Detection of Mastitis
Changes in milk composition
Table 1. Changes in milk composition associated with mastitis.

<table>
<thead>
<tr>
<th></th>
<th>Normal milk</th>
<th>Mastitic milk</th>
<th>% of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid-not-fat</td>
<td>8.98</td>
<td>8.8</td>
<td>99</td>
</tr>
<tr>
<td>Fat</td>
<td>35.0</td>
<td>3.20</td>
<td>91</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.90</td>
<td>4.40</td>
<td>90</td>
</tr>
<tr>
<td>Total protein</td>
<td>3.61</td>
<td>3.56</td>
<td>99</td>
</tr>
<tr>
<td>Total casein</td>
<td>2.8</td>
<td>2.30</td>
<td>82</td>
</tr>
<tr>
<td>Whey protein</td>
<td>0.8</td>
<td>1.30</td>
<td>162</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.02</td>
<td>0.07</td>
<td>350</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.02</td>
<td>0.10</td>
<td>500</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>0.10</td>
<td>0.60</td>
<td>600</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.06</td>
<td>0.105</td>
<td>184</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.09</td>
<td>0.147</td>
<td>161</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.17</td>
<td>0.157</td>
<td>91</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.12</td>
<td>0.04</td>
<td>33</td>
</tr>
</tbody>
</table>

Changes in milk composition
Physical examination

- Signs of inflammation
- Empty udder
- Differences in firmness
- Unbalanced quarters
Taste Test

• 60% of salty quarter milk have clinical alterations

• Normal milk (0.08-0.12% NaCl)

• Mastitic milk (over 1.4% NaCl)
Mastitic milk
The Stripcup Test

• The stripcup or strip plate is for determining the presence of clinical mastitis.

• Few streams of the foremilk are squirted onto the strip cup and are visually examined for milk abnormalities.
Strip cup
Sub-clinical and clinical mastitis

- Clinical cases: 1
- Subclinical infections: 20-40
- Uninfected cows: ????
Mastitis Diagnostic Tests

• Reduced synthetic ability of mammary gland
  —Lactose determination

• Disease-combating response of animal
  —Somatic cell counting
Mastitis Diagnostic Tests

• Tissue damage and blood capillary permeability
  – Bovine serum albumin
  – Na, K, Cl
  – Enzymes
Somatic cell counting

• Direct monitoring
  — Direct microscopic counting
  — Automated machine counting

• Fluoro-opto-electronic cell counting
• Particle size analysis
Enzymes

• Catalase test

• NAG-ase determination (N-acetyl-β-D-glucosaminidase)

• Antitrypsin capacity test
Direct microscopic somatic cell count (Breed’s method)
DMSCC

• Levowitz-Weber stain reagent (Modified Newmann-Lampert stain)

• Preparing the milk slide

• Staining

• Determining MF, counting and calculation
FOECC

- Sample, sampling
- Preservatives
- Principle
SOMACOUNT FLUID FLOW DIAGRAM

To Waste

Valve #3

Syringe Pump #2

Valve #2

Valve #1

Syringe Pump #1

Flow Cell Block Assy

Figure Fluid Flow

Dye Intake

FOECC
Figure: Flow Cell
Whiteside Test

- Modified Whiteside test
- Field Whiteside test
Modified Whiteside Test (MWT)

- Performing the test
- Interpretation
- Principle
NaOH

Nucleic acid

Sodium salt (gelatinous mass)

Viscid mass formation

Characteristic precipitate

Phase II reaction (fat)

Serum solid and fat adsorbed

Breakdown into white flakes and shreds in translucent fluid background

Phase I reaction (cells)
MWT ± (Milky, Fine particles)
MWT 1+
(milky, less opaque, coagulated particles)
MWT 2+ (whey-like, large coagulated particles, gel)
MWT 3+
(very watery, large coagulated particles, gel)
MWT 4+

(a tenacious coagulum)
The modified Whiteside test on bucket milk of a small herd of 21 cows.
- Field Whiteside Test

- 10ml milk

- 2ml of 4% NaOH solution with 0.0025% cresol red (w/v)
California Mastitis Test (CMT)
Advantages of the CMT

* CMT is fairly accurate in measuring the somatic cell concentration in milk.

* CMT correlates well with other tests

* CMT is sensitive, inexpensive, and simple.

* The CMT Paddle is easy to clean up – simply rinse with water.
Advantages of the CMT

- The CMT is fairly accurate in measuring somatic cell concentration in milk, correlating well with other tests.
- It is sensitive.
- Foreign material, such as hair or other matter, does not interfere with the test.
• It is inexpensive.

• The test is simple, and little equipment is needed.

• Easy clean-up after each test--simply rinse with water.
• Environmental temperature changes have little effect on the CMT as long as the milk has been refrigerated and is not over two days old.
Disadvantages of the CMT

- Scoring the test may vary between individual testers. It is necessary to be as consistent as possible to insure uniform results.

- Scores represent a range of leucocyte content rather than an exact count.
False positive reactions occur frequently on cows that have been fresh less than 10 days, or on cows that are nearly dry. These animals should be tested closer to the middle of the lactation.
• Occasionally, acute clinical mastitis milk will not score positive due to the destruction of leucocytes by toxins from the infecting organism.
Procedure

Step 1: Take about 1 teaspoon (2 cc) milk from each quarter.

This is the amount of milk that would be left in the cups if the CMT Paddle were held nearly vertical.
Procedure

Step 2: Add an equal amount of CMT solution to each cup in the paddle.
Procedure

Step 3: Rotate the CMT Paddle in a circular motion to thoroughly mix the contents.

Do not mix more than 10 seconds.
Procedure

Step 4: “Read” the test quickly.

Visible reaction disintegrates after about 20 seconds.

The reaction is visually scored.

The more gel formation, the higher the score.
HOW THE CMT WORKS

- CMT reagent is a detergent with a pH indicator added (reason for purplish color). When milk and CMT reagent are mixed in equal amounts.
• CMT reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leucocyte, which are primarily fat (detergent dissolves fat).

• DNA is now released from the nuclei.

• DNA will string or gel together to form a stringy mass.
• As the number of leucocytes increase in a quarter, the amount of gel formation will increase in a linear fashion.
Cells

CMT reagent

Cells ruptured And DNA release

Gel

pH ≥ 7

DNA-ase
Interpretation of CMT Scores

CMT scores are directly related to average somatic cell counts.

Any reaction of T (trace) or higher indicates that the quarter has subclinical mastitis.
CMT Score Somatic Cell Range Interpretation

N (Negative): 0 – 200,000 Healthy Quarter
T (Trace): 200,000 – 400,000 Subclinical Mastitis
1: 400,000 – 1,200,000 Subclinical Mastitis
2: 1,200,000 – 5,000,000 Serious Mastitis Infection
3: Over 5,000,000 Serious Mastitis Infection
• N - (negative=100,000 SCC)
• T - (trace=300,000 SCC)
• 1 - (900,000 SCC)
• 2 - (2.7 million SCC)
• 3 - (8.1 million SCC).
• Readings of 1, 2 and 3 are definite *positives*. The quarter(s) is infected.

• A trace (T)reading indicates a possible infection.
• If all 4 quarters read “trace”, there is no infection.
• If one to two quarters read “trace”, infection is possible.
• A negative (N) reading indicates no infection.
A simple method of scoring

• Negative (N), Suspect (S) and Positive (P).

• **Negative** corresponds to 0 on the traditional system.

• **Suspect** corresponds to *Trace and 1*.

• **Positive** corresponds to Scores of 2 *and* 3 on the traditional method.
• Factors other than infection that tend to increase leukocyte numbers and to increase CMT reactions:
• Positive reaction one to two weeks following treatment.

• Very early (colostrum) and late lactation.

• Teat end injury.
• Fluctuating and irregular milking vacuum.
• Injury to the udder.
• Periods of estrus.
• Abnormal health of cow such as foot rot or uterine infection.
WHEN TO RUN A CMT

• The CMT should be run on foremilk. Foremilk contains the fewest leucocytes.

• If the CMT is positive on foremilk, you can be assured that the rest of the milk from that quarter will be higher.
• Strippings will have a SCC that is 2 to 3 times higher than foremilk because leucocytes tend to stay with milk fat.
• The milk fat content of foremilk is approximately 1% in Holsteins while strippings will be 10% to 13% fat.
• Dirt, manure and other particles do not interfere with the CMT reading; because there is no DNA.
California mastitis test (CMT)
<table>
<thead>
<tr>
<th>ออนไลน์</th>
<th>数</th>
<th>จำนวน SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>น้อยกว่า 10 มม.</td>
<td>น้อยกว่า 500,000</td>
<td></td>
</tr>
<tr>
<td>10-20 มม.</td>
<td>500,000-900,000</td>
<td></td>
</tr>
<tr>
<td>มากกว่า 20 มม.</td>
<td>มากกว่า 1 ล้าน</td>
<td></td>
</tr>
</tbody>
</table>
Brabant mastitis test (BMT)

<table>
<thead>
<tr>
<th>อัตราการไหล</th>
<th>ค่าเฉลี่ย SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>น้อยกว่า 5 วันที่</td>
<td>250,000</td>
</tr>
<tr>
<td>5-10 วันที่</td>
<td>800,000</td>
</tr>
<tr>
<td>มากกว่า 10 วันที่</td>
<td></td>
</tr>
<tr>
<td>มากกว่า 60 วันที่</td>
<td>สูงมาก</td>
</tr>
</tbody>
</table>
FIG. Catalase test as conducted in Smith fermentation tubes showing from zero to 40% gas.
Rapid Field Catalase Test

• 5-6 drops of milk on a glass over a dark background

• 2-3 drops of 9% fresh cool H2O2 solution

• Bubbles within a few minutes after mixing…….POSITIVE (better use hand lens)
Electrical conductivity test (EC)

Illustration and Function
各部の名称と機能

- Temperature Compensator 温度センサー
- Small Electrode 電極
- Sampling Cup 測定槽
- Digital Display 表示部
- Push Button 電源スイッチ
- Battery Box 電池ボックス
- Calibration 調整
### Illustration and Function
各部の名称と機能

- **Temperature Compensator**
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  電池ボックス
- **Calibration**
  調整

### Specification

<table>
<thead>
<tr>
<th>Measuring Method</th>
<th>Measurement of electrical conductivity</th>
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<tbody>
<tr>
<td>表示</td>
<td>3桁ディジタル</td>
</tr>
<tr>
<td>測定範囲</td>
<td>0−13.0 mS/cm</td>
</tr>
<tr>
<td>精度</td>
<td>3% ± 1 digit</td>
</tr>
<tr>
<td>自動温度補償</td>
<td>3〜40 C (convertd at 25 C) (25℃補正)</td>
</tr>
<tr>
<td>電源</td>
<td>Dry cell AA (40 hours) 乾電池 (単3×2本)</td>
</tr>
<tr>
<td>消費電力</td>
<td>0.58 mA</td>
</tr>
<tr>
<td>本体寸法</td>
<td>75(W) × 40(H) × 165(D)</td>
</tr>
<tr>
<td>重量</td>
<td>Ca. 200 g</td>
</tr>
<tr>
<td>Electrical Conductivity （電気伝導度）</td>
<td>Infected 乳房炎</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Absolute Conductivity (In Japan) （伝導度絶対値）</td>
<td>&gt; 6.2 mS/cm</td>
</tr>
<tr>
<td>Differencial Conductivity (In Japan) （分房間差値）</td>
<td>&gt; 0.5 mS/cm</td>
</tr>
</tbody>
</table>
EC used in New Zealand condition

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected qtr</td>
<td>ECR &lt; 1.15</td>
</tr>
</tbody>
</table>
| Infected qtr   | EC $\geq 7000 \ \mu S$
|                | Or ECR $\geq 1.15$ |
Bromthymol blue
pH indicator paper
pH 6.6-6.7 pale-green
pH 6.8 moderate green
pH 7.1 green
pH 7.4 dark blue-green
NAG-ase

• N-acetyl-\(\beta\)-D-glucosaminidase

• A lysosomal enzyme in cells

• As an indicator for severity or degrees of cell damage
Milk antitrypsin capacity

• $\alpha_1$-proteinase inhibitor ($\alpha_1$-antitrypsin) derived from blood

• As an indicator for serum leakage into the milk
End of this topic