THE INVENTION OF PLASTIC PLATE FOR THE SEMIQUANTITATIVE DETECTION OF SOMATIC CELLS IN FARM BULK TANK MILK

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การนับจำนวนเซลล์โซมาติกในน้ำนมถังรวมของฟาร์มเป็นตัวชี้วัดคุณภาพน้ำนมและเต้านมอักเสบแบบไม่แสดงอาการในฟาร์มโคนม หลายประเทศใช้การตรวจนับจำนวนเซลล์โซมาติกเป็นเกณฑ์คัดกรองคุณภาพและกำหนดราคาน้ำนม ประเทศไทยกำหนดน้ำนมถังรวมของฟาร์มที่ระดับ 500,000 เซลล์ต่อมิลลิลิตร และคัดกรองน้ำนมดิบคุณภาพเยี่ยม (premium grade milk) ที่จำนวนเซลล์โซมาติกน้อยกว่า 100,000 เซลล์ต่อมิลลิลิตร ศูนย์รวบรวมน้ำนมดิบส่วนมากใช้การตรวจซีเอ็มที (California Mastitis Test) ในการตรวจคัดกรองคุณภาพน้ำนมดิบ แต่การตรวจมีข้อความผิดพลาดและความแปรรวนสูง การศึกษานี้มีวัตถุประสงค์เพื่อประดิษฐ์แผ่นพลาสติกสำหรับการตรวจนับจำนวนเซลล์โซมาติกแบบกึ่งปริมาณสำหรับการใช้งาน ณ ศูนย์รวบรวมน้ำนมดิบ และประเมินประสิทธิภาพแผ่นพลาสติกสำหรับตรวจนับจำนวนเซลล์โซมาติกแบบกึ่งปริมาณโดยเปรียบเทียบกับเครื่องตรวจนับจำนวนเซลล์โซมาติก (Fossomatic™ FC) และการตรวจซีเอ็มที ผลการศึกษาพบว่าการใช้แผ่นพลาสติกร่วมกับน้ำยาจำเพาะ เมื่อผสมกับตัวอย่างน้ำนมถังรวมของฟาร์มสามารถตรวจนับจำนวนเซลล์โซมาติกแบบกึ่งปริมาณได้ โดยกำหนดให้อ่านคะแนนจำแนกเป็น 0,1,2 และ 3 สอดคล้องตามปริมาณจำนวนเซลล์โซมาติกในน้ำนม วิธีการนี้มีความสอดคล้องไปในทิศทางเดียวกันกับเครื่อง Fossomatic™ FC ($r=0.80$, $p<0.05$) และการตรวจด้วยซีเอ็มที ($r=0.94$, $p<0.05$) ในระดับห้องปฏิบัติการ การทดสอบกับตัวอย่างน้ำนมถังรวมของฟาร์ม ณ ศูนย์รวบรวมน้ำนมดิบ พบว่ามีความสอดคล้องไปในทิศทางเดียวกันกับเครื่อง Fossomatic™ FC ($r=0.85$, $p<0.05$) โดยมีความถูกต้องของการทดสอบเท่ากับ 96.41% (95%CI = 93.66% – 98.19%) ที่ระดับจำนวนเซลล์โซมาติกน้อยกว่า 100,000 เซลล์ต่อมิลลิลิตรและเท่ากับ 91.18% (95%CI = 87.42% – 94.10%) ที่ระดับจำนวนเซลล์โซมาติกมากกว่าหรือเท่ากับ 500,000 เซลล์ต่อมิลลิลิตร

สาขาวิชา อายุรศาสตร์สัตวแพทย์ ลายมือชื่อนิสิต .....

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Piyarat Jiarpinitnun : THE INVENTION OF PLASTIC PLATE FOR THE SEMIQUANTITATIVE DETECTION OF SOMATIC CELLS IN FARM BULK TANK MILK. Advisor: Assoc. Prof. Dr. KITTISAK AJARIYAKHAJORN, D.V.M., M.Sc., Ph.D. Co-advisor: Asst. Prof. Dr. CHAIDATE INCHAISRI, D.V.M., M.Sc., Ph.D.

Bulk tank milk somatic cell count (BTM SCC) is worldwide acceptable as an indicator of subclinical mastitis and milk quality at the farm level. Several countries determine the BTM SCC level for quality control and milk pricing. In Thailand, BTM SCC standard is set at 500,000 cells/ml and premium milk quality is set at less than 100,000 cells/ml. Milk collecting centers (MCCs) use CMT (California Mastitis Test) for screening raw milk quality. However, CMT has an error and high variation. This study aims to invent a new semiquantitative device for determining BTM SCC and to validate the device in comparison with Fossomatic™ FC and CMT. The results showed that the use of a specifically designed plastic plate with a mixture of specific chemical reagents and BTM is able to discriminate a semiquantitative BTM SCC according to reading scores 0,1,2 and 3. The reading scores is significantly correlated with Fossomatic™ FC (r=0.80, p<0.05) and CMT (r=0.94, p<0.05) at the laboratory testing. At MCC, this new semiquantitative device is able to determine BTM SCC in which the test reading is correlated with the Fossomatic™ FC (r=0.85, p<0.05). The accuracies of the test device are equal to 91.18% (95%CI = 93.66% – 98.19%) at SCC level less than 100,000 cells/ml and equal to 96.41% (95%CI = 87.42% – 94.10%) at SCC level more than or equal to 500,000 cells/ml.

Field of Study:	Veterinary Medicine	Student's Signature .....
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CHAPTER 1

INTRODUCTION

1. Importance and Rationale

Most of the Thai dairy farms are smallholders. The majority of dairy farms size ranges 21 to 50 cows (Figure 1) (Information and Communication Technology center, 2018). The bucket type milking system is generally practiced for raw milk collecting in smallholder farms of Thailand. The farm bulk tank milk (BTM) is sent to the nearest milk collecting center (MCC) for storing and distributing to the dairy processing factories. The good quality dairy products depend on quality raw milk from MCC. Thai MCC is an essential operation for raw milk quality monitoring and controlling. Thai MCC determines BTM including physical appearance, milk composition, bacterial count, antibiotic residuals and somatic cells (National Bureau of Agricultural Commodity and Food Standards, 2010).

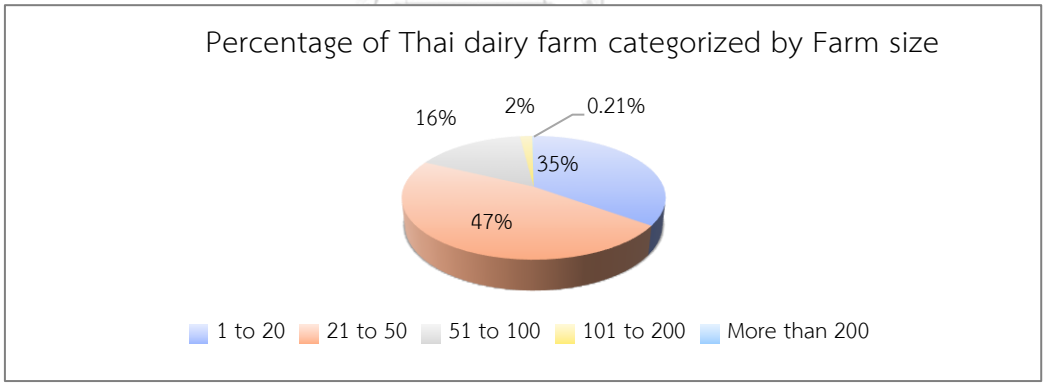


Figure 1 Percentage of Thai dairy farm categorized by farm size according to data of Thai Information and Communication Technology center (2018)

Mastitis is an inflammation of the mammary gland which is affecting the whole chain of dairy products (Khan and Khan, 2006; Hagnestam-Nielsen and Østergaard, 2009; Sharma et al., 2011). Mastitis can changes milk quantity and milk composition (Ogola et al., 2007), affect dairy product processing (Barbano et al., 1991; Auldist and Hubble, 1998; Khan and Khan, 2006; Fernandes et al., 2007). The

pathogenesis has two processes including intramammary infection and mammary gland inflammation. Mastitis is generally classified into two forms. First, clinical mastitis is the udder inflammation with noticeable signs including abnormal milk appearance, pain, redness, swelling and loss of mammary gland function. Moreover, systemic signs such as fever, shock, and sudden death can be found in some cases. The second form of mastitis is subclinical mastitis in which clinical signs are not observed, in particular abnormal milk appearance. Farmers have difficult to observe the subclinical form. Subclinical mastitis affects both milk quantity and quality. Subclinical mastitis occurs more than clinical mastitis in which causes high economic loss (Seegers et al., 2003b). Early detection of subclinical mastitis can reduce economic losses and shorten recovery periods (Janzen, 1970; Halasa et al., 2007).

Somatic cells compose of leukocytes and epithelium cells. Somatic cell count (SCC) is the indicator of intramammary inflammation. The SCC method is divided into direct and indirect count. SCC is worldwide acceptable as the single indicator for milk quality control because SCC reflects the health status of the mammary gland and the risk of non-physiological changes to milk productions (Pyörälä, 2003; Hamann, 2005). Direct somatic cell count is the standard method in which microscopic count or slide count is a conventional standard method for milk SCC. Nowadays, the Fossomatic™ FC machine which counts somatic cells based on recognition of nucleus of somatic cells by using fluorescent and flow cytometry technique. This method is acceptable as a direct microscopic counting for bovine milk somatic cells. This machine is valid with high sensitivity and specificity because the design of the flow cell ensures that only one single somatic cell can pass through and detected at a time. However, this machine is very expensive, time consume with dairy calibration and professional skill required. The indirect count is the estimation of SCC by measuring the appearance of reaction caused by milk somatic cells and specific reagents such as Wisconsin Mastitis Test, White Side Test and California Mastitis Test (CMT). CMT is a cow-level indirect somatic cell determination in order to detect subclinical mastitis. Thai MCCs use CMT for milk quality grading and pricing despite the slide count and Fossomatic™ FC machine because of cost concern and personal skill. However, a study found CMT is not

suitable for milk quality and milk grading that requires a more precise SCC level because CMT has variation among scores, readers and reagent (Read et al., 1969). Therefore, Thai MCCs require a new method that is practical, inexpensive, less time-consuming, and comparable to standard count method. This study was aimed to invent a new semiquantitative method for somatic cell count in bulk tank milk samples and to determine the precision, accuracy, and validity for this method.

2. Objectives
    1. To invent a new semiquantitative device for somatic cell count in bulk tank milk sample
    2. To validate the new semiquantitative device for somatic cell count at milk collecting center

3. Hypothesis

New semiquantitative plastic plate device has the ability to determine bulk tank milk somatic cell count at milk collecting center in which has comparable validity to Fossomatic™ FC somatic cell count.

CHAPTER 2

LITERATURE REVIEWS

1. Somatic cells count (SCC)

1.1 Definition of somatic cell count

Somatic cell count (SCC) is the number of somatic cells in a milliliter of milk. The predominant cells in milk somatic cells are leucocytes and mammary gland epithelium cells. Main leucocytes are including macrophages, polymorphonuclear neutrophils cells (PMNs), and lymphocytes (Boutinaud and Jammes, 2002; Ezzat Alnakip et al., 2014). SCC in milk increases whenever injury or infection of the mammary gland occurs. Therefore, the number of somatic cells presented in a sample of milk from the mammary gland can determine the likelihood of mastitis even though all other visible signs of udder inflammation are absent (Sharma et al., 2011)

BTM quality is very important because it indicates pricing, dairy products and customer health. The high SCC BTM affect dairy products quality, product shelf-life, and product processing such as pasteurized milk, cheese, and yogurt (Barbano et al., 1991; Auldist and Hubble, 1998; Khan and Khan, 2006; Fernandes et al., 2007). Many studies found that high SCC in BTM associated with the antibiotic residuals in milk (Ruegg and Tabone, 2000; Schaik et al., 2002; Jayarao et al., 2004; Khan and Khan, 2006). Farms with high SCC levels of BTM also showed more often had high bacterial plate count levels. Farms with BTM SCC higher than 750,000 cells/ml showed a much higher rate of antibiotic residue violations (Schaik et al., 2002). Therefore, the study to determine the proportion of farms with higher than SCC threshold and discriminate high BTM SCC is important to improve Thai milk quality and customer health guarantee.

SCC is accepted as the international standard measurement of milk quality and identification of mammary gland health status in the herd, cow, and quarter level (National mastitis council, 2001). In many countries, the results of somatic cells are used in acceptance or rejection of milk samples for processing or consumption. The BTM quality thresholds based on standards which vary from country to

country. The thresholds are 1,000,000 cells/ ml, 750,000 cells/ ml, and 500,000 cells/ml for Brazil, the USA, and South Africa, respectively (Ruegg and Pantoja, 2013). In Canada, England, EU countries, China, India, New Zealand, and Australia set the threshold at 400,000 cells/ml (Ruegg and Pantoja, 2013). Countries in the South East Asia area also use BTM SCC threshold as milk quality control. Vietnam and Indonesia set the thresholds at 400,000 cells/ ml. Malaysia set the threshold at 450,000 cells/ ml. (Bureau of Quality Control of Livestock Product Department of Livestock Development, 2013).

Thai milk board is an official Thai milk quality control organization. BTM SCC together with milk appearance and milk composition are used for milk quality control, grading and pricing in Thailand. Thailand milk quality SCC threshold is set at 500,000 cells/ ml (Table 1) (Thai Dairy cows and Dairy Products Board: Thai Milk Board, 2016). The Thai MCCs start to put the price penalty if farm BTM SCC threshold is more than 500,000 cells/ml. Thai farm BTM is rejected when BTM SCC is more than or equal to 1,000,000 cells/ml. CMT score 3+ also use as an indicator for Thai BTM rejection (Thai Dairy cows and Dairy Products Board: Thai Milk Board, 2016). The Thai school milk quota which is valuable and one of the most important marketplaces for Thai dairy farms. The MCCs are eligible to get school milk quota when the average of BTM SCC of all farm members less than 500,000 cells/ml for a period of four months consecutively (Thai Dairy cows and Dairy Products Board: Thai Milk Board, 2016).

Table 1 The Thai standard of BTM SCC for milk pricing per kilogram (Thai Dairy cows and Dairy Products Board: Thai Milk Board, 2016)

SCC (cells/ml)	**Price (Baht/kg)**
<200,000	+0.5
200,000-300,000	+0.3
300,000-400,000	+0.2
400,000-500,000	Unchanged
500,000-700,000	-0.2
700,000-1,000,000	-0.3
>1,000,000	rejected

Measurement of SCC at cow-level is necessary to estimate the presence of subclinical mastitis. Measurement of SCC at a quarter level is necessary to identify pathogens that cause mammary gland infection and the occurrence of subclinical mastitis detection (Sharif and Muhammad, 2008). At cow-level, to indicate mastitis from quarter samples, somatic cells more than 200,000 cells/ ml are the most practical threshold to separate healthy from infected quarter (Dohoo and Leslie, 1991) and somatic cells more than 200,000 cells/ml in composite milk also indicate the mastitis cow (Schepers et al., 1997).

1.2 Factors affecting milk somatic cell count

There were several important factors affecting cow-level SCC. Infection status (Meek et al.; Godden et al., 2002; Mamache et al., 2014), animal factors including age, stage of lactation and parity (Laevens et al., 1997) and environmental factors were associated with SCC in quarters and individual cow milk (Harmon, 1994; Skrzypek et al., 2004). For the BTM SCC level, there were also several factors associated with BTM

SCC. A number of production cows and dry cows in the herd, and length of dry period were associated with BTM SCC. The number of cows result herd production dilution effects that was associated with BTM SCC (Cicconi-Hogan et al., 2013; Vissio et al., 2018). Unhygienic milking techniques, contaminated pathogens while milking, unhygienic farm management and BTM SCC methods were also result high BTM SCC in herds (Eardmusic, 2011). The use of teat disinfection or teat dipping is the most effective method to reduce BTM SCC (Wagner and Ruegg, 2002). There was an association between low SCC and an increased level of hygiene and frequency of cleaning of the holding yard, passageways, and cubicles (Kelly et al., 2009).

The BTM SCC was affected by many factors. Besides, the high SCC of BTM level can result from the number of infected cows in the herd (Middleton et al., 2004) BTM SCC, BTM composition, and BTM quantity were affected by year, seasonal, farm location, farm size. The Large farm size trends to have higher somatic cell counts than small and medium farm size. (Yeamkong et al., 2010). At the herd level, it is especially important to follow trends over time and interfere when the cell counts appear to increase above a threshold. Moreover, high SCC which affects milk composition and milk yield per cow, resulting in loss of money and economic losses, including treatment cost, culling cost, and replacement cost (Seegers et al., 2003a). Monitoring milk quality over time provides an opportunity to evaluate the progress of the disease, study relationships between milk composition and milk SCC. Then, estimate the efficacy of mastitis control programs (Schukken et al., 1992; Sargeant et al., 1998). The convenience and acceptable accuracy of SCC methods for milk quality monitoring will encourage the efficient mastitis control program.

1.3 Methodology for somatic cell count

Milk somatic cells were first described in 1838 (Boutinaud and Jammes, 2002). There are two methodologies for somatic cell counting. First is direct somatic cell count, which directly measures somatic cells in milk. Second is indirect somatic cell count, which estimates milk somatic cells by the measure of interaction between somatic cells and specific reagents depending on methods.

1.3.1 Direct somatic cell count

Direct microscopic somatic cell count (DMSCC) or slide count, which is the standard method for SCC (Park and Humphrey, 1986). DMSCC using a microscopical smear and staining slide to differentiate and to determinate the staining somatic cells in milk. This method consists of manually counting and staining techniques. For each slide, the enumeration is concerned in field or areas. The DMSCC is the preferred gold standard counting somatic cells method in laboratories in many countries (National mastitis council, 2001). However, there are several problems in manual cell counting by DMSCC. The dilution and volume of reagent, pipetting errors, human perception of cell definition and variation among readers are DMSCC disadvantages. Moreover, the experiences and professional skills are the requirements for precise interpretation of DMSCC (Zajac et al., 2016).

The Fossomatic™ SCC method was first developed in the early 1980's. (FOSS Foss Allé 1 DK-3400 Hilleroed, Denmark). The flow cytometry technique and fluorescent staining are used in Fossoamtic™ FC SCC. The flow cytometry machine is very accurate because it performs only one single cell flows in a narrow stream in front of a laser beam. Fossomatic™ FC SCC machine is the current automatic direct somatic cells counting method. According to the manufactory procedure and machine performance, Fossomatic™ FC SCC measurement ranges 0 - 10 million cells/ ml. The percentage of covariance (CV) that represents the repeatability of Fossomatic™ FC SCC are CV < 6% at 100,000 to 299,999 cells/ml, CV < 4% at 300,000 to 499,999 cells/ml and CV < 3% at 500,000 to 1,500,000 cells/ml (appendix 3). This machine is strongly correlated with the result of DMSCC (Gunasekera et al., 2003). Thus, the efficiency is acceptable and similar to the standard direct SCC method. Fossomatic™ FC SCC has been proved and agreed to use instead of using DMSCC. Fossomatic™ FC SCC might count large nuclear fragments as cells, whereas DMSCC would omit such particles from counts, this may cause Fossomatic™ FC SCC too high depending on the frequency of particles (Miller et al., 1986). Fossomatic™ FC SCC reduces

subjective errors among readers. However, this machine is currently costly, oversize for field testing, requiring professional skills, and time-consuming in daily calibration (Viguier et al., 2009). Therefore, this machine may not be suitable for working in MCCs.

1.3.2 Indirect somatic cell count

There are several indirect SCC methods depend on techniques. The examples of techniques are detection of chemical reaction between somatic cells and chemical reagents, and the detection of enzymes which produced from somatic cells in milk. The popular indirect SCC technique is a chemical reaction.

Whiteside test (WST), which is a cow-side screening test for subclinical mastitis. WST uses sodium hydroxide (NaOH) solution as a specific reagent to estimate the SCC level in milk. The positive test result is recorded when the milk thickens, separates into flakes or shreds, and the solution shows semi-opaque to clear whey. The normal and mastitis milk both tend to be positive to this test after a few minutes. This test is inconvenient in the field practice in terms of the handling of glassware equipment. Moreover, a false positive test can occur due to fat globules in mastitis milk (Gordon et al., 1980).

The most popular indirect SCC is the California Mastitis Test (CMT). CMT is a cow-side level indirect SCC method modified from the WST. CMT principle is using the gel formation detection to estimate SCC level in milk. The gel formation comes from the reaction between proteins and deoxyribonucleic acid (DNA) in milk and detergent reagent. The test is a quick and simple method to predict SCC in both composite and quarter milk samples (Middleton et al., 2004). This method has high sensitivity for the detection of subclinical mastitis at cow-side. However, the only eye visible technique was used to CMT score interpretation. Studies found that CMT scoring has high subjective errors and variations when comparing with DMSCC. (Kroger and Jasper, 1967; Gordon et al., 1980; Eardmusic, 2011). There is a

large gap between SCC numbers within only one CMT score scale. There was false-negative of CMT in which is up to 20% (Dingwell et al., 2003). Moreover, CMT is more difficult to standardize between analysts and laboratories (Read et al., 1969). Nowadays, there are several CMT reagent formulations. So, the use of different concentration reagent solutions may result in the difference in gel formation. Thus, CMT reading results can vary among CMT reagents.

Wisconsin mastitis test (WMT) uses the same principle as the CMT. However, the amount of remaining gel formation is measured in quantitative as millimeters (mm) in a calibrated tube. The research found that this method is more precise than CMT but still showed variability (Thompson and Postle, 1964). WMT provides complexity procedures and using too many types of equipment composed of the clear plastic test tube with cap, the tube rack, and syringe for pipetting milk samples, which are resulting in an inconvenience in field condition working. The WMT correlates well with the DMSCC and has a high degree of repeatability. However, the study suggested a WMT reading could be used for screening out milk supplies with over 500,000 cells/ml. A confirmatory test should be made to determine whether the sample in question exceeded the somatic cell standard of 1.5 million somatic cells per ml (Thompson and Postle, 1964).

Many countries invented both direct and indirect SCC devices by using several technologies implication to improve device performance. Example of current SCC devices details are shown in appendix 1. The comparison of some current direct and indirect SCC device examples is shown in Table 2.

There are several portable direct SCC machines such as NucleoCounter (ChemoMetec A/S, Denmark), Lactoscan (MILKOTRONIC LTD, Bulgaria) and DeLaval (DeLaval, Sweden). Techniques of portable SCC to analyze milk samples is diluted milk somatic cells in staining solution (with ethidium bromide or propidium iodide). The DeLaval showed significant coefficients of regression ($b = 0.91$ to 1.01) and correlation ($r > 0.99$) when compared with the DMSCC and Fossomatic™ FC SCC methods. Moreover, the DCC gave repeatability values similar to the DMSCC, and their log SCC means did not differ from the reference value (Gonzalo et al., 2006).

The use of portable direct cell counter cassette (DeLaval, Tumba, Sweden) with the C-Reader system (Digital Bio-Technology Co. , Seoul, Korea) are new technologies that utilize electronic counting of somatic cells by mobile application. A study in 2017 showed several advantages of developed miniaturized cell counting platform for rapid and portable enumeration of somatic cells in milk. The platform provided several advantages including automatic sample delivery, integrated, on-chip sample preparation, and simple, accurate cell counting to determine milk quality and manage mastitis occurrence in dairy herds (Kim et al., 2017).

The PortaSCC (PortaScience, Portland) is a qualitative test that uses an algorithm to convert the enzymatic reaction into milk SCC. The PortaSCC results can present in digital numbers. However, according to manufactory procedure, test requires 45-minute incubation period. PortaSCC Quick, which is indirect SCC based on colorimetry or the intensity of the blue color. PortaSCC Quick estimates the amount of SCC by comparing the strip to the color chart. The researcher suggested that this method was rapidity to provide results for subclinical mastitis detection in farm level. PortaSCC Quick has 94.12% and 87.30% sensitivity and specificity respectively. PortaSCC Quick is a substantial agreement (k = 0.70) compared with the Fossomatic^{TM} FC machine (Salvador et al., 2014).

SCC Dunk from Japan is now launch in the market. SCC Dunk is screening test kit for neutrophils in individual milk and bulk milk. The test is based on a chemical reaction between a dye on the test strip and an enzyme found in the cells. This reaction makes the test strip sample well change to blue color. The darker the blue, the higher the cell count. Reaction time is about 5 to 6 minutes at room temperature in summer and 6 to 8 minutes in winter. The study of this test in my reviewed has never been studied.

There are several modified formal indirect SCC methods to improve test performance, user convenience and reduce subjective issues.

Surf Field Mastitis Test (SFMT) which is an indirect SCC method similar to CMT. SFMT provided sensitivity, specificity, predictive values, and kappa index which is similar diagnostic efficiency to CMT (Muhammad et al., 2010). Moreover, this study suggested that SFMT can be used as a cheaper, user-friendly alternative animal-side subclinical mastitis diagnostic test in developing countries because of the inexpensive and ready availability of the SFMT reagent (Muhammad et al., 2010).

Somaticell (Madrasa, Sao Paulo, Brazil) which is modified from the WMT. Somaticell showed similar results to electronic counting devices with a high degree of agreement (Rodrigues et al., 2009). Somaticell is an adequate efficiency of determining quarter milk SCC in quantitative results and may be considered as an alternative subclinical mastitis detection at the farm level. This device is portable, rapid to perform in a few minutes, and inexpensive are preferred (Rodrigues et al., 2009).

Table 2: The comparison of some current direct and indirect SCC with techniques, advantages and disadvantages

	Techniques	**Test**	**Advantages and disadvantages**
Direct SCC	1. Detect SCC in milk samples diluted in staining solution 2. Mobile application SCC	1. NucleoCounter 2. Lactoscan 3. DeLaval Cell counter 4. DeLaval 5. Cell counter Cell Counter ICC Mobile 6. Dairy Quality RT10^{TM} SCC tester	- Accuracy - Electricity needed - Laboratory bench equipment - Costs
Indirect SCC	1. Modified CMT 2. Modified Wisconsin Mastitis Test 3. Chemical reaction between a dye on the test strip and an enzyme found in the cells in the milk.	1. Surf Field Mastitis 2. Somaticell 3. Porta Quick SCC 4. SCC Dunk	- Reader variation - Reagents variation - Single-use

Current world and Thailand trends are focused on Artificial agricultural intelligence (AI), sensors, and big data analysis. Sensors for on-farm analysis of milk composition have been developed either for replacing visual inspection of foremilk

by the milker (color and image sensors) or for monitoring indicators in milk that have a high informational value but are not recognizable directly by the milker (Brandt et al., 2010). The main areas of application of the latter are feed adaptation, reproduction management, and early detection of udder infections. Ultrasonic or electromagnetic waves can realize the rapid and nondestructive analysis of multiple components. Essential sensing techniques for enzymatic changes in milk are biosensors, whereas chemical sensors are used for detecting several mostly volatile metabolites of pathogens (Brandt et al., 2010). In addition, techniques for counting somatic cells and automated CMT were recently developed by implemented automatic viscosity sensors at the main milk-line on every fourth milking stall. The study found the tendency to improve in the installed sensor system (Neitzel et al., 2014). The technological methods can also be adapted to Thai indirect SCC in the future.

2. Mastitis

Mastitis is an inflammation of the mammary gland, regardless of causes. It affects the whole chain of dairy products including decreased milk yield at the farm level (Hagnestam-Nielsen et al., 2009), change in milk compositions (Schultz, 1977; Ogola et al., 2007), and alteration of dairy product processing (milk pasteurization, yogurt processing, and cheese processing) (Khan and Khan, 2006; Mazal et al., 2007; Fernandes et al., 2008). Mastitis is characterized by physical, chemical, and usually bacteriological changes in the milk from pathological changes in the udder. Early recognition and prompt mastitis treatment are important for limiting tissue damage, production losses, and economic losses (Sharif et al., 2009).

Mastitis pathogenesis combines two processes, including intramammary infection and mammary gland inflammation. Mastitis is generally classified into two forms. First, clinical mastitis is the udder inflammation with noticeable signs including abnormal milk appearance, pain, redness, swelling, and loss of function of the udder. Clinical mastitis severity may during the disease appeared. Systemic signs such as fever, shock, and sudden death can be found in some cases. Clinical cases can be

defined as subacute (mildly clinical) when symptoms include only minor alterations in the milk and the affected quarter such as clots, flakes, or discolored secretion. The quarter may also be slightly swollen and tender. Acute mastitis cases are characterized by sudden onset, pain, heat, swelling, redness and reduced as well as altered milk secretion from affected halves. Abnormal secretion in the form of clots, flakes, or watery milk is the clinical sign most consistently observed. Depending upon the severity and the causative agent, acute mastitis cases may have significant systemic involvement characterized by fever, depression, and weakness. In its most severe form, it can be fatal. The second form of mastitis is subclinical mastitis in which clinical signs are not be observed, particularly in abnormal milk appearance. Subclinical mastitis affects milk quantity and milk quality. Subclinical mastitis occurs more frequently than clinical mastitis and causes more economic loss than clinical form (Seegers et al., 2003a), but farmers have difficulty in recognizing the subclinical form. Early detection of subclinical mastitis can reduce economic losses and shorten recovery periods (Halasa et al., 2007). Subclinical mastitis is less obvious and may only be detectable by measures of the milk's cellular content or milk somatic cells and milk bacterial culture.

Mastitis stills the most concerned problem of Thai farmers (Kampa et al., 2010). The occurrences of subclinical mastitis were very depended on the study area, seasonal, study design, and pathogen infection in the herds. There was a subclinical mastitis incidence rate equal to 3.37 per cows per year in Chiang Mai. The prevalence of subclinical mastitis in Chiang Mai small dairy farms ranged from 0 to 33.3% (Boonyayatra and Chaisri, 2003). Nakornpathom, the central part of Thailand, was found about 7.69-75% (Ajariyakhajorn et al., 2003). The prevalence of subclinical mastitis in smallholder dairy farms in Khon Kaen which located the northeast of Thailand was up to 62.8% (Aiumlamai et al., 2000). The percentage of new infections in herd often are due to milking technique or hygiene condition of farm (Schukken et al., 2009). The high SCC (threshold more than 200,000 cells/ml) in the quarter or (threshold more than 250,000 cells/ml) in the cow-level can represent subclinical mastitis or the incidence of intramammary gland infection (Sharma et al., 2011).

There was a higher prevalence of subclinical mastitis in dairy farms more than clinical mastitis (Biffa et al., 2005; Mdegela et al., 2009; Rahman et al., 2010).

Monitoring subclinical mastitis at herd level requires longitudinal SCC data over time because of the variability of time in inflammatory responses between cows in a herd (Schukken et al., 2003). The individual SCC and production records should be obtained to identify which cow is the most substantial contributor to the BTM SCC and likely candidates for individual cultures. Whole-herd only screening testing like CMT is a cheaper but less informative option (Plummer and Plummer, 2012). Routine SCC and milk composition analysis in individual cow milk are more essential than whole-herd CMT.

3. Viscosity of fluid

Rheology is defined as the study of material deformation and flow. Rheology represents the properties or characteristics of both solid and liquid foods (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Rheology of liquid foods like milk is the viscosity. The viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. Viscosity can be conceptualized as quantifying the frictional force that arises between two adjacent layers of fluid that are in relative motion. Fluid Viscosity sometimes referred as dynamic viscosity or absolute viscosity. Dynamic viscosity is the fluid's resistance to flow, which is caused by shearing stress within a flowing fluid and between a flowing fluid and its container. There are many systems for viscosity units. The unit in the English system are lb sec/ft² or Slug/ft.sec and and the SI system is Ns/m² or kg/ms. The Pascal unit (Pa) is specified pressure or stress force per area. Pascals can be combined with seconds to define dynamic viscosity (1.00 Pas = 10 Poise = 1000 Centipoise). Centipoise (cP) is commonly used to describe dynamic viscosity because water at a temperature of 20°C has a viscosity of 1.002 Centipoise. The viscometer can divide into several types depended on measuring viscosity types such as Capillary viscometer, Rotational viscometer, Rotating cylinder viscometer, and Cone-on-plate viscometer (Kamrich and Schoff, 1999).

The viscosity of milk is twice as high as that of water due to the friction of fat in the milk (emulsified in milk) (Park, 2007). Whole milk and skim milk display viscosities of 2.0–2.1 and 1.5–1.8 cP (or mPa/sec) at 20°C, respectively. Whey has a viscosity of 1.2 cP. The viscosity value for cow milk at 5 °C is a function of the fat content and ranges from 2.96×10^{-3} Pas (skim milk) to 3.25×10^{-3} Pas (whole milk), whereas at 20 °C the ranges for skim and whole milk are 1.79×10^{-3} Pas and 1.3×10^{-3} Pas (Park, 2007). The viscosity of livestock ruminant milk is sheep (2.48 cPas), Egyptian Camel (2.2 cPas), Buffalo (2.2 cPas), Goat (2.12cPas), and Cow (1.7 cPas), respectively (Park, 2007). In normal milk, viscosity is affected by milk composition and milk appearance such as fat and protein, milk temperature, pH, and age of the milk (Jenness and Patton, 1974). The casein micelles of milk affect the viscosity of milk more than any other components. The fat contribute to viscosity less than casein but greater than whey proteins (Davies and Law, 1983). Viscosity varies not only with changes in the physical of fat but also with the hydration of proteins (Davies and Law, 1983). External factors that affect milk viscosity are processing methods, additional enzymes, and processing temperatures by heating (Manji et al., 1986). When fat globules are greatly subdivided by homogenization, an increase in viscosity is observed. The viscosity of milk and cream creates the impression of "richness" to the consumer. From an organoleptic standpoint, viscosity contributes to mouthfeel and flavor release (Davies and Law, 1983).

4. Semiquantitative test

Qualitative examinations are the measure of presence or absence of a substance or evaluate cellular characteristics such as morphology. The results are not expressed in numerical terms, but in descriptive or qualitative terms such as "positive," "negative," "reactive," "non-reactive," "normal," or "abnormal". The examples of qualitative examinations include microscopic examinations for cell morphology or presence of parasitic organisms, serologic procedures for presence or absence of antigens and antibodies, some microbiological procedures, and some molecular techniques (International Health Regulations, 2015).

Semiquantitative examinations are similar to qualitative examinations that testing does not measure the precise quantity of a substance. The difference is that the results of these tests are expressed as an estimate of how much a measured substance is present. This estimate is sometimes reported as a number or a range. Therefore, test results for semiquantitative tests may be shown as “trace amount”, “1+, 2+, or 3+”, or positive at any dilution or titer. Quantitative examinations are the tests that measure the precise quantity of a substance. The quantitative test is given a numerical or exact amount of results (International Health Regulations, 2015). Examples of semiquantitative examinations are urine dipsticks, tablet tests for ketones, serological agglutination and indirect SCC.

In summary, quantitative examinations give exact numerical results. Semiquantitative examinations are estimate amount and present in non-numerical results. Qualitative examinations indicate only the presence or absence of a substance (positive or negative results) or evaluate cellular characteristics such as morphology. Semi-quantitative examinations provide an estimate of how much of the measured substance is present (International Health Regulations, 2015).

CHAPTER 3
MATERIALS AND METHODS

The new semiquantitative device for BTM SCC has two principles including chemical appearance and physical appearance. The chemical appearance using the reaction between somatic cells and specific reagent that cause gel formation. The physical appearance was defined by gels adherence to plastic plate surface. The high SCC in BTM results in viscosity of gel formation. Thus, the higher gel viscosity can adhere and leave the higher amount of gel on plastic plate surface. The designed plastic plate patterns are able to differentiate the level of BTM SCC. Semiquantitative reading scores are given depending on the amount of gel formation that remain on the plastic plate surface.

The experimental study divided into three experiments including 1) plastic plate pattern design and chemical reagent optimization 2) Plastic prototype testing and calibration in the laboratory and 3) Final model prototype field testing and validation, which is conducted at a milk collecting center. Experiment 1 and experiment 2 were parallelly studied and followed by experiment 3.

Experiment 1: Plastic plate design and specific chemical reagent optimization

1) Plastic plate design

The plastic color, plastic materials and plastic plate patterns were reviewed and discussed with plastic material and 3D printer engineers. The proper design of plastic plate prototype and material selection were invented according to the physical appearance of gel formation and the adherence to the plastic plate surface. The patterns of plastic plate prototypes were designed according to the principles of tension force, adhesion force, capillary effect, and surface area of plastic plate. The design aims to create proper gel formation, gel viscosity, and gel adherence on the plastic plate prototypes. Tinkercad program (Autodesk, Inc., San Rafael, California, U.S.A) was used to design and to sketch the plastic plate prototypes. The 3D plastic

prototypes were printed by using the Stratasys F170 3D printer (Stratasys, Ltd., Eden Prairie, Minnesota, United States) at Siam Innovation District, Siam Square One Building, Bangkok, Thailand. Many different materials can be used in 3D printers (appendix 2A).

2) Specific chemical reagent optimization

The specific chemical reagent was formulated and prepared according to the protocol described by Laboratory of Livestock hospital, Faculty of Veterinary Science, Chulalongkorn University, Nakhon Pathom province, Thailand. This solution was designed in order to create the proper amount of gel formation which can discriminate bulk tank milk somatic cell count level. BTM samples were measured Fossomatic^{TM} FC SCC and classified according to SCC levels into four levels 1) Low (SCC less than 100,000 cells/ml), 2) Moderate (SCC 100,000 to 299,999 cells/ml), 3) High (SCC 300,000 to less than 499,999 cells/ml) and 4) Very High (SCC more than or equal to 500,000 cells/ml).

Ten BTM samples per SCC level were selected to optimize specific chemical reagent concentration. The dilutions of half, normal and double concentration (0.5x, 1x and 2x) were formulated and prepared. Each dilution mixed with each level of BTM samples. The optimization of the mixed solution was proved by using mixed solution viscosity. The viscosity of the mixed solution was determined by using sine wave viscometer SV-100 (A&D Company, Limited, Tokyo, Japan) at Pharmaceutical Laboratory, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in the day after BTM SCC was measured. The appropriate concentration of mixed solution was selected by the correlation between SCC and mixed solution viscosity values and SCC levels categorization. The appropriate amount of specific chemical reagent and BTM sample volume was also determined.

Experiment 2: Laboratory plastic plate prototype testing and calibration

Farm BTM samples were randomly selected from farm members of a milk collecting center in Nakhon Ratchasima province. At the milk collecting center, 30 ml of farm BTM was collected. Samples were labeled, properly ice packed and

submitted to the milk quality laboratory, Center of Learning Network for the regions, Chulalongkorn University, Kangkoi, Saraburi province, Thailand. All BTM sample was stored in 4-8-degree Celsius immediately after laboratory arrival. The experiment was performed within 24 hours after the samples collected.

A BTM sample was divided into three parts: 10 ml of milk were used in Fossomatic™ FC machine SCC according to manufacturers' instructions (FOSS Foss Allé 1 DK-3400 Hilleroed, Denmark) (appendix 3A), 3 ml of milk was used for CMT testing (Schalm and Noorlander, 1957) (appendix 3B), and 10 ml of milk was used for plastic plate prototype testing. After Fossomatic™ FC SCC BTM samples were classified into four levels as the same range of SCC in experiment 1. Fifteen BTM samples per each SCC level were randomly selected and estimated SCC by using the plastic prototypes and CMT. All printed prototypes were testing in the same manner and reader. All printed prototypes were error and trial depend on the principle of chemical and physical appearance. The application of each plastic plate prototypes was also invented. The reading scores were recorded after plastic plate prototypes can clearly discriminate the SCC levels and showed difference pattern in each SCC levels. All reading scores of plastic plate prototypes were calculated correlation with Fossomatic™ FC SCC and CMT scoring. The percentage of false results of each plastic plate prototype was also calculated. The chemical appearance, physical appearance, percentage of false results and correlation between tests were used as the criteria for plastic prototype design improvement and the final model plastic plate prototype chosen. After the final model plastic plate prototype was selected, the final model was then used in field testing in experiment 3.

Experiment 3: The final model plastic prototype field testing and validation

At one milk collecting center in Nakhon Ratchasima province, 305 BTM samples were randomly selected from farm members of the milk collecting center in July 2019. Duplicated 30 ml samples were collected. First duplicated 30 ml samples were used for final model plastic plate prototype testing at the milk collecting area of the MCC. The final model plastic plate prototype was testing in the same manner

and reader as experiment 2. The reading scores were recorded. Second duplicated 30 ml samples were sent to milk quality laboratory and divided into two parts. The first part was for Fossomatic™ FC SCC (FOSS Foss Allé 1 DK-3400 Hilleroed, Denmark) (appendix 3A). The Fossomatic™ FC SCC has used the same manner as Experiment 2. The second part was for Milk composition including fat, protein, casein, lactose, total solids, SNF and urea were analysis by Fourier transform infrared spectroscopy (MilkoScanTM FT2 Hilleroed, Denmark) (appendix 3C). The SCC and milk composition analysis were according to manufacturers' instructions. BTM samples were classified into four levels according to the same range of SCC in experiment 1. Test performances of the final model plastic plate prototype were calculated for test validation comparing with Fossomatic™ FC SCC which was used as a standard method. Test performances were including the percentage of true positive, true negative, false positive, false negative; sensitivity, specificity, positive and negative predictive values, and accuracy were determined in every reading scores. The correlation between Fossomatic™ FC SCC and final model plastic plate prototype SCC scoring was also calculated.

Data analysis

All the descriptive statistics including mean, standard error (SE), and the range were calculated. The normal distribution of the SCC data was examined by using the Kolmogorov - Smirnov test (K-S test) and using normal probability plots by the QQ plot. Skewness and Kurtosis values were also obtained. In order to normalize SCC data, a base-10 logarithmic scale (log10) was performed. The log SCC data were compared among groups using Kruskal Wallis nonparametric one-way ANOVA (Kruskal-Wallis H test). Post Hoc test was obtained from each log SCC level and compared the difference between log SCC levels. All statistics calculation was performed by using IBM® SPSS® software version 22.0 (International Business Machines Corp, New York, USA).

All test performances of plastic plate devices, including sensitivity, specificity, predictive values (both positive and negative), and accuracy were calculated

according to formulas which are shown in Table 3. Fossomatic™ FC SCC was used as a standard method. The Fossomatic™ FC SCC cut-off thresholds for test performances are 100,000 cells/ml, 300,000 cells/ml and 500,000 cells/ml. All test performances were calculated by using EpiTools (Sergeant, ESG, 2018. Epitools epidemiological calculators. Ausvet Pty Ltd. Available at http://epitools.ausvet.com.au).

The relationships between milk composition, plastic plate prototype SCC scoring, CMT scoring, and Fossomatic™ FC SCC were evaluated by using Pearson's correlation coefficient and Kendall rank correlation coefficient. The variation among scores of plastic plate prototypes scoring were also calculated and presented in percentage of covariance (%CV). The statistical significance level was considered at $p<0.05$.

Table 3: The calculation of test performances (Šimundić, 2009)

		Fossomatic™ FC SCC		
		Positive	Negative	Measure
Plastic plate results	Positive	True positive (TP)	False positive (FP)	Positive predictive value (PPV) $\frac{TP}{TP+FP}$
	Negative	False negative (FN)	True negative (TN)	Negative predictive value (NPV) $\frac{TP}{TN+FN}$
	Measure	Sensitivity $\frac{TN}{TP+FN}$	Specificity $\frac{TN}{FP+TN}$	Accuracy $\frac{TP+TN}{TP+FP+TN+FN}$

TP = True Positive, FP = False Positive, TN = True negative, FN = False Negative

Cut off thresholds Fossomatic™ SCC = 100,000 / 300,000 / 500,000 cells/ml

CHAPTER 4

RESULTS

Experiment 1: Plastic plate design and specific chemical reagent optimization

1) Plastic plate design

According to the engineers' consultation, the white color ABS material was selected for plastic material in this study. Six teen plastic plate prototypes were developed and improved according to the principles of chemical appearance and physical appearance. Three patterns of plastic plate prototype (hole, grid and slits) were designed and printed. The characteristics of plastic plate prototypes is shown in appendix 2B. There are four hole-pattern plastic prototypes (1A to 1D), five grid patterns plastic plate prototypes (2A to 2E) and seven slits patterns plastic plate prototypes (3A to 3G) (appendix 2B).

2) Specific chemical reagent optimization

The active ingredients of chemical reagent are 1.2% w/w of Linear Alkylbenzene Sulfonate and Potassium Salt with 0.86% w/w of Sodium Lauryl Ether Sulfonate. Bromocresol purple was used as a pH indicator reagent. Fluid viscosities of three specific chemical concentrations (0.5x, 1x and 2x) were compared. The appropriate concentration of specific chemical reagent was 1x. The mixed solution of 10 ml of 1x specific chemical reagent and 10 ml of BTM samples produced the best gel formation. The fluid viscosity of 1x mixed solution was clearly discriminated the SCC in levels and was significantly correlated with Fossomatic^{TM} FC SCC ($p<0.05$ data is not shown). The appropriate amount of mixed solution was composed of 10 ml of specific chemical reagent and 10 ml of BTM sample volume (ratio 1:1). The preparation of specific chemical reagent is described in appendix 2C.

Experiment 2: Laboratory plastic plate prototype testing and calibration

Sixteen plastic prototypes were tested and improved by using trial and error testing. The criteria used for plastic plate prototypes testing and final model plastic plate prototype selection is shown in Table 4. From the experiment study, the first

prototype pattern, hole plastic plate prototypes (1A to 1D) (appendix 2B) can indicate gel formation but gels can not adhere on any patterns of plastic plate surface. The second prototype pattern, grid plastic plate prototypes (2A to 2E) (appendix 2B) began to show the gel formation adherence at SCC more than 300,000 cells/ml (high SCC level). This plastic plate prototypes can discriminate less than 100,000 cells/ml and 500,000 cells/ml (low and very high level). However, this plastic plate prototype cannot detect the difference between SCC 100,000 to 500,000 cells/ml (moderate and high levels). The third prototype pattern is slits plastic plate prototype pattern. BTM SCC was clearly classified into levels in the model 3C (appendix 2B). However, this model 3C was still subjective reading due to the mixed solution pouring position. This obstacle was solved by designed pouring chamber in which the mixed solution was allowed to automatically reach the end of plastic plate as shown in models 3D to 3G (appendix 2B).

Table 4: The criteria of final model selection and sixteen patterns of plastic plate prototypes comparison

Pattern	Chemical appearance	Physical appearance	SCC levels	Correlation		%False results
				Fossomatic™ FC SCC	CMT	
1. Hole patterns (1A to 1D)	+	-	-	N/A	N/A	N/A
2. Grid patterns (2A to 2E)	+	+	-	N/A	N/A	N/A
3. Slits patterns 1) 3A	+	+	-	N/A	N/A	N/A
2) 3B	+	+	-	N/A	N/A	N/A
3) 3C	+	+	+	r = 0.77 (p<0.05)	r = 0.90 (p<0.05)	25.00
4) 3D	+	+	+	r = 0.70 (p<0.05)	r = 0.92 (p<0.05)	30.25
5) 3E	+	+	+	r = 0.73 (p<0.05)	r = 0.92 (p<0.05)	28.33
6) 3F	+	+	+	r = 0.76 (p<0.05)	r = 0.93 (p<0.05)	23.33
7) 3G	+	+	+	r = 0.80 (p<0.05)	r = 0.94 (p<0.05)	21.67

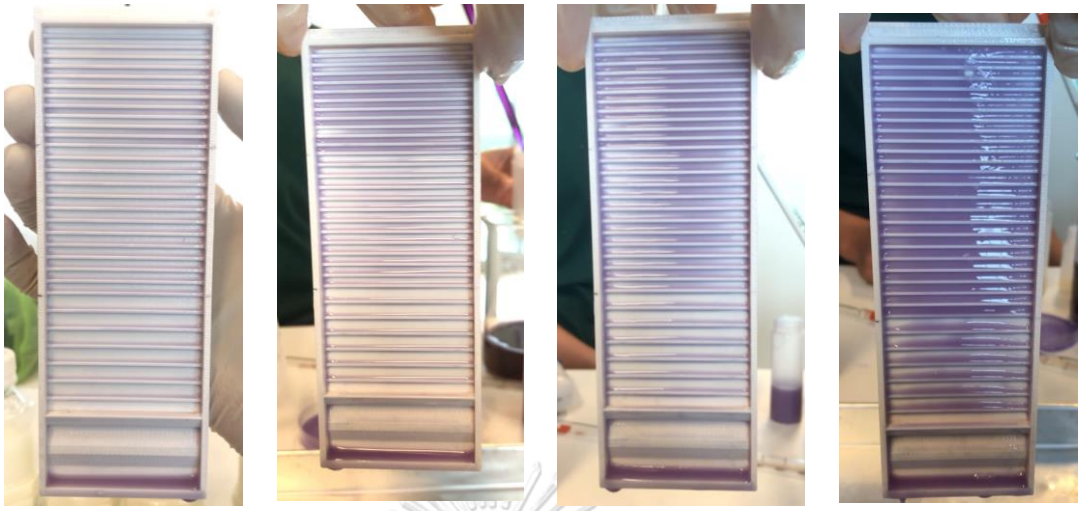
+ observed, - not observed, N/A not applicable

The best plastic plate prototype for final model is model 3G (appendix 2B). The proper plastic plate prototype dimension is 158 x 53 x 3 mm (Length x Width x Height), and depth from the edge of plastic is equal to 2.2 mm. There are three clusters of slits in the plastic plate. Each slit dimension is 50x1x1 mm (Length x Width x Height). The first cluster of slits consists of 7 plastic slits with spaces between slits are equal to 5 mm. The second cluster of slits consists of 12 plastic slits with spaces between slits are equal to 3 mm. The third cluster of slits consists of 12 plastic slits with spaces between slits are equal to 2 mm. At the end of the plastic plate has one pouring chamber in which dimension is 50x25x8 mm (Length x Width x Height) with 45-degree slopes at the bottom of the chamber in order to allow mixed solution automatically reach the end of plastic plate. The repeatability of all plastic prototypes SCC scoring was comparable to Fossomatic™ FC (data not shown). There was no significant difference between percentage of Fossomatic™ FC CV and the final model 3G ($p<0.05$). The CV percentages of final model 3G were equal to 5.48% at less than 100,000 cells/ml, 2.27% at 100,000 to 299,999 cells/ml, 1.14% at 300,000 to 499,999 cells/ml and 3.70% in more than 500,000 cells/ml.

The reading scores and plastic plate SCC scale for final model 3G is shown in figure 2. The application procedure of plastic plate final model 3G is followed by these steps

1. The 10 ml BTM sample is added into bottle of 10 ml of specific chemical reagent.

   The bottle is hand mixed for 3 seconds
2. Pour the mixed solution into plastic plate chamber and wait until the mixed solution reaches another end of plastic plate
3. Pulled plastic plate up appendicular to floor level.
4. Plastic SCC scoring is read at 10 second



Score 0 Score 1 Score 2 Score 3

Plastic plate model 3G reading scale

SCC < 100,000 cells/ml	=	0
SCC 100,000 – 299,999 cells/ml	=	1
SCC 300,000 – 499,999 cells/ml	=	2
SCC ≥ 500,000 cells/ml	=	3

Figure 2: The pictures of reading score of final model 3G and the plastic plate SCC scales

The final model 3G showed the correlations with Fossomatic™ FC SCC (r= 0.80, p<0.01) and with CMT (r= 0.94, p<0.01), respectively. The false result was equal to 21.97%. The percentage CV of plastic plate 3G was equal to 5.65%, 4.82%, 2.83%, and 4.07% in score 0, score 1, score 2, and score 3, respectively.

This study indicated that recommendation reading time was 10 seconds after sample was mixed with specific chemical reagent and poured into chamber because it showed the highest agreement with Fossomatic™ FC SCC (r=0.69, p<0.01).

Experiment 3: The final model plastic prototype field testing and validation

A total of 305 BTM samples were determined SCC by using Plate SCC scoring at milk collecting center comparing to laboratory Fossomatic™ FC SCC. The BTM SCC and milk composition data are shown in table 5. The average SCC was 740,102 ± 41,693.31 cells/ml (log10 = 5.685 ± 0.02). Maximum SCC was 5,355,000 cells/ml (log10 = 6.73). Minimum SCC was 16,000 (log10 = 4.20). The average percentage of milk fat was 4.10 ± 0.02. The average percentage of protein was 2.95 ± 0.01. The average percentage of casein was 2.142 ± 0.01. The average percentage of lactose was 4.33 ± 0.01. The average percentage of total solid was 12.41 ± 0.03, and the percentage of solids not fat was 8.39 ± 0.01.

Table 5: The milk quality of BTM samples was determined by Fossomatic™ FC and Milkoscan™ FT2 (n=305). (Mean ± Standard Error, Min and Max). The milk data was shown here, including SCC (cells/ml), log SCC, percentage of Fat (%), Protein (%), Casein (%), Lactose (%), Total solid (%) and Solid not fat (%).

	Mean ± SE	Min	Max
SCC (cells/ml)	740,101.64 ± 41,693.31	16,000	5,355,000
log SCC	5.69 ± 0.02	4.20	6.73
Fat (%)	4.09 ± 0.02	3.20	5.31
Protein (%)	2.95 ± 0.01	2.50	3.86
Casein (%)	2.14 ± 0.01	1.77	2.84
Lactose (%)	4.33 ± 0.01	3.90	4.60
Total solid (%)	12.41 ± 0.03	11.28	14.39
Solid not fat (%)	8.39 ± 0.01	7.80	9.27

The box plots of BTM SCC samples are shown in Figures 3. Our study BTM SCC showed a skew right distribution (Skewness = 2.37 ± 0.14, Kurtosis = 8.26 ± 0.28) with significant Kolmogorov - Smirnov test (K-S test) ($p<0.05$). Outliers and the distribution graph are presented in Figure 3A. The median of 305 BTM SCC was equal to 520,500 cells/ml (Figure 3A). The average SCC was equal to 740,102 ± 41,693.31 cells/ml. The 10-based logarithm of 305 BTM SCC is shown in Figure 3B. The log SCC was normal distribution. The median equal to 5.72. The average log SCC was equal to 5.69 ± 0.42 (Figure 3B).

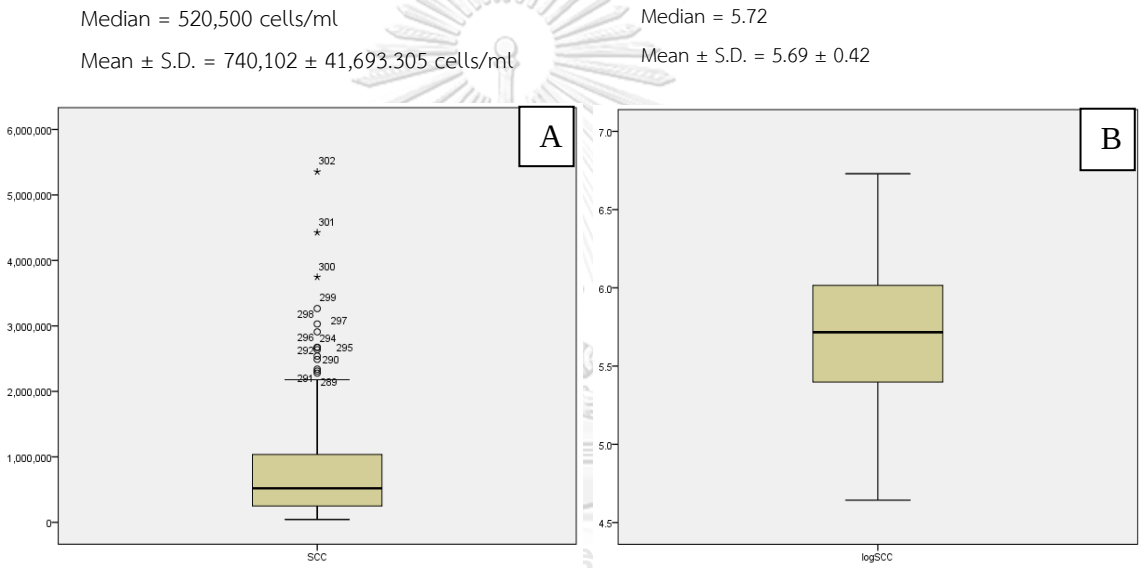


Figure 3: The box plot of BTM SCC (A) and 10-base logarithm BTM SCC (B) measured by Fossomatic™ FC (n=305)

The 305 BTM samples were categorized by final model 3G into SCC levels including low (SCC < 100,000 cells/ml), moderate (SCC 100,000 to 299,999 cells/ml), high (SCC 300,000 to 499,999 cells/ml) and very high (SCC ≥ 500,000 cells/ml). This plastic plate SCC method was compared to laboratory Fossomatic™ FC. The results are shown in Figure 4. BTM SCC was clearly categorized into SCC levels by Fossomatic™ FC. There were BTM SCC over standard (SCC ≥ 500,000 cells/ml or logSCC ≥ 5.69). There were variations in low and very high SCC levels. However, the final model 3G cannot clearly categorized BTM SCC into groups. The result showed overlapping values between scores, and there were variations among scores greater than Fossomatic™ FC SCC.

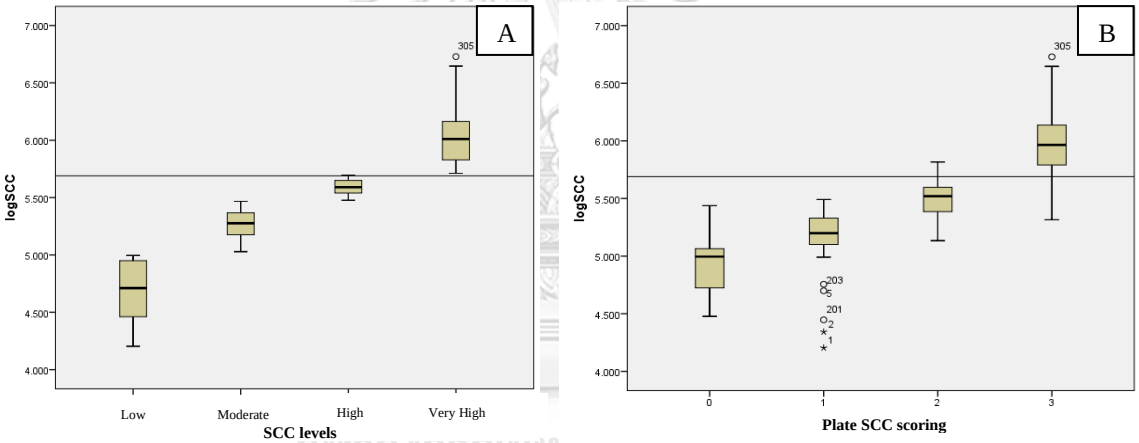


Figure 4: The box plot between base-10 logarithm Fossomatic™ FC SCC and SCC levels with cut off line equal to 5.69 (SCC = 500,000 cells/ml) (A) and the box plot between base-10 logarithm Fossomatic™ FC SCC and the Plate SCC scoring with cut off line equal to 5.69 (SCC 500,000 cells/ml) (B).

The descriptive data of BTM samples SCC categorized by Fossomatic™ FC into SCC levels are shown in table 6. The average BTM SCC in each level were 56,333.33 ± 8,879.10 (log SCC = 4.68 ± 0.08), 192,024.39 ± 5,685.26 (log SCC = 5.27 ± 0.01), 395,315.79 ± 7,667.89 (log SCC = 5.59 ± 0.09) and 1,212,831.17 ± 61574.06 (log SCC = 6.02 ± 0.02) in low, moderate, high and very high SCC levels, respectively. The average SCC in each BTM Fossomatic™ FC SCC was significantly different among SCC

levels (p<0.05) accept in low and moderate levels (p>0.05). The average log SCC in each BTM Fossomatic™ FC SCC was significantly different among SCC levels (p<0.05). The lowest SCC was 16,000 cells/ml (log SCC = 4.204) which was in a low-level group (SCC less than 100,000 cells/m). The highest SCC was 5,355,000 cells/ml (log SCC = 6.729), which was in the very high group. The highest frequency of BTM SCC samples was equal to 154 samples which was also in the very high SCC level.

Table 6: The descriptive data of level categorization of BTM samples (n= 305) by Fossomatic™ FC determination (Number of samples in each level, Mean ± Standard Error, Min and Max)

SCC level (cells/ml) (logSCC)	n	Mean ± SE (cells/ml)	Min (cells/ml)	Max (cells/ml)
Low (<100,000cells/ml)	12	56,333.33 ± 8,879.10[a] (4.68 ± 0.08)[a]	16,000 (4.20)	99,000 (4.99)
Moderate (100,000-<300,000 cells/ml)	82	192,024.39 ± 5,685.26[a] (5.27 ± 0.01)[b]	107,000 (5.03)	293,000 (5.47)
High (300,000-<500,000 cells/ml)	58	395,315.79 ± 7,667.89[b] (5.59 ± 0.01)[c]	300,000 (5.48)	497,000 (5.69)
Very High (≥500,000 cells/ml)	154	1,212,831.17 ± 61574.06[C] (6.02 ± 0.02)[d]	515,000 (5.71)	5,355,000 (6.73)

[a,b,c,d] The mean difference among SCC level is significant at the 0.05 level.

The descriptive data of BTM samples SCC categorized by final model 3G scoring into SCC levels are shown in table 7. The average of SCC in each level were 108,222.22 ± 24,888.52 (log = 4.95 ± 0.10), 164,754.39 ± 8,680.03 (log = 5.16 ± 0.03), 335,277.78 ± 13,924.63 (log = 5.50 ± 0.02) and 1,145,065.87 ± 59,533.43 (log = 5.98 ± 0.02) in score 0, 1, 2 and 3, respectively. The average SCC in score 3 was significantly different from other scores ($p<0.05$). The average log SCC in each BTM Plate SCC scoring was significantly different among scores in score 1,2 and 3 ($p<0.05$) but scores 0 and 1 was not significantly different ($p>0.05$). The highest frequency of BTM SCC samples were also in the score 3. The lowest SCC was 16,000 cells/ml (log SCC = 4.204, which was found in the score 0. The highest SCC was 5,355,000 (log SCC = 6.729), which was found in the score 3 group.

Table 7: The descriptive data of level categorization of BTM samples (n= 305) by plate SCC scoring determination (Number of samples in each level, Mean ± Standard Error, Min and Max)

SCC level (cells/ml)	Score	n	Mean ± SE (cells/ml)	Min (cells/ml)	Max (cells/ml)
Low (<100,000cells/ml)	0	9	108,222.22 ± 24,888.52[a] (4.95 ± 0.10)[a]	30,000 (4.48)	274,000 (5.44)
Moderate (100,000-<300,000 cells/ml)	1	58	164,754.39 ± 8,680.03[a] (5.16 ± 0.03)[a]	16,000 (4.20)	310,000 (5.49)
High (300,000-<500,000 cells/ml)	2	72	335,277.78 ± 13,924.63[a] (5.50 ± 0.02)[b]	136,000 (5.13)	656,000 (5.82)
Very High (≥500,000 cells/ml)	3	166	1,145,065.87 ± 59,533.43[b] (5.98 ± 0.02)[c]	332,000 (5.52)	5,355,000 (6.73)

[a,b,c,d] The mean difference among SCC level is significant at the 0.05 level.

By using the Thai Dairy Cows and Dairy Products Board announcement BTM SCC cut off (Thai Dairy cows and Dairy Products Board: Thai Milk Board, 2016), the Fossomatic™ FC SCC indicated 54.43% (n= 154) of farm BTM samples were below quality standard (500,000 cells/ml) or penalty grade milk. Moreover, the premium grade milk which SCC less than 100,000 cell/ml was equal to 3.95% . In the other hand, plastic plate final model 3G indicated 50.49% of farm BTM samples were the penalty grade milk (score 3) and 2.95% were premium grade milk (score 0).

The test performance of plastic plate final model 3G SCC scoring of 305 BTM SCC is shown in table 8 and table 9. The highest overall true result was found in score 0 (96.72%). The highest overall false result was found in score 1 (81.64%). The highest true positive was found in score 3 (48.20%). The highest true negative was found in score 0 (94.75%). For the false results, the highest false positive was found in score 2 (9.18%) and the highest false negative was also found in score 1 (79.34%). Accuracies of this final model 3G was ranged from 18.3% to 96.41% . The highest accuracy was found in score 0. The lowest accuracy was found in score 1. The accuracy of score 0 was equal to 96.41% (CI = 93.66 to 98.19). The sensitivity was equal to 41.67% (CI = 15.17% to 72.33%), specificity was equal to 98.64% (CI = 96.55% to 98.19%) which was the highest sensitivity of this plate scoring. The predictive values were 88.02% (CI = 82.99% to 91.72%) and 94.96% (CI = 90.12% to 97.50%) in positive predictive value and negative predictive value, respectively. The accuracy of score 1 was equal to 18.30% (CI = 14.13% to 23.10%) which was the lowest sensitivity of this plate scoring. The sensitivity is equal to 41.67% (CI = 15.17% to 72.33%), specificity was equal to 87.93% (CI = 80.95% to 92.59%). The predictive values were 87.93% (CI = 80.95% to 92.59%) and 2.02% (CI = 1.04% to 3.87%) in positive predictive value and negative predictive value, respectively. The score 1 showed the lowest percentage of sensitivity, specificity, negative predictive value and accuracy compared to other scores.

The accuracy of score 2 was equal to 35.95% (CI = 30.57% to 41.60%). The sensitivity was equal to 20.75% (CI = 15.50% to 26.84%), specificity was equal to 70.21% (CI = 59.90 to 79.21). The predictive values were 61.11% (CI = 51.13% to 70.24%) and 28.21% (CI = 25.30% to 31.31%) in positive predictive value and

negative predictive value, respectively. The accuracy of score 3 was equal to 91.18% (CI = 87.42 to 94.10). The sensitivity was equal to 95.45% (CI = 90.86 to 98.15) which was the highest sensitivity in this plate scoring. The specificity was equal to 86.84% (CI = 80.41 to 91.77). The predictive values were 88.02% (CI = 82.99 to 91.72) and 94.96% (CI = 90.12 to 97.50) in positive predictive value and negative predictive value, respectively.

Table 8: The test performance of Plate SCC scoring (n=305) (percentage of true positive, true negative, false positive and false negative)

Reading scores	Score 0	Score 1	Score 2	Score 3
Overall True results (%)	96.72	18.36	36.07	91.48
	(n = 293)	(n = 56)	(n = 110)	(n = 279)
- True positive (%)	1.31	16.72	14.43	48.20
	(n = 4)	(n = 51)	(n = 44)	(n = 147)
- True negative (%)	94.75	1.64	21.64	43.28
	(n = 289)	(n = 5)	(n = 66)	(n = 132)
Overall False results (%)	3.94	81.64	63.93	8.53
- False positive (%)	(n = 12)	(n = 249)	(n = 195)	(n = 26)
	1.64	2.30	9.18	6.23
	(n = 5)	(n = 7)	(n = 28)	(n = 19)
- False negative (%)	2.30	79.34	54.75	2.30
	(n = 7)	(n = 242)	(n = 167)	(n = 7)

The test performance was using Fossomatic™ FC SCC as the gold standard. Cut off thresholds: 100,000 cells/ml at score 0, 200,000 cells/ml at score 1, 300,000 cells/ml at score 2 and 500,000 cells/ml at score 3

Table 9: The test performance of plate SCC scoring (n=305) (percentage of sensitivity, specificity, negative predictive value, positive predictive value, and accuracy)

Reading scores	Score 0 % (95% CI interval)	Score 1 % (95% CI interval)	Score 2 % (95% CI interval)	Score 3 % (95% CI interval)
Sensitivity	41.67 (15.17 to 72.33)	17.35 (13.20 to 22.17)	20.75 (15.50 to 26.84)	95.45 (90.86 to 98.15)
Specificity	98.64 (96.55 to 99.63)	41.67 (15.17 to 72.33)	70.21 (59.90 to 79.21)	86.84% (80.41 to 91.77)
PPV	55.56 (27.72 to 80.29)	87.93 (80.95 to 92.59)	61.11 (51.13 to 70.24)	88.02 (82.99 to 91.72)
NPV	97.64 (96.25 to 98.53)	2.02 (1.04 to 3.87)	28.21 (25.30 to 31.31)	94.96 (90.12 to 97.50)
Accuracy	96.41 (93.66 to 98.19)	18.30 (14.13 to 23.10)	35.95 (30.57 to 41.60)	91.18 (87.42 to 94.10)

The test performance was using Fossomatic™ FC SCC as the gold standard. Cut off thresholds: 100,000 cells/ml at score 0, 200,000 cells/ml at score 1, 300,000 cells/ml at score 2 and 500,000 cells/ml at score 3.

CHAPTER 5
DISCUSSION

In 1999, Thai Department of Livestock Development (DLD) announced a raw milk quality standard for Thai dairy farms (Kampa et al., 2010). The results of milk quality testing have been used to set the price of milk buying for Thai small dairy farms since the official standard started. Thai milk broad announcement is an official milk quality standard for Thailand. Many standards were continually launched to encourage Thai farmers had to be aware of losses due to mastitis and low BTM quality. In Thailand, raw milk used for human consumption should have SCC less than 500,000 cells/ml. However, more than 40% of the Thai dairy herds still had BTM SCC higher than the standard (National Bureau of Agricultural Commodity and Food Standards, 2010). The data of northern Thailand from September 2010 to April 2012 showed the percentage of farms BTM SCC over 500,000 cells/ml equal to 24.6% (Suriyasathaporn et al., 2012) while more than 50% in our study which studied in July 2019. The variation of percentage of BTM SCC not only affected by farms mastitis occurrence but also number of cows, farm management, climate and seasonal (Sharma et al., 2011).

ABS plastic material was selected in this study because of its characteristics and low-cost of production. American Food and Drug Administration (FDA) compliant ABS is available for good grade products. ABS is an engineering polymer that is easy to machine and fabricate. ABS holds useful toughness and strength properties. ABS is a widely plastic material for structural applications and pre-production prototypes when impact resistance, strength, and stiffness are required. It is suitable for plastic prototypes developing because it has excellent dimensional stability and it is easy to assemble and decorate (Rosato et al., 1991). The selection color was white because of excellent visibility for gel detection.

The basis of our plate SCC scoring is a combination of the mixed solution viscosity and the physical appearance of the plastic plate. The active chemical ingredients that use in study was used as standard CMT reagents. Research has

proved that the use of this formula chemical reagent as CMT reagent can discriminate SCC in levels and associated with Fossomatic™ FC SCC (Eardmusic, 2011). The CMT gel viscosity detection of begins with potassium salt destroys cell membrane of somatic cells in milk. Proteins and DNA leak out of cell then bind with Sodium Lauryl Ether Sulfonate and Linear Alkylbenzene Sulfonate the Fibrilla gel network are formed. The fibrillar gel network caused gel formation detection and gel viscosity (Whyte et al., 2005). CMT scores based on gel formation and the viscosity of the mixing solution changed. The sample can be semiquantitatively scored to allow for sample comparison and to facilitate communication of the severity of milk quality (Plummer and Plummer, 2012). The reagent also contains a pH indicator that will turn from blue to yellow in acidic milk to identify the acidity of BTM.

The previous study focused on the fundamental biochemistry of CMT gel formation to investigate the gel structure. They found that CMT gel is a detergent bond with DNA and histone complex which is extremely difficult to control and to quantify. However, the gel formation has the potential to be used as the basis of a reliable estimating the SCC of milk from individual cows or quarters or from the bulk milk tank provided that carefully controlled. (Whyte et al., 2005). Our plastic plate uses the same principal as CMT. However, the design of plastic plate with clusters of plastic slits give objective scoring that can reduce variation among readers of CMT, which using only eye visible on the pain plate. Besides, our plate SCC scoring added the physical appearance of the plastic plate. The detection of gel remaining on the slits can reduce the human validation among the scoring. Moreover, samples can semiquantitative score to communicate the severity of milk quality easier and more precise than CMT. The mixed solution viscosity of was clearly discriminated the SCC in levels ($p<0.05$) and was significantly correlated with Fossomatic™ FC SCC ($r= 0.15$, $p<0.05$). Our results are showed the same correlation direction as the study in 2012.The previous study found the correlation high correlation ($r = 0.78$, $p<0.01$) and no significant difference between SCC by using direct microscopic method (DMSCC) and viscosity values somatic cell count (VMSCC), assessed by a viscosity meter for determination of SCC of BTM samples collected from smallholder dairy farms (Atasever et al., 2012).

At laboratory, three patterns of plastic plate prototype (hole, grid and slits) were designed according to the principles of physical and chemical appearance. The hole patterns can indicate gel formation but gels could not adhere to any hole patterns on plastic plate. The failure of gel adhesion may affect by adhesive force of gel and hole surface area. This result may cause by the force between holes surface and gels were low. The holes of plastic plates were resized but it still cannot hold the gels of high SCC level. The patterns had changed to grid patterns. Gels can attach in the grids but plastic plate prototypes still cannot clearly discriminate SCC in levels. The grid plastic plate patterns (2A to 2E) (appendix 2B) began to show the gel formation adherence at SCC more than 300,000 cells/ml. This plastic plate prototype discriminates 300,000 cells/ml and 500,000 cells/ml. However, this plastic plate prototype could not detect the difference between 100,000 cells/ml and 300,000 cell/ml. This may because the high capillary force. The third plastic plate prototype was designed to slits pattern in order to reduce the tension force between the mixed solution and plastic plate. BTM SCC was clearly classified into levels in the model 3C (appendix 2B). However, this model was still subjective reading due to the mixed solution pouring position. This obstacle was solved by designed pouring chamber in which the mixed solution was allowed to automatically reach the end of plastic plate as shown in model 3D to 3G (appendix 2B). The design of milk chamber used the principle of dike to let mixed solution automatically reach the end of plastic plate. Our plate SCC scoring also show the high correlation (r=0.80, $p<0.05$) between plate SCC scoring and Fossomatic™ FC SCC and the high correlation (r=0.95, $p<0.05$) with California Mastitis Test (CMT). The correlation coefficient can indicate our plate SCC scoring is consistent the same way with Fossomatic™ FC SCC and CMT. However, Kappa value also depends upon the number of categories (Sabour et al., 2017). The correlation of Plastic plate scoring and can improve by rescoring.

At MCC, the average SCC of studied samples was more than Thai milk quality standard but milk compositions were in the normal range. Our study performed in July which is the raining season of Thailand. The raining season may cause dirty of bedding and contaminated pathogens in milk. These factors may result high SCC in some farm BTMs (Sharma et al., 2011). The test performances of plastic plate final

model 3G SCC scoring in laboratory and field condition are acceptable. The highest true positive, which means farm BTM samples were correctly identified as that SCC level was found in score 3 (48.20%). The highest true negative, which means farm BTM samples were correctly identified as not that SCC level was found in score 0 (94.75%). For the false results, the highest false positive was found in score 2 (9.18%), which means 9.18% of farm BTM at SCC 300,000 to 499,999 cells/ml incorrectly identified as this plastic plate. For the false negative, the highest false negative was also found in score 1 (79.34%), which means 79.34% of farm BTM at SCC 100,000 to 299,999 cells/ml incorrectly identified in this plastic plate. Our plate SCC scoring still found the variation among scores especially in score 1 (SCC 100,000 to less than 300,000 cells/ml) and score 2 (SCC 300,000 to less than 500,000 cells/ml). The variation of SCC might affect by the chemical reaction limitation and time-dependent. We found scoring results can be changed if time over 20 seconds after mixing. The study about the association of chemical reaction and SCC less than 500,000 cells/ml especially SCC ranged 100,000 to 300,000 cells/ml must be investigated. How the reaction is created and a suitable amount of chemical reagent to lysis somatic cells and blind with DNA in each SCC level must be investigated more. The molecular-scale on the gel structure in the interaction, which can help to discriminate SCC in the level clearly.

A diagnostic test accuracy study provides evidence on how well a test correctly identifies or rules out disease (Mallett and Halligan, 2012). The acceptable sensitivity of test preferably more than 50% to evaluate the validity of a single test compared to a gold standard (Sabour et al., 2017). Our study revealed that the sensitivity and specificity of the Plate SCC scoring are high and acceptable at score 0 and score 3. The sensitivity of the test is the ability to detect the presence of a disease, and it is calculated as the proportion that had a disease and a positive test. The specificity of the test is the ability to detect that did not have a disease, and it is calculated as the proportion of non-disease that had a negative test. Generally, as the sensitivity of a test increases, the specificity will decrease (Parikh et al., 2008). This was not demonstrated clearly in this study. The low sensitivity of score 1 and 2 probably due to failure of interaction between the chemical reagent and somatic

cells give less gel formation to detection. The predictive values of the test reflect how the test results could be interpreted in the field. Positive predictive value (PPV) is the probability of disease given a positive test, and negative predictive value (NPV) is the probability of no disease following a negative test. These values are highly useful for clinical purposes because they give the clinician an indication of the likelihood of disease or a specific event such as death (Trevethan, 2017). The PPV in this study indicates the likelihood that a BTM SCC below the standard with a positive to Plate SCC scoring results. Conversely, the NPV indicates the likelihood that a BTM in each SCC level, which test indeed wrong level of SCC. The predictive value of any test is influenced not only by the test sensitivity and specificity but also by the prevalence within the population (Lalkhen and McCluskey, 2008). Thus, the change of population and prevalence can affect test predictive values.

The sampling number in field study is also the limitation of this study. Number of samples can result in the wide range of standard deviation values (Sabour et al., 2017). This affected test the variation of SCC among scores. BTM should be enough sampled to ensure that the variation of results affected by the plastic plate scoring method. The further study should establish efficient sampling method to control the effects of sampling number to ensure that the variation among scores come from plate SCC scoring itself.

Our plastic plate scoring is the semiquantitative test because testing does not measure the precise quantity of somatic cells in bulk tank milk. Our tests are expressed as an estimate of how much of measured somatic cell is present by the amount of gel formation. Test results were shown as Negative, 1, 2, or 3. Qualitative and semiquantitative testing must be monitored by quality control processes. These processes should use controls that mimic samples as much as possible (International Health Regulations, 2015). A quality control program for all of the time using this semiquantitative test should be further established. Moreover, we focused on test validity only Intra-rater reliability aspect in this study, which was showed the degree of agreement among repeated administrations of a diagnostic test performed by a single reader. Further study should be done on the inter-rater reliability,

which is the degree of agreement among readers. It will indicate a score of how much homogeneity or consensus of test exists in the ratings given by various readers.

Thai standards must be standardized. Thai MCCs must be using the same threshold for milk grading, milk pricing, and milk quality control. Thai MCC must strictly follow the rules to eliminate under standard milk for adverse consequences of high SCC milk on the dairy product quality and customer health. The milk quality control must be based on BTM SCC need whole chain cooperation including farmer routine cow or quarter checking, MCC routine BTM SCC checking, and milk factory checking. Veterinary service should be emphasized, and the official standard or the law must be strictly followed. The development of plate design must be focused on how more precise SCC can fit on the Thai standard. The methods for bulk tank milk SCC device invention and application for quantitative and qualitative propose of SCC at milk collecting center has rarely been studied. This may because the milk production chain of Thailand and South East Asia characteristic are difference from other countries that has no MCCs. Invented machines from western countries still are impractical and not suitable for Thai MCC because of economics and on-site working. For example, many kinds of portable somatic cell counters are electricity needed and unfitted size for milk quality grading at a milk collecting point. Hence, at the MCC, it seems not practical to use. Moreover, the counters machine maintenance cost is too high for our country because counters are imported from western countries, spares are difficult to find in Thailand and must be sent to the headquarters to repair. For mobile application cell counters, the device is accurate, and sizes are practical for MCCs SCC testing, but the reagents, cassettes, pipettes, or accessories are single-use. Fluency accessories may result in a high cost for every additional batch (about £100 or about ฿400 per sample).

Technology that enables the detection of more than a human can detect is presumed to show the most potential for comprehensive use in more than one application field. In addition, computers and robots can help human work more comfortable than in the past. Artificial intelligence and Humans working together might bring significant productivity gains in the future.

CHAPTER 6
CONCLUSION

The plastic plate prototype with dimension 15.8x5.3x0.8 cm (Length x Width x Height) is designed. There are 3 clusters of slits on a plastic plate with one milk tank with dimension at the bottom. This plate is made from acrylonitrile butadiene styrene (ABS) filament material using the 3D printer. BTM semiquantitative SCC, which called "Plate SCC scoring," was determined by mixing BTM with the specific chemical reagent in ratio 1:1 (10:10 ml) then pouring into a plastic plate tank. After 10 seconds, pulling plastic plate up pedicular and scoring BTM SCC depending on gel formation. The plate SCC scoring can use at Thai milk collecting centers to grade premium milk and penalty milk. The premium-grade milk can be discriminated at score 0 while the penalty milk can discriminate at score 3. This plate SCC scoring can be used as a new indirect semiquantitative device for BTM SCC at Thai MCC and cooperatives, which grading milk at 100,000 cells/ml and 500,000 cells/ml with acceptable test performances, including the percentage of high accuracy, sensitivity, specificity, predictive values, and repeatability. BTM SCC by plate SCC scoring is highly correlated with Fossomatic™ FC SCC and highly correlated with CMT. However, the variation among scores 1 and 2 must be improved in the future.

This plastic plate is now on patent registration process, pending number 1903000472. We hopefully Thai MCCs can use this new semiquantitative test to grade premium grade and penalty grade milk in the next few months.

APPENDIX

Appendix 1 Currently somatic cell count methods

A. Portable direct somatic cell count

1. Florescence staining and flow cytometry methods

1.1. The NucleoCounter® SCC-100™ Somatic Cell Counter (ChemoMetec A/S, Gydevang 43, DK-3450 Allerod, Denmark)

TECHNICAL SPECIFICATIONS	
Measurement range	10,000 to 2,000,000 Cells/ml with an optimal measurement range of between 10,000 to 1,000,000 Cells/ml
Analysis time	30 seconds
Capacity	Up to 100 samples per hour
WF	Working Factor = 1,000
Size	38 x 26 x 22 cm (W x H x D)
Weight	3 kg / 6.7 lb
Printer	External printer for documentation – optional
Software	SomaticView™ computer software for documentation and presentation – optional

1.2. Lactoscan Somatic cell counter

(MILKOTRONIC LTD, 4, Narodni Buditeli Street, 8900 Nova Zagora, Bulgaria)

TECHNICAL SPECIFICATIONS	
Measurement range	0 – 10,000,000 cell/ml
Analysis time	20 to 60 seconds
Size	20.5 x 29.0 x 30.5 cm (W x H x D)
Weight	lower than 9.0 kg
Software	The results are color-coded. Takes up to 60 images by computer-controlled X: Y movements and then processes these with special image analysis software. Stores an unlimited number of records in the database. Automatic software update and remote service
Price	Cost: €2,973.44 (฿118,937.6) Lactoscan SCC Kit x4 €132 (฿5,280)

1.3. DeLaval Cell Counter (DCC)

(DeLaval International AB, Stockholm, Sweden)

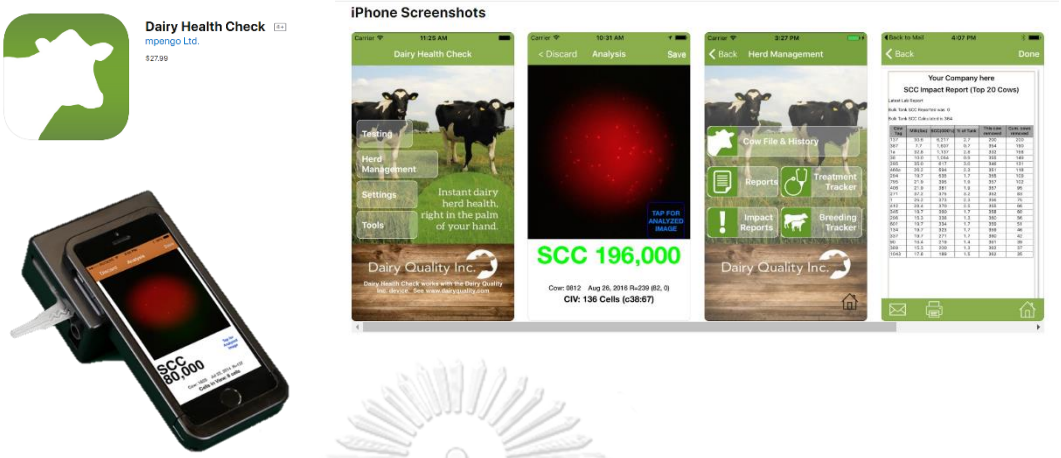
TECHNICAL SPECIFICATIONS	
Measurement range	10,000 to 4,000,000 cell/ml
Analysis time	Less than 45 seconds
Sample volume	Approx. 60 µl in the cassette
Measuring volume	Approx. 1 µl in the cassette
Size	23.5 x 24.9 x 23.6 cm (W x H x D)
Weight	4.1 kg
Software	DeLaval database kit DCC to store SCC data, the possibility to list and print SCC, easy way to compare SCC in bulk milk on day to day basis and to compare SCC on cow and quarter level over lactations
Price	Cost: € 4,487.58 (฿179,503.2) A box of DCC cassettes (72 cassettes) € 96.25 (฿3,840)

2. Connect with Mobile Application

1.1. DeLaval Cell Counter ICC Mobile SCC Detection

- Use with DeLaval cassette
- Connect with DeLaval Cell Counter ICC mobile application
- It has features to help track results of the herd, storing dates and results by cow tag. Information can be sent from the iPod Touch as reports to desktop or veterinarian or can be used in tandem with herd management software.
- SCC results in 45 seconds
- Price:
  - Device US $2,195.00 (฿66,804.64)
  - Application US $27.99 (฿851.87)
  - Cassette (72 cassettes) € 96.25 (฿3,840)

1.2. Dairy Quality RT10™ Somatic Cell Count Tester (Dairy Quality Inc.)



- Use with The DeLaval cassettes (DeLaval Inc.)
- Connect with Dairy Health Check (mpengo Ltd.) mobile application
- The device cans work with an iPod touch or iPhone to deliver test results.
- Device provides SCC analysis and an image of cells in test samples in seconds.
- Real-time Data Storage - Store images, results and records automatically
- Price:
  - Device US $2,195.00 (฿66,804.64)
  - Application US $27.99 (฿851.87)
  - Cassette (72 cassettes) € 96.25 (฿3,840)

B. Indirect somatic cell count

1. Relative viscosity methods

1.1. Ekomilk Horizon Unlimited

(Ekomilk, Promishlena Str., Industrial Area, 6000, Stara Zagora, Bulgaria)

TECHNICAL SPECIFICATIONS	
Measurement range	80,000 – 15,000,000 with accuracy ± 8%
Analysis time	Approx. 1 min
Size	27 x 32 x 32 cm (W x H x D)
Weight	6.5 kg
Software	A hybrid milk analyzer using the ultrasonic method for milk parameters measuring and worldwide accepted viscosity method to determine the Somatic Cell Count (SCC). The measuring results are automatically transferred to the Animal Monitoring Platform (AMP) for easy visualization, monitoring and analyzing.
Price	Cost €3,400.00 (฿136,000)

2. Chemical reaction

b. The Porta SCC milk test (PortaScience, Portland)

- The test is based on a chemical reaction between a dye on the test strip and an enzyme found in the cells in the milk. This reaction makes the test strip sample well change to blue color. The darker the blue, the higher the cell count. After 45 mins estimate the somatic cell count by comparing the strip to the Color Chart or by using the Digital Reader (Multiply by 1,000,000 to obtain the number of somatic cells/ml)
- The product has proven to be able to determine accurate data below 100,000 cells/ml.
- The PortaSCC Milk test can be used to:

- Identify problem cows or quarters.
- Monitor response to treatment.
- Check cows at freshening and dry off.
- Monitor udder health.
- Screen a herd or a group of cows.

- Price 24 test per box €314.60 (฿12,584)

c. PortaSCC Quick Test (PortaScience, Portland)

- The test is based on a chemical reaction between a dye on the test strip and an enzyme found in the cells in the milk. This reaction makes the test strip sample well change to blue color. The darker the blue, the higher the cell count.
- A quick 5-minute test to estimate the somatic cell count in cow milk without a reader.
- Check for

- problem cows or quarters
- response to treatment
- cows at freshening and dry off
- a group of cows

- Price 40 tests per box €64.13 (฿2,565.2)

2.3 SCC Dunk milk test (AR Brown Co., Ltd Specialty Chemical dep Agri Food safety FCF team, Japan)

- Purpose: Screening Test Kit for neutrophils in individual and bulk milk
- The test is based on a chemical reaction between a dye on the test strip and an enzyme found in the cells in the milk. This reaction makes the test strip sample well change to blue color. The darker the blue, the higher the cell count.
    - Low: 100,000 to 200,000 grayish slightly white
    - Moderate: 300,000 to 400,000 light blue
    - High: more than 500,000 dark blue
- Reaction time: 5 to 6 minutes at Room Temperature in summer and 6 to 8 minutes in winter
- Reaction Temperature of Milk: Form Room Temperature to 40℃ Most Suitable Temperature for Reaction: 25℃ and more.
- It is no problem with using milk at room temperature, but it is more obviously color reaction by warming milk.

Appendix 2 Plastic plate designs

A. Plastic materials characteristics (Bell, 2019)

1. ABS

   Ease of use: Expert

   Print temperature: 210°C – 250°C

   Print bed temperature: 80°C – 110°C

   Advantages: high strength, better UV resistance for outdoor applications, commonly used in household goods, relatively heat-resistant compared to PLA and phenomenal layer adhesion.

   Disadvantages: Noticeable odor and requires venting while printing, considerable warping or shrinkage issues, heat bed required, concern over Volatile Organic Compound (VOC) emissions (especially for students with respiratory ailments) and full enclosure needed for heat regulation and ensuring proper ventilation.

2. PETG

   Ease of use: Medium

   Print temperature: 220°C – 250°C

   Print bed temperature: 50°C – 75°C

   Advantages: Incredible print bed adhesion, improved flexibility over PLA, high strength, minimal warping or shrinking, resulting prints are relatively heat-resistant compared to PLA and great layer adhesion.

   Disadvantages: Some odor, the filament absorbs moisture if stored in the open (leading to poor print performance), requires a heated print bed and print bed separator recommended (painters tape or glue stick) to prevent permanent bond.

3. PLA

   Ease of use: Easy

   Print temperature: 180°C – 230°C

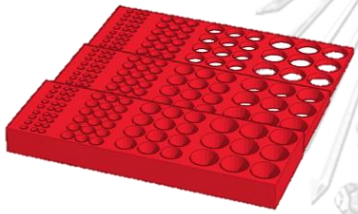
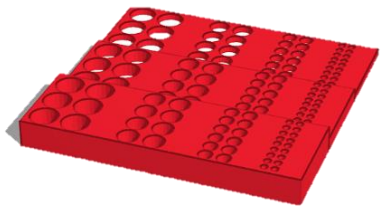
   Print bed temperature: No heat required, 20°C – 60°C (optional)

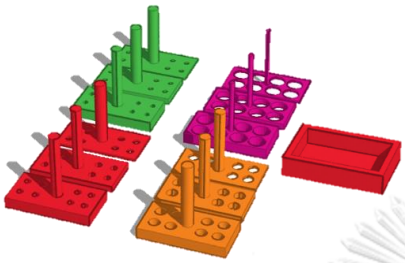
Advantages: Relatively odorless, minimal warping or shrinkage, an incredible number of filament variations, inexpensive, heated print bed not required, limited biodegradable and limited recyclable.

Disadvantages: Brittle prints with relatively low mechanical strength compared to other materials and melts easily under high heat

B. Plastic plate patterns were divided into 3 subgroups:

1) Hole patterns

Plastic Model	Characteristics
Model 1A 	- 3 plastic plates size 200x68 mm with plastic thickness: 5 mm, 10 mm and 15 mm - Each plate contains holes with diameter 20 mm (9 holes), 15 mm (12 holes), 10mm (18 holes) and 5 mm (54 holes) - Application: pour mixture solution cover every hole and pull plates up pedicular - Estimate SCC level by gel formation remaining in holes.
Model 1B 	- 3 plastic plates sizes 198x65 mm with 3 thickness: 5 mm, 10 mm and 15 mm - Each plate contains holes with diameter 20 mm (6 holes), 15 mm (8 holes), 10 mm (12 holes) and 5 mm (18 holes) - Application: pour mixture solution cover every hole and pull plates up pedicular

	- Estimate SCC level by gel formation remaining in holes
Model 1C 	- 3 plastic plates size 75x30 mm with 3 thickness: 5 mm, 3 mm and 1 mm - Each plate contains holes with diameter 20 mm (1 hole), 15 mm (1 hole), 10 mm (2 holes), 5 mm (4 holes) and 3 mm (4 holes) - Application: Put plates in a plastic tank then pour mixture cover every plate and pull plates up pedicular. - Estimate SCC level by gel formation remaining in holes
Model 1D 	- Plastic plates size 40x20 mm with handle. - Plastic plate thickness: 5 mm, 3 mm and 1 mm - Each plate contains 4 holes with diameter: 2 mm, 3 mm, 5 mm and 8 mm. - Application: Put plates in a plastic tank then pour mixture cover every plate and pull plates up pedicular. - Estimate SCC level by gel formation remaining in holes

2) Grids patterns

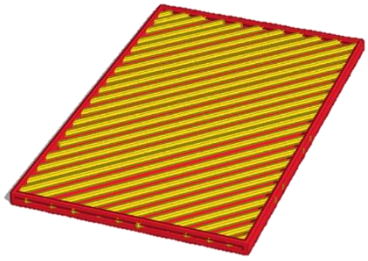
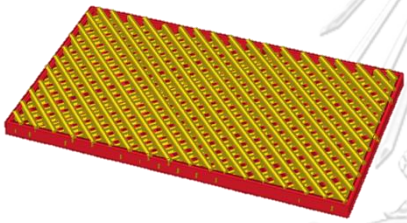
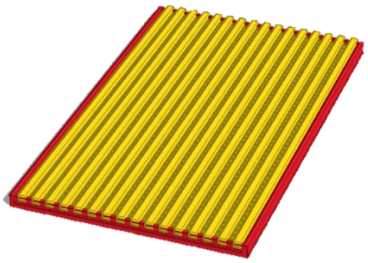
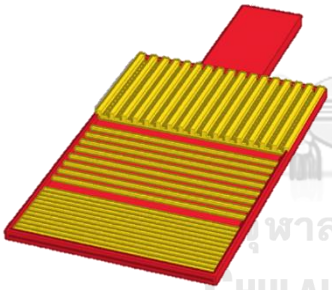
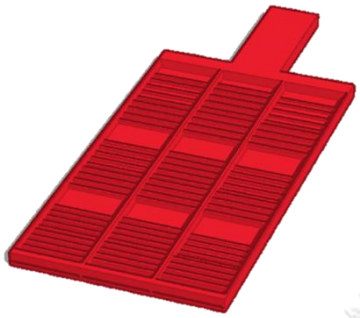
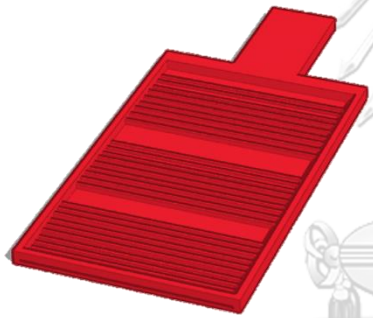
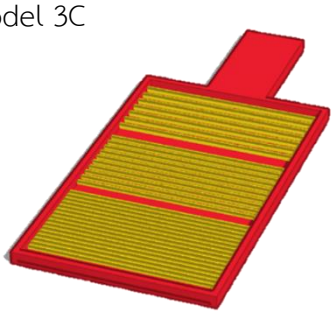
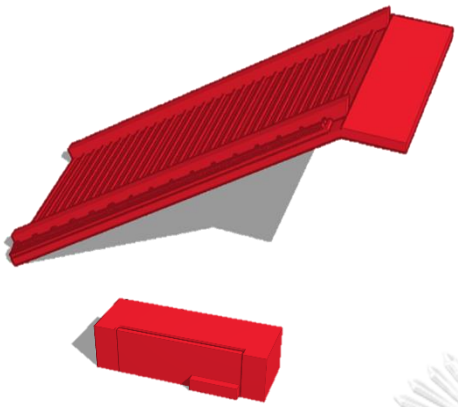
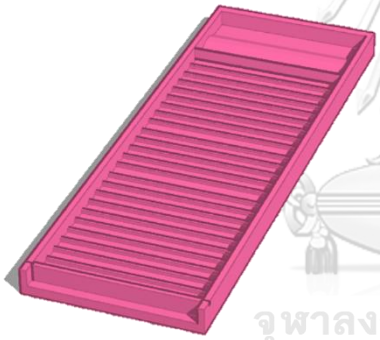
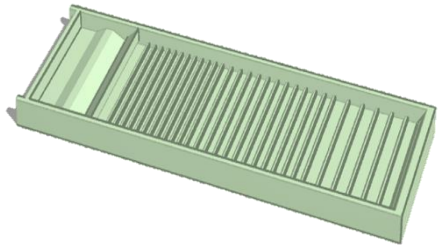
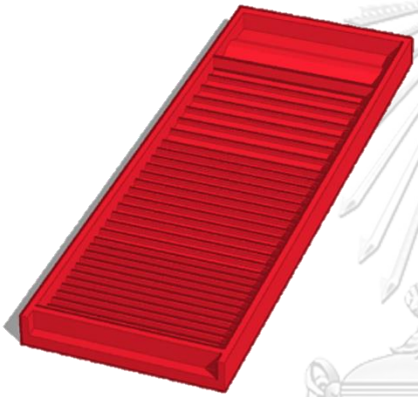
Plastic Model	Characteristics
Model 2A 	- Plastic plate size 110x60x3.5 mm - The plastic plate contains 1 mm thick diagonal lines with 5 mm space between lines. - Application: Put plastic plates in a plastic tank then pour mixture cover the plate and pull the plate up pedicular. - Estimate SCC level by gel formation remaining in the channels between lines
Model 2B 	- Plastic plate size 110x60x3.5 mm - The plastic plate contains 2 layers of 1 mm thick diagonal lines with 1.5 mm space between layers and 3 mm space between lines in the 1st layer and 5 mm space between lines in the 2nd layer. - Application: Put plates in a plastic tank then pour mixture cover the plate and pull the plate up pedicular. - Estimate SCC level by gel formation remaining in channels between lines
Model 2C 	- Plastic plate size 110x60x3.5 mm - The plastic plate contains 2 layers of 1 mm thick lines with 1.5 space between layers and 3 mm space between lines in the 1st layer and 5 mm space between lines in the 2nd layer. - Application: Put plates in a plastic tank then pour mixture cover the plate and pull the

	plate up pedicular. - Estimate SCC level by gel formation remaining in channels between lines
Model 2E 	- Plastic plate size 100x60x4 mm with a 20x40x4 mm handle. - The plastic plate contains 3 holes with 25 mm diameter which were covered by lines in different spaces. - Application: Pour mixture cover the plate and pull the plate up pedicular. - Estimate SCC level by gel formation remaining in holes
Model 2F 	- Plastic plate size 100x60x4 mm with a 20x40x4 mm handle. - The plastic plate contains 3 clusters: grids with 5 mm space between 3.5x1.5x1 mm lines, 1x1x60 mm crosslines with 5 mm space between lines and 1x1x60 mm crosslines with 3 mm space between lines. - Application: Pour mixture cover the plate and pull the plate up pedicular. - Estimate SCC level by gel formation remaining in plate

3) Slits patterns

Plastic Model	Characteristics
Model 3A 	- Plastic plate size 100x60x4 mm with a 20x40x4 mm handle. - The plastic plate contains 3 clusters of 1x1x1 crosslines with 5 mm, 3mm and 1 mm space between lines mixed together. - Application: Pour mixture cover the plate and pull the plate up pedicular. - Estimate SCC level by gel formation remaining in slits
Model 3B 	- Plastic plate size 100x60x4 mm with a 20x40x4 mm handle. - The plastic plate contains 3 clusters of 1 mm thickness crossline with space between line equal to 1.5, 2 and 2.5 mm. - Application: Pour mixture cover the plate and pull the plate up pedicular. - Estimate SCC level by gel formation remaining in slits
Model 3C 	- Plastic plate size 100x60x4 mm with a 20x40x4 mm handle. - The plastic plate contains 3 clusters of crossline 1.5 mm, 2 mm and 2.5 mm thickness with space between line equal to 1, 1.5 and 0.5 mm. - Application: Put plates in a plastic tank then pour mixture cover every plate and pull plates up pedicular. - Estimate SCC level by gel formation

	remaining in slits
Model 3D 	- Plastic plate size 100x60x4 mm with a 20x40x4 mm handle. - The plastic plate contains 3 clusters of 1x1x1 crosslines with 5 mm, 3mm and 1 mm space between lines mixed together. - Application: Pour mixture in milk tank and open the opener, milk automatically pours down the plastic plate. Then, pull the plate up pendicular. - Estimate SCC level by gel formation remaining in slits
Model 3E 	- Plastic plate size 158x50x3 mm (depth 2.2 from the edge) with milk tank sizes 50x25x8 mm with slopes at the bottom of the tank to let the mixture down to slits automatically. - The plastic plate contains 1x1x50 mm crosslines with a 5 mm space between lines. - Application: Pour mixture in milk tank and wait until mixture touch the opposite end of the plate, then pull the plate up pedicular. - Estimate SCC level by gel formation remaining in slits
Model 3F	- Plastic plate size 158x50x3 mm (depth 2.2 from the edge) with milk tank sizes 50x25x8 mm at a cluster of 2 mm space with slopes at the bottom of the tank to let the mixture down to slits automatically. - The plastic plate contains 3 clusters of

	1x1x50 mm crosslines with 5 mm, 3 mm and 2 mm space between lines in each cluster. - Application: Pour mixture in milk tank and wait until mixture touch the opposite end of the plate, then pull plate up pedicular. - Estimate SCC level by gel formation remaining in slits.
Model 3G 	- Plastic plate size 158x50x3 mm (depth 2.2 from the edge) with milk tank sizes 50x25x8 mm at the cluster of 5 mm space with slopes at the bottom of the tank to let the mixture down to slits automatically. - The plastic plate contains 3 clusters of 1x1x50 mm crosslines with 5 mm, 3 mm and 1 mm space between lines in each cluster. - Application: Pour mixture in milk tank and wait until mixture touch the opposite plate, then pull plate up pedicular. - Estimate SCC level by gel formation remaining in slits.

C. Specific chemical reagent preparation

1) Formulation

The active ingredient of chemical reagents of specific chemical reagent was 1.2% w/w of Linear Alkylbenzene Sulfonate and Potassium Salt combined with 0.86% w/w of Sodium Lauryl Ether Sulfonate. Bromocresol purple was used as a chemical reagent pH indicator.

2) Preparation of 1-liter specific chemical reagent

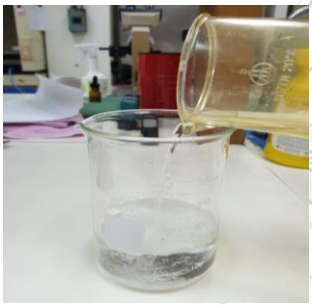
2.1) Prepared 120 ml of solution composed of

- 1.2% w/w Linear Alkylbenzene Sulfonate, Potassium salt
- 0.86% w/w Sodium Lauryl Ether Sulfate

2.2) Adjust volume to 1-liter with 880 ml of distilled water

2.3) Add 0.1 g of Bromocresol purple for a pH indicator

2.4) Adjust chemical reagent pH to 7.0 with 1M NaOH



Appendix 3 The information on machines and tests

A. Fossomatic™ FC somatic cell count (FOSS Foss Allé 1 DK-3400 Hilleroed, Denmark).

SCC (cells/ ml) was automatically measured by Fossomatic™ FC machine (FOSS Foss Allé 1 DK-3400 Hilleroed, Denmark). In the beginning, the machine must do the daily preparation process including reagent preparation, machine calibration, and machine performance testing according to the manufacturer protocol. The milk samples are warmed in the water bath at 37-40 degrees Celsius for 15 minutes and mixed carefully by turn bottle up and down.

Samples will put under pipette one by one. The results of SCC were automatically interpreted and recorded in the program.

Performance	
Measuring range	0 to 10,000,000 cells/ml
Performance range	0.1 to 1.5 ml
Repeatability	CV < 6% at 100,000 to 299,999 cells/ml CV < 4% at 300,000 to 499,999 cells/ml CV < 3% at 500,000 to 1,500,000 cells/ml
Repeatability with precision setup in use	CV < 3.5% at 100,000 to 299,999 cells/ml CV < 2.5% at 300,000 to 499,999 cells/ml CV < 2% at 500,000 to 1,500,000 cells/ml
Accuracy	< 10% relative mean difference from Direct Microscopic Somatic Cell Count
Carry-over	< 1% relative usually below 0.4%

B. California Mastitis Test (Schalm, 1957)

California Mastitis Test or CMT was carried out by mixing an equal volume of bulk tank milk samples with Linear Alkylbenzene Sulfonate and Potassium Salt combined with 0.86% w/w of Sodium Lauryl Ether Sulfonate. Bromocresol purple was used as a chemical reagent pH indicator and pH was adjusted to 7. Each milk sample was placed in one clean well of a white plastic test paddle divided into four separate wells, one for each sample. As the plate was rotated gently for 20 seconds to make sure that the milk sample and

solution were well mixed. Any color changes or formation of a viscous gel were interpreted as by the authors above: in brief, scores were given within the range 0 to + 3, with 0 for no reaction or negative result, + 1 for a trace, + 2 for a weak positive and +3 for a strong positive.

	Score	Meaning	Description of reaction	Individual Quarter Sample	Bucket Milk Sample
	N	**Negative**	Mixture remains liquid. No slime or gel form. It can drip out of the paddle well.	No Mastitis	No Mastitis
	T	**Trace**	Mixture becomes slimy or gel like. It's seen to best advantage by tipping paddle back and forth, observing mixture as it flows over the bottom of cups.	Trace of mastitis	Mastitis in one or more quarters
	1	**Weak Positive**	Mixture distinctly forms a gel.	Mastitis	Define mastitis - Check quarters
	2	**Distinct Positive**	Mixture thickens immediately, tends to form jelly. Swirling cup moves mixture in toward center exposing outer edges of the cup.	Mastitis	Serious Mastitis – Check quarters

From Macdonald Campus Farm Cattle Complex Standard Operating Procedure # DC-617

C. MilkcoScan milk composition analysis (MilkoScan™ FT2)

Milk composition including fat, protein, casein, lactose, total solids, SNF and urea by Fourier transform infrared spectroscopy (MilkoScan™ FT2 Hilleroed, Denmark). In the beginning, the machine must do the daily preparation process, including reagent preparation, machine calibration, and machine performance testing according to the manufacturer protocol. The milk samples are warmed in the water bath at 37-40 degrees Celsius for 15 minutes and mixed carefully by turn bottle up and down. Samples will put under pipette one by

one. The results of milk composition were automatically interpreted and recorded in the program.

Feature	**Specification**
Calibration range	Up to 50% fat Up to 7% protein Up to 7% lactose Up to 55% total solids
Included calibrations	- Milk & Concentrated milk - Cream - Whey and permeate - Concentrated whey - Yoghurt & Fermented products - Dessert and ice cream
Accuracy	≤ 1% CV on major raw cow milk components (Fat, Protein, Lactose, Total solids)
Repeatability	≤ 0.25% CV on major raw cow milk components (Fat, Protein, Lactose, Total solids)
Analysis time	30 seconds for milk
Sample volume	8 ml
Sample temperature	5 to 55 ℃ (the sample must be homogeneous)

Aiumlamai S, Chanlun A, Sakolapong V, van Oosteveen F, Jarassaeng C, Krajangpo C and Pattanawong J 2000. Prevalence of organisms causing subclinical mastitis in Amphur Muang, Khon Kaen. Proceedings of the 26th Annual conference the Thai Veterinary Medical Association Udder the Royal Patronage, Bangkok, Thailand:15-17.

Ajariyakhajorn K, Samngamnim S, Boonserm T, Inchaisri C, Thirapatsakun T and Farnsworth R 2003. Mastitis in small dairy holders of Nakhonpathom province, Thailand. Proceedings of the 11th International Symposium of the Word Association of Veterinary Laboratory Diagnosticians and OIE Seminar on Biotechnology:9-13.

Atasever S, Erdem H and Kul E 2012. Using viscosity values for determining somatic cell count in cow milk. Asian J. Anim. Vet. Adv. 7: 441-445.

Auldist M and Hubble I 1998. Effects of mastitis on raw milk and dairy products. Aust. J. Dairy Technol. 53(1): 28-36.

Barbano DM, Rasmussen RR and Lynch JM 1991. Influence of milk somatic cell count and milk age on cheese yield1. J. Dairy Sci. 74(2): 369-388.

Bell D 2019. 3D Printing Materials Guide for Classrooms. Retrieved May, 2018, from https://blog.tinkercad.com/3d-printing-materials-guide.

Biffa D, Debela E and Fekadu B 2005. Prevalence and risk factors of mastitis in lactating dairy cows in southern Ethiopia. Intern. J. Appl. Res. Vet. Med. 3(3): 189-198.

Boonyayatra S and Chaisri W 2003. Prevalence of subclinical mastitis in smallholder dairy farms of Chiang Mai province, Thailand. Chiang Mai V J. 2547(2): 6.

Boutinaud M and Jammes H 2002. Potential uses of milk epithelial cells: a review. Reprod. Nutr. Dev. 42(2): 133-147.

Brandt M, Haeussermann A and Hartung E 2010. Invited review: technical solutions for analysis of milk constituents and abnormal milk. J. Dairy Sci. 93(2): 427-436.

Bureau of Quality Control of Livestock Product Department of Livestock Development 2013. Standard for milk quality of Thailand and trading partner countries. Retrived October 16, 2019, from

http://dairydevelopmentprogram.weebly.com/blog-361536343619366036173626364035 86/3.

Cicconi-Hogan KM, Gamroth M, Richert R, Ruegg PL, Stiglbauer KE and Schukken YH 2013. Associations of risk factors with somatic cell count in bulk tank milk on organic and conventional dairy farms in the United States. J. Dairy Sci. 96(6): 3689-3702.

Davies DT and Law AJ 1983. Variation in the protein composition of bovine casein micelles and serum casein in relation to micellar size and milk temperature. J. Dairy Res. 50(1): 67-75.

Dingwell RT, Leslie KE, Schukken YH, Sargeant JM and Timms LL 2003. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can. Vet. J. 44(5): 413-416.

Dohoo IR and Leslie K 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Prev. Vet. Med. 10(3): 225-237.

Eardmusic S 2011. Factors associated with high bulk tank somatic cell count in small dairy holders. (Master's thesis) Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

Ezzat Alnakip M, Quintela-Baluja M, Böhme K, Fernández-No I, Caamaño-Antelo S, Calo-Mata P and Barros-Velázquez J 2014. The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions. Vet. Med. 2014: 1-31.

Fernandes AM, Bovo F, Moretti TS, Rosim RE, Lima CGd and Oliveira CAFd 2008. Casein fractions of ultra high temperature milk with different somatic cell counts. Pesq. agropec. bras. 43(1): 149-152.

Fernandes AM, Oliveira C and Lima CG 2007. Effects of somatic cell counts in milk on physical and chemical characteristics of yoghurt. International Dairy Journal. 17(2): 111-115.

Godden SM, Bey RF, Farnsworth RJ, Reneau JK and LaValle M 2002. Field Validation of a Milk Line Sampling Device for Monitoring Milk Quality and Udder Health. Journal of Dairy Science. 85(6): 1468-1475.

Gonzalo C, Linage B, Carriedo JA, de la Fuente F and Primitivo FS 2006. Evaluation of the overall accuracy of the DeLaval cell counter for somatic cell counts in ovine milk. J. Dairy Sci. 89(12): 4613-4619.

Gordon WA, Morris HA and Packard V 1980. Methods to Detect Abnormal Milk – A Review. Journal of Food Protection. 43(1): 58-64.

Gunasekera T, Veal D and Attfield P 2003. Potential for broad applications of flow cytometry and fluorescence techniques in microbiological and somatic cell analyses of milk. Int. J. Food Microbiol. 85(3): 269-279.

Hagnestam-Nielsen C, Emanuelson U, Berglund B and Strandberg E 2009. Relationship between somatic cell count and milk yield in different stages of lactation. J. Dairy Sci. 92(7): 3124-3133.

Hagnestam-Nielsen C and Østergaard S 2009. Economic impact of clinical mastitis in a dairy herd assessed by stochastic simulation using different methods to model yield losses. Animal. 3(2): 315-328.

Halasa T, Huijps K, Østerås O and Hogeveen H 2007. Economic effects of bovine mastitis and mastitis management: a review. Vet Q. 29(1): 18-31.

Hamann J 2005. Mastitis in dairy production: current knowledge and future solutions. Vol 1. 1st ed. In: Wageningen Academic Publishers, Netherland. 82-90.

Harmon R 1994. Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci. 77(7): 2103-2112.

Information and Communication Technology center 2018. Livestock data in Thailand 2018. Page 191. Department of Livestock Development, ed, Bangkok.

International Health Regulations 2015. Laboratory quality management system handbook. Pages 91-100 *in* Procedure Control: Quality Control of Qualitative and Semi-Quantitative Procedures. Wold Health Organization, ed. WHO Press, World Health Organization, France.

Janzen J 1970. Economic losses resulting from mastitis. A review. Journal of Dairy Science. 53(9): 1151-1160.

Jayarao BM, Pillai SR, Sawant AA, Wolfgang DR and Hegde NV 2004. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci. 87(10): 3561-3573.

Jenness R and Patton S 1974. Fundamentals of dairy chemistry. 3 ed. In: Physical properties of milk. Aspen Publishers, Inc., New York, USA. 402-441.

Kampa J, Sukolapong V, Chaiyotwittakun A and Polpakdee A 2010. Chronic mastitis in small dairy cattle herds in Muang Khon Kaen. Thai J. Vet. Med. 40(3): 265-272.

Kamrich JP and Schoff CK 1999. Rheological Measurements Vol 2. In: Encyclopedia of Chemical Technology. 1760-1764.

Kelly PT, O'Sullivan K, Berry DP, More SJ, Meaney WJ, O'Callaghan EJ and O'Brien B 2009. Farm management factors associated with bulk tank somatic cell count in Irish dairy herds. Ir. Vet. J. 62: 45-51.

Khan MZ and Khan A 2006. Basic facts of mastitis in dairy animals: a review. Pak. Vet. J. 26(4): 204-208.

Kim B, Lee YJ, Park JG, Yoo D, Hahn YK and Choi S 2017. A portable somatic cell counter based on a multi-functional counting chamber and a miniaturized fluorescence microscope. Talanta. 170: 238-243.

Kroger D and Jasper D 1967. Relationships between Wisconsin mastitis test scores and cell counts in milk. J. Dairy Sci. 50(8): 1226-1233.

Laevens H, Deluyker H, Schukken YH, L DM, Vandermeersch R, De Muêlenaere E and De Kruif A 1997. Influence of parity and stage of lactation on the somatic cell count in bacteriologically negative dairy cows. J. Dairy Sci. 80(12): 3219-3226.

Lalkhen AG and McCluskey A 2008. Clinical tests: sensitivity and specificity. BJA Education. 8(6): 221-223.

Mallett S and Halligan S 2012. Interpreting diagnostic accuracy studies for patient care. Retrieved November, 12, from https://doi.org/10.1136/bmj.e3999.

Mamache B, Rabehi S and Meziane T 2014. Bacteriological study of subclinical mastitis in Batna and Setif governorates Algeria. J. Vet. Adv. 4(2): 364-373.

Manji B, Kakuda Y and Arnott D 1986. Effect of storage temperature on age gelation of ultra-high temperature milk processed by direct and indirect heating systems. J. Dairy Sci. 69(12): 2994-3001.

Mazal G, Vianna P, Santos M and Gigante M 2007. Effect of somatic cell count on Prato cheese composition. J. Dairy Sci. 90(2): 630-636.

Mdegela R, Ryoba R, Karimuribo E, Phiri E, Løken T, Reksen O, Mtengeti E and Urio N 2009. Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. Journal of the South African Veterinary Association. 80(3): 163-168.

Meek AH, Barnum DA and Newbould FHS Use of total and differential somatic cell counts to differentiate potentially infected from potentially non-infected quarters and cows and between herds of various levels of infection. J. Food Prot. 43(1): 10-14.

Middleton JR, Hardin D, Steevens B, Randle R and Tyler JW 2004. Use of somatic cell counts and California mastitis test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. J. Am. Vet. Med. Assoc. 224(3): 419-423.

Miller R, Paape M and Acton J 1986. Comparison of milk somatic cell counts by Coulter and Fossomatic Counters1. J. Dairy Sci. 69(7): 1942-1946.

Muhammad G, Naureen A, Asi MN and Saqib M 2010. Evaluation of a 3% surf solution (surf field mastitis test) for the diagnosis of subclinical bovine and bubaline mastitis. Tropical animal health and production. 42(3): 457-464.

National Bureau of Agricultural Commodity and Food Standards 2010. Thai Agricultural Standard (TAS) 6003-2010: Raw Cow Milk. Page 11 Vol. 127. National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

National mastitis council 2001. Normal and abnormal raw milk based on SCC and signs of clinical mastitis. 40th Annual Meeting Proceedings 2001, Ontario, Canada.

Neitzel A-C, Stamer E, Junge W and Thaller G 2014. Calibration of an automated California mastitis test with focus on the device-dependent variation. Springerplus. 760(760): 1-6.

Ogola H, Shitandi A and Nanua J 2007. Effect of mastitis on raw milk compositional quality. J. Vet. Sci. 8(3): 237-242.

Parikh R, Mathai A, Parikh S, Chandra Sekhar G and Thomas R 2008. Understanding and using sensitivity, specificity and predictive values. Indian J Ophthalmol. 56(1): 45-50.

Park Y 2007. Rheological characteristics of goat and sheep milk. Small Ruminant Res. 68(1-2): 73-87.

Park Y and Humphrey R 1986. Bacterial cell counts in goat milk and their correlations with somatic cell counts, percent fat, and protein. J. Dairy Sci. 69(1): 32-37.

Plummer PJ and Plummer C 2012. Diseases of the mammary gland. In: Sheep and Goat Medicine. 2nd ed. D. G. Pugh and A. N. Baird (eds). Saint Louis: W.B. Saunders. 442-465.

Pyörälä S 2003. Indicators of inflammation in the diagnosis of mastitis. Vet. Res. 34(5): 565-578.

Rahman M, Islam M, Uddin M and Aktaruzzaman M 2010. Prevalence of subclinical mastitis in dairy cows reared in Sylhet district of Bangladesh. International Journal of Bio Research. 1: 23-28.

Read R, Reyes A, Bradshaw J and Pleeler J 1969. Evaluation of seven procedures for detection of abnormal milk due to mastitis. J. Dairy Sci. 52(9): 1359-1367.

Rodrigues ACO, Cassoli LD, Machado PF and Ruegg PL 2009. Short communication: evaluation of an on-farm test to estimate somatic cell count. J. Dairy Sci. 92(3): 990-995.

Rosato DV, Di Mattia DP and Rosato DV 1991. The properties of plastics. In: Designing with plastics and composites: a handbook. Springer, Boston, MA. 405-406.

Ruegg P and Pantoja J 2013. Understanding and using somatic cell counts to improve milk quality. Irish J. Agr. Food Res.: 101-117.

Ruegg PL and Tabone TJ 2000. The relationship between antibiotic residue violations and somatic cell counts in Wisconsin dairy herds. J. Dairy Sci. 83(12): 2805-2809.

Sabour S, Abbasnezhad O, Mozaffarian S and Kangavari HN 2017. Accuracy and precision in medical researches; common mistakes and misinterpretations. WJRR. 4(4): 58-60.

Salvador R, Soliven R, Balagan E, Abes N, Gutierrez C and Mingala C 2014. Evaluation of a portable somatic cell counter in the diagnosis of bubaline subclinical mastitis. Thai Journal of Agricultural Science. 47(4): 205-209.

Sargeant JM, Schukken YH and Leslie KE 1998. Ontario bulk milk somatic cell count reduction program: progress and outlook. J. Dairy Sci. 81(6): 1545-1554.

Schaik GV, Lotem M and Schukken YH 2002. Trends in somatic cell counts, bacterial counts, and antibiotic Residue violations in New York state during 1999–2000. J. Dairy Sci. 85: 782-789.

Schalm O 1957. Experiments and observations leading to development of the California mastitis test. J. Am. Vet. Med. Assoc. 130: 199-204.

Schepers A, Lam T, Schukken Y, Wilmink J and Hanekamp W 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J. Dairy Sci. 80(8): 1833-1840.

Schukken YH, González RN, Tikofsky LL, Schulte HF, Santisteban CG, Welcome FL, Bennett GJ, Zurakowski MJ and Zadoks RN 2009. CNS mastitis: nothing to worry about. Vet. Microbiol. 134(1): 9-14.

Schukken YH, Leslie K, Weersink A and Martin S 1992. Ontario bulk milk somatic cell count reduction program. 1. Impact on somatic cell counts and milk quality. J. Dairy Sci. 75(12): 3352-3358.

Schukken YH, Wilson DJ, Welcome F, Garrison-Tikofsky L and Gonzalez RN 2003. Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34(5): 579-596.

Schultz L 1977. Somatic cells in milk-physiological aspects and relationship to amount and composition of milk. J. Food Prot. 40(2): 125-131.

Seegers H, Fourichon C and Beaudeau F 2003a. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 34(5): 475-491.

Seegers H, Fourichon C and Beaudeau F 2003b. Production effects related to mastitis and mastitis economics in dairy cattle herds. Veterinary research. 34(5): 475-491.

Sharif A and Muhammad G 2008. Somatic cell count as an indicator of udder health status under modern dairy production: a review. Pakistan Vet. J. 28(4): 194-200.

Sharif A, Umer M and Muhammad G 2009. Mastitis control in dairy production. J. Agric. Soc. Sci. 5: 102-105.

Sharma N, Singh N and Bhadwal M 2011. Relationship of somatic cell count and mastitis: an overview. Asian-australas. J. Anim. Sci. 24(3): 429-438.

Šimundić A-M 2009. Measures of diagnostic accuracy: basic definitions. EJIFCC. 19(4): 203-211.

Skrzypek R, Wojtowski J and Fahr RD 2004. Factors affecting somatic cell count in cow bulk tank milk–a case study from Poland. J Vet Med A Physiol Pathol Clin Med. 51(3): 127-131.

Suriyasathaporn W, Chupia V, Sing-Lah T, Wongsawan K, Mektrirat R and Chaisri W 2012. Increases of antibiotic resistance in excessive use of antibiotics in smallholder dairy farms in northern Thailand. Asian-australas. J. Anim. Sci. 25(9): 1322.

Tabilo-Munizaga G and Barbosa-Ca´novas GV 2005. Rheology for the food industry. J. Food Eng. 67(2005): 147–156.

Thai Dairy cows and Dairy Products Board: Thai Milk Board 2016. Thai standard for raw milk purchasing. Ministry of Agriculture and Cooperatives, ed. Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

Thompson D and Postle D 1964. The Wisconsin mastitis test–an indirect estimation of leucocytes in milk. J Milk Food Technol. 27(9): 271-275.

Trevethan R 2017. Sensitivity, specificity, and predictive values: foundations, pliabilities, and pitfalls in research and practice. Public Health Front. 5: 307-307.

Viguier C, Arora S, Gilmartin N, Welbeck K and O'Kennedy R 2009. Mastitis detection: current trends and future perspectives. Trends Biotechnol. 27(8): 486-493.

Vissio C, Bouman M and Larriestra AJ 2018. Milking machine and udder health management factors associated with bulk milk somatic cell count in Uruguayan herds. Prev. Vet. Med. 150: 110-116.

Wagner A and Ruegg P 2002. The effect of manual forestripping on milking performance of Holstein dairy cows. J. Dairy Sci. 85(4): 804-809.

Whyte D, Walmsley M, Liew A, Claycomb R and Mein G 2005. Chemical and rheological aspects of gel formation in the California mastitis test. J. Dairy Res. 72(1): 115-121.

Yeamkong S, Koonawootrittriron S, Elzo M and Suwanasopee T 2010. Milk quantity, quality and revenue in dairy farms supported by a private organization in central Thailand. Livestock Res. Rural Dev. 22(2). Retrieved October 16, 2019, from http://www.lrrd.org/lrrd22/2/yeam22033.htm.

Zajac P, Zubricka S, Capla J and Zelenakova L 2016. Fluorescence microscopy methods for the determination of somatic cell count in raw cow's milk. Vet. Med. - Czech. 61(11).

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