figure 2: California mastitis test [16].

- Rotate the CMT Paddle in a circular motion to mix the milk contents do not mix more than 10 seconds.
- The result is observed after about 20 seconds. The result is more gel formation

Somatic cell count (SCC)

Somatic cells which found in milk are composed of white blood cell and sloughed epithelial cells. Cell white blood cell found in normal milk from uninfected glands include neutrophils, macrophages, lymphocytes and epithelial cells [16,17]. SCCs are a measure of the number of cells present in the milk. In healthy udder the scc is made up predominantly of epithelial cells. In the inflammation of the udder, white blood cells enter the udder to combat the infection and the scc rises. Numerical scc are measure using the automatic somatic methods, which is used to determine sccs in bulk milk and individual cow samples. sccs can either be performed by bulk milk presented monthly, three monthly and annual average or on an individual cows [17]. The macrophages have an important role in providing surveillance in the uninfected gland. When bacteria invade and colonize the mammary gland, the macrophages respond by initiating the inflammatory response that attracts polymorphonuclear cells (PMNs) into the milk to engulf and destroy the bacteria. The largest factor that influences the SCC of milk is mastitis. The SCC of a cow that is not infected with mastitis is usually less than 200,000 cells/ml and many cows maintain SCC values < 100,000 cells/ml [18].

Physical examination of milk

The milk can be examined physical by observing the consistency, color and odor or given milk samples, it is summarized as following [19-21]:

Color of Milk

- ✓ Normal: white in buffalo and whitish yellow in cow
- ✓ Yellow: ratio containing carotene, colostrum, some breed like Jersey cow

Abnormal:

- ✓ Blood stained: Highly produced animal's pathological discoloration

- ✓ Red: Dicumarol toxicity, Leptospirosis, Staphylococcosis, Bacterium erythrogene
- ✓ Green: Corynebacterium
- ✓ Yellow: most of bacterial Mastitis, Pseudomonas xantha.

Odor

- ✓ Normal milk: milky odor

Abnormal:

- ✓ Fetid (putrefied) odor: Gangrenous mastitis, Dry cow mastitis
- ✓ Fecal odor: Coliform mastitis
- ✓ Rancid odor: Milking in bad ventilated place or leaving milk for 2 days in environment
- ✓ Antiseptic odor: Excessive dipping of teat
- ✓ Acetone odor: Ketosis

Consistency

- ✓ Normally: milk consistency

Abnormally: Watery milk (decrease consistency): Streptococcal mastitis

- ✓ Increased consistency: Corynebacterial mastitis

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