Determination of Lactose in Lactose-free and Low-lactose Milk, Milk Products, and Products Containing Dairy Ingredients by the LactoSens®R Amperometry Method: First Action 2020.01

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Abstract

Background: The AOAC Stakeholder Panel on Strategic Food Analytical Methods approved Standard Method Performance Requirements (SMPR®) 2018.009 for lactose in low-lactose or lactose-free milk, milk products, and products containing dairy ingredients. The LactoSens®R Method is a biosensor assay kit developed for the determination of lactose in a variety of lactose-free or low-lactose milk, dairy, and infant formula products produced with yeast neutral lactases. *Objective:* In response to a call for methods, the LactoSensR method was validated in single laboratory study with comparison to SMPR 2018.009. *Methods:* The LactoSensR method was evaluated for calibration, interference, repeatability, recovery, and robustness. In a method comparison study samples naturally containing low levels of lactose were evaluated using LactoSensR and an accredited high-performance anion-exchange chromatography with pulsed amperometric detection. *Results:* Calibration with lactose standard solutions was shown to be linear and the method was shown to be free of interference from a variety of sugars, vitamins, alcohols, flavorings, and other compounds. Matrix studies, including 85 spiked materials, 55

products naturally containing lactose, and 13 reference materials, resulted in RSD_r of 0–10.5% at 8–100 mg lactose/100 g and 0.2–5.4% at >100 mg lactose/100 g for milk and dairy products and 1.0–6.8% for infant formula, in compliance with SMPR 2018.009 with few exceptions. Recovery was 85.0–110.3% at 8–100 mg lactose/100 g and 85.6–109.7% at >100 mg lactose/100 g for milk and dairy products and 91.1–97.0% for infant formula, also meeting the performance requirements with few exceptions. The method was shown to be robust to changes in ambient temperature, sample temperature, and sample volume. *Conclusions:* The LactoSensR method compares favorably with the requirements of SMPR 2018.009 and should be adopted as a First Action AOAC *Official Method*™. *Highlights:* The LactoSensR method is a fast, easy to use method that meets the requirements of SMPR 2018.009.

Introduction

Lactose intolerance is a condition in which the small intestine produces too little lactase enzyme and, therefore, cannot digest all of the lactose consumed from dairy products (1). Symptoms of lactose intolerance include bloating, diarrhea, gas, nausea, and abdominal pain. The treatment for this condition is the limitation or total avoidance of dairy products in the diet, which can in turn reduce the needed calcium and vitamin D intake. Low-lactose and lactose-free dairy products have been developed to accommodate the dietary needs of lactose intolerant individuals. These dairy products are treated with lactase enzyme during production to reduce or eliminate lactose, thus allowing lactose intolerant individuals to manage their symptoms without eliminating dairy products completely from their diets.

In order to monitor label claims for lactose-free and low-lactose milk and dairy products, new methods are needed that are accurate and precise. The LactoSens®R method is a biosensor assay kit intended for the determination of lactose levels in a variety of lactose-free or low-

lactose milk and dairy products. An enzyme immobilized on a disposable test strip oxidizes the lactose in the test portion and the resulting electrons are detected amperometrically by the LactoSensR Reader. Proprietary software converts the electrical signal into a lactose concentration according to the factory-set calibration function. Each test strip is labelled with a QR code for sample tracking and batch-specific information and a ready-to-use positive control is provided for quality assurance. The LactoSensR method was certified by NordVal in 2018 (3).

Single-Laboratory Validation Study Designs and Methodology

The LactoSensR method was validated to demonstrate conformity with AOAC Standard Method Performance Requirements (SMPR®) 2018.009 (2) for determination of lactose in lactose-free and low-lactose milk, milk products, and products containing dairy ingredients (Table 1). The validation included calibration, interference, and robustness studies as well as matrix studies including spiked samples, naturally contaminated samples, and reference materials. All studies were performed by DirectSens (Vienna, Austria). The reference high-performance anion-exchange chromatography with pulsed amperometric detection HPAEC-PAD method (4) was carried out by an accredited contract laboratory (LAZBW,Wangen, Germany).

Calibration Study

Stock solution A was prepared by addition of 0.21 g lactose monohydrate (CAS No. 64044-51-5, ≥99 %), 2.64 g D(+)-glucose monohydrate (CAS No. 14431-43-7, ≥ 99.5%), and 2.4 g D-(+)-galactose (CAS No. 59-23-4, ≥99.0%) to a 100 mL volumetric flask. The contents were brought to volume with deionized water and the flask was capped and inverted to mix. Stock solution B (artificial milk, AFM) was prepared by adding 2.64 g D(+)-glucose monohydrate (CAS No. 14431-43-7, ≥99.5%) and 2.4 g D-(+)-galactose (CAS No. 59-23-4, ≥99.0%) to a 100

mL volumetric flask. The contents were brought to volume with deionized water and the flask was capped and inverted to mix. Stock solution A was diluted with stock solution B so as to produce AFM solutions containing 0, 5.0, 8.0, 14, 20, 60, 100, and 200 mg/100 g lactose. Duplicate 1 mL test portions of each concentration were randomized and blind coded. Each blinded test portion was itself analyzed in duplicate according to the LactoSensR Amperometry Method from step **D**, *Sample Preparation* following instructions for low fat liquids.

Selectivity Study

Thirty-two substances including sugars, alcohols, vitamins, and flavorings were evaluated for potential interference in the LactoSensR method (*see* Supplemental information Table 1). Both positive and negative interference were evaluated by testing each compound in the absence and presence of lactose, respectively. Potentially interfering substances were tested at concentrations relevant to dairy matrixes with and without lactose at 50 mg/100 g in AFM or at 71 mg/100 g and <8 mg/100 g in pasteurized milk with 3.5 % fat. AFM was prepared as above except LactoSensR dilution buffer was used in place of water as the diluent. To prepare a stock solution of lactose at 475 mg/100 g, 5 g lactose monohydrate (CAS No. 64044-51-5, ≥99%) was added to a 100 mL volumetric flask, brought to volume with LactoSensR dilution buffer, capped, and inverted to mix. To prepare 50 mg/100 g lactose in AFM, 2.64 g D(+)-glucose monohydrate, 2.4 g D-(+)-galactose, and 10.527 mL lactose stock solution were added to a 100 mL volumetric flask, brought to volume with LactoSensR dilution buffer, capped, and inverted to mix. Pasteurized milk (3.5% fat, 71 mg/100 g lactose) and pasteurized lactose-free milk (3.5% fat, <8 mg/100 g lactose) were purchased from a local market.

Solutions of water-soluble substances (as indicated in Table 2) were prepared by adding an appropriate weight of substance to a volumetric flask, bringing to volume with deionized water, capping the flask, and inverting to mix. Fat-soluble substances (as indicated in Table 2) were prepared at 100x concentration by adding an appropriate weight of substance to a volumetric flask, bringing to volume with sunflower oil, capping the flask, and inverting to mix. Some solutions required heat to aid in solubility. For water-soluble preparations requiring less than 0.1 g in 100 mL, a 10x solution was first prepared and then diluted 10-fold in order to minimize weighing errors. For water-soluble substances, 300 µL of prepared solution was mixed with 300 µL of AFM or with 300 µL of 50 mg/100 g lactose in AFM in a 1.5 mL microcentrifuge tube and vortexed. For fat-soluble substances, 10 µL of prepared solution (in oil) was mixed with 990 µL pasteurized milk or 990 µL pasteurized lactose-free milk in a 1.5 mL microcentrifuge tube and vortexed. The resulting milk samples were then diluted 1:2 with LactoSensR Dilution Buffer. Samples were tested following the LactoSensR Amperometry Method from step **E**, *Determination*

The enzyme β-galactosidase was tested only in the absence of lactose for potential positive interference. It was tested at a level 10-fold above the level used in producing low lactose and lactose-free products. All samples were randomized and blind coded for testing. Each blind coded sample was tested in duplicate.

Matrix Studies

The matrix studies in total included a wide variety of commercially available milk, milk products, and dairy products (Agro Tami, Agropur, Berlandmilch, Carrefour, Francia, Hipp, Kärntner Milch, Kaslink Food, Maresi, Mevgal, Milupa, Mondelez, Moravia, Nestle, NÖM,

Novalac, Nutricia, Obersteirische Molkerei, Pinzgau Milch, Rajo, SMA Nutrition, Spar, Tesco, Valio) with a wide range of fat content, products with flavorings, products with fruit, and powdered milk and infant formula products.

To assess the influence of fat content on the LactoSensR results, cream sample sets at 3 fat concentrations (based on label claim) were analyzed in quintuplicate in a blind coded randomized order. Each sample set included a lactose-free and a lactose-positive sample. All sample sets were analyzed by an HPAEC-PAD method (4) at an accredited laboratory for comparison. Sample preparation followed the standard procedure for low-fat liquid samples of 1+1 dilution with LactoSensR Buffer.

Spiked samples were prepared by spiking 17 products with 0, 20, 50, 100, and 150 mg lactose/100 g, producing 85 materials for analysis. Matrixes included a variety of liquid, semisolid, solid, and powdered products with fat content ranging from 0.1–36% (*see* Table 3). Samples were processed and analyzed according to the LactoSensR method. To calculate recovery, the background endogenous lactose concentration was subtracted from each of the spiked results, then compared to the spike concentration.

To assess the utility of the method with real-world natural samples, a variety of lactose-free and low lactose milk, dairy, and infant formula products (55 samples) were analyzed without spiking. Some dairy products containing regular lactose concentrations were hydrolyzed using beta-galactosidase (Chr. Hansen) to produce samples with a wide range of low lactose concentrations. The results were compared to the HPAEC-PAD method.

Thirteen reference materials were obtained from muva (Kempten, Germany), National Milk Laboratories as distributed by Deutsches Referenzbüro für Ringversuche und Referenzmaterialien (DRRR, Waltenhofen, Germany), and National Institute of Standards and

Technology (NIST, Gaithersburg, MD) and analyzed according to the LactoSensR method. NIST SRM 2383a, as mentioned in SMPR 2018.009, was not available at the time of testing, but NIST SRM 1869 was approved as a suitable substitute. Five replicate test portions of each material were analyzed.

LOQ

The LactoSensR method is factory calibrated for the range 8–200 mg lactose/100 g for milk and dairy products and 5–200 mg lactose/100 g for infant formula. To validate the milk and dairy LOQ of 8 mg lactose/100 g and the LOQ of 5 mg lactose/100 g for infant formula, 10 replicates each of a variety of products containing lactose close to the LOQ values were analyzed.

Robustness

The idea for the robustness testing is to deliberately introduce minor, reasonable variations in a test kit method and observe the effects. These minor variations should be of a magnitude that might well be expected to occur when the test kit is used by an end user. The parameters and values tested for the LactoSensR method included ambient temperature (20°C, 22°C, 24°C), test portion temperature (4°C, 20°C, 40°C), and sample volume (80, 100, 120 µL). The experiment was carried out in a factorial design of the high and low values of each parameter as well as an additional treatment combination of all of the intermediate (method-specified) values. Each treatment combination was tested with 50 mg lactose /100 g in calibration solution (AFM) in duplicate.

Statistical Analyses

Outliers were determined by the Grubbs test using GraphPad (https://www.graphpad.com/quickcalcs/Grubbs1.cfm). Removal of statistical outliers prior to

subsequent statistical analyses is noted in table footnotes. All other statistical analyses were performed in Microsoft® Excel.

AOAC Official Method 2020.01

Lactose in Low-Lactose and Lactose-Free Milk, Milk Products, and Products Containing Dairy Ingredients

LactoSens®R Amperometry Method

First Action 2020

[Applicable for determination of lactose in a variety of liquid, semi solid, solid, and powdered milk and dairy products in the range 8–200 mg/100 g and powdered infant formula in the range 5–200 mg/100 g produced with yeast neutral lactases.]

Caution: Use appropriate personal protective equipment such as a laboratory coat, safety glasses, and gloves. Dispose of all materials according to federal, state, and local regulations.

A. Principle

The LactoSensR Method is a biosensor assay kit intended for the determination of lactose levels in a variety of lactose-free or low-lactose milk and dairy products. An enzyme immobilized on a disposable test strip oxidizes the lactose in the test portion and the resulting electrons are detected amperometrically by the LactoSensR Reader. Proprietary software converts the electrical signal into a lactose concentration according to the factory-set calibration function. Each test strip is labelled with a QR code for sample tracking and lot-specific information and a ready-to-use positive control is provided for quality assurance and calibration check.

B. Apparatus and Reagents

(**a**) *LactoSensR Reader Kit, LR10, DirectSens*.—Including software version 2.0 or higher.

(**b**) *LactoSensR Biosensor Assay Kit, LK1225, DirectSens.*—Includes 25 LactoSensR Biosensors; LactoSensR Buffer - 2 bottles 125 mL each, pH 5.6, ready-to-use, LB10, DirectSens; LactoSensR positive control - 1 vial 2 mL, ready-to-use, LC12 POC, DirectSens; User Manual; and Quality Assurance Certificate.

(**c**) *Laptop or PC.*—For connection with LactoSensR Reader.

(**d**) *Vortex mixer.*

(**e**) *Sample vials.*—Polypropylene tubes with caps.

(**f**) *Pipets and tips.*—1 mL and 100 µL.

(**g**) *Centrifuge.*—With rotor capable of 10,000 × *g*, not refrigerated

(**h**) *Paddle blender.*—Stomacher® 400 (available from major laboratory suppliers) or equivalent, with sample bags.

(**i**) *Mortar and pestle.*

C. General Preparation

(**a**) Bring the sample(s), LactoSensR Buffer, and required number of biosensors to room temperature (22 ± 2°C).

(**b**) Connect the LactoSensR Reader to the laptop or PC.

(**c**) Turn on the LactoSensR Reader.

(**d**) Start the LactoSens software. A green icon in the lower left corner of the software window indicates a connected reader.

D. Sample Preparation

Note: Fat content is based on label claims. When fat content is unknown, determine using AOAC OMA **989.05** (IDF-ISO-AOAC Method).

(**a**) *Low fat liquids (<20% fat).*—Mix product by shaking or vortexing. Dilute a 1 mL test portion with 1 mL LactoSensR Buffer in a sample vial and cap. Mix by shaking or vortexing.

(**b**) *High fat liquids (≥20% fat).*—Centrifuge 10 min at 10,000 × *g* and transfer the aqueous phase to a new container. Dilute a 1 mL test portion of the aqueous phase with 1 mL LactoSens **R** buffer in a sample vial and cap. Mix by shaking or vortexing.

(**c**) *Low fat semi-solids (<20% fat).*—Homogenize a representative sample using a mortar and pestle or paddle blender. Accurately weigh a 2 g test portion into a sample vial, note exact weight and add an equivalent amount of LactoSensR Buffer. Cap the vial. Mix by shaking or vortexing.

(**d**) *High fat semi-solids (≥20% fat).*—Homogenize a representative sample using a mortar and pestle or paddle blender. Centrifuge homogenized product 10 min at 10,000 × *g* and transfer the aqueous phase to a new container. Dilute a 1 mL test portion of the aqueous phase with 1 mL LactoSensR Buffer in a sample vial and cap. Mix by shaking or vortexing.

(**e**) *Solids.*—Finely chop with a knife and macerate product with a mortar and pestle. Accurately weigh a 2 g test portion into a clean mortar or a paddle blender bag, note exact weight and add an equivalent amount of LactoSensR Buffer. Homogenize for 1 min.

(**f**) *Infant formula and powdered milk.*—Pre-warm tap water to 45°C in a water bath. Measure appropriate volume of pre-warmed water in a graduated cylinder, then add appropriate amount of powder and dissolve completely by mixing. Reconstitute powder according to

package instructions for water volume and powder mass, such as 25 g infant formula powder dissolved in 200 g pre-warmed water. Dilute a 1 mL test portion with 1 mL LactoSensR Buffer in a sample vial and cap. Mix by shaking or vortexing.

E. Determination

(**a**) In the software window, type the sample name or code and click on "Scan sensor QR code". Hold the scanner 10–20 cm from the QR code on the sensor package with the red light pointing directly on the QR code. An acoustic signal indicates successful scanning of the QR code.

(**b**) In the "Settings" menu, choose either the "infant formula" setting with a quantitation range of 5–200 mg/100g lactose or "standard" setting with a quantitation range of 8–200 mg/100g lactose depending on the samples being analyzed.

(**c**) Open the sensor package being careful to not touch the sensitive sample application area.

(**d**) With the LactoSens logo facing upwards, insert the end with silver contacts into the reader as far as it will go. Confirm by pressing "Next".

(**e**) For viscous samples, cut the pipet tip prior to using to avoid clogging. Transfer a 100 µL aliquot of prepared sample onto the sensitive sample application area. Use the pipet tip to distribute the aliquot and ensure the sensitive area is completely covered. Correct application of the aliquot is crucial for an accurate measurement. Aliquot application must be completed within 15 s.

(**f**) The LactoSensR Reader will automatically detect the sample and the measurement will start after 15 s.

(**g**) Select the desired measurement units (g/L or %).

(**h**) Choose output file and click "Save and Next".

(**i**) If the result is above the upper limit of the method range, dilute the sample (e.g., 10-fold) with LactoSensR Buffer and repeat the analysis. Multiply the result by the dilution factor (e.g., 10).

(**j**) Analyze next prepared sample.

F. Correction Factors

(**a**) *High fat*.—Samples with a fat content ≥20 % must be centrifuged for 10 min at 10,000 × *g* prior to analysis. The aqueous phase is used for dilution with the dilution buffer and subsequent analysis. The obtained results must be corrected with the respective fat concentration. Measured values of samples with a fat content ≥20% are multiplied by a factor of 100% - x% fat content.

(**b**) *Calibration with "artificial milk" (AFM).*—LactoSensR is factory calibrated with solutions containing varying concentrations of lactose and constant concentrations of glucose and galactose (24 g/L each) mimicking the major carbohydrate composition of lactose-free milk products. These solutions are termed "Artificial Milk" (AFM). Since the calibration function for LactoSensR subtracts a constant milk hydrolysis background equivalent to 9.2 mg/100 g of lactose, this concentration must be added to the results for calibration solutions (AFM).

G. System Suitability

(**a**) *LactoSensR Positive Control.*—A positive control is provided with the kit in a ready-to-use format and can be used to verify the calibration status and correct handling of the sensor. Test 100 µL of positive control solution and verify that the result is 20 ± 5 mg/100 g.

Results and Discussion

Calibration Study

The method range (calibration) study determines the applicable range of the method. SMPR 2018.009 identifies three separate analytical ranges, dependent on the type of product evaluated: 5–100 mg/100 g lactose for infant formula and adult nutritionals; 10–100 mg/100 g lactose for lactose-free milk, milk product,s and products containing dairy ingredients; and >100–2000 mg/100 g lactose for low-lactose milk, milk products, and products containing dairy ingredients. The LactoSensR has a range of 8–200 mg/100 g (0.008–0.200%) lactose for milk, milk products, and products containing dairy ingredients and a range of 5–200 mg/100 g (0.005–0.200%) lactose for infant formula. Higher concentrations can be determined after dilution of the sample to within the range of the method. LactoSensR is factory calibrated with AFM solutions containing varying concentrations of lactose and constant concentrations of glucose and galactose (24 g/L each).

Results are presented in Table 4. At all non-zero concentrations, relative standard deviation (RSD_r) was less than 10% and residual was less than 18%. A plot of mean result versus known lactose concentration (Figure 1) yields a slope very close to 1 and an intercept very close to zero with a correlation coefficient (r^2) of 0.999. When residual is plotted against concentration in Figure 2, a random pattern is observed, indicating good linearity with no systematic error.

Selectivity Study

The results of the selectivity study are presented in Table 2. None of the substances caused positive interference (false positive results) as evidenced by the data for all substances in the

absence of lactose yielding results of <8 mg/100 g, below the limit of quantitation for milk, milk products and products containing dairy ingredients. When the potentially interfering substances were tested in the presence of lactose, recoveries ranged from 86–110 %, indicating no significant effect of any of the substances.

Matrix Studies

Influence of fat content.—Results are shown in Table 5. High fat content leads to a blocking of the sensor surface which can lower the recovery to 86%. Therefore, an additional sample preparation step was added for cream and other dairy products with a fat content ≥20%. These products must be centrifuged for 10 min at 10,000 × g prior to analysis. The aqueous phase is used for dilution with the dilution buffer and subsequent analysis. The obtained results are then corrected for the respective fat concentration. Table 6 shows the results using the high fat protocol for fat content ≥20%. Recoveries increased to 93 to 95% for the samples ≥20% fat. This supports use of the high-fat sample preparation protocols as described in the method.

Spiked matrixes.—Table 3 presents the data for spiked matrixes. There was one statistical outlier based on the Grubbs test at the highest spike concentration of 36% fat cream. Overall, the repeatability of the LactoSensR method varied between 0.7 to 10.5% RSD_r and recovery varied between 85.0 to 106.9%.

For spiked milk and dairy matrixes in the range of 8 to 100 mg/100 g lactose (49 materials), repeatability ranged from 1.2 to 10.5% RSD_r and recovery ranged from 85.0 to 109.6%. These values meet the requirements of SMPR 2018.009, except for 3 materials, including pasteurized milk at 8.2 mg lactose/100 g (10.2% RSD_r), curd at 10.2 mg lactose/100 g (10.1% RSD_r), and

coffee caramel macchiato at 9.0 mg lactose/100 g (10.5% RSD_r), all near the LOQ and barely above the criterion of ≤10%.

For spiked milk and dairy matrixes in the range of >100 to 200 mg lactose/100 g (31 materials), repeatability ranged from 0.7 to 4.7% RSD_r and recovery ranged from 85.6 to 109.0%. Repeatability met the acceptance criterion of ≤7%. For recovery, of 31 materials there were 3 instances of results just below 90% recovery, including cream (36% fat, 139.8 mg lactose/100 g) at 85.6% recovery; yogurt (1% fat, 143.4 mg lactose/100 g) at 88.8% recovery; and cottage cheese (2.2% fat, 145.0 mg lactose/100 g) at 89.3% recovery.

For infant formula, repeatability was 1.1 to 6.8% RSD_r and recovery was 91.1 to 97.0%, meeting the SMPR criteria.

Matrixes were further analyzed by plotting the known spike concentrations against the mean results with background subtracted and regression analysis was performed. Figure 3 shows 6 representative plots and all regression results are presented in Table 7. All matrixes demonstrated good correlation of results with the lactose spike concentration ($r^2 > 0.98$) regardless of matrix type (liquid, semi solid, solid, or powder) or fat content.

Natural samples.—All samples with undetectable lactose by the LactoSensR method were also undetectable by the HPAEC-PAD method (Table 8). Overall, the samples with detectable levels of lactose varied from 12.4 to 6655 mg lactose/100 g with repeatability in the range 0 to 9.2% RSD_r and recovery relative to the HPAEC-PAD method in the range 89.7 to 110.3%. For milk and dairy samples in the range 8 to 100 mg lactose/100 g, repeatability ranged from 0 to 9.2% RSD_r and recovery ranged from 89.7 to 110.3%. For milk and dairy samples >100 mg

lactose/100 g, repeatability ranged from 0.2 to 5.4% RSD_r and recovery ranged from 90.2 to 107.2%. Infant formula samples ranged from 1.0 to 4.4% RSD_r and 96.3 to 107.8% recovery. These values all meet the requirements of SMPR 2018.009.

Reference materials.—There was one statistical outlier removed from the NIST SRM 1869 replicates by the Grubbs test. Analyses yielded 0.7 to 3.9 % RSD_r (Table 9) overall. Recoveries were 93.7% to 109.7% overall. By SMPR 2018.009 category and range, RSD_r values were 1.3%, 1.4%, and 0.7 to 3.9% for infant formula, low range dairy products, and high range dairy products, respectively, meeting the SMPR criteria. Recoveries were 97.3, 93.7, and 94.2 to 109.7% for infant formula, low range dairy products, and high range dairy products, respectively, meeting the SMPR criteria.

LOQ

Table 10 presents the data from 10 replicates analyses of a variety of products containing lactose near the LOQ. Lactose concentration varied from 8.0 to 12.7 mg/100 g for milk and dairy products and was 5.4 mg/100 g for reconstituted infant formula powder. The repeatability was ≤10.5% RSD_r for all matrixes, which is within acceptable limits for the LOQ.

Robustness

Table 11 shows the results of the robustness testing. The mean value for the nominal conditions (treatment combination 9) was 50.0 mg lactose/100 g and the variations of ambient temperature, sample temperature, and sample volume (in a factorial design of experiment) resulted in mean values ranging from 49.5 to 54.5 mg/100 g. The repeatability within each treatment combination varied from 0 to 9.4% and the repeatability among treatment

combinations was 2.8%. These results demonstrate that the method is robust to changes in ambient temperature, sample temperature, and sample volume over the ranges tested.

Summary of LactoSensR Performance Compared to SMPR 2018.009

In total, 17 samples spiked at 5 lactose concentrations, 55 natural samples, and 13 reference materials were analyzed in the matrix study. The precision and recovery data from these analyses were combined and compared to the requirements of SMPR 2018.009 in Table 12. Infant formula samples were validated in the range 5 to 200 mg lactose/100 g. Within this range, the recovery values varied from 91.1 to 97.3% and repeatability precision (RSD_r) varied from 1.0 to 6.8%. These values are well within the acceptable criteria of 85–115% recovery and ≤10% repeatability.

For milk, milk products, and products with dairy ingredients, the results were divided into 2 ranges, 8 to 100 mg lactose/100 g and >100 mg lactose/100 g. At the lower range, recovery was 85 to 110.3% and repeatability was ≤10.5%. As noted earlier, there were 3 instances of repeatability between 10.0 and 10.5%, all in unspiked low level matrixes (Table 3). Two of these instances, pasteurized milk, and coffee caramel macchiato, were at concentrations below the lower limit of the SMPR range, so one could argue that the criteria do not apply. The third was at 10.2 mg/100 g, just at the lower limit of the SMPR range. At the higher range, recovery overall was 85.6 to 109.7% and repeatability was 0.2 to 5.4%. As discussed previously, the 3 instances of recovery below 90% were in spiked matrixes at the highest levels. Thus, all of the natural samples, all of the reference materials, and all of the infant formula materials met all SMPR criteria. In the spiked matrix study of milk and dairy samples, 3 samples led to slightly high repeatability for the low range and 3 samples led to slightly low recovery for the high range. Overall, the LactoSensR results compared favorably with the SMPR criteria.

Conclusion

The LactoSensR method for determination of lactose in milk, milk products, products with dairy ingredients and infant formulas produced with yeast neutral lactases was validated in a single laboratory study. With very few exceptions, the method performance was within acceptable limits as described in SMPR 2018.009, supporting adoption as a First Action AOAC *Official Method*™.

References

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Table 1. SMPR 2018.009 performance requirements[a]			
	Infant formula	Milk, milk products, and products containing dairy ingredients	
Analytical range, mg/10 g	5–100	10–100	>100–2000
Recovery, %	85–115	85–115	90–110
RSD_r, %	≤10	≤10	≤7
RSD_R, %	≤15	≤15	≤10
[a] Requirements are for foods and beverages as received. For infant formula and adult nutritional products, concentrations apply to “ready-to-feed” liquids, reconstituted powders (for infant formula products, 25 g into 200 g water).			

Table 2. Selectivity study results

			Testing in absence of lactose			Testing in Presence of Lactose						
Substance	Concn, g/L	Substance solution	Lactose-free sample	Rep 1, mg/100 g	Rep 2, mg/100 g	Lactose sample	Rep 1, mg/100 g	Rep 2, mg/100 g	Mean, mg/100 g	Recovery, %	s_r	$RSD_{r,\%}$
Glucose	24	H_2O	AFM[a]	<8	< 8	50 mg/100 g AFM	56	54	55	110	1.4	2.6
Fructose	40	H_2O	AFM	<8	< 8	50 mg/100 g AFM	56	52	54	108	2.8	5.2
Galactose	24	H_2O	AFM	<8	< 8	50 mg/100 g AFM	51	51	51	102	0.0	0.0
Sucrose	40	H_2O	AFM	<8	< 8	50 mg/100 g AFM	50	52	51	102	1.4	2.8
Maltose	10	H_2O	AFM	<8	< 8	50 mg/100 g AFM	47	47	47	94	0.0	0.0
Amylose	40	H_2O	AFM	<8	< 8	50 mg/100 g AFM	52	50	51	102	1.4	2.8
Allolactose	10	H_2O	AFM	<8	< 8	50 mg/100 g AFM	52	52	52	104	0.0	0.0
Lactulose	1	H_2O	AFM	<8	< 8	50 mg/100 g AFM	51	53	52	104	1.4	2.7
Ascorbic acid	0.25	H_2O	AFM	<8	< 8	50 mg/100 g AFM	46	44	45	90	1.4	3.1
Vitamin A	0.005	Oil[b]	Milk[c]	<8	< 8	71 mg/100 g Milk	68	67	68	95	0.7	1.0
Vitamin D	0.000045	Oil	Milk	<8	< 8	71 mg/100 g Milk	68	67	68	95	0.7	1.0
Vitamin B6	0.12	H_2O	AFM	<8	< 8	50 mg/100 g AFM	49	51	50	100	1.4	2.8
Vitamin E	0.06	Oil	Milk	<8	< 8	71 mg/100 g Milk	71	68	70	98	2.1	3.1
Sorbic acid	0.25	H_2O	AFM	<8	< 8	50 mg/100 g AFM	55	53	54	108	1.4	2.6
Glycerol	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	51	50	51	101	0.7	1.4
Erythritol	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	53	54	54	107	0.7	1.3
Xylitol	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	50	51	51	101	0.7	1.4
Sorbitol	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	50	53	52	103	2.1	4.1
Mannitol	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	53	48	51	101	3.5	7.0
Maltitol	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	46	46	46	92	0.0	0.0
Lactitol	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	49	49	49	98	0.0	0.0
Isomalt	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	51	49	50	100	1.4	2.8
Sucralose	50	H_2O	AFM	<8	< 8	50 mg/100 g AFM	43	43	43	86	0.0	0.0
Sodium Chloride	8	H_2O	AFM	<8	< 8	50 mg/100 g AFM	46	47	47	93	0.7	1.5
Glucosamine HCl	1.5	H_2O	AFM	<8	< 8	50 mg/100 g AFM	51	50	51	101	0.7	1.4
Coffee	3	H_2O	AFM	<8	< 8	50 mg/100 g AFM	46	47	47	93	0.7	1.5
Cacao	10	H_2O	AFM	<8	< 8	50 mg/100 g AFM	46	47	47	93	0.7	1.5
Vanilla	10	H_2O	AFM	<8	< 8	50 mg/100 g AFM	48	50	49	98	1.4	2.9
Banana	170	H_2O	AFM	<8	< 8	50 mg/100 g AFM	48	45	47	93	2.1	4.6

Peach/apricot	170	H_2O	AFM	<8	< 8	50 mg/100 g AFM	46	48	47	94	1.4	3.0
Strawberry	170	H_2O	AFM	<8	< 8	50 mg/100 g AFM	46	49	48	95	2.1	4.5
Cherry	170	H_2O	AFM	<8	< 8	50 mg/100 g AFM	48	49	49	97	0.7	1.5
Beta-galactosidase	8	H_2O	AFM	<8	< 8							
Na-Caseinate	100	H2O	AFM	<8	< 8	50 mg/100 g AFM	50	46	48	95	2.3	5.0

[a]AFM = Artificial milk.
[b]Oil = Sunflower oil.
[c]Milk = Pasteurized milk with 3.5% fat.

Table 3. LactoSensR results for spiked matrixes

				LactoSensR results				
Matrix	Fat content, %	Spike, mg/100 g	N	Mean, mg/100 g	s(r)	RSD(r), %	Mean - Bkgd, mg/100 g	Recovery, %
Pasteurized milk	1.8	0	5	8.2	0.8	10.2	0	NA
		20	5	27.2	1.1	4.0	19.0	95.0
		50	5	62.6	2.6	4.2	54.4	108.8
		100	5	117	3.3	2.8	109	109.0
		150	5	163	1.7	1.0	155	103.5
UHT[a] milk	0.5	0	5	9.2	0.8	9.1	0	NA
		20	5	28.6	0.5	1.9	19.4	97.0
		50	5	59.8	1.1	1.8	50.6	101.2
		100	5	111	2.7	2.4	102	102.0
		150	5	165	6.9	4.2	156	103.9
UHT milk	3.5	0	5	9.2	0.4	4.9	0	NA
		20	5	29.8	1.8	6.0	20.6	103.0
		50	5	59.8	0.8	1.4	50.6	101.2
		100	5	113	3.3	2.9	104	104.2
		150	5	161	6.5	4.0	152	101.2
Cream	10	0	5	8.8	0.8	9.5	0	NA
		20	5	29.4	1.1	3.9	20.6	103.0
		50	5	62.6	1.8	2.9	53.8	107.6
		100	5	117	4.9	4.2	109	108.6
		150	5	167	1.9	1.1	158	105.5
Cream[d]	36	0	5	11.4	1.1	10.0	0	NA
		20	5	33.2	1.3	3.9	21.8	109.0
		50	5	56.0	1.2	2.2	44.6	89.2
		100	5	113	5.3	4.6	102	101.8
		150	4[c]	140	1.0	0.7	128	85.6
Yogurt	0.1	0	5	8.8	0.8	9.5	0	NA
		20	5	25.8	1.3	5.1	17.0	85.0
		50	5	52.8	1.3	2.5	44.0	88.0
		100	5	99.8	2.8	2.8	91.0	91.0
		150	5	154	3.3	2.1	145	96.5
Yogurt	1.0	0	10	10.2	1.0	9.6	0	NA

		20	5	29.6	1.7	5.7	19.4	97.0
		50	5	58.4	2.9	4.9	48.2	96.4
		100	5	101	0.9	0.9	91.2	91.2
		150	5	143	4.0	2.8	133	88.8
Greek yogurt	10	0	10	12.7	1.2	9.1	0	NA
		20	5	31.0	0.7	2.3	18.3	91.5
		50	5	63.6	0.9	1.4	50.9	101.8
		100	5	112	4.5	4.0	98.9	98.9
		150	5	155	4.0	2.6	142	94.7
Sour cream	15	0	10	8.3	0.7	8.1	0	NA
		20	5	28.0	1.2	4.4	19.7	98.5
		50	5	61.2	1.3	2.1	52.9	105.8
		100	5	114	3.1	2.7	106	105.7
		150	5	157	3.4	2.2	149	99.3
Cream cheese[d]	24	0	5	9.6	0.9	9.3	0	NA
		20	5	31.4	2.2	7.0	21.8	109.0
		50	5	64.4	1.9	3.0	54.8	109.6
		100	5	117	2.4	2.0	107	107.2
		150	5	161	3.0	1.9	152	101.2
Cottage cheese	2.2	0	10	11.1	0.6	5.1	0	NA
		20	5	28.6	1.1	4.0	17.5	87.5
		50	5	56.6	1.8	3.2	45.5	91.0
		100	5	107	5.0	4.7	95.7	95.7
		150	5	145	5.2	3.6	134	89.3
Curd	0.2	0	10	10.2	1.0	10.1	0	NA
		20	5	29.2	1.1	3.8	19.0	95.0
		50	5	57.6	0.9	1.6	47.4	94.8
		100	5	107	3.2	3.0	96.4	96.4
		150	5	155	4.3	2.8	145	96.7
Mozzarella cheese	19	0	10	9.5	0.7	7.4	0	NA
		20	5	28.0	1.4	5.1	18.5	92.5
		50	5	58.2	2.4	4.1	48.7	97.4
		100	5	107	1.5	1.4	97.1	97.1
		150	5	157	3.6	2.3	148	98.6
Coffee caramel	1.1	0	10	9.0	0.9	10.5	0	NA
		20	5	27.0	1.2	4.5	18.0	90.0

machiato		50	5	55.4	3.4	6.1	46.4	92.8
		100	5	100	1.1	1.1	91.2	91.2
		150	5	144	3.1	2.2	135	90.1
Strawberry yogurt	5.5	0	10	10.7	0.8	7.7	0	NA
		20	5	29.4	1.5	5.2	18.7	93.5
		50	5	59.0	0.7	1.2	48.3	96.6
		100	5	107	1.9	1.8	96.7	96.7
		150	5	148	4.0	2.7	137	91.4
Milk powder[b]	1.5	0	10	9.0	0.3	2.9	0	NA
		20	5	30.8	0.7	2.3	21.8	109.2
		50	5	62.9	1.7	2.6	53.9	107.8
		100	5	114	2.9	2.5	105	104.5
		150	5	159	5.5	3.4	150	100.3
Infant formula[e]	2.8	0	10	5.4	0.4	6.8	0	NA
		20	5	24.8	1.6	6.4	19.4	97.0
		50	5	51.5	0.6	1.1	46.2	92.3
		100	5	96.6	4.3	4.5	91.3	91.3
		150	5	142	5.6	3.9	137	91.1

[a]UHT = Ultra high temperature pasteurization.
[b]Reconstituted 1/10. Data are for reconstituted product.
[c]One statistical outlier removed based on Grubbs' test.
[d]High fat protocol used. Results corrected for fat content.
[e]Reconstituted according to package instruction. Data are for ready-to-feed liquid.

Table 4. Calibration study results

Lactose, mg/100 g	Replicate results, mg/100 g				Mean, mg/100 g	s_r	RSD_r, %	Residual
	1	2	3	4				
0	0.89	-0.13	-0.32	0.63	0.27	NA	NA	0.08
5	5.53	5.28	6.01	5.72	5.63	0.31	5.5	0.46
8	6.51	6.89	7.59	6.13	6.78	0.62	9.1	-1.39
14	13.60	11.19	12.96	11.83	12.40	1.09	8.8	-1.76
20	18.72	18.47	20.30	20.24	19.43	0.97	5.0	-0.71
60	59.83	55.21	62.54	63.62	60.30	3.75	6.2	0.25
100	100.61	107.00	106.05	109.41	105.77	3.71	3.5	5.81
200	195.79	197.56	197.50	197.12	196.99	0.82	0.4	-2.74

Table 5. Effect of fat content on LactoSensR results without centrifugation

Sample ID	Fat, %	N	LactoSensR results, mg/100 g			HPAEC-PAD, mg/100 g	Recovery, %
			Mean	s_r	RSD_r, %		
C36S8	36	5	61	2	4	71	86
C36S11	36	5	<8	NA	NA	<10	NA
C22S9	22	5	55	1	3	64	86
C22S11	22	5	<8	NA	NA	<10	NA
C10S10	10	5	63	2	3	66	95
C10S12	10	5	<8	NA	NA	<10	NA

Table 6. LactoSensR results using high fat protocol for samples with ≥20% fat

			LactoSensR results[a], mg/100 g				
Sample ID	Fat, %	N	Mean	s_r	RSD_r, %	HPAEC-PAD, mg/100 g	Recovery, %
C36S8	36	5	68	2	3	71	96
C36S11	36	5	<8	NA	NA	<10	NA
C22S9	22	5	60	1	2	64	94
C22S11	22	5	<8	NA	NA	<10	NA
C10S10	10	5	66	2	4	66	100
C10S12	10	5	<8	NA	NA	<10	NA

[a]Corrected for fat content.

Table 7. Regression results for spiked matrixes

Matrix	Regression equation	Correlation coefficient (r^2)
Pasteurized milk, 1.8% fat	y = 1.0505x + 0.2874	0.9983
UHT[a] milk, 0.5% fat	y = 1.0395x - 0.9657	0.9998
UHT milk, 3.5% fat	y = 1.0170x + 0.3105	0.9996
Cream, 10% fat	y = 1.0627x + 0.2284	0.9996
Cream, 36% fat	y = 0.8796x + 3.0257	0.9849
Yogurt, 0.1% fat	y = 0.9628x – 2.2584	0.9982
Yogurt, 1.0% fat	y = 0.8854x + 1.7351	0.9991
Greek yogurt, 10% fat	y = 0.9560x + 0.8574	0.9986
Sour cream, 15% fat	y = 1.0056x + 1.0831	0.9979
Cream cheese, 24% fat	y = 1.0179x + 1.9721	0.9982
Cottage cheese, 2.2% fat	y = 0.9084x + 0.3812	0.9977
Curd, 0.2% fat	y = 0.9679x - 0.3845	1.0000
Mozzarella cheese, 19% fat	y = 0.9865x - 0.6987	0.9999
Coffee caramel macchiato, 1.1% fat	y = 0.9027x + 0.3850	0.9999
Strawberry yogurt, 5.5% fat	y = 0.9231x + 1.0810	0.9984
Milk powder[b]	y = 1.0042x + 1.8632	0.9989
Infant formula powder[c]	y = 0.9075x + 0.6226	0.9999

[a]UHT = Ultra high temperature pasteurization.

[b]Reconstituted 1/10. Data are for reconstituted product.

[c]Reconstituted according to package instruction. Data are for ready-to-feed liquid.

Table 8. LactoSensR results for natural matrixes

			LactoSensR results				
Matrix sample	Fat, %	N	Mean, mg/100 g	s_r	RSD_r, %	HPAEC-PAD, mg/100 g	Recovery, %
Milk 1, pasteurized	0.8	5	<8	NA[a]	NA	<10	NA
Milk 2, pasteurized	1.8	10	<8	NA[a]	NA	<10	NA
Milk 3, pasteurized	3.5	5	12.4	1.1	9.2	12	103.3
Milk 4, pasteurized	3.5	5	69.6	3.9	5.6	71	98.0
Milk 5, pasteurized	1.5	5	134	7.2	5.4	128	104.8
Milk 6, pasteurized	0.8	5	187	4.7	2.5	189	98.7
Yogurt 1	1.0	5	47.0	1.9	4.0	44	106.8
Yogurt 3	3.5	5	92.2	4.4	4.8	86	107.2
Yogurt 4	0.1	5	271	4.0	1.5	301	90.2
Yogurt 5	0.1	5	29.8	2.4	8.0	28	106.4
Yogurt 7	0.1	5	<8	NA	NA	<10	NA
Yogurt 8	0.1	5	<8	NA	NA	<10	NA
Yogurt 9	1.8	2	14.0	0.0	0.0	14	100
Yogurt 10	1.8	5	<8	NA	NA	<10	NA
Coffee yogurt	1.8	5	<8	NA	NA	<10	NA
Vanilla yogurt	1.8	5	<8	NA	NA	<10	NA
Banana yogurt	1.8	5	<8	NA	NA	<10	NA
Peach yogurt	1.8	5	<8	NA	NA	<10	NA
Strawberry yogurt	1.8	5	<8	NA	NA	<10	NA
Cherry yogurt	1.8	5	<8	NA	NA	<10	NA
Sour cream	15	5	<8	NA	NA	<10	NA
Hard cheese	45	2	<8	NA	NA	<10	NA
Mozzarella 1	23.3	2	<8	NA	NA	<10	NA
Mozzarella 2	15	2	150	5.7	3.8	148	101.4
Vanilla drink 1	1.4	3	99.0	4.6	4.6	100	99.0
Vanilla drink 2	1.4	3	47.7	0.6	1.2	48	99.3
Vanilla drink 3	1.4	3	<8	NA	NA	<10	NA
Banana drink 1	0.2	3	91.0	3.6	4.0	94	96.8
Banana drink 2	0.2	3	46.3	2.1	4.5	50	92.7

Cacao drink 1	1.5	3	166	0.6	0.3	172	96.3
Cacao drink 2	1.5	3	104	4.6	4.4	101	103.0
Cacao drink 3	1.5	3	65.0	1.4	2.2	61	106.6
Cacao drink 4	1.5	3	46.3	1.2	2.5	45	103.0
Cacao drink 5	1.5	3	28.7	0.6	2.0	26	110.3
Iced coffee 1	2.5	3	189	8.7	4.6	193	97.9
Iced coffee 2	2.5	3	129	0.6	0.4	120	107.2
Iced coffee 3	2.5	3	73.7	3.1	4.1	81	90.9
Iced coffee 4	2.5	3	23.3	0.6	2.5	26	89.7
Iced coffee 5	2.5	3	17.7	0.6	3.3	19	93.0
Iced coffee 6	2.5	3	<8	NA	NA	<10	NA
Milk powder 1[b]	0.1	2	4939	19.3	0.4	4895	100.9
Milk powder 2[b] (Sweet whey)	ND[c]	2	6328	182	2.9	6433	98.4
Milk powder 3[b] (Whey permeate)	ND	2	6655	15.0	0.2	6675	99.7
Milk powder 4[b] (Whey Protein Concentrate - 34% Protein)	0.3	2	4646	68.6	1.5	4425	105.0
Milk powder 5[b] (Whey Protein Concentrate – 80% Protein)	0.6	2	497	13.9	2.8	482	103.3
Milk powder 6[b] (Whey Protein Concentrate – 70% Protein)	0.1	2	1277	41.2	3.2	1308	97.6
Milk powder 7[b] (Milk Protein Isolate – 85% Protein)	0.1	2	532	6.9	1.3	518	102.7
Milk powder 8[b]	ND	2	457	1.5	0.3	489	93.5
Infant formula 1[d,e] (maltodextrin, milk proteins)	2.6	2	<5	NA	NA	2.0	NA
Infant formula 2[d,e] (maltodextrin, GOS, milk proteins)	2.7	4	2466	28	1.2	2379	103.7

Infant formula 3[d,e] (maltodextrin, milk proteins)	2.6	3	2521	110	4.4	2617	96.3
Infant formula 4[d,e] (glucose, GOS, milk proteins)	2.5	3	2495	25	1.0	2314	107.8
Infant formula 5[d,e] (glucose syrup, amino acids)	2.5	2	<5	NA	NA	0.8	NA
Infant formula 6[d,e] (glucose syrup, amino acids)	2.5	2	<5	NA	NA	0.9	NA
Infant formula 7[d,e] (maltodextrin, milk proteins)	2.5	2	<5	NA	NA	1.0	NA

[a]NA = Not applicable.
[b]Reconstituted 1/10. Data are for reconstituted product.
[c]ND = Not determined.
[d]Reconstituted according to package instructions. LactosensR data are based on analysis of ready-to-feed liquid.
[e]HPAEC-PAD results are based on analysis of powdered infant formula and adjusted for dilution to ready-to-feed liquid.

Table 9. Analysis of available reference materials

			LactoSensR results					
Reference material	Sample type	N	Mean mg/100 g	s_r	RSD_r, %	Ref. value, mg/100 g	Ref. uncert., mg/100 g	Recovery, %
muva ML-2308	UHT Milk	5	295	2.2	0.8%	306[a]	41	96.0
muva ML-2309	UHT Milk	5	38.1	0.7	1.8%	41[a]	NA[c]	93.0
muva ML-2310	UHT Milk	5	200	6.9	3.5%	212[a]	NA	94.2
muva ML-2311	UHT Milk	5	<8	NA	NA	7[a]	NA	NA
muva ML-2312	UHT Milk	5	211	5.3	2.5%	217[a]	30	97.3
muva ML-2313	UHT Milk	5	<8	NA	NA	7[a]	6	NA
DRRR 1121007 RM CP L MP MOP 1	Whey powder[e]	5	7040	134	1.9%	6864[b]	611	102.6
DRRR 1141003 LKM-PAM K3 19-049	Milk	5	4644	101	2.2%	4747[b]	24	97.8
DRRR 1141001 LKM-PAM K1 19-048	Milk	5	4647	182	3.9%	4843[b]	22	95.9
DRRR 1101002 RM CP L MR MLAC 1	UHT milk	5	<8	NA	NA	< 100[b]	5	NA
DRRR 1121064 RM CP L MR MMG 1	Milkshake	5	6272	165	2.6%	5720[b]	147	109.7
DRRR 1111001 RM CP L MR SK 15	Processed cheese	5	6830	137	2.0%	7040[b]	250	97.0
NIST[d] SRM 1869	Infant formula[e]	4[f]	50.6	0.6	1.3%	52.0	12.0	97.3

[a]MUVA samples report reference values according to multiple methods. Values reported from HPLC analysis are included here. MUVA = muva kempten GmbH, Kempten, Germany.
[b]DRRR materials report guidance values. DRRR = Deutsches Referenzbüro für Ringversuche und Referenzmaterialien, Waltenhofen, Germany.

[c]NA = Not applicable.
[d]NIST = National Institute of Standards and Technology, Gaithersburg, MD, USA.
[e]Reconstituted according to instructions. Data are for ready-to-feed liquid.
[f] One statistical outlier removed based on Grubbs' test.

Table 10. Validation of the claimed LOQ

			LactoSensR results		
Matrix	Fat, %	N	Mean, mg/100 g	s_r	RSD_r, %
Pasteurized milk	1.8	10	8.0	0.7	9.0
UHT milk	0.5	10	9.9	0.9	8.8
UHT milk	3.5	10	9.1	0.6	6.2
Yogurt	1.0	10	10.2	1.0	9.6
Greek yogurt	10	10	12.7	1.2	9.1
Sour cream	15	10	8.3	0.7	8.1
Cream	36	10	12.0	1.2	9.6
Cottage cheese	2.2	10	11.1	0.6	5.1
Curd	0.2	10	10.2	1.0	10.1
Mozzarella	19	10	9.5	0.7	7.4
Coffee caramel macchiato	1.1	10	9.0	0.9	10.5
Strawberry yogurt	5.5	10	10.7	0.8	7.7
Milk powder	1.5	10	9.0	0.3	2.9
Infant formula powder	2.8	10	5.4	0.4	6.8

Table 11. Robustness testing with Factorial Design

	Parameter value			LactoSensR results, mg/100 g					Global (TC 1-8)		
TC[a]	Ambient temp., °C	Test portion temp., °C	Sample vol., µL	Rep1	Rep2	Mean	s_r	RSD_r, %	Mean, mg/100 g	s_r	RSD_r, %
1	20	4	80	52	50	51.0	1.4	2.8	52.1	1.5	2.8
2	20	4	120	48	51	49.5	2.1	4.3			
3	20	40	80	51	54	52.5	2.1	4.0			
4	20	40	120	51	53	52.0	1.4	2.7			
5	24	4	80	54	52	53.0	1.4	2.7			
6	24	4	120	51	53	52.0	1.4	2.7			
7	24	40	80	51	58	54.5	4.9	9.1			
8	24	40	120	56	49	52.5	4.9	9.4			
9	22	20	100	50	50	50.0	0.0	0.0			

[a]TC = Treatment combination.

Table 12. LactoSensR Performance Compared to SMPR 2018.009

	Infant formula		Milk, milk products, and products containing dairy ingredients			
Parameter	SMPR	LactoSensR	SMPR	LactoSensR	SMPR	LactoSensR
Analytical range, mg/100 g	5–100	5–200	10–100	8–100	>100–2000	>100–6655
Recovery, %	85–115	91.1–97.0	85–115	85.0–110.3	90–110	85.6–109.7
RSD_r, %	≤10	1.0–6.8	≤10	0.0–10.5	≤7	0.2–5.4

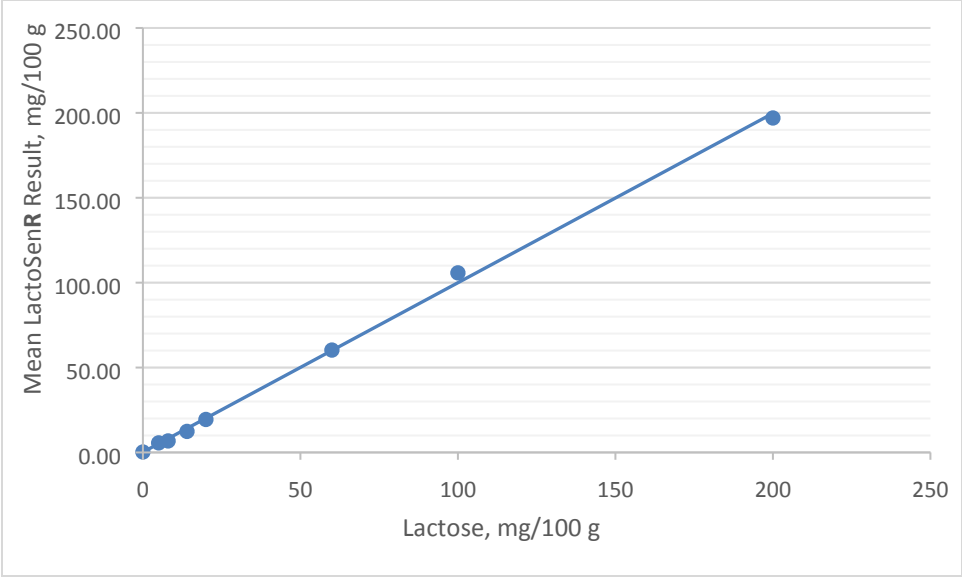


Figure 1. Calibration Plot using AFM Calibration Solutions

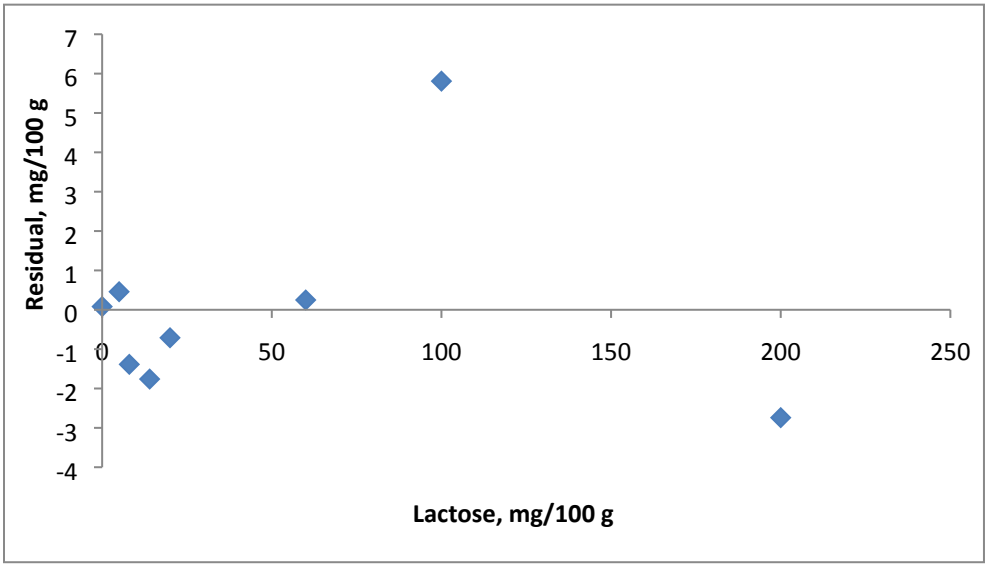
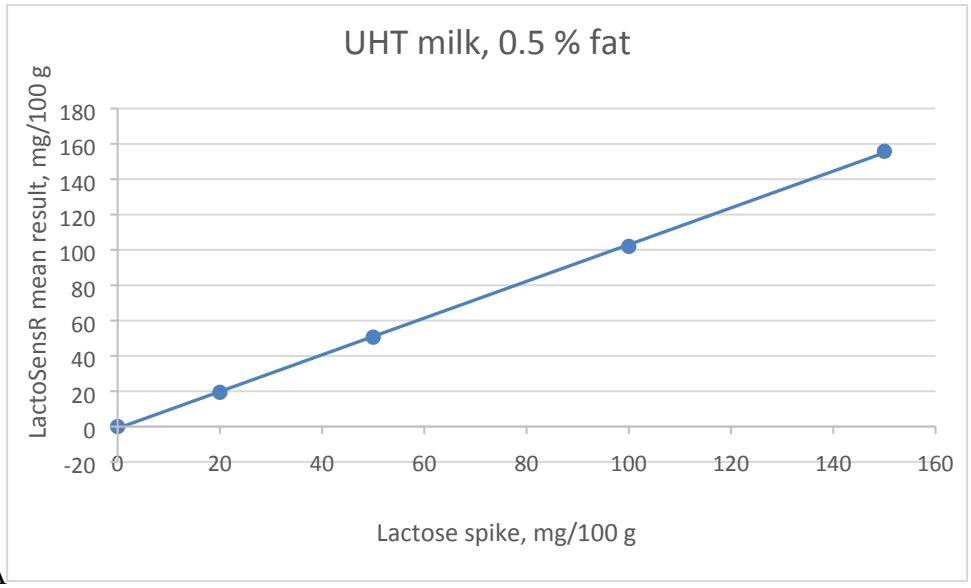
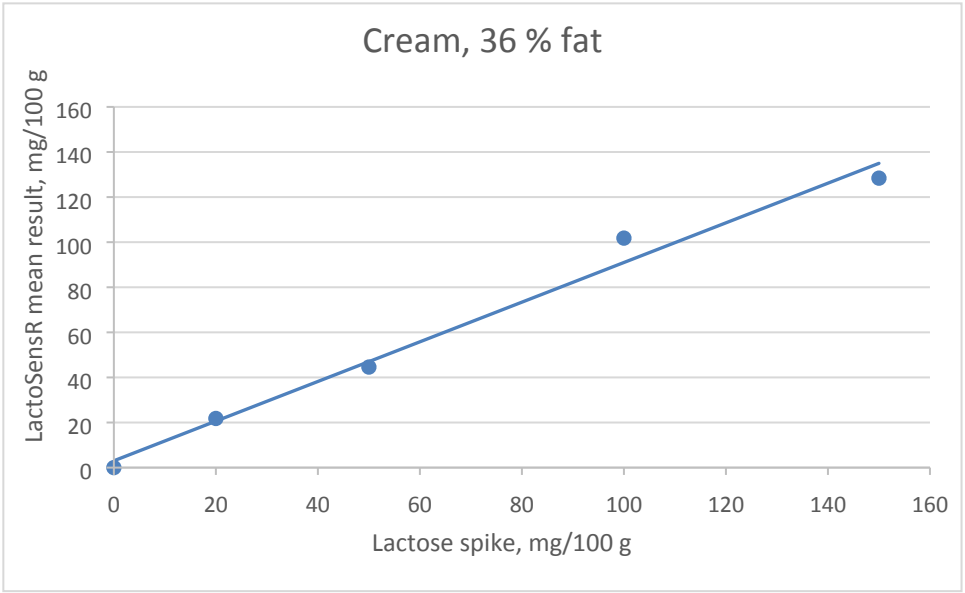


Figure 2. Residual Plot using AFM Calibration Solutions

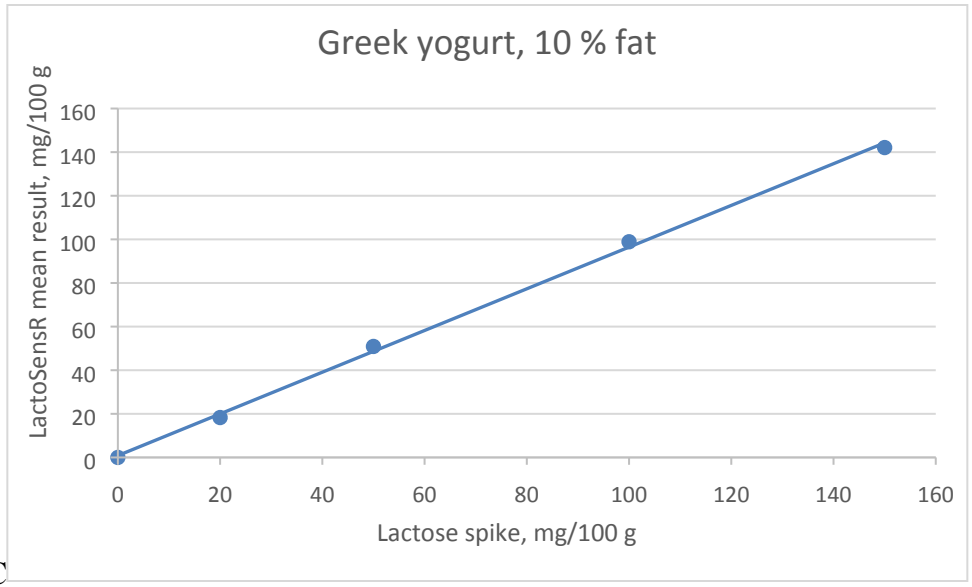


A

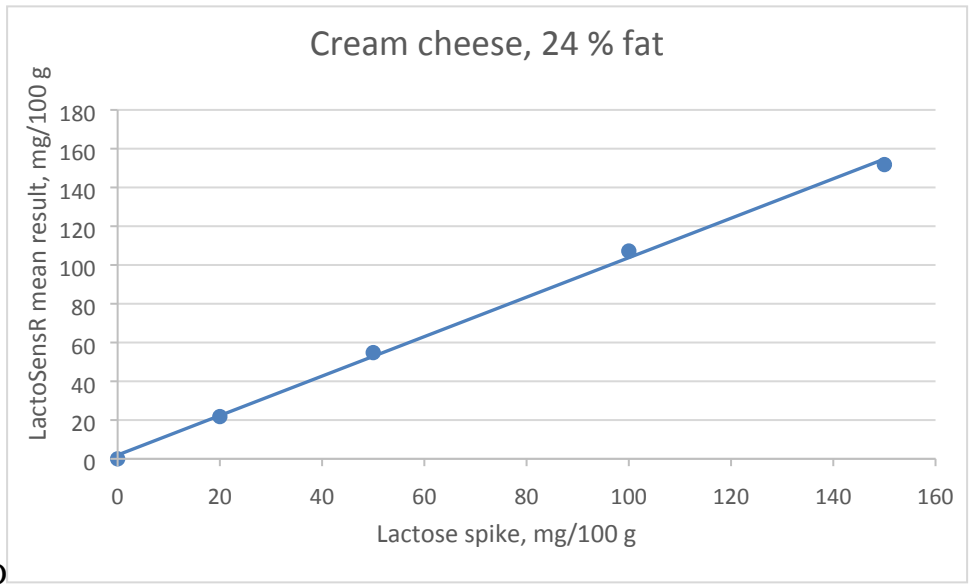


B

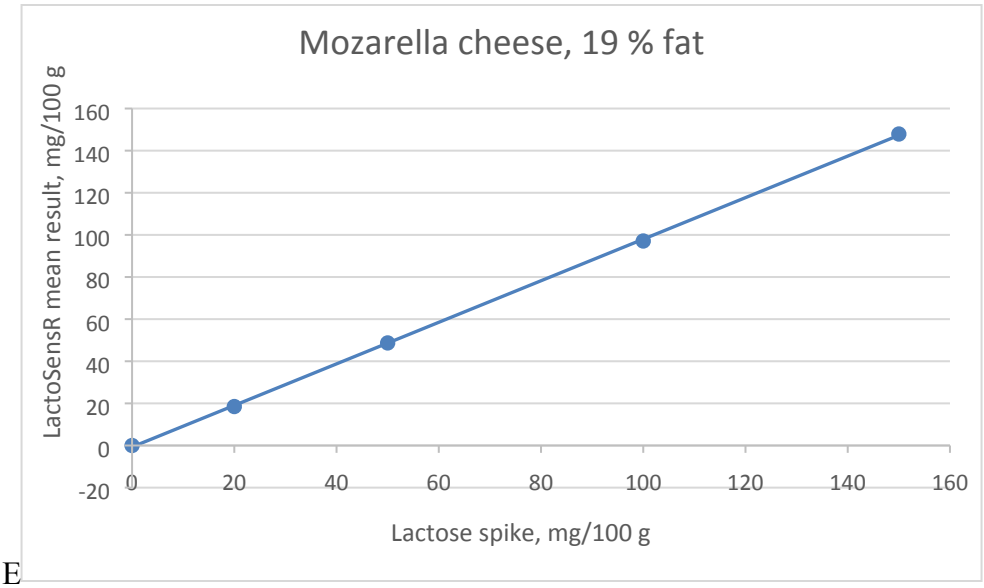
C



D



E



F

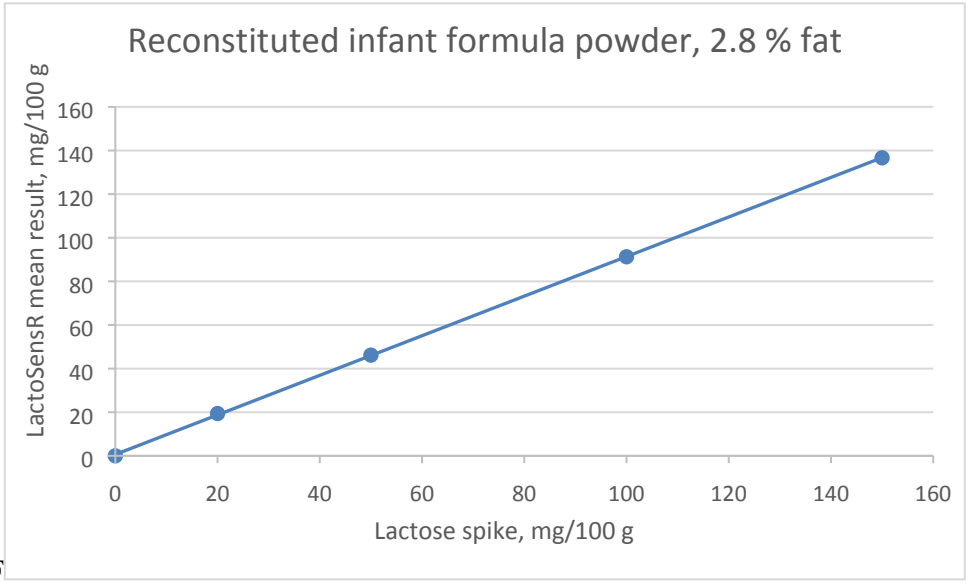


Figure 3. Regression plots for A) UHT milk, B) cream, C) Greek yogurt, D) cream cheese, E) Mozzarella cheese, and F) reconstituted infant formula powder.