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Preparation and stability of milk somatic cell reference materials[1]

Larissa Di Marzo, Karen L. Wojciechowski, and David M. Barbano[2]
Northeast Dairy Food Research Center, Department of Food Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Our objectives were to develop a method to produce milk somatic cell count (SCC) reference materials for calibration of electronic somatic cell count (ESCC) using gravity separation and to determine the effect of refrigerated storage (4°C) and freeze-thaw stability of the skim and whole milk SCC reference materials. Whole raw milk was high-temperature short-time pasteurized and split into 2 portions. One portion was gravity separated at 4°C for 22 h and the second portion was centrifugally separated to produce skim milk that was also gravity separated with somatic cells rising to the surface. After 22 h, stock solutions (low SCC skim milk, high SCC skim milk, high SCC whole milk) were prepared and preserved (bronopol). Two experiments were conducted, one to compare the shelf-life of skim and whole milk SCC standards at 4°C and one to determine the effect of freezing and thawing on SCC standards. Both experiments were replicated 3 times. Gravity separation was an effective approach to isolate and concentrate somatic cells from bovine milk and redistribute them in a skim or whole milk matrix to create a set of reference materials with a wider and more uniformly distributed range of SCC than current calibration sets. The liquid SCC reference materials stored using the common industry practice at 4°C were stable (i.e., fit for purpose, no large decrease in SCC) for a 2-wk period, whereas frozen and thawed reference materials may have a much longer useful life. A gradual decrease occurred in residual difference in ESCC (SCC × 1,000/mL) versus original assigned reference SCC over duration of refrigerated storage for both skim and whole milk SCC samples, indicating that milk ESCC of the preserved milks was gradually decreasing during 28 d of storage at 4°C by about 15,000 SCC/mL. No difference in the ESCC for skim milk was detected between refrigerated and frozen storage, whereas for whole milk the ESCC for frozen was lower than refrigerated samples. Future work is needed to determine the time and temperature of longer term frozen storage over which the SCC results are stable.
Key words: gravity separation, somatic cell, reference materials

INTRODUCTION

Milk SCC is an indicator of udder health of lactating cows (Schukken et al., 2003). When the bacteria enter the mammary gland and an infection is established, inflammation occurs and white cells from the bloodstream migrate to the mammary gland to combat the infection, leading to an altered (i.e., changes in the volume and composition of milk) secretory function (Jain, 1979; Craven and Williams, 1985; Harmon, 1994). In a healthy cow, milk SCC is normally <100,000 cells/mL. A SCC greater than 200,000 cells/mL indicates that inflammation may be present in the udder (National Mastitis Council, 2001). Milk SCC is also a basis for a portion of the payment for milk to dairy farmers in the United States and provides an incentive to reduce SCC levels (van Asseldonk et al., 2010). An elevated SCC negatively affects farm profitability, and the main economic consequences are caused by treatment, milk production loses, product quality, culling, veterinary services, and the risk of other diseases (Halasa et al., 2007).

Different techniques are used to determine somatic cell levels in milk. They are divided into direct method: direct microscopy (**DMSCC**) developed by Prescott and Breed (1910), and indirect methods: California mastitis test developed by Schalm and Noorlander (1957) and later standardized by Schneider and Jasper (1964), Wisconsin mastitis test developed by Thompson and Postle (1964), and electronic analyzers (ESCC) described by Madsen (1975). The DMSCC is the validated reference methodology for SCC in milk (Fitts and Laird, 2004: method number 10.010). Electronic analyzers are used to simplify monitoring and measurement of milk SCC on large numbers of samples (Silveira et al., 2005).

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[1]Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, Cornell University.
[2]Corresponding author: dmb37@cornell.edu

Electronic somatic cell counters are based on different analysis principles, such as the Coulter Counter, in which electrical impulses generated by the passage of particles between 2 electrodes are counted (Mattern et al., 1957; Read et al., 1967), and the optical fluorescence. In the latter method (AOAC International, 2000; method 17.13.01; 978.26), the DNA of cells is stained with a fluorescent dye and when the dye-DNA complex is stimulated by a light source of one wavelength, then the dye-DNA complex emits fluorescence at another wavelength and that is measured and correlated with reference SCC data (Schmidt-Madsen, 1975).

Different organizations use milk SCC data for different purposes. The 3 primary organizations in the United States that produce and use milk SCC data are the National Dairy Herd Improvement Association (**NDHIA**), the Food and Drug Administration (**FDA**), and the USDA Federal Milk Markets. In addition, the European Union (**EU**) has separate standards that are also relevant, particularly in the context of international trade.

The NDHIA in the United States has the objective of promoting accuracy, credibility, and uniformity of NDHIA records, represents the NDHIA system on issues involving other national and international organizations, and organizes industry activities that benefit members (NDHIA, 2013, 2014). The NDHIA SCC program helps the dairy farmer monitor subclinical mastitis status of individual cows using the SCC data to make decisions (i.e., cow segregation, milking order, culling, and so on) and also provides to the farmer a report of current and previous SCC history of individual cows, permitting evaluation of the success or failure of the herd's mastitis control program (NDHIA, 2002). The NDHIA considers SCC normal when it is <100,000 cells/mL (NDHIA, 2014).

The goal of the FDA Pasteurized Milk Ordinance (**PMO**) is to ensure the safety of grade A milk and milk products. The PMO is controlled by the National Conference of Interstate Milk Shipments, which is directed by the US Department of Health and Human Services, Public Health Service, FDA. The FDA has regional milk specialists all around the United States to help the states have consistency in the inspections of bulk tank somatic cell count (**BTSCC**) of the grade A milk shipments (IDFA, 2013). The maximum legal BTSCC for grade A milk of an individual producer outlined by PMO is 750,000 cells/mL. If a producer sells milk with the BTSCC value over the limit, a notice is issued and additional samples are tested within 3 wk. If in 5 consecutive mo, 3 counts exceed the maximum, the producer may have the permission to sell grade A milk suspended or may have to pay a monetary penalty (FDA, 2013).

The Federal Milk Market Administrators (**FMMA**) of the USDA laboratories were implemented to validate testing accuracy to ensure fair and equitable payment among producers and processors for milk through the use of Standard Procedures and Official Methods (USDA, 2009). Four of these FMMA-USDA orders also incorporated a per hundredweight adjustment based on the SCC of producer milk (USDA, 2013). The quality adjustment is added to the price if the somatic cell count is below 350,000 cells/mL and subtracted from the price if above this value (USDA, 2011a; Code of Federal Regulations, 2015).

The dairy market in EU is regulated by the Common Market Organization for milk and milk products through the Common Agricultural Policy, which has the objective to support raw milk and dairy product prices, and the incomes of dairy farmers (European Commission, 2011). The BTSCC limit for milk established by the EU is 400,000 cells/mL for each individual farm. A geometric mean BTSCC is calculated based on the last 3 mo of BTSCC data, and if it exceeds the legal limit the herd is placed on a watch list and is removed from it only if the next 3 tests are within the limit established. If the geometric mean BTSCC is above the limit for all 3 additional tests, then the herd cannot market milk until corrective action is taken (EUR-Lex, 2004; USDA, 2011b).

The EU and the 3 US organizations described above have some common and some different individual needs regarding analytical accuracy and operational procedures for controlling and validation of the accuracy of milk SCC testing for payment. A common need is the agreement on calibration of the instruments done with a set of certified SCC reference materials with a consistent matrix and range of concentration. An example of a different need is the need in the USDA-FMMA to have accurate SCC across the range of test values because a payment value is determined at each 1,000 SCC above or below 350,000 SCC per mL versus the FDA-PMO system that only needs to correctly classify if the milk from a farm is greater than or less than 750,000 SCC. Thus, the needs for analytical performance (i.e., repeatability and reproducibility) of the reference procedures and the rapid analytical measures are different for the 2 programs due the difference in the use of SCC data. No set of certified reference materials for calibration of ESCC is available (Orlandini, 2012) because the shelf-life of the materials is too short. Typically commercial sets of milk SCC standards in the United States contain 4 different milks because of the amount of work to assemble different milks with different SCC over the desired range and to run a large number of direct microscope SCC. From a statistical analysis perspective, 4 levels of SCC across the range