DAIRY PRODUCTS

Interlaboratory Assessment of Dry Calibration Milk Powders for Calibrating Infrared Milk Analyzers

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An interlaboratory study was carried out to assess the performance of preformulated, preanalyzed, dry calibration milk powders designed for calibrating infrared milk analyzers. The calibration powders can be reconstituted to produce consistent calibrations within laboratories. The powders met AOAC specifications in terms of accuracy and repeatability, and provide calibrations with performance characteristics comparable to those produced with conventional calibration milks. The reconstituted solutions were shown to be stable for up to 6 h at 40°C, and can be stored under refrigerated conditions and used for repeated analyses for up to 21 days without apparent deterioration in calibration performance. In general, the calibration powders perform as well as, or better than, the conventional calibrants used by the laboratories participating in the study, and the calibrations could be switched without significantly changing the analytical results. Simulation and cross-calibration analyses indicated that the powdered calibrants produced more consistent results overall than fluid calibration milks. The powdered calibrants, as formulated, are shown to be suitable for any application requiring calibrations that meet AOAC specifications, including payment applications. The calibration powders have the stability and performance characteristics to serve as a consistent, stable reference standard for monitoring instrument performance, and would be a useful tool for interlaboratory standardization or accrediting payment and dairy herd analysis laboratories.

Infrared milk analyzers are the instruments of choice for payment/dairy herd analysis laboratories, and are used in most dairy plant laboratories for routine quality control purposes (1–4). Calibration is a major requirement for the effective use of this rapid method of analysis, yet the precise chemical analyses depend on the proper facilities, a qualified work force, and the availability of chemically preanalyzed milks. The Central Milk Testing Laboratory (CMTL) in Guelph, ON, supplies preanalyzed calibration milks to many Canadian dairies and payment/herd analyses laboratories (5). These standards, however, have a limited shelf life and are susceptible to oiling off after 1 or 2 heating cycles (6). As a result of these concerns, we have developed stable, dry calibration powders that can be reconstituted on site to produce calibration milks immediately. The composition (fat, 0.12–5.49%; protein, 2.48–4.50%; and lactose, 3.68–5.50%), preparation, and an AOAC performance assessment of the 12 powder calibrants were previously reported (7).

As a consequence of the favorable results obtained for the powder calibrants relative to AOAC performance standards, a comprehensive interlaboratory study was undertaken to subject them to the additional variability associated with diverse analytical settings (i.e., instruments, procedures, personnel, etc.). This paper presents the protocol and results of the interlaboratory study, and discusses the general suitability of preformulated powder calibrants for calibrating infrared milk analyzers.

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Experimental

Nine laboratories agreed to participate in this study. Each received a Procedures Manual detailing the experimental protocol, and a calibration kit containing the following: 12 calibration powders (ca 75 g of each powder), a Braun mixer (Consumers Distributors Ltd, Montreal, PQ.), potassium dichromate preservative tablets (Pharma Science, Montreal, PQ), 12 plastic mixing cups, 12 screw-capped solution bottles, a spatula, and a chemical data specification sheet detailing the chemical data for each of the 12 powders. The experimental protocol required that each laboratory have available "conventional" calibration milks (their own or supplied by a third party), and a series of "unknowns" or milks routinely analyzed. All instruments were assured to be in good working order, and each laboratory was instructed to use AOAC calibration procedures (8). The manual called for the reconstitution of 3 separate sets of powders, a series of calibration trials, and various temperature and storage experiments. Clear instructions were provided, with photographs used to illustrate common operations. The manual included 16 pages structured specifically for the data of each experiment or operation. Other than the basic instructions given in the manual, the participants were asked to use procedures that were routine to their laboratory. In the selection of the participating laboratories, no attempt was made to differentiate among instrument type, wavelengths used for fat measurement, or the calibration method used (i.e., slope adjustment or multiple regression) (4), although this information was requested and provided. In addition, a general questionnaire was included in the manual that asked for comments about possible problems encountered, preparation times, general concerns, and the overall suitability of the calibration powders for their laboratory.

The assessment protocol was split up into 3 phases: calibration comparisons, reconstitution reproducibility trials, and temperature and storage stability trials. The instructions for each phase are described in detail in (7), and summarized below.

Phase 1–Calibration Comparisons

Reconstitute a set of calibration powders (set No. 1). Pass first run of reconstituted powder calibrants through instrument (data 1). Pass first run of unknowns through instrument

(data 2). Pass second run of unknowns through instrument (data 3). Pass first run of conventional calibration milks through instrument (data 4). Operator option: (*a*) recalibrate instrument with conventional milks (data 5); or (*b*) run second pass of reconstituted powder calibrants through instrument (data 5). Run third (or second) pass of reconstituted powder calibrants (data 6) through instrument. Pass third run of unknowns (data 7).

Phase 2–Reconstitution Reproducibility Trials

Reconstitute a new set of calibration powders (set No. 2). Pass through instrument (data 8). Reconstitute a new set of calibration powders (set No. 3). Pass through the instrument (data 9). Refrigerate sets Nos. 2 and 3 for further use.

Phase 3a–Temperature Stability Trials

Pass set No. 2 through instrument (data 10). Leave in bath at 40°C for 3 h. After 3 h, pass set No. 2 through instrument (data 11). Leave in bath at 40°C for 3 h. After 3 h, pass set No. 2 through instrument (data 12).

Phase 3b–Time Stability Trials

Pass set No. 3 (data 13) through instrument. After 7 days, repeat (data 14). After 7 more days, repeat (data 15). After 7 more days, repeat (data 16).

Data Analysis

The data recorded on data sheets 1–16 were entered, stored, and processed using Statgraphics (STSC Inc., Rockville, MD), a statistical and graphics package run on a personal computer. Multiple regression analysis was the most common technique used to obtain the calibration equations required to calculate the chemical estimates from instrument signals:

$$F_{ce} = f_1F_i + p_1P_i + l_1L_i$$

$$P_{ce} = f_2F_i + p_2P_i + l_2L_i$$

$$L_{ce} = f_3F_i + p_3P_i + l_3L_i$$

where F_{ce}, L_{ce}, and P_{ce} are the instrumental chemical estimates and f_{1-3}, p_{1-3}, and l_{1-3} are the multiple regression coefficients. The regressions were forced through the origin to provide equations that are comparable between data sets (4). In addition, mean differences (MD_a) and standard deviation of the differences (SDD_a) for accuracy and MD_r and SDD_r for repeatability (9) were calculated depending on the experiment. The accuracy parameters were calculated to determine how well the instrumental estimates reflected the chemical values for calibration samples, while the repeatability parameters were used to compare the results obtained for the unknowns, powder calibrant reconstitutions, temperature, and storage stability trials.

Results

Five of the 9 laboratories that participated in the interlaboratory study adhered rigorously to the study protocol as outlined in the *Experimental* section. The balance of the laboratories carried out only selected portions of the experimental protocol, and although useful and comparable results were obtained from these laboratories, only the data from the fully documented and properly conducted studies were analyzed, to allow for direct performance comparisons. The participants represented a mix of industrial, payment, and dairy herd analysis laboratories and used a variety of instruments (Foss 104, 605, Multispec MK 2, and DairyLab). These instruments make use of the common forms of fat wavelength measurement based on the ester linkage

Table 1. Accuracy parameters, multiple regression coefficients, and statistical parameters obtained by Laboratories 1–5 for fat calibrations using powder sets 1–3

L/S	MD_a	SDD_a	F_{coef}	P_{coef}	L_{coef}	R^2	SE
1/1	-0.0006	0.042	1.0239	-0.0133	-0.0038	0.9998	0.047
1/1	-0.0006	0.044	1.0285	-0.0141	-0.0050	0.9998	0.049
1/2	0.0003	0.057	1.0316	0.0091	-0.0170	0.9998	0.054
1/3	-0.0006	0.056	1.0367	0.0388	-0.0390	0.9997	0.060
2/1	0.0002	0.044	1.0110	-0.0021	0.0011	0.9998	0.049
2/1	0.0006	0.042	1.0090	-0.0014	-0.0005	0.9998	0.047
2/2	0.0005	0.042	1.0130	-0.0114	0.0053	0.9998	0.046
2/3	0.0003	0.046	1.0065	0.0025	-0.0013	0.9998	0.051
3/1	-0.0025	0.088[a]	1.0476	-0.0429	0.0190	0.9993	0.098
3/1	-0.0015	0.089[a]	1.0515	-0.0559	0.0239	0.9992	0.099
3/2	-0.0003	0.058	1.0374	-0.0151	-0.0002	0.9997	0.065
3/3	0.0010	0.040	1.0426	-0.0141	-0.0008	0.9998	0.046
4/1	0.0010	0.029	0.9800	-0.0457	0.0406	0.9999	0.032
4/1	0.0030	0.037	0.9427	-0.0469	0.0310	0.9999	0.041
4/2	0.0008	0.035	0.9403	-0.0274	0.0291	0.9999	0.038
4/3	0.0004	0.032	0.9423	-0.0221	0.0225	0.9999	0.032
5/1	0.0010	0.056	1.0108	-0.0436	0.0206	0.9997	0.062
5/1	0.0006	0.054	1.0033	-0.0556	0.0263	0.9997	0.060
5/2	-0.0004	0.044	0.9858	-0.0145	0.0061	0.9998	0.049
5/3	0.0005	0.051	0.9876	-0.0208	0.0108	0.9997	0.057

[a] Out of AOAC specifications.

Table 2. Accuracy parameters, multiple regression coefficients, and statistical parameters for powder calibrants obtained by Laboratories 1–5 for protein calibrations using powder sets 1–3

L/S	MD_a	SDD_a	F_{coef}	P_{coef}	L_{coef}	R^2	SE
1/1	-0.0010	0.034	0.0004	0.9388	0.0534	0.9999	0.038
1/1	0.0001	0.032	0.0001	0.9399	0.0536	0.9999	0.002
1/2	0.0010	0.031	0.0027	0.9534	0.0393	0.9999	0.034
1/3	0.0006	0.029	0.0026	0.9415	0.0489	0.9999	0.032
2/1	0.0002	0.032	0.0092	1.0085	0.0068	0.9999	0.036
2/1	-0.0001	0.030	0.0092	0.9945	0.0138	0.9999	0.033
2/2	-0.0001	0.027	-0.0062	1.0025	0.0079	0.9999	0.029
2/3	-0.0002	0.025	-0.0055	1.0086	0.0044	0.9999	0.028
3/1	-0.0005	0.035	0.1476	0.8739	-0.0073	0.9999	0.039
3/1	0.0010	0.032	0.1537	0.8725	-0.0117	0.9999	0.036
3/2	0.0010	0.037	0.1517	0.8671	0.0112	0.9999	0.042
3/3	0.0050	0.038	0.1522	0.8692	0.0119	0.9999	0.040
4/1	0.0000	0.022	0.0077	0.9930	0.0010	1.0000	0.023
4/1	0.0001	0.019	0.0106	0.9728	0.0080	1.0000	0.020
4/2	0.0000	0.022	0.0083	0.9849	0.0144	0.9999	0.025
4/3	-0.0000	0.029	0.0079	0.9810	0.0179	0.9999	0.033
5/1	-0.0004	0.025	-0.0050	1.0032	0.0090	0.9999	0.028
5/1	-0.0006	0.028	-0.0096	1.0260	0.0070	1.0000	0.011
5/2	-0.0008	0.023	-0.0053	1.0327	0.0005	0.9999	0.025
5/3	-0.0006	0.032	-0.0088	1.0239	0.0109	0.9999	0.033

(5.73/5.58 μm) and the C–H stretch (3.48/3.56 μm), either alone or in combination (10–13).

Tables 1–3 present the parameters for accuracy, the multiple regression coefficients for equations, and the statistical parameters for the powder calibrant sets 1–3 obtained by Laboratories 1–5 for fat, protein, and lactose, respectively. The first set of results for each laboratory are duplicate passes of the same sample through the instrument carried out in Phase 1 (data 2 and 6) to provide an indication of the reproducibility within a set. Tables 1–3 compare the performance of the powders relative to AOAC specifications (14) for accuracy ($MD_a < 0.05\%$, $SDD_a < 0.06\%$) for fat, protein, and lactose. The statistical parameters and coefficients provide an indication of the fit of the calibrations and the degree of consistency obtainable for the regression coefficients, within and between laboratories.

The data in Table 1 represent the 3 sets of reconstituted powders in relation to fat for 5 laboratories; that data also incorporate all the interlaboratory variables that could affect the results (i.e., instruments, weighing errors, sample handling, etc.). The most apparent feature of the data presented is the consistency of the regression coefficients, not only within any one laboratory but also between laboratories. This interlaboratory consistency is remarkable considering instrument variability and potential sources of error one would expect in such a comparison. The consistency within any one instrument was not surprising, and we have used this feature of the powder calibrants to monitor cell wear, window fouling, and water vapor changes within the instrument. All 5 laboratories met MD_a specifications of $< 0.05\%$ for fat, and only Laboratory 3 did not meet the specifications for SDD_a of $< 0.06\%$, but only in their first set. This result was considered an anomaly, because 2 additional laboratories, in addition to the 5 reported here, were also within specifications.

The data for protein and lactose (Tables 2 and 3) show similar trends noted for fat, and, in general, the results are somewhat better in terms of MD_a and SDD_a, with the exception for the lactose result of set 3 from Laboratory 1. This anomaly is not readily explained, considering the overwhelming conformity of the rest of the data. Overall, the MD_a, SDD_a, and the regression data in Tables 1–3 show that individual laboratories were able to reconstitute 3 separate sets of powder calibrants and obtain calibrations that were within AOAC specifications. A key feature associated with all the data is that most laboratories have primary coefficients near unity for each respective component equation, indicating that the instruments were linearized and the primary slope was adjusted to obtain optimal response for each component. All of the chemical estimates derived for the duplicate runs for set 1 were also assessed for MD_r and SDD_r (data not shown). The results met AOAC specifications (14) for reproducibility ($MD_r < 0.02$; $SDD_r < 0.02$) and were of the same order as obtained in our previous study (7). The ability to reconstitute the powder calibrants and obtain consistent, reproducible results, both within and between laboratories, suggests that the powders may be a useful vehicle for comparing interlaboratory calibrations.

Table 4 compares the calibrations obtained from powder calibrant set 1 for fat to those obtained with conventional calibration milks, prepared either in-house or supplied by a third party. All calibrations meet the MD_a specifications. Only Laboratory 3 exceeded specifications for SDD_a (on its initial trial), as noted earlier. The conventional calibrants from Laboratories 1 and 2 generally performed better in terms of SDD_a; however, Laboratory 5 was outside specifications. The performance of the powder calibrants is slightly better for Laboratories 4 and 5, and all the calibration coefficients once again show a general similarity, with the obvious exception of Laboratory 5. Only the fat calibration milks for Laboratory 5 differed from the conventional laboratory calibration milks; protein and lactose milks had a range of $< 0.25\%$. Such a narrow range of variability for 2 components relative to each other and to fat result in an anomalous regression equation. Tables 5 and 6 present parallel data for protein and lactose resulting in calibrations that all met MD_a and SDD_a AOAC specifications. Laboratory 5 again pro-

duced quite different equations. The general conclusion based on these data is that the calibration powders produced good results within AOAC specifications, although on average the conventional calibrations were marginally better. These results concur with our previous work (7) when a direct comparison was made to CMTL calibration milks. The minor difference in calibration fit is probably the result of the instructed use of a less accurate top loading balance, designed to simplify the reconstitution process.

Calibration Comparisons

Considering the general similarity of the calibration equations, with the exception of Laboratory 5, the question arises as to the overall relative performance of the conventional and powder calibrants. Such an assessment was carried out with 3 basic tests: simulation, cross-calibration, and with the unknowns. Simulation assumes the existence of an ideal instrument that has a perfect mechanical calibration, so that its instrumental estimates exactly match the chemical values. Using a hypothetical set of instrumental signals, the values can then be processed by each calibration equation (conventional or powder) to obtain the predicted chemical estimates and subsequently compared them to the chemical data using the MD_a and SDD_a to determine how well each calibration performs as a predictor. The data in Table 7 (generated by the calibration equations presented in Table 4) presents the results of such a simulation. The data set consisted of 10 values for fat, protein, and lactose, with ranges of 0.5–5.0%, 3.5–4.4%, and 4.1–5.0% in increasing increments of 0.5, 0.10, and 0.10, respectively.

On the basis of the simulation, the powdered calibrations on the whole perform better than the conventional calibrations, with the majority of the results meeting AOAC specifications. Laboratory 3, which had marginal calibrations, performed poorly with both calibrations. The calibration from Laboratory 5 has major problems handling this data set because protein and lactose vary outside its calibration range and the large coefficients impact strongly on the fat result. A cross-calibration was also carried out using the instrumental calibration data supplied by each laboratory. The original instrument specific signals recorded by each laboratory for the powder calibrant milks were processed by equations derived from the conventional calibrants. The process was then reversed, using the conventional calibration milk signals and processing them with the powder calibration equations (7), in effect exchanging calibrations. The results obtained followed a similar pattern as that shown in Table 7, although the overall MD_a and SDD_a were roughly double because of the compounding of the error associated with each calibration.

Both approaches have limitations: the simulation ignores inherent instrument variability, while the calibration exchange confounds calibrant differences and accumulates the inherent error associated with the 2 calibrations. Regardless of these limitations, both the simulation and the calibration exchange procedures indicate that the powder calibrants perform better than the conventional calibration milks overall. The implication of these results is that the powder calibrants are more consistent in predicting the chemical estimates and, therefore, are more transferable between instruments because of their compositional consistency. The conventional calibration milks used by the laboratories varied in source or batch, resulting in a variable calibration reference point. Any one specific batch of these calibration milks could have similar consistency characteristics and calibration transferability as the powders; however, a batch degrades and, therefore, can not be used for an extended period of time.

Assessing the reproducibility characteristics of the unknowns relative to each calibration is another method of evaluating the performance of the calibrations, and this method avoids the limitations of the simulation and cross-

Table 3. Accuracy parameters, multiple regression coefficients, and statistical parameters for powder calibrants obtained by Laboratories 1–5 for lactose calibrations using powder sets 1–3

L/S	MD_a	SDD_a	F_{coef}	P_{coef}	L_{coef}	R^2	SE
1/1	0.0010	0.040	0.0015	0.0433	0.9577	0.9999	0.045
1/1	0.0010	0.042	0.0031	0.0293	0.9644	0.9999	0.045
1/2	0.0005	0.043	0.0042	0.0497	0.9500	0.9999	0.048
1/3	-0.0003	0.092[a]	0.0260	0.0244	0.9596	0.9996	0.102
2/1	-0.0007	0.032	-0.0351	0.0220	0.9886	0.9999	0.036
2/1	-0.0008	0.044	-0.0355	0.0103	1.0064	0.9999	0.050
2/2	-0.0009	0.031	-0.0345	0.0203	1.0034	0.9999	0.035
2/3	-0.0010	0.032	-0.0335	0.0295	0.9973	0.9999	0.036
3/1	0.0005	0.041	-0.0262	-0.0056	1.0113	0.9999	0.047
3/1	0.0010	0.053	-0.0230	-0.0229	1.0149	0.9998	0.059
3/2	0.0008	0.043	-0.0166	-0.0114	1.0166	0.9999	0.048
3/3	-0.0004	0.026	-0.0160	-0.0035	1.0010	1.0000	0.030
4/1	-0.0009	0.032	-0.0145	0.0617	0.9701	0.9999	0.036
4/1	-0.0008	0.035	-0.0087	0.0661	0.9501	0.9999	0.039
4/2	-0.0008	0.031	-0.0158	0.0620	0.9523	0.9999	0.034
4/3	-0.0010	0.036	-0.0175	0.0609	0.9550	0.0999	0.040
5/1	-0.0010	0.041	-0.0353	0.0613	0.9752	0.9999	0.046
5/1	0.0010	0.044	-0.0383	0.0529	0.9853	0.9999	0.050
5/2	0.0010	0.046	-0.0401	0.0492	0.9887	0.9999	0.049
5/3	-0.0010	0.043	-0.0334	0.0498	0.9878	0.9999	0.048

[a] Out of AOAC specifications.

calibration analysis discussed previously. Any major changes caused by recalibrating the instrument in Phase 1 should be detectable by comparing the MD_r and SDD_r of the unknowns when switching calibrations. All participants chose to recalibrate their instruments in Phase 1. Table 8 presents the MD_r and SDD_r based on replicates for the unknowns analyzed using powder and conventional calibrations. To facilitate the comparison, the chemical estimates for the unknowns were assessed for their MD_r and SDD_r based on the powder calibration and the 2 sets of predictions averaged. The MD_r and SDD_r for the third run of the unknowns under the new calibration was then calculated against the averaged data set from the first 2 runs. The MD_r was judged to have changed significantly if the mean value changed by more than the acceptable standard deviation of ± 0.05% (15). Judged by these standards, calibration changes shifted 4 of the 15 means just out of specification; lactose accounted for 3 of the shifts (Table 8). In general, lactose is one of the least accurate calibrations, because most natural milk blends cover only a narrow lactose range. The range is much wider in the powder calibrants, and a shift in this mean can be expected. Overall, calibration changes had little effect on the results of the un-

Table 4. Comparison of accuracy parameters, multiple regression coefficients, and statistical parameters for fat in powder calibrants (P) and the conventional calibration milks (C) obtained by Laboratories 1–5

L/Calibrant	MD_a	SDD_a	F_{coef}	P_{coef}	L_{coef}	R^2	SE
1/P	-0.0006	0.042	1.0239	-0.0133	-0.0038	0.9998	0.047
1/C	-0.0002	0.012	1.0180	-0.1180	0.0619	1.0000	0.021
2/P	0.0002	0.044	1.0110	-0.0021	0.0011	0.9998	0.049
2/C	-0.0010	0.012	0.9968	0.0021	-0.0004	1.0000	0.022
3/P	-0.0025	0.088[a]	1.0476	-0.0429	0.0190	0.9993	0.098
3/C	0.0002	0.052	1.0408	0.0841	-0.0820	0.9997	0.059
4/P	0.0010	0.029	0.9800	-0.0457	0.0406	0.9999	0.032
4/C	-0.0001	0.032	1.0354	0.0197	-0.0133	0.9999	0.036
5/P	0.0010	0.056	1.0108	-0.0436	0.0206	0.9997	0.062
5/C	-0.0005	0.063[a]	1.0271	2.3224	-1.5187	0.9997	0.076

[a] Out of AOAC specifications.

Table 5. Comparison of accuracy parameters, multiple regression coefficients, and statistical parameters for protein in powder calibrants (P) and the conventional calibration milks (C) obtained by Laboratories 1–5

L/Calibrant	MD_a	SDD_a	F_{coef}	P_{coef}	L_{coef}	R^2	SE
1/P	-0.0010	0.034	0.0004	0.9388	0.0534	0.9999	0.038
1/C	0.0000	0.001	0.0040	0.6328	0.2264	1.0000	0.020
2/P	0.0002	0.032	-0.0092	1.0085	0.0068	0.9999	0.036
2/C	0.0000	0.022	0.0085	0.9533	0.0227	0.9999	0.025
3/P	-0.0005	0.035	0.1476	0.8739	-0.0073	0.9999	0.039
3/C	-0.0001	0.029	0.1563	0.8099	0.0125	0.9999	0.031
4/P	-0.0004	0.022	0.0077	0.9930	0.0010	1.0000	0.023
4/C	0.0000	0.013	0.0164	0.9389	0.0372	1.0000	0.015
5/P	-0.0004	0.025	-0.0050	1.0032	0.0090	0.9999	0.028
5/C	0.0000	0.008	-0.0046	0.1742	0.5241	1.0000	0.010

Table 6. Comparison of accuracy parameters, multiple regression coefficients, and statistical parameters for lactose in powder calibrants (P) and the conventional calibration milks (C) obtained by Laboratories 1–5

L/Calibrant	MD_a	SDD_a	F_{coef}	P_{coef}	L_{coef}	R^2	SE
1/P	0.0010	0.040	0.0015	0.0433	0.9577	0.9999	0.045
1/C	0.0000	0.008	-0.0046	0.8165	0.4775	1.0000	0.014
2/P	0.0007	0.032	-0.0351	0.0220	0.9886	0.9999	0.036
2/C	0.0002	0.026	0.0010	-0.1443	1.0982	1.0000	0.024
3/P	0.0005	0.041	-0.0262	-0.0056	1.0113	0.9999	0.047
3/C	0.0001	0.038	-0.0137	-0.0086	1.0269	0.9999	0.042
4/P	-0.0009	0.032	-0.0145	0.0617	0.9701	0.9999	0.036
4/C	0.0000	0.023	0.0011	-0.0330	1.0559	1.0000	0.024
5/P	-0.0010	0.041	-0.0353	0.0613	0.9752	0.9999	0.046
5/C	-0.0001	0.017	-0.0127	0.3714	0.7710	1.0000	0.019

knowns, indicating that the conventional and powder calibrants are essentially compatible and interchangeable with the conventional calibration milks used at each laboratory.

Temperature Stability

Temperature stability trials were carried out with powder set 2 by keeping the samples in a 40°C H_2O bath for 6 h, and determining the chemical estimates of the components at t = 0, 3, and 6 h. Table 9 presents the MD_r and SDD_r values for the fat component as a function of time relative to the results at time zero. Significant differences are an indication of oiling off over time as the calibration standards stay in the H_2O bath, a problem commonly encountered with conventional calibration milks. No major changes are apparent and, with the exception of Laboratory 5, all of the samples stay within specifications over the 6 h period. The samples for Laboratory 5 may not have been shaken adequately before presentation to the instrument. Protein and lactose are also stable; increases and decreases in their respective means generally indicate microbial spoilage.

The regression equations for each time were also examined and found to be stable in terms of the regression coefficients and standard error (SE), with the exception of Laboratory 5, where the primary fat coefficient and SE both showed a significant change.

Table 7. MD_a and SDD_a for fat, protein, and lactose obtained by simulation using powder and conventional calibration equations from Tables 4–6 on hypothetical instrumental data

	Powder calibrants		Conventional calibrants	
Laboratory	MD_a	SDD_a	MD_a	SDD_a
		Fat		
1	0.006	0.032	−0.140[a]	0.015
2	−0.022	0.017	−0.007	0.004
3	−0.045	0.066[a]	−0.098[a]	0.053
4	−0.055[a]	0.030	0.110[a]	0.053
5	0.054[a]	0.009	2.230[a]	0.285[a]
		Protein		
1	0.001	0.003	−0.414[a]	0.037
2	−0.034	0.009	−0.037	0.313[a]
3	0.130[a]	0.180[a]	0.271[a]	0.181[a]
4	−0.008	0.010	0.032	0.017
5	−0.035	0.005	−0.835[a]	0.098[a]
		Lactose		
1	−0.022	0.004	−0.831[a]	0.081[a]
2	−0.066[a]	0.049	0.126[a]	0.012
3	−0.047	0.037	−0.045	0.015
4	−0.062[a]	0.013	−0.121[a]	0.008
5	−0.027	0.043	0.385[a]	0.022

[a] Out of AOAC specifications.

Storage Stability

The storage stability trials combine both repeated heating and refrigerated storage effects, which can lead to increased susceptibility to oiling off and provide time for psycrophyllic microbial growth to take place, the latter resulting in acid production and proteolysis. Table 10 presents the MD_r and SDD_r values for fat, protein, and lactose obtained over a 21 day period relative to day 0. There is no perceptible trend or indication that the fat results deteriorate, even after 21 days, and only Laboratory 3 was out of specifications to any significant degree. All lactose and protein stabilities met the AOAC specifications, with the exception of Laboratory 3, where microbial growth is evident in the regression analysis and in the shift in the means and standard deviation. The general conclusion related to temperature and storage time data is that the reconstituted powder calibrants can take a reasonable amount of temperature abuse and can have a shelf life of up to 21 days.

Discussion

All the laboratories were able to obtain calibrations within AOAC specifications with the powder calibrants supplied. These could be reconstituted in a reproducible fashion within a laboratory and between laboratories. The powders were shown to be stable to temperature abuse and could be stored for up to 21 days in liquid form without significant changes in calibration results. These 2 factors are important from a practical standpoint by reducing the worry about oiling off when keeping samples warm and providing a substantial extension in shelf life relative to conventional calibration milks. One of the most common positive responses to our follow-up questionnaire was the convenience of making samples at any time rather than placing an order and waiting. Some common qualifiers regarding the routine use of powders were: confidence in the powders, the calibrations prepared from them, oiling off stability, and solution shelf life.

In addition to concerns regarding the convenience and stability of the powders, a key issue associated with infrared milk analysis is devising a universal, uniform, and accurate calibration system. Although workable calibration milks can be derived from blending herd milks, 2 fundamental limitations are associated with this approach: (*a*) the need to continually prepare and analyze new batches because of their inherently limited shelf life, and (*b*) the resulting lack of a standard reference calibrant that can be used to monitor instrument calibration over an extended period of time. The latter problem is particularly crucial in terms of assessing or regulating high volume payment and dairy herd analysis laboratories, because the calibration is performed against a variable reference.

Powder-based calibrants provide a means of producing bulk, stable, preanalyzed calibrants that can provide a consistent calibration reference for an extended period of time when refrigerated in the dry form. Objections may be raised as to whether such calibration powders take into account the natural correlations between components, seasonal changes in the composition of butterfat, or feeding practices. With relatively few suppliers of calibration milks in North America blending selected herd milks, or skimming or adding fat to bulk milks, such milks can at best only typify milks found in a particular region (5) and can certainly not typify individual

cow milks. In addition, these "natural" methods still limit the variability attainable in protein and lactose composition, and consequently the flexibility of the calibration. As a result, these milks can run into problems when component values are at the extremes of the calibration range. This situation sometimes leads to outliers, especially for individual cow milks.

Of the concerns described above, the calibration powders appear to be the weakest in terms of the seasonal variability of cows milk, which changes in its average molecular weight and degree of unsaturation (10). This factor did not appear to affect the results of this study, which covered a wide range of locations over the spring and late summer, the 2 periods when these variations are most prominent. This problem should have become apparent in comparing Phase 1, where regular milks normally being analyzed on a day-to-day basis were passed through the instruments calibrated using either calibrant system, yet produced essentially the same results. Although this is an indirect indication of seasonal variation and does not bias the results in any significant manner, definitive proof can only be obtained by carrying out concurrent chemical analyses of those samples. Unfortunately, this additional procedure could not be imposed on the participants in this study.

The problem of seasonal variation and its influence on instrumental results has been examined by a number of researchers (10, 16). Biggs and McKenna have recently published an extensive quantitative analysis of these effects and shown that the changes in molecular weight and unsaturation can be minimized through the combined use of A (C=O) and B (C–H) filters, with their signals used in specific proportions (0.27 and 0.73). An earlier submission of this study to AOAC has resulted in a recommendation (17, 18) to revise the AOAC infrared calibration procedure so that any seasonal effects can be accounted for more readily, thus minimizing recalibration. Laboratories specifically concerned about this problem are advised to use the A + B filter approach, or if this is not possible, to evaluate for themselves whether seasonal effects cause any significant biases relative to the powder calibrants.

Considering all the variables a calibration theoretically has to account for (breed, feed, season, and individual cow variations), and the practical problems associated with conventional calibration milks (variability, shipping, oiling off, and stability), laboratories gain more by calibrating to a preformulated, exhaustively analyzed dry calibrant that is stable and accounts for a broader range of component concentrations than herd milks. The consistency of the calibration

Table 8. MD_r and SDD_r for duplicate unknowns run through the instrument initially calibrated with the powder calibrant (P) and for the third pass after conventional calibration compared with the average of the first 2 runs (C)

	MD_r			SDD_r		
L/Calibrant	Fat	Protein	Lactose	Fat	Protein	Lactose
1/P	-0.009	-0.009	-0.005	0.005	0.010	0.013
1/C	-0.014	-0.007	0.002	0.003	0.008	0.004
2/P	0.008	-0.002	-0.002	0.017	0.006	0.006
2/C	0.005	0.003	0.010	0.010	0.001	0.003
3/P	0.001	0.005	0.004	0.043	0.038	0.026
3/C	0.002	-0.049	0.052[a]	0.048	0.008	0.022
4/P	-0.002	-0.001	-0.004	0.013	0.005	0.007
4/C	0.056[a]	-0.015	-0.055[a]	0.011	0.006	0.008
5/P	-0.005	-0.001	-0.005	0.012	0.013	0.006
5/C	0.012	-0.024	-0.055[a]	0.020	0.015	0.002

[a] Out of AOAC specifications.

Table 9. MD_r and SDD_r for the reconstituted calibration solutions, maintained at 40°C as a function of time relative to time 0 for Laboratories 1–5

		MD_r			SDD_r		
Laboratory	Time	Fat	Protein	Lactose	Fat	Protein	Lactose
1	0–3	0.0008	0.0000	-0.0002	0.0390	0.0070	0.0100
1	0–6	0.0010	0.0001	0.0000	0.0240	0.0080	0.0090
2	0–3	0.0001	0.0001	-0.0001	0.0310	0.0050	0.0370
2	0–6	-0.0005	0.0001	0.0000	0.0400	0.0050	0.0080
3	0–3	0.0000	0.0004	-0.0007	0.0190	0.0210	0.0180
3	0–6	0.0002	0.0001	-0.0004	0.0190	0.0300	0.0110
4	0–3	0.0005	0.0000	-0.0002	0.0330	0.0110	0.0150
4	0–6	0.0007	0.0002	0.0005	0.0430	0.0170	0.0250
5	0–3	0.0006	0.0001	-0.0001	0.0750[a]	0.0070	0.0360
5	0–6	0.0010	-0.0005	-0.0004	0.0990[a]	0.0150	0.0240

[a] Out of AOAC specifications.

Table 10. MD_r and SDD_r for the reconstituted calibration solutions refrigerated and analyzed as a function of time relative to day 0 for Laboratories 1–5

		MD_r			SDD_r		
Laboratory	Time	Fat	Protein	Lactose	Fat	Protein	Lactose
1	0–7	0.0009	0.0010	0.0002	0.0370	0.0350	0.0080
1	0–14	0.0005	0.0009	–0.0000	0.0320	0.0330	0.0080
1	0–21	0.0020	0.0008	0.0007	0.0610	0.0360	0.0110
2	0–7	–0.0001	–0.0001	–0.0002	0.0140	0.0070	0.0090
2	0–14	0.0001	–0.0001	–0.0001	0.0110	0.0100	0.0060
2	0–21	–0.0001	–0.0001	–0.0001	0.0120	0.0060	0.0060
3	0–7	0.0030	–0.0051	–0.0005	0.0100	0.0020	0.0400
3	0–14	0.0020	–0.0053	–0.0005	0.0482	0.0030	0.0400
3	0–21	0.0030	0.0520[a]	0.0270	0.0713[a]	0.0040	0.0730[a]
4	0–7	0.0004	–0.0003	–0.0002	0.0160	0.0070	0.0090
4	0–14	–0.0001	0.0000	0.0006	0.0140	0.0060	0.0240
4	0–21	0.0004	0.0000	0.0002	0.0080	0.0040	0.0130
5	0–7	–0.0001	0.0001	0.0002	0.0240	0.0040	0.0310
5	0–14	0.0008	0.0001	0.0004	0.0260	0.0040	0.0170
5	0–21	0.0001	–0.0001	0.0001	0.0280	0.0100	0.0280

[a] Out of AOAC specifications.

equations (16) also provides a very useful means for monitoring instrument performance over time (i.e., cell wear, protein buildup, etc.) and allows comparison of instruments within a laboratory. More importantly, powdered calibrants could serve as a means of regulating or standardizing analyses that have financial implications (payment/dairy herd improvement) and could, in effect, serve as a potential laboratory accreditation tool.

Conclusion

This study has confirmed that the powdered calibrants designed (7) for calibrating infrared milk analyzers perform within AOAC specifications on an interlaboratory basis. Glenarry Biotech (Cornwall, ON) now produces these powders, providing a simple and practical alternative to the perishable calibration milks routinely used. These powders could serve as a standard for interlaboratory comparisons.

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