What techniques can be used to identify bacteria other than milk culture?

Although there has been some interest in developing ‘cow-side’ tests to identify the species of mastitis bacteria, there is currently no other system that is as reliable and economic as routine culture of milk samples.

**Identification systems available overseas**

Test systems that are in use overseas (but not available in Australia) are:

**ProStaph (ProScience Corp, Sterling, Virginia)**
ProStaph is an ELISA test that detects *Staphylococcus aureus* antigens in milk (Adams et al 1988). Snoep et al (1995) found that the test was unreliable for cows at less than 30 days lactation and for cows producing less than 14 kg of milk. Outside these limits, the technique was reported as superior to culture for detecting infection, with the added advantage of not requiring a sterile milk sample (it can be used on herd recording samples).

**HyMast (Pharmacia and Upjohn)**
HyMast is a paddle with selective media for contagious and environmental pathogens. The paddle is dipped into an aseptically collected milk sample and then incubated for 24 hours. The colony characteristics and colour, and the change in colour of the selective media can indicate the pathogen present. In studies in Italy, Canada, the United States and Israel, the test was more sensitive than most laboratory culture tests for detection of environmental pathogens except *Strep dysgalactiae*. The sensitivity for *Staph aureus* is variable (Bahar 1995, Leslie et al 1995).

**Linmast (Mybach-Vettech, Stockholm)**
Linmast is used to differentiate coliform mastitis from other aetiologies. It is based on the phenomenon that endotoxin coagulates Limulus-crab’s blood. The test can be used ‘cow-side’ because the mixing of endotoxin in milk with test reagents results in development of a yellow colour within 15 minutes. It is widely used in the pharmaceutical industry for quality control, and is in use on farms in Scandinavia.

Technote 4.3 describes collecting and submitting milk samples for culture.
Polymerase chain reaction

A research tool that has diagnostic potential to identify mastitis bacteria in milk is the polymerase chain reaction (PCR). It works by amplifying a specific segment of the genetic code of bacteria, and different test reagents are used to identify bacterial species. Preliminary assessment of PCRs to identify Staph aureus, Strep agalactiae, Strep dysgalactiae and Strep uberis in bulk tank milk samples are underway (Glenn Browning personal communication) and some work has been done on identification of Mycoplasma bovis (Ghadersohi et al 1999).

PCR will only be a valuable diagnostic tool if:
• milk samples containing pathogenic bacteria give test positive results;
• those containing non-pathogenic bacteria are test negative; and
• the test correctly identifies the bacterial species present in the sample.

An advantage of the technique is it can be applied to milk samples that are not collected aseptically.

As with any test confirming the presence of bacteria, it is important to decide whether the pathogens identified are likely to be responsible for a mastitis problem in the herd and whether further action needs to be taken. For example, Strep uberis isolated from a vat sample may have originated from the surface of teats rather than the udder tissue itself.

Key papers

Blackspot is the common name for lesions that look like necrotic craters with raised edges and a black spot of ulceration or scab in the centre. The ulcers commonly extend to include the teat opening, and the damaged tissue provides a site for infection (e.g. with Fusobacterium necrophorum) and bacterial multiplication (e.g. Staphylococcus aureus) (Radostits 1994).

Although blackspot is not an infectious disease, tissue damage extending to the teat opening and bacterial multiplication at the teat end makes affected cows very susceptible to mastitis. Teat openings become occluded and cows often become slow milkers.

Diagnosis is made on the clinical appearance of lesions. If only one or two cows are affected, poor teat conformation may be contributing to teat damage at milking. Milking machine function should be thoroughly checked if more than one or two cows are affected because blackspot is often associated with failure of pulsation, excessive vacuum or over milking.

Management of blackspot in a herd involves:

- treating the lesions with antiseptic ointments or chemicals such as hydrogen peroxide or iodine;
- using teat disinfection to minimise bacterial infection of lesions; and
- checking the milking machine function.

Veterinarians may consider incising lesions of very badly affected teats (e.g. with a concealed teat-knife) to increase the size of the occluded teat opening and increase blood supply to the area. Severe lesions may be intractable to treatment.

**Key paper**

Bovine herpes mammillitis

How does bovine herpes mammillitis spread?

Bovine herpes mammillitis (BHM) is an ulcerative dermatitis of the teats, udder and area between the vulva and anus (Turner et al 1976). Outbreaks may make milking very difficult, and are often accompanied by concurrent mastitis.

BHM is caused by bovine herpesvirus 2 (BHV-2). The virus can live away from the host, persisting for more than 100 days at room temperature, and surviving freezing and thawing. It is inactivated at pH of 3 and by iodophor disinfectants.

Two epidemiological patterns are seen: in some outbreaks most of the herd is affected over a period of up to four months; in others the disease appears only in first calf heifers (indicating that older cows have been exposed and established some immunity).

Observations in the United Kingdom and Australia have indicated that insects may act as vectors. The virus must be deposited in the deep layers of the skin, so it is unlikely that a correctly functioning milking machine would be an important predisposing factor for spread. Nevertheless, liners will carry large quantities of virus shed at the peak of the disease and could introduce the virus to uninfected cows if teat lesions were present.

It is presumed that the virus spreads by local extension on the skin. Affected cattle usually show no systemic illness although a mild fever may occur.

Teats develop multiple, raised, oedematous plaques of 1-2 cm with or without vesiculation. The lesions may coalesce and cover a large part of the teat. The surface then sloughs leaving raw ulcers with sharp edges, which are subsequently covered with dark coloured scabs. The scabs dry and finally detach, often without leaving scars. If the whole surface of the teat has been affected, the detachting scab may come away in a single cast that resembles a thimble in appearance. Mild lesions heal in about 10 days but some ulcers may persist for months. Lesions that occur on the udder or above the vulva tend to be more diffuse and superficial. Mastitis may occur if the teat-ends are affected.

Like all herpes viruses, BHV-2 remains latent and may recur (e.g. after corticosteroid treatment). Higher incidence around calving may be associated with the immunosuppression associated with parturition.
Clinical diagnosis is made on the presence of characteristic lesions (but must be differentiated from pseudocowpox). Confirmation can be achieved by virus isolation, or demonstration of virus in vesicle fluid, swabs or biopsy material taken at the peak of the infection. Isolation can be difficult if lesions are more than seven days old, or iodophors have been used. Recovered animals may have a raised titre for up to two years.

There is no specific treatment for BHM although application of crystal violet dye has a good reputation in helping alleviate signs (Radostits et al 1994). To facilitate milking, a water miscible antibiotic ointment may be applied before the teatcups are put on (ensuring milk is withheld from the vat), followed by an astringent after milking.

Natural infection leads to immunity that lasts for about one year, but no commercial vaccine is available.

Humans do not appear to be susceptible to infection with BHV-2.

Key papers


What cow-side tests are useful in detecting mastitis?

Tests used in the dairy shed to detect subclinical mastitis in cows include the Rapid Mastitis Test and measuring the electrical conductivity or pH of milk samples.

The Rapid Mastitis Test

The Rapid Mastitis Test (or Californian Mastitis Test) is a cow-side test that detects subclinical mastitis in individual quarters by the presence of cells in milk samples. A small amount of milk from each quarter is squirted into a dish at milking and an equal amount of detergent reagent is added. The solution is swirled to mix it, and the amount of gel reaction is assessed.

It is critical to differentiate trace reactions from test negative samples to identify cows with subclinical mastitis. Experience is required to obtain accurate readings of trace reactions as the slime layer may not be obvious in some samples.

Gel reaction scores in the Rapid Mastitis Test

<table>
<thead>
<tr>
<th>Score</th>
<th>Visible change</th>
<th>Approximate cell count (cells/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Fluid mixture</td>
<td>&lt; 200,000</td>
</tr>
<tr>
<td>Trace</td>
<td>Slight slime formation most noticeable when the mixture is gently swirled in the dish</td>
<td>150,000 – 500,000</td>
</tr>
<tr>
<td>1+, 2+ or 3+</td>
<td>Distinct slime formation which coats the side of the container when the mixture is swirled</td>
<td>&gt;400,000</td>
</tr>
</tbody>
</table>

Commercial Rapid Mastitis test kits, such as Mastest, from DLC Australia Pty Ltd, are available from veterinarians and dairy product suppliers.
Electrical conductivity of milk

Normal milk contains a small amount of salt that allows an electric current to pass through it. In damaged udder tissue, more salt leaks into the milk and the electrical conductivity of milk increases. Changes in electrical conductivity in inflamed mammary glands may precede visible changes in milk, and assist early identification of subclinical and clinical cases of mastitis. Evaluations of conductivity tests to diagnose intramammary infection have given widely varying results, and are summarised in a comprehensive review by the International Dairy Federation (Hamann and Zecconi 1995).

There are two different approaches to measuring conductivity:
- hand-held, portable instruments used for occasional strategic cow-side testing; and
- in-line units that provide conductivity measurements throughout each milking (e.g. units with conductivity electrodes implanted in the cluster and coupled to a computer).

Although the proportion of herds in Australia that take in-line conductivity measures at each milking is very low, it is likely that the use of this diagnostic system will increase in the future, especially in large herds.

There are several hand-held meters on the Australian market. It is important to assess foremilk at the start of the milking process (Woolford et al 1998), and testing this milk immediately after it is removed from the udder reduces variations in readings due to changes in temperature of the sample.

The accuracy of conductivity meters varies with the type of pathogen causing the mastitis and the duration and extent of tissue damage. There is substantial overlap between the conductivities of milk from normal and mastitic quarters and a lot of variation in the natural conductivity during milking for individual cows and between cows (Hillerton and Walton 1991, Milner et al 1996). The ability of meters to discriminate between normal and infected quarters depends on consistent collecting and testing techniques, the availability of base-line data for cows, and the method of interpretation of results.

To find a quarter with damaged tissue, it is best to compare between quarters in the same cow at the same time rather than look for a particular absolute conductivity level. (An assumption is made that the quarter with the lowest value has ‘normal’ tissue, which is not always the case.) Before interpreting the result of a hand-held device it is important to check whether the display reports electrical conductivity or electrical resistance – the two measures are inversely related with resistance decreasing as conductivity increases.

Selecting an optimal threshold to classify cows as infected can vary between herds and is influenced by the prevalence of mastitis in the herd and the relative cost of misdiagnosis (Mansell 1998, Sheldrake et al 1983). Even under ideal conditions conductivity meters are only considered as an aid to mastitis diagnosis.
pH of milk

Another simple diagnostic test based on changes in milk ion concentrations is pH measurement. In inflamed quarters, bicarbonate ions entering the milk from the bloodstream can cause increases in the pH (from a normal level of 6.6) to levels of 6.9 or higher and this is detectable by indicator dyes or conventional electrode procedures. In isolation, changes in pH are considered a poor test. Indicators have been combined with Rapid Mastitis Test reagents so that they demonstrate a colour change due to change in ion concentration as well as gel formation from elevations in somatic cell counts.

Key papers


Herd improvement organisations provide milk recording (herd testing), artificial breeding and herd management services in Australia.

These services are available throughout Australia from private companies and co-operatives. Twelve laboratories test milk samples for milk recording, and 16 herd improvement organisations provide advice, data analysis and reporting using one of five data processing systems (listed below).

Many of the companies (listed in the table on the following page) are members of the National Herd Improvement Association of Australia (NHIA).

Features of the five data processing systems in Australia

<table>
<thead>
<tr>
<th>Data processing systems</th>
<th>Marketed by</th>
<th>Companion products for use on farms¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>AusHerd</td>
<td>Victorian Dairy Industry Authority</td>
<td>AusStock, PCFarm</td>
</tr>
<tr>
<td>CHISWA</td>
<td>Joint venture between Consolidated Herd</td>
<td>CHISPC_DAIRY</td>
</tr>
<tr>
<td></td>
<td>Improvement Services Co-operative (CHIS) and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Herd Improvement Services of Western Australia</td>
<td></td>
</tr>
<tr>
<td>Dairy Herd Recording</td>
<td>Dairy Express</td>
<td>PCExpress</td>
</tr>
<tr>
<td>Scheme (DHRS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MISTRO</td>
<td>Maffra Herd Improvement Co-operative</td>
<td>MISTRO_FARM</td>
</tr>
<tr>
<td>WinHerd</td>
<td>Tasmanian Dairy Industry Authority (TDIA)</td>
<td>WinFarm, SortaCow²</td>
</tr>
</tbody>
</table>

¹ Most data can be transferred between systems by standard Data Interchange Formats (DIF files)
² SortaCow is used to organise sample bottles on test day and identify missing cows

Technote 23 lists advantages of using milk recording data in mastitis control programs.

Agriculture Victoria Ellinbank operates a commercial somatic cell count service that enables milk testing laboratories to assess the accuracy of their somatic cell counts.
Herd improvement organisations regularly forward their herd recording data to the Australian Dairy Herd Improvement Scheme (ADHIS) to generate state and national statistics and to assist farmers to benchmark their herd's performances. The statistics are published annually by National Herd Improvement Association of Australia.

The national statistics:
- include herds that have at least 30 cows;
- include cows that have a 305 day lactation for the fiscal year being reported, and those that completed a 305 day lactation from the previous test period;
- count cows once only for each season; and
- exclude cows from production figures for a variety of reasons such as when the interval between tests is greater than 150 days, heifers calve before 18 months of age, the first test is more than 100 days after calving, or the lactation length is less than 120 days.

**Key papers**

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**Sixteen dairy herd improvement services in Australia that provide milk recording statistics (NHIA 1999)**

<table>
<thead>
<tr>
<th>Centre</th>
<th>Location</th>
<th>Region serviced</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian Herd Recording Services</td>
<td>Kenilworth</td>
<td>New South Wales, Queensland</td>
<td>Kenilworth¹, Melbourne²</td>
</tr>
<tr>
<td>Bovine Inseminations</td>
<td>Tongala</td>
<td>Northern Victoria</td>
<td>Tongala¹, Melbourne²</td>
</tr>
<tr>
<td>Colac HI Co-op</td>
<td>Colac</td>
<td>Western Victoria</td>
<td>Colac¹, Melbourne²</td>
</tr>
<tr>
<td>Consolidated HI Services Co-op</td>
<td>Kyabram (eight sub-centres in NSW, Tasmania, Western Victoria)</td>
<td>Northern Victoria, New South Wales, Kyabram</td>
<td></td>
</tr>
<tr>
<td>Dairy Express</td>
<td>Armidale, Wacol</td>
<td>NSW, Queensland</td>
<td>Armidale, Wacol</td>
</tr>
<tr>
<td>Maffra Herd improvement Co-op</td>
<td>Maffra</td>
<td>Gippsland</td>
<td>Maffra</td>
</tr>
<tr>
<td>HI Services of SA Co-op (HISCOL)</td>
<td>Yankalilla</td>
<td>South Australia</td>
<td>Yankalilla</td>
</tr>
<tr>
<td>HI Service WA (HISWA)</td>
<td>Bunbury</td>
<td>Western Australia</td>
<td>Bunbury</td>
</tr>
<tr>
<td>Northern Herd Development Co-op</td>
<td>Cohuna</td>
<td>Northern Victoria, South Australia</td>
<td>Cohuna</td>
</tr>
<tr>
<td>South Gippsland HI Inc</td>
<td>Korumburra</td>
<td>Gippsland</td>
<td>Korumburra</td>
</tr>
<tr>
<td>Tasmanian Dairy Industry Authority</td>
<td>Hadspen</td>
<td>Tasmania</td>
<td>Hadspen</td>
</tr>
<tr>
<td>Timboon HI Co-op</td>
<td>Timboon</td>
<td>Western Victoria</td>
<td>Timboon</td>
</tr>
<tr>
<td>Victorian Herd Management Services</td>
<td>Leongatha (one sub-centre in Warragul)</td>
<td>Gippsland</td>
<td>Leongatha¹, Melbourne²</td>
</tr>
<tr>
<td>Western HI Co-op</td>
<td>Warrnambool (regional office at Terang)</td>
<td>South Australia, Tasmania, Western Victoria</td>
<td>Warrnambool Melbourne²</td>
</tr>
<tr>
<td>West Gippsland HI Co-op</td>
<td>Warragul</td>
<td>Gippsland</td>
<td>Warragul¹, Melbourne²</td>
</tr>
<tr>
<td>Yarram HTA Inc</td>
<td>Yarram</td>
<td>Gippsland</td>
<td>Yarram</td>
</tr>
</tbody>
</table>

¹ Site of weigh station  
² Site of milk analysis
Does post-milking teat disinfection with iodophors lead to iodine residues in milk?

Moderate levels of iodine in milk can make a significant and healthy contribution to the recommended daily dietary requirement of people. The recommended dietary allowance for iodine is 150 µg/day for adults, 70-120 µg/day for children, and 40-50 µg/day for infants (National Research Council 1980). In the United States, a nation of high consumption of dairy products, milk and dairy products contributed 56% of total food iodine intake for most age groups according to 1978 Food and Drug Authority survey data. Supplemental iodine in rations for dairy cows has been documented as the main contributing factor to high milk iodine levels in the United States.

Iodophor sanitisers and teat disinfectants also increase milk iodine levels. Their contribution depends on type and concentration, and milking practices. Iodophor post-milking teat disinfectants have consistently increased iodine in milk. The results of an Australian study is summarised in the table below. For those who would like a comprehensive review, see Galton et al (1986).

In a study of iodine concentrations in milk from individual quarters that were teat-dipped after milking, Sheldrake et al (1980) concluded that the bulk of iodine residue in milk came directly from unwashed teat skin. Although other researchers have concluded that the primary mode of increased iodine is absorption through the skin and entry into the milk by the milk synthesis process rather than by direct contamination from the teat skin (Conrad and Hemken 1978), it is evident that both pathways are important.

### Post-milking teat dips and iodine levels in individual quarters (Sheldrake et al 1980)

<table>
<thead>
<tr>
<th>Pre-milking preparation</th>
<th>Available iodine concentrations in milk (µg per quarter per milking)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial post-dip concentration of 0.1%</td>
<td>Initial post-dip concentration of 0.5%</td>
</tr>
<tr>
<td>Not washed or wiped</td>
<td>143</td>
<td>291</td>
</tr>
<tr>
<td>Thoroughly washed and dried</td>
<td>70</td>
<td>99</td>
</tr>
</tbody>
</table>
The Foods Standards Code specifies an iodine limit of 500 µg/L, and this is the maximum acceptable level used by the dairy industry. Surveillance by state dairy authorities has shown no problems occurring with normal use of iodophor, even when cows are strategically washed prior to milking. Hubble and Mein (1986) found that iodine residues in milk, on farms using iodophors for teat disinfection only and various washing techniques, averaged levels of 160 µg/L in summer-autumn and 110 µg/L in winter-spring.

Monitoring in recent years in Victoria and NSW found very few milk supplies to register milk iodine levels above the legal limit of 500 µg/L. In the few instances where elevated iodine occurred it was due to excessive dietary iodine supplementation of cows or inappropriate use of iodine-based milking machine sanitisers. Other circumstances that may increase iodine levels in milk include high iodine content of water (especially in drought conditions), and iodine from sanitisers that has adhered to rubber components of the milking machine.

Countdown Downunder recommends washing teats only if they are dirty. Minimising use of water on udders and teats is beneficial for teat skin health and also generally leads to better milk quality (coliform counts, sediment) unless very careful drying techniques are used. If it were necessary, iodine levels in milk could be reduced markedly by careful pre-milking teat preparation (washing and wiping of teats) when post-milking iodophor teat disinfectants were used.

If particular dairy farming areas do experience milk iodine levels that are unacceptably high at certain times of the year, alternatives to iodophor teat disinfectants could be chosen if farmers are not prepared to wash and wipe the teats of cows before milking.

Although iodine residues in milk can be reduced by lowering the concentration of available iodine in teat disinfectants, there is no point in post-milking teat disinfection if the product is too dilute to be effective.

**Key papers**


Pre-dipping teats means applying a quick-acting disinfectant just before milking to reduce the bacterial population on teat skin especially in the region of the external teat orifice. In the United States, it is common to use 0.1-0.5% iodophor as a pre-dip. The addition of an emollient is neither necessary nor desirable for pre-milking teat disinfection.

Pre-dipping is widely accepted as being most effective against environmental rather than contagious pathogens, reducing new environmental streptococcal infections by as much as 50% in some studies (Pankey et al, 1987, Smith and Hogan, 1997). However, at least one recent paper indicated that dipping teats both pre- and post-milking with a disinfectant containing chlorous acid and chlorine dioxide reduced Staph aureus infections by nearly 70% and Strep uberis infections by 65% when compared with post-dipping alone (Oliver et al, 1993).

Australian farmers could consider pre-dipping teats if they had high numbers (more than 5 per 100 cows per month) of clinical cases due to environmental bacteria (for example Strep uberis). Because no teat disinfectants are registered for use as pre-dips in Australia at present, no recommendations are given to farmers in the Countdown Downunder Farm Guidelines for Mastitis Control. Nevertheless, veterinarians could make recommendations for off-label use in appropriate situations provided that the local milk processor is aware of the advice given to one or more of their suppliers, and that people take precautions to minimise chemical residues in milk. A list of sanitisers approved for pre-dipping in the United States is published in the National Mastitis Council proceedings each year and updated regularly (National Mastitis Council, 1998).

The major concern is the potential for increased iodine residues in milk. Although pre-dipping with 0.5% or even 1% iodophor did not affect milk iodine residues as long as teats were wiped dry with a paper towel, residues increased significantly when teats were not wiped (Galton et al, 1986).
The ‘Iodine milk residues’ FAQ sheet discusses the impact of post-milking teat disinfection on iodine residues.

The general technique, as recommended in United States, is part of a pre-milking udder preparation routine that can be summarised as: ‘Strip, Dip, Dry and Apply’. That is:
- Strip: Wipe loose dirt from each teat and pre-strip foremilk;
- Dip: Pre-dip and wait for at least 15 sec, preferably 20-30 sec;
- Dry: Wipe each teat with a single-use paper towel;
- Apply: Apply teatcups 60-90 sec after the first touch of each udder.

An effective alternative method of pre-milking disinfection could suit typical Australian milking routines. It involves a commercially available, disposable paper towel pre-moistened with an ethanol/chlorhexidene disinfectant. The ‘dip’ and ‘dry’ in the list above can be combined when this product is used, provided that the operator takes care to wipe across each teat orifice with a clean section of the sanitary wipe.

Key papers


Features of pre- and post-milking teat disinfection (adapted from Blowey and Edmonson 1995)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Pre-dip disinfection</th>
<th>Post-dip disinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season of use</td>
<td>High-risk periods</td>
<td>Essential throughout the year</td>
</tr>
<tr>
<td>Speed of action</td>
<td>Must be rapid</td>
<td>Not important</td>
</tr>
<tr>
<td>Primary target</td>
<td>Environmental mastitis</td>
<td>Contagious mastitis</td>
</tr>
<tr>
<td>Effect on total plate count</td>
<td>Decreases if plate counts are high due to dirty teats</td>
<td>No effect unless Strep agalactiae is involved</td>
</tr>
<tr>
<td>Effect on milk residues</td>
<td>Teats must be wiped dry</td>
<td>May increase the potential for milk residues (although this is unlikely)</td>
</tr>
</tbody>
</table>
Pseudocowpox infection is common in cattle in Australia, although it causes few significant problems in herds. People contacting infected materials, including the teats, contaminated teatcups, udder cloths or suckling calves may develop localised lesions similar to those seen on the teats of cattle (i.e. ‘milkers nodules’).

Pseudocowpox is a virus of the paravaccinia group (closely related to ‘scabby mouth’ of sheep). Calves suckling teats of cows with pseudocowpox may develop stomatitis and muzzle lesions (bovine papular stomatitis) (Snowdon 1982).

Acute infection may be most apparent in young cows after calving or cows introduced to a herd that has the virus infection. Spread of infection through the herd can be relatively slow. Immunity to pseudocowpox is short lived, lasting four to six months, so infections can recur in individual animals and be a chronic problem in some herds.

In cattle, early acute lesions are localised, red, oedematous and painful. Affected animals resent being milked. Small, raised, circumscribed lesions (papules) may develop in a couple of days and form rough dark-red centres. In some cases a reside forms in the centre of the papule, although in contrast to bovine herpes mammillitis, this is rare with pseudocowpox. When the crusts fall away they leave a characteristic ring or ‘horseshoe’ shaped scab that joins with scabs of adjacent lesions. The lesions usually heal without scarring in 3-6 weeks if there is no interference. Healing can be protracted in milking cows if milking removes the scabs leaving reddened bleeding areas on the teats. Bacteria may infect lesions near the teat-end, enter the teat canal and cause mastitis.

Clinical diagnosis is based on the observation of characteristic lesions in or on the hands of people. The cause can be readily confirmed, and differentiated from herpes virus, by submitting biopsy samples for virus isolation.

There is no specific treatment. Spread of infection can be minimised by milking infected cattle at the end of the run, wearing gloves in the milking shed, and thoroughly washing teats, udders, hands and milking equipment.

Cowpox, which also causes teat lesions and is a member of the same family of viruses, is rarely recognised in Australia and it is likely that many of the early cases were actually pseudocowpox (Stevenson and Hughes 1980).

Key papers


Quality assurance programs increase consumer confidence in the quality and safety of dairy products. A common approach is to identify critical points during milk production on dairy farms that (1) may impact on milk quality and (2) can be controlled by farmers. Identification and control of key points during product manufacture is known as Hazard Analysis Critical Control Point (HACCP).

Dairy farmers who choose to participate in quality assurance programs must:
• identify relevant control points for milk production on their farms;
• design a flow diagram linking the critical activities that affect milk production;
• specify what they will do at each point;
• describe how their actions and results will be monitored;
• establish what will happen if a result falls outside the acceptable limit; and
• maintain records of key activities in sufficient detail to satisfy auditors.

Examples of activities identified as critical to the production of good quality raw milk are: animal identification systems, livestock sales and purchases, livestock transport, animal health and treatment, drug and chemical registers, mastitis control, water quality, stock feeds purchases, milking practices, milk cooling and storage, milking machine maintenance, staff training, cleaning and sanitation programs, environment and waste management, and record keeping.

For each activity the farmer states:
• the objective;
• what procedures will be implemented to ensure compliance; and
• what ‘quality tests’ will be used to demonstrate compliance.

Rather than have everyone start from scratch, in 1995 the Australian dairy industry developed a food safety and quality management program for farms known as Dairy First (Darmody 1998). Following a pilot trial of 80 dairy farms in Victoria and South Australia in 1997, a variety of these programs using components of Dairy First to varying degrees began to emerge. In May 1998 the Australian Dairy Industry Council, concerned by the plethora of programs, developed a nationally agreed set of required elements that were considered essential for any on-farm quality assurance program to demonstrate that appropriate care had been taken in the production of safe milk.
The programs have a good level of voluntary adoption by dairy farmers.

Some dairy companies pay incentives (e.g. an extra 0.5 cents/L) to accredited suppliers. In the future it is possible that farms will be required to have a quality assurance program in place for milk pick-up, payment of quality premiums, or access to vat rebate schemes. It is likely that quality assurance programs will become a benchmark.

**Mastitis control quality assurance**

The following is an example of a mastitis control component of a quality assurance program for dairy farms. It follows ‘best practice’ for mastitis control as recommended by Countdown Downunder.

This example assumes other elements essential to effective mastitis control (such as animal identification, good treatment records, staff training as described above) are covered in the relevant sections of the quality assurance program.

The Countdown Downunder Farm Guidelines for Mastitis Control is a detailed and comprehensive toolkit for farmers interested in milk quality relating to mastitis. For this reason, the action advised when mastitis control activities do not comply with the standard is to “seek professional advice”.

Information in the Farm Guidelines can also be used to identify the standards necessary for mastitis control for other schedules, such as milking machine maintenance, milking hygiene etc.

**Key papers**

### Example of a mastitis control schedule for a farm quality assurance program

#### Objective

All milk supplied to factory to have bulk milk cell counts less than 400,000 cells/mL.

#### Farm procedures

Follow the Countdown Downunder Farm Guidelines for Mastitis Control. Seek technical advice on milk quality and mastitis.

#### Schedule of control activities

<table>
<thead>
<tr>
<th>Critical control activity</th>
<th>Action required (Farm Guideline)</th>
<th>When to take action</th>
<th>Person responsible</th>
<th>Minimum standard</th>
<th>What to do if a problem occurs (non-compliance)</th>
<th>Records</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detect and treat cows with clinical mastitis</td>
<td>1, 4, 8, 10, 18, 19, Fact sheets A, B, E</td>
<td>Lactating and dry periods.</td>
<td>Milkers</td>
<td>Heifers: &lt; 3 cases in the last 50 calvings. First month of lactation: &lt; 5 cases/100 cows milking. Subsequent months: &lt;2 cases/100 cows milking.</td>
<td>Seek professional advice.</td>
<td>Stock treatment record and clinical case records.</td>
</tr>
<tr>
<td>Manage teat cracks and sores</td>
<td>9</td>
<td>In lactating cows.</td>
<td>Milkers</td>
<td>Improvement of teat condition within 3 weeks of observing problems.</td>
<td>Seek professional advice.</td>
<td>Teat scoring chart.</td>
</tr>
<tr>
<td>Management at drying-off to protect milk quality</td>
<td>16</td>
<td>Two weeks either side of drying-off</td>
<td>Manager</td>
<td>Do not milk cows yielding &lt; 5L per day.</td>
<td>Seek professional advice.</td>
<td>Herd recording data.</td>
</tr>
<tr>
<td>Implement Dry Cow Treatment strategy</td>
<td>14, 17, Fact sheets B, C</td>
<td>At drying-off</td>
<td>Manager</td>
<td>First month of lactation: 5 cases/100 cows milking. 70% of cows infected during lactation are cured by the following lactation.</td>
<td>Seek professional advice.</td>
<td>Stock treatment record.</td>
</tr>
<tr>
<td>Cull cows with persistent infection</td>
<td>15</td>
<td>At least annually.</td>
<td>Owner</td>
<td>No cows on the farm have been infected for more than two consecutive lactations despite receiving Dry Cow Treatment.</td>
<td>Seek professional advice.</td>
<td>Clinical case records and herd recording data.</td>
</tr>
<tr>
<td>Minimize spread from cows with mastitis</td>
<td>4, 5, 8, 11, 12 (if applicable)</td>
<td>Throughout lactation.</td>
<td>Milkers</td>
<td>No more than 1% of additional heifers should have a peak IC CC &gt;250,000 cells/mL each months. (In herds that do not herd record – an average BMCC during the past 6 months of &lt;250,000 cells/mL, and &lt;2 clinical cases/100 cows from day 30 to the end of lactation.)</td>
<td>Seek professional advice.</td>
<td>Reports from specialist IC CC analysis.</td>
</tr>
<tr>
<td>Monitor BMCC</td>
<td>11, (12 if applicable)</td>
<td>Each time results are received from the dairy company.</td>
<td>Manager</td>
<td>Average BMCC for the past 6 months of &lt;250,000 cells/mL.</td>
<td>Seek professional advice.</td>
<td>Company reports (filed). Graph of BMCC in herd book.</td>
</tr>
<tr>
<td>Practice biosecurity when purchasing stock</td>
<td>21</td>
<td>Whenever stock are purchased.</td>
<td>Manager</td>
<td>No purchased cows with IC CC &gt; 250,000 cells/mL. No purchased from herds where average BMCC in the past 6 months exceeds 200,000 cells/mL.</td>
<td>Seek professional advice.</td>
<td>Written information from vendors (filed).</td>
</tr>
</tbody>
</table>

This example assumes other schedules cover animal identification systems, animal health and treatment, drug and chemical registers, water quality, milking practices, milk cooling and storage, milking machine maintenance, staff training, cleaning and sanitation programs, environment and waste management, and record keeping.
Lesions due to photosensitisation are largely confined to non-pigmented areas of skin exposed to sunlight and may therefore be evident on the outer surfaces of light coloured teats of affected cows.

Photosensitisation usually occurs when photodynamic agents are retained in the bloodstream rather than being excreted at normal rates in the bile. The most common agent is a breakdown product of chlorophyll called phylloerythrin. Phylloerythrin circulates through the blood and interacts with ultraviolet light in poorly pigmented skin. Cows can be affected by photosensitisation when grazing lush spring pastures due to a high chlorophyll intake. Events that disrupt liver function or cause bile stasis may also trigger an episode. Examples of conditions where photosensitisation is secondary to liver damage include lantana poisoning and facial eczema.

Cows with early photosensitisation of the teats may be restless and kick at their abdomens (because the affected areas are very itchy). Affected skin becomes red and oedematous but changes may not be noticed until the top layers of skin die and become hard, dry and leathery, or sheets of dead skin flake off.

Diagnosis is based on clinical presentation, especially the type and distribution of lesions. Veterinarians may take blood samples to check the liver enzymes and determine whether there is on-going liver damage. Although photosensitisation is usually associated with liver dysfunction, liver enzymes may not be elevated even in cows with severe photosensitive dermatitis because:

- high levels of circulating phylloerythrin can precede liver damage; and
- not all liver dysfunction manifests as overt changes in liver tissue or liver enzymes.

The incidence of photosensitisation may be higher in cows induced to calve with corticosteroids. Sometimes the dermatitis is so severe that milking is virtually impossible.
Teat photosensitisation

Management of cows with photosensitisation generally involves:
- ensuring that the cows have access to shade at all times, and possibly housing severely affected animals in shed;
- administering anti-histamines and anti-inflammatory drugs (e.g. flunixin) in early cases;
- treating teat lesions as indicated – some lesions may become infected and require antibiotic treatment;
- applying black photosensitisation ointment to teats and exposed parts of the udder;
- preventing new cases – by changing the diet if the feed is thought to be contributing to the problem, or possibly applying teat ointment to darken light-coloured skin. In districts where facial eczema is likely to occur, specific measures to reduce the incidence of facial eczema should be discussed with a veterinarian;
- further investigation – if the cause of an outbreak is unclear.
How do teat sealants protect cows during the dry period?

Teatseal (Bimeda (NZ) Ltd) is a commercial product that is infused into each quarter at drying-off to physically block the teat canal and prevent infections entering the udder. It is a non-antibiotic approach to protecting uninfected quarters during the dry period.

The product is registered in New Zealand and may become available on the Australian market in future.

The concept of using a physical barrier at the teat end originated from observations that the teat canal ‘closed’ at drying-off through the formation of a keratin plug. The initial research for a suitable artificial teat sealant began in Ireland in the 1970s and was modified in New Zealand in the 1990s. The substances used commercially are inert, have the consistency of plasticine, and do not harden or set. After infusion, teat sealants remain in the teat sinus and canal until they are removed by suckling calves or by manually stripping the quarter.

Teatseal is composed largely of bismuth subnitrate in a liquid paraffin base (Woolford et al 1998). It is infused into the udder from individual dose plastets using a similar mode of administration as for Dry Cow Treatments. The compound remains lodged in the lower teat for at least 3-4 weeks after drying-off, and some dispersion eventually occurs in dry cow secretions in the udder. Residual levels of bismuth in bulk milk have been reported to be 10 parts per million at the first milking, declining to less than 1 parts per million within five days.

Confidence – Low
Teat sealants may be useful tools but there is no local experience of their application in Australian dairy herds.

Research priority – Moderate
Information about the use of teat sealants on commercial farms in Ireland (where the formulation contains antibiotic) and in New Zealand (no antibiotic) would be useful.

<p>| Intramammary infections in 528 cows treated with Teatseal (adapted from Woolford et al 1998) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Time of infection</th>
<th>Teatseal</th>
<th>Teatseal + Dry Cow Treatment</th>
<th>Dry Cow Treatment</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>During the dry period</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
<td>3.4</td>
</tr>
<tr>
<td>At calving</td>
<td>2.3</td>
<td>1.5</td>
<td>2.3</td>
<td>12.7</td>
</tr>
<tr>
<td>Total</td>
<td>2.5</td>
<td>1.9</td>
<td>2.7</td>
<td>16.1</td>
</tr>
</tbody>
</table>

a. Quarters that developed clinical mastitis.
b. Quarters that were milk culture positive (of which 56% of infections were Strep uberis).
c. Untreated quarters had significantly more new infections than other treatments (P<0.01).
In contrast to the parent product developed in Ireland in 1977 (Osmonds’ TeatSeal, Cross-Vetpharm Group), the formulation evaluated in New Zealand is more viscous and does not contain antibiotics.

Studies in New Zealand found that Teatseal performed similarly to Dry Cow Treatments and that there was no advantage in including antibiotics with the Teatseal treatment (Woolford et al. 1998).

Only cows without mastitis should be treated with Teatseal, as Teatseal contains no antibiotic and will not cure infected quarters. The absence of antibiotic in the formulation also means that special care should be taken to ensure sterile administration of Teatseal. Introduction of bacteria into the mammary gland at the time of the infusion due to poor technique can have severe consequences.

**Key papers**

Bovine papilloma viruses cause teat warts. Six separate virus strains have been identified that differ in appearance and cause warts in different anatomical areas (Radostits et al 1994). On teats, different strains cause ‘rice grain’ flat white warts (BVP-5), frond-like papillomas that protrude in a ragged fringe of up to one centimetre in length (BVP-6) and fibropapillomas that protrude from the teat surface (BVP-1).

In general, young animals are very susceptible to papilloma viruses, and usually build up immunity (from apparent or inapparent infection) before they enter the milking herd. In older cattle, papillomas are usually confined to the udder and teat and tend to increase in frequency with age.

Spread is from one animal to another, with virus usually entering through skin abrasions. Teatcup inflations and milkers’ hands help transfer the virus from one cow to the next. The live virus is relatively robust, and will remain fully viable at room temperature for over three weeks.

Warts can interfere with the function of the inflations and can, in some cases, block the teat canal. If they become damaged, they can serve as home for a number of mastitis pathogens (particularly Staph aureus and Strep dysgalactiae). They can also make it difficult to keep the teat clean.

Most warts are self-limiting and disappear within 5-6 months. The frond type can be physically removed. If there is a major problem in a herd, an autogenous vaccine can be made from wart tissue from cows in the herd. Type-specificity is high, so vaccines must include all serotypes and tissue types responsible for the outbreak. The response of the low, flat warts to vaccination is relatively poor.

Iodine teat disinfectants with emollients are recommended to keep teat skin healthy and keep damaged warts clean.

Key papers


What do teat warts look like and how are they treated?
What tests, apart from bulk milk cell counts, are useful in assessing milk quality?

A number of tests are conducted on milk samples from farm vats. Vat tests that relate specifically to mastitis are bulk milk cell counts and bulk-tank milk cultures. Other tests, such as a total plate count or Bactoscan, are primarily related to milk quality issues.

**Bulk-tank milk cultures**

Bulk-tank milk cultures may help identify organisms present in the vat and provide information on the cleanliness of milk harvesting techniques and equipment, and the adequacy of milk cooling. Dairy advisers sometimes submit vat samples to laboratories as part of mastitis investigations.

To obtain samples that are representative, the vat must be well mixed by turning on the agitator 10 minutes before sampling and samples taken from both the top and bottom of the tank (Mackie 1997). The methods used to collect and store samples are critical to prevent overgrowth with micro-organisms, and appropriate arrangements must be made to ensure samples are maintained at refrigeration temperatures. It is not advisable to make diagnostic decisions based on a single test — a series of at least three is recommended.

The procedures for culture of vat samples for bacteria originating from within the udder has been developed and refined in the United States over the past 15-20 years (Guterbock and Blackmer 1984), but few formal analyses of its usefulness as a diagnostic test for mastitis have been conducted. Vat culture is reported to have a low sensitivity for the major mastitis pathogens. Although herds infected with Strep agalactiae are expected to have high numbers of bacteria in milk, Godkin and Leslie (1990) found they were only detected reliably by repeated cultures. This experience has also been observed in the field in Australia.

Further studies are required to identify methods to increase the sensitivity of this screening test, including establishing appropriate sampling regimens on farm, and selective media and inocula sizes in laboratories. Although it is tempting to use bulk-tank milk to identify potential pathogens, isolated organisms do not necessarily originate from mastitic cows. For example Strep uberis is ubiquitous in the environment and able to multiply in raw milk cooled below 10°C, so isolation from the vat does not necessarily indicate infected quarters. More direct links between the bacteria isolated from vat milk samples and the cause of mastitis can be established by sampling individual cows.

**Technote 4.3 discusses milk cultures from individual cows.**
Total plate count

Total plate counts provide accurate counts of bacteria in vat milk. The presence of bacteria is established by incubating a diluted sample of vat milk on agar plates (with special growth media) for 72 hours at 30°C. The bacterial that grow are counted and the total number in the original sample is estimated according to the dilution factor used.

Counts may be high if:
- there are problems with washing equipment or refrigeration of the milk; and
- wet or dirty cows are milked, with bacteria from the cows’ skin and hair washing into the milk.

Although mastitis can cause an increase in total plate counts, this situation is not common. High total plate counts are occasionally seen in herds with Strep agalactiae infections.

Bactoscan

The Bactoscan has largely replaced total plate counts because the number of living bacteria in a milk sample can be estimated in five minutes (rather than the three days required for cultures). The Bactoscan machine counts bacteria with the aid of a fluorescent dye. These machines are subject to regular documented calibrations to ensure their accuracy.

Thermoduric count

Thermoduric counts are the number of bacteria per millilitre of milk that survive laboratory heat treatment. High thermoduric counts indicate poor equipment cleaning and sanitation problems.

Milk samples are held at 62.8°C for 30 minutes, cooled for 10 minutes, then incubated for 72 hours at 30°C. The equivalent diagnostic test in the United States is the Laboratory Pasteurised Count (LPC), where samples are incubated for 48 hours at slightly higher temperature (32°C).
Using multiple tests to troubleshoot milk quality problems with high bacterial counts

High bacterial counts can arise from organisms passed in the milk or from bacteria contaminating equipment. Problems of milk quality due to high bacterial counts can be investigated using a combination of tests such as total plate counts, thermoduric counts and coliform counts (where coliforms specifically are estimated). The approach is to take milk samples from the bulk tank at different times during milking and from different locations in the milking plant. This helps to differentiate high bacterial counts arising from problems of pre-milking hygiene, equipment cleaning and sanitation, and incubation of bacteria in the milk handling system from mastitis pathogens (Reinemann et al 1997).

Guide to troubleshooting milk quality (Mein 1999)

<table>
<thead>
<tr>
<th>Test</th>
<th>Examples of milk quality categories</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMCC (cells/mL)</td>
<td>Excellent: &lt;200,000; Adequate: 200,000 – 400,000; Warning or penalty: &gt;400,000</td>
<td>Mastitis may be a cause of high bacteria counts if both the BMCC and Bactoscan readings/ total plate counts are high.</td>
</tr>
<tr>
<td>Bactoscan band (estimated bacteria/mL)</td>
<td>&lt;80,000 – ≥80,000</td>
<td></td>
</tr>
<tr>
<td>Total plate count (colony forming units/mL)</td>
<td>&lt;10,000 – 10,000 – 20,000 – &gt;20,000</td>
<td>Elevated thermoduric counts generally result from equipment cleaning and sanitation problems.</td>
</tr>
<tr>
<td>Thermoduric (count/mL)</td>
<td>&lt;1,500 – 1,500 – 3,000 – &gt;3,000</td>
<td>Medium elevations in coliform counts generally result from inadequate milking hygiene (cups on wet teats, manure on cups, etc). High elevations often result from incubation in the milking system during long milking shifts (e.g. bacterial growth on milk filters).</td>
</tr>
<tr>
<td>Coliform (count/mL)</td>
<td>&lt;100 – (low) – 100 – 500 – (medium) – &gt;1,000 – (high)</td>
<td></td>
</tr>
</tbody>
</table>

Key papers


Vat milk tests
An Index to the contents of the Countdown Downunder Technotes and associated FAQs can be found between the Introduction section and Technote 1.