

**A NEW RAPID, ON-SITE TEST FOR DETECTION AND MAJOR CLASSIFICATION OF BACTERIA IN MILK FROM COWS WITH MODERATE AND SEVERE CLINICAL MASTITIS**

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A new simple diagnostic test has been developed for rapid on-site detection of bacteria in bovine milk samples. The procedure can be performed in less than 5 minutes, and detects bacteria in milk samples, in addition to providing information about Gram staining features.

Prior to application on a filter device, a small volume of milk (500 µl) is mixed with an equal volume of a solubilising reagent and heated for a few second under hot tap water to increase filterability. The mixture is pre-filtered through a hydrophobic mesh to remove clumps or debris, and to reduce fat content. A drop of the pre-filtered mixture is thereafter filtered through a porous polysulfone membrane, placed in a vertical flow-through device, with a pore size retaining and concentrating bacteria from the sample. Vertical flow through the polysulfone membrane is achieved by placing an absorptive media directly under the membrane.

Any bacteria concentrated on the surface of the membrane are visualized by staining with a cationic dye (toluidine blue) and can be directly read as a blue coloured spot, by the naked eye. Following staining, and confirmation of bacteria in the sample, a drop of ethanol-acetic acid mixture (pH 2.8-3.0) is put on the same membrane, to cause decolourisation of Gram-negative bacteria, whereas Gram-positive bacteria retain the stain. The method is simple to perform, and does not require heat fixation, electrical power, microscopic examination or specially trained personnel.

Detection limit was determined using over-night cultures of *S.aureus* and *E. coli*, diluted in milk from a mastitis free cow, and was found to be  $5 \times 10^6$  for *S.aureus* and  $1 \times 10^6$  bacteria/ml for *E. coli*, respectively. The test described, identified correctly bacteria in 51 of 55 milk samples from cows with moderate or severe clinical mastitis, giving an accuracy of 93%, and a sensitivity of Gram classification in the same samples found to be positive for bacteria (48 samples), of 94%.

Using a flow through device, instead of a syringe filter, makes the assay more user-friendly and enables immediate confirmation of a positive staining and Gram status, without the need of cumbersome opening and remounting of a syringe-filter housing. This new rapid method should therefore be a useful on-site aid for the veterinarian in optimising the use of antibiotics in therapy of moderate and severe clinical mastitis.

For additional information, reference is also made to the oral presentation by Siamak P. Yazdankhah; "A rapid method for detection of Gram-positive and -negative bacteria in milk from cows with moderate and severe clinical mastitis."