



GUIDELINE FOR CONTROLLING THE ACCURACY OF ELECTRONIC TESTING INSTRUMENTS FOR MILK COMPONENTS

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Guideline Preparation and Review Process

Guideline development within Dairy Practices Council (DPC) is unique and requires several levels of peer review. The first step in the process of guideline development starts with a Task Force subcommittee comprised of individuals from industry, regulatory and education interested in and knowledgeable about the subject to be addressed. Drafts, referred to as ‘white copies,’ are circulated until all members are satisfied with the text. The final white copy may then be distributed to the entire Task Force, DPC Executive Vice President and whoever the Task Force Director feels would add to the strength of the review. Following final white copy review and correction, the next step in the process requires a yellow cover draft that is circulated to the member Regulatory Agency representatives that are referred to as “Key Sanitarians.” The Key Sanitarians may suggest changes and insert footnotes if their state standards and regulations differ from the text. After final review and editing the guideline is distributed in the distinctive DPC green cover to people worldwide. These guidelines represent the state of the knowledge at the time they are written.

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INTRODUCTION

Testing milk for butterfat as a basis for milk payment and for standardizing dairy products has occurred for many years. Currently, percentages of other milk components, such as true protein, other solids, solids not fat and somatic cell count are being determined for payment or for standardization purposes. This task has been greatly simplified by the development of electronic testing instruments for estimating the percentages of various components in milk. These instruments have dramatically improved laboratory efficiency by increasing the number of samples that can be analyzed, and with the appropriate instruments, by providing for the determination of percentages of several components simultaneously.

There are several different makes and models of electronic testing instruments currently available or in use for testing milk or other dairy products. Instruments using the absorption of infrared or near-infrared energy at specific wavelengths as the method of analysis can analyze for many other components in addition to butterfat. Infrared analyzers are more commonly used in producer payment programs and individual cow testing programs.

The infrared absorption procedure is approved by the AOAC International and is recognized by most regulatory agencies for determining the component results of milk samples when used as a basis for payment of the milk. The procedures set forth in the "Official Methods of Analysis" (AOAC) or in "Standard Methods for the Examination of Dairy Products" (APHA) are often cited. When used to test components for public record, for payment purposes or for official inspection, any electronic instrument or method must be approved by the controlling regulatory agency and the person who operates the instrument may be required to be licensed or certified.

As with all electronic instruments, control of the accuracy of the instrument's output is imperative. Since these machines provide indirect measurements, they must be routinely calibrated and adjusted to agree with the results of chemical reference methods to produce accurate, reproducible readings.

The purpose of this guideline is to provide general procedures and requirements for adjusting and controlling the accuracy of electronic testing instruments. It should be noted that the technology and the procedures used to control their accuracy are constantly evolving. It is important that the industry uses these instruments and responds to change in a standardized manner. This standardization of procedures ensures equitable accounting for dairy products that move freely between today's markets.



DEFINITIONS

Calibration – the operation of performing an ordinary least square linear regression to set the secondary slope and intercept to optimize the instruments results to that of known reference results on a set of samples.

Difference (d) = averaged instrument results (x) minus reference results (y) for each sample

$$d = x - y$$

Mean Differences (MD) = average of the differences (d) of the full sample set

$$MD = \frac{\sum d}{n}$$

n is the number of samples

Standard Deviation of the Differences (SDD) – the standard deviation of the sample differences (d) for the full sample set.

$$SDD = \sqrt{\frac{\sum (d - \bar{d})^2}{n - 1}}$$

n is the number of samples

Standard Deviation of repeatability (S_r) – square root of the average sample variance from instrument results for a full sample set.

$$S_r = \sqrt{\frac{\sum_{i=1}^n \left(\frac{\sum (x_{i \text{ rep}} - \bar{x}_{i \text{ rep}})^2}{n_{\text{rep}}} \right)}{n}}$$

n is the number of samples

n_{reps} is the number of reps per sample

UF – Ultrafiltered

RO – Reverse Osmosis

AOAC International – Association of Official Analytical Collaboration International

SMEDP – Standard Methods for the Examination of Dairy Products

ISO – International Organization for Standardization



GUIDELINE CONTENT

Control and Calibration Procedures

Following are guidelines and procedures for maintaining the control and accuracy of electronic testing instruments for milk components. Because instruments vary, as do the requirements of different regulatory agencies, precise control and calibration procedures will not be covered in this document. To use this guideline effectively, the following are required:

1. An instrument approved by the controlling regulatory agency, in working condition.
2. The instructional manual for operation, calibration, and maintenance procedures.
3. Documentation of the requirements of the controlling regulatory agency, which may include:
 - Laboratory Requirements
 - Calibration and Control Samples
 - Accepted Reference Tests
 - Precalibration Procedures
 - Instrument Calibration
 - Required Control Tests
 - Frequency of Control Tests
 - Required Records

Laboratory Requirements

The following equipment, supplies, facilities, and records should be available:

1. An approved electronic testing instrument, all required accessories and reagents, an instruction manual for operation and calibration of the instrument, and an instrument maintenance record.
2. Suitable laboratory facilities with temperature and humidity conditions meeting those specified by the manufacturer of the electronic instrument. The laboratory should be free from drafts, dust, noise, and vibrations. Laboratory should meet safety standards for both equipment installation and chemical storage.
3. Adequate lighting and sufficient counter space for all essential equipment.
4. A power supply specified for the electronic testing instrument(s) in use.
5. Prepared control milks with component concentrations established by the official reference methods, may be obtained commercially or from the controlling regulatory agency. Control milk samples must cover component ranges established by the regulatory agency or expected sample range.
6. A thermostatically controlled water bath, having proper temperature distribution to maintain samples at the temperature specified for the testing instrument or procedure in use (water bath temperatures generally 40-43°C).¹
7. A supply of distilled or deionized water.
8. Refrigeration at 0.5-4.5°C for milk sample storage.
9. Hot and cold water, wash sinks, and cleansing agents to clean equipment.
10. An adequate waste system to safely dispose of all milk, chemicals and wastewater as required by the local ordinance(s).
11. Thermometer with accuracy of $\pm 0.5^{\circ}\text{C}$ and with a response time of less than 15 seconds.

¹ New York: 36.7-37.8°C (98-100°F)



Calibration and Control Samples

Calibration and/or control milks used for calibration and performance checks of electronic instruments must cover the component ranges established by the controlling regulatory agency and/or the expected ranges of the samples to be tested. Generally, a minimum of 8 samples is required for calibration.

It is essential that samples used for controlling the accuracy of an electronic instrument be prepared and handled to assure their integrity and accuracy. Homogeneity of the sample set should be checked. Calibration and control samples should be made to best represent the type of milk for which the instrument will be used. For example, if the instrument is used for testing producer herd samples, the control samples should be made from raw whole milk herd samples. Blended samples of milk from more than one herd are acceptable. Alternate methods of production that utilize separation methods such as UF, RO, etc. may be used to manufacture sets of calibration samples with a robust component range covering the expected range for the samples to be tested.

Milk used to make up control and calibration standards should be of acceptable quality as determined by somatic cell counts, bacteria counts, free fatty acids and other appropriate quality control procedures. In addition, milk sources used should be monitored on an ongoing basis to assure normal response with the electronic testing instruments. Certain herd feeding programs or abnormalities can influence milk composition in a manner that may bias instrument readings. Such milks should not be used as reference standards.

Accepted Reference Tests

The following are the reference tests and requirements for establishing component levels in calibration milks.²

1. Butterfat (Fat) – The Modified Mojonnier Ether Extraction Method (AOAC standard method 989.05) is the recommended butterfat reference method. For preparation of calibration and control samples, a sub-sample of each milk should be tested in duplicate (triplicate may be required under some regulatory agencies). Mojonnier results should be read to the nearest 0.001%. For all chemical reference tests, the individual results and the average for each control or calibration milk sample shall be recorded.
2. True Protein (TP) – The Kjeldahl True Protein method (AOAC standard method 991.22) is the protein reference test in most areas. For preparation of calibration and control samples, a sub-sample of each milk should be tested in duplicate (triplicate may be required under some regulatory agencies). Individual results should be read to the nearest 0.001%. Record the individual result and the average for each control milk sample.
3. Lactose, anhydrous (Lact) – Lactose in Milk: Spectrophotometric Enzymatic Method Using Weight Additions and Path Length Adjustment (AOAC standard method 2006.06). For preparation of calibration and control samples, a sub-sample of each milk should be tested in duplicate (triplicate may be required under some regulatory

² Procedures for these reference tests can be found in latest editions of AOAC International Official Methods of Analysis.



agencies). Individual results should be read to the nearest 0.001%. Record the individual result and the average for each control milk sample.

NOTE: The above method for lactose reports results as anhydrous lactose, rather than lactose monohydrate. Anhydrous lactose is a more accurate measurement of the true lactose content in a milk sample. Methods that measure lactose monohydrate will result in lactose measurements approximately 5% higher, as 5% of the weight of lactose monohydrate is due to water:

$$\text{AnhyLact (\% w/w)} = 0.95 * \text{LactMonohyd (\% w/w)}$$

Lactose data is becoming of growing importance in dairy processing. Reporting of lactose measurements as anhydrous lactose is strongly encouraged, as it will support accurate and consistent lactose data across the industry.

4. Total Solids (TS) – Solids (Total) in Milk by Direct Forced Air Oven Drying (AOAC standard method 990.20). For preparation of calibration and control samples, a sub-sample of each milk should be tested in duplicate (triplicate may be required under some regulatory agencies). Individual results should be read to the nearest 0.001%. Record the individual result and the average for each control milk sample.
5. Solids Not Fat (SNF) – Solids Not Fat is determined by mathematical calculation.
$$\text{SNF} = \text{TS} - \text{Fat}$$

Determinations should be reported to the nearest 0.001%.
6. Other Solids (OS) – Other Solids is determined by mathematical calculation.
$$\text{OS} = \text{TS} - \text{Fat} - \text{TP}$$

Determinations should be reported to the nearest 0.001%.
7. Milk Urea Nitrogen (MUN) – Milk Urea Nitrogen in Milk: Spectrophotometric Enzymatic Method Using Weight Additions and Path Length Adjustment (AOAC standard method 202x.xx) or ISO 14637/IDF 195 - Milk - Determination of urea content Enzymatic method using difference in pH.

Precalibration Procedures

The instrument must be under control to be able to properly calibrate and run samples. Precalibration is a step to help assure that the instrument is under control before starting the calibration. Listed below are the recommended and/or required quality assurance checks.

1. Mechanical Checks and Homogenization Efficiency: The Mechanical Checks that should be checked are what the instrument manufacturer recommends. For Homogenization Efficiency, the mid infrared electronic testing instruments rely on uniform, efficient homogenization of milk fat globules in order to get repeatable results. Insufficient homogenization may result in light scattering that may influence readings for fat as well as for other components. Current tests for homogenization efficiency require that a sample of raw milk be run through the system, read and collected as the sample exits. The collected sample is then reheated and run through the system again and read (taking care that the milk is not rediluted in systems where diluent is added). This should be repeated with several samples, run in triplicate. If the average difference between first run and repeat run butterfat test is >0.05%, then the homogenizer should be replaced or repaired. It should be noted that with this procedure, the absence of



homogenization will not be detected; repeat results will be the same. Other inconsistencies may also exist.

2. **Linearity Check:** This check is normally only done upon installation or if major parts of the instrument are replaced, such as the IR box or detector. Procedures for checking whether an instrument's output is linear are covered in SMEDP and the AOAC Official Methods of Analyses. Generally, they involve testing a range of prepared solutions of cream, calcium propionate and/or lactose in water for testing linearity of the fat, protein and/or lactose readings, respectively. Instrument readings are plotted against the relative concentrations of the prepared solutions. A non-linear plot requires adjustment of the instrument.
3. **Zero Shift:** A zero shift check is used to verify the stability of the readings of the instrument, generally using zero solution or water. Clean the system using recommended cleaning procedures. Zero the machine for all components, as prescribed by the manufacturer's instructions. Run a vial of zero solution through the instrument to establish Initial Zeros. Run a raw milk sample(s) through the machine at least 12 times. Run another vial of zero solution through the instrument to flush out the flow system. Run another new vial of zero solution through the instrument to get Final Zeros. The zero shift (Initial Zeros minus Final Zeros) should be within ± 0.02 . If the instrument fails this test, it is generally an indication that the cuvette may need to be replaced.

NOTE: The Zero Shift test can be run in combination with the Repeatability check by adding the running of Initial Zeros, Flush and Final Zeros samples.

4. **Repeatability Check:** This procedure is used to verify the reproducibility of readings of the instrument. Run either a single, well mixed beaker of raw milk or several vials of raw samples to get 11 or more consecutive readings from the instrument. Determine the range and/or standard deviation of repeatability (S_r) of the 2nd through the last readings (10 or more results) for each component. If the range is greater than 0.04 or the S_r is 0.01 or greater, discontinue operation until the cause of the variability is determined and corrected.

NOTE: For better statistical interpretation, 18-21 samples should be run.

NOTE: A difference of +0.03 or greater between the first and second milk readings on fat, protein or lactose on the repeatability check might suggest insufficient purging efficiency.

5. **Purging Efficiency:** Efficient purging of the system is required to prevent carry over from one sample to the next. A complete procedure for testing purging efficiency is described in the AOAC International. This involves taking readings of 2 vials of zero solution followed by readings of 2 vials of pasteurized, homogenized, whole milk until a total of 20 readings are made [zero solution(W1), zero solution(W2), milk(M1), milk(M2), zero solution(W1), zero solution(W2), milk(M1), milk(M2), ...]. The purging efficiency is calculated as:

Purging Efficiency (water to milk) = $(\Sigma M1 - \Sigma W2) / (\Sigma M2 - \Sigma W2) \times 100$

Purging Efficiency (milk to water) = $(\Sigma M2 - \Sigma W1) / (\Sigma M2 - \Sigma W2) \times 100$



Values less than 99% suggest insufficient purging that might require that the system pump be repaired or adjusted to improve sample purging. Carry over has been shown to occur due to residual milk on the sample stirrer or probes that can also influence this test. If the initial test fails, it should be repeated by manually mixing and running the samples to prevent contamination.

Instrument Calibration

Calibration of electronic testing instruments should be done according to the manufacturer's instructions using reference samples prepared as described. A set of at least 8 calibration milk samples shall be used of the same type of sample to be run on the machine and shall represent a variety of component levels within the anticipated range of official samples to be tested.

Conditions Requiring Calibration:

- The instrument should be calibrated when initially installed.
- The instrument should be calibrated when the performance check or accuracy check (described below) fails.
- The instrument should be calibrated whenever any part that may affect proper operation of the instrument is replaced, rebuilt, or adjusted.

A machine shall be considered properly calibrated when the MD and the SDD on the sample set used for calibration are less than the values described in Table 1. Please note that these are maximum, not to exceed, values.

Table 1.

Maximum Allowable MD and SDD Between Results of the Electronic Tester and the Reference Test Method				
Reference Method	Machine Evaluated On			
	Sample set used for calibration		Other set used to verify instrument calibration	
	MD	SDD	MD	SDD
Mojonnier	±0.02%	≤0.04	±0.04%	≤0.04
Kjeldahl TP	±0.02%	≤0.04	±0.04%	≤0.04
Enzymatic Lactose	±0.02%	≤0.04	±0.04%	≤0.04
Oven Solids	±0.07%	≤0.09	±0.09%	≤0.12
Milk Urea Nitrogen	±0.5	≤1.0	±1.5	≤1.5

Should either the mean difference or the standard deviation of difference exceed the values shown in the “Sample set used for calibration” on Table 1, the instrument must be adjusted, and the calibration evaluation procedure repeated with another set of calibration or control samples.



Figure 1. Example of Calculation of MD and SDD of Instrument vs. Reference Test Results

Location:	Sampleville, WI - Foss MilkoScan FT+(ser # 11111111) and Foss Fossmatic FC(ser # 22222222)										
Operator:	J. Doe						Date:	Wednesday, July 6, 2022			
Evaluation of Instrument Results				Fat				TP			
				Ref	Instr	Instr. Mean	Diff.	Ref	Instr	Instr. Mean	Diff.
				Mean	4.172	4.172		3.219		3.211	
				MD			0.000				-0.007
				SDD			0.012				0.022
				SE _p			0.013				0.024
				R ²			0.055				0.005
Quality Assurance				S _e			0.003				0.004
				Zero Shift							
				PE Warning (W>M)							
				PE Warning (M>W)							
Samples From	Sample Set ID	Sample ID	Rep	Ref	Instr	Instr. Mean	Diff.	Ref	Instr	Instr. Mean	Diff.
MA TPC	6/20/2022	1	1	3.558	3.532	3.537	-0.021	3.068	3.034	3.042	-0.026
			2		3.539				3.045		
			3		3.541				3.045		
MA TPC	6/20/2022	2	1	3.723	3.716	3.720	-0.004	3.011	3.016	3.020	0.009
			2		3.720				3.023		
			3		3.723				3.023		
MA TPC	6/20/2022	3	1	3.906	3.914	3.915	0.010	3.041	3.033	3.032	-0.009
			2		3.915				3.029		
			3		3.917				3.033		
MA TPC	6/20/2022	4	1	4.880	4.869	4.868	-0.012	3.368	3.347	3.346	-0.022
			2		4.866				3.345		
			3		4.870				3.346		
MA TPC	6/20/2022	5	1	5.003	5.018	5.015	0.013	3.655	3.639	3.643	-0.012
			2		5.014				3.648		
			3		5.014				3.640		
MA TPC	6/20/2022	6	1	3.529	3.540	3.539	0.010	2.952	2.955	2.955	0.003
			2		3.539				2.958		
			3		3.540				2.951		
MA TPC	6/20/2022	7	1	3.957	3.945	3.947	-0.010	3.082	3.082	3.079	-0.004
			2		3.947				3.075		
			3		3.949				3.078		
MA TPC	6/20/2022	8	1	4.272	4.283	4.284	0.012	3.350	3.309	3.308	-0.041
			2		4.285				3.310		
			3		4.284				3.306		
MA TPC	6/20/2022	9	1	4.718	4.720	4.723	0.005	3.441	3.476	3.476	0.034
			2		4.724				3.474		
			3		4.724				3.477		

Preparation of Control Samples (Pilot Samples)

This is one area with the most variation in recommendations. Different regulatory agencies may have different requirements. As a practical approach, a minimum of one control/pilot sample is needed to show that the instrument is under control. The use of only one control/pilot sample will show if a shift has happened in the instrument. Additional control/pilot samples could help diagnose what type of problem has caused this shift. Make up enough of the samples to allow for performance checks to be done for a week. These samples may be from the calibration set or made separately from different milks.

1. When using one control/pilot milk sample, it should be a commingled sample of unhomogenized milk with approximately 4% butterfat. This butterfat range is approximately the average butterfat for the testing range. When using more than one control/pilot sample, make samples that cover the range of testing for the desired components (from 2.5 to 6.0% butterfat and 2.5 to 4.0% true protein).
2. An approved preservative shall be added at the required rate and mixed thoroughly if the control sample is to be used more than 72 hours after preparation. Mixing should be done in a manner that avoids churning. Divide each sample into sub-samples of adequate size, keeping the sample thoroughly mixed.
3. The homogeneity of the sub-samples should be tested on an instrument to prove repeatability of the splitting procedure.
4. A minimum of 4 sub-sample of each control milk shall be tested in duplicate (triplicate if required) by each instrument, soon after the calibration has been verified to be accurate, and the averages for each control milk sample shall be recorded as the reference values for that instrument.
5. The remaining preserved control sub- samples should be stored at 0.5-4.5°C until used.
6. Prior to the expiration date or before the last sub-sample of the control/pilot milk is used, whichever comes first, preparation or purchase of a new set of control/pilot milk samples shall be completed.

Routine Inspection and Control

Each electronic testing instrument should be inspected before each day's use in accordance with the manufacturer's instructions. Any deficiencies found during that inspection should first be recorded in the instrument maintenance record and appropriate repairs or adjustments should be made before the instrument is used to test milk. The following procedures should be followed each day before routine testing begins.

1. Repeatability Check: Ten or more consecutive readings on a single source, well-mixed commingled sample of raw milk (can be multiple sips from several vials) shall be made and recorded as a permanent record. The standard deviation shall be less than 0.01% for fat, true protein, or lactose component. If the standard deviation is 0.01% or greater, discontinue operation of the machine until the cause is determined and corrected.
2. Accuracy Check: At least one sub-sample of control milk shall be tested (in triplicate where required) using the electronic instrument (NYS requires 2 control samples, one low and one high; 4 control samples are recommended by NYS). The operator shall read the tests to the nearest 0.01%. If the result is within +0.05% from the reference for fat, true protein or lactose components (+0.09% for total solids), the operator may continue. (Where required: The first reading should be disregarded. If the difference between the average of the second and the third readings obtained from the electronic instrument and the average of the results obtained by the reference method is 0.05% or less (0.09% for T.S.), the operator shall proceed with testing).



3. If the difference is more than 0.05% on fat, true protein, or lactose components (0.09%, for total solids), test another sample of the control milk. If the difference of the additional sample exceeds 0.05% for fat, true protein, or lactose components (0.09% for total solids), the operator shall discontinue operation of the machine, determine the reason for the difference, and correct the deficiencies before resuming operation.
4. An accuracy check shall be performed at least once each hour during the time the instrument is in operation. Ideally, a control/pilot sample should be run before normal testing of batches and then either during or after a batch has finished. If the control/pilot samples pass, the operator knows that the instrument was under control while the batch was tested.

Required Records

Records of the operation and maintenance of each electronic instrument should be kept on paper or electronically. Record forms should be essentially like the sample form in Figure 1 and should contain the following information.

1. Performance Check (refer to Figure 1)
 - Laboratory name and machine identification.
 - Averaged individual sample test results by reference method.
 - Individual sample test results by electronic method and average result.
 - Differences between results of reference method(s) and electronic method(s) for each sample.
 - Results for Mean Differences (MD).
 - Results for Standard Deviation of Differences (SDD).
 - Performance test results (pass/fail) and description of any corrective adjustments made.
 - Date, technician name (signature and license number where required).
2. Routine Inspections
 - Name of laboratory and machine identification.
 - Precalibration performance test results (pass/fail) and description of any corrective adjustments made.
 - Date, technician name (signature and license number where required).
3. Instrument Maintenance Records
 - Name of laboratory and machine identification.
 - Instrument sample count and/or hours of machine operation on reporting date.
 - Rebuilding of homogenizer and/or other required maintenance procedure per manufacturer's recommendations.
 - Any other maintenance. Copies of service call reports for repairs or part replacement shall be kept with the maintenance records.



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- “AOAC International.” www.aoac.org/.

APPENDIX

None.

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