FOOD COMPOSITION AND ADDITIVES

Evaluation of a Chemiluminescence Method for Measuring Alkaline Phosphatase Activity in Whole Milk of Multiple Species and Bovine Dairy Drinks: Interlaboratory Study

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Alkaline phosphatase (ALP) is a ubiquitous enzyme in milk with time–temperature destruction similar to that of certain pathogens destroyed in pasteurization. Measurement of ALP to indicate proper pasteurization is a common practice. Recently the public health level for ALP was decreased to 350 mU/L, a level below the sensitivity of older colorimetric ALP methods. This study was conducted within the structure of the International Dairy Federation and the International Organization for Standardization to evaluate the reproducibility of the chemiluminescence method (Charm PasLite) for ALP at 50, 100, 350, and 500 mU/L in whole milk of multiple species to meet new regulations in the United States and proposed regulations in the European Union (EU). Fifteen laboratories from 8 countries evaluated bovine, goat, sheep, and buffalo milk, bovine skim milk, 20% fat cream, and 2% fat chocolate milk. At ALP levels of 350 and 500 mU/L, the average relative standard deviation for repeatability (RSD_r) was 7.5%, and the average relative standard deviation of reproducibility was (RSD_R) 15%. For ALP at 100 and 50 mU/L, the average RSD_r values were 10.5 and 12.6%, respectively, and the average RSD_R values were 18 and 25%, respectively. The limit of detection was 20 mU/L. Results are comparable to those obtained with other enzymatic photo-activated system methods such as the fluorometric method. Results indicate that the method is suitable for measuring ALP in the milk of multiple species and in dairy drinks at U.S. and proposed EU levels.

Alkaline phosphatase (ALP) is an enzyme in milk that has been used as a marker for the effectiveness of time-temperature pasteurization for more than 50 years (1). The legislation in many countries still requires the use of the Aschenburg-Mueller and Sharer colorimetric methods both for phosphatase detection and as standards that industry is required to use for pasteurization (2, 3). These methods detect as little as 0.1% raw milk in pasteurized products. Since 1990, 2 instrument-based methods that use enzyme substrates, enzyme photo-activated systems (EPAS), to produce fluorescence (Fluorophos™ Test, Advanced Instruments, Norwood, MA) and luminescence (Charm ALP-PasLite™ Charm Sciences Inc., Lawrence, MA) signals have been developed to improve the speed, precision, and detection of phosphatase (4–6). These instrumental methods provide measurements in milliunits per liter (mU/L) and have been shown to detect <0.3% raw milk in various dairy drinks (4; E. Zomer, Charm Sciences, Inc., unpublished collaborative study, 1996). In 2003, the U.S. Pasteurized Milk Ordinance (PMO) specification for pasteurization was revised to "350 mU/L using instrument based methods" by the National Conference on Interstate Milk Shipments (7, 8). References to colorimetric methods were deleted because these methods were not considered sufficiently sensitive to detect subregulatory levels and were problematic for some matrixes (9). Similarly, the European Union (EU) Laboratory Workshop has recommended that the EU adopt a 350 mU/L public health level and a 100 mU/L internal investigation level that are proposed to be effective in 2006 (10). These lower specifications have led to collaborative studies within the International Dairy Federation (IDF) and the International Organization for Standardization (ISO) to determine the precision and accuracy of the EPAS methods at these lower action levels (11; R. Salter, Charm Sciences Inc., IDF, unpublished data, 2003 and 2004).

This paper reports the results of the 2005 interlaboratory study of the chemiluminescence (Charm ALP-Paslite) method for measuring ALP in cow, goat, sheep, and buffalo milk as well as in 20% fat cream, 2% fat chocolate milk, and skim milk from cows. The study was conducted in 15 laboratories in 8 countries. Data were incorporated into the pending

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Table 1. ALP levels (mU/L) determined by 15 laboratories in cow whole milk spiked at 4 levels

	Spike level, mU/L									
	Negative		50		100		350		500	
Lab.	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	0	0	53	52	117	116	456	327	634	516
2	22	33	70	64	125	115	358	307	487	471
3	14	17	66	71	115	122	398	357	518	476
4	35	41	34	43	86	130	251	258	341	370
5	6	2	46	45	89	119	391	257	378	413
6	22	20	60	55	100	109	333	278	356	393
7	4	6	44	47	83	92	279	248	414	378
8	15	14	51	53	98	105	289	245	421	391
9	34	35	83	77	147	124	388	324	533	494
10	0	0	49	67	103	109	361	377	543	460
11	0	5	36	32	102	124	332	284	424	455
12	8	10	62	51	78	85	310	252	365	401
13	54[a]	34[a]	83	71	128	107	411	376	510	559
14	17	26	79	71	109	125	338	318	462	451
15	23	15	68	59	126	104	363	297	449	456

[a] Cochran straggler.

ISO-22160/IDF-209 standard "Milk and Milk-Based Drinks—Determination of Alkaline Phosphatase Activity—Enzyme Photo-Activated System (EPAS) Method."

Interlaboratory Study

Preparation of Samples

In a first round of testing, raw cow, goat, and buffalo milks were heated to 95 C and held at that temperature for 5 min to produce ALP-free milk. Sheep milk required 63 C for 40 min to produce an ALP-free milk with no protein precipitation. Milks were cooled to 4 C on ice, and antibiotics were added as preservatives to final concentrations: 100 ppb cephapirin, 1000 ppb gentamicin, and 15 ppm proclyn 3000. Drinks were split and spiked with various amounts of species-specific raw milk to produce ALP concentrations of 50, 100, 350, and 500 mU/L. Bulk samples were tested for ALP level and homogeneity by using the PasLite method and then divided into 5 mL portions and stored under nitrogen in stoppered amber bottles and along with a set of ALP-negative samples. Portions (90 mL) of negative sample were also stored under nitrogen in stoppered 100 mL glass bottles for use as negative milk samples during method calibration. Duplicates at each level, i.e., 0, 50, 100, 350, and 500 mU/L, were randomly selected, labeled, and sent under refrigeration at <4 C to the 15 participating laboratories.

In a second round of testing, ultra high temperature (UHT) light cream (20% fat), UHT 2% fat chocolate milk, and UHT skim milk were heated to 95 C for 5 min to ensure the absence of ALP activity. The samples were preserved and spiked with raw cow milk, aliquots were taken, and duplicates were randomly selected and sent under refrigeration to the participating laboratories.

Table 2. Precision data calculated for the determination of ALP in cow whole milk

Target ALP level, mU/L	Average ALP level, mU/L	s_r	RSD_r, %	r	s_R	RSD_R, %	R
0	17	5.1	29.6	14.1	14.6	85.5	40.8
50	58	5.7	9.8	16.0	14.4	24.8	40.3
100	110	13.5	12.3	37.8	16.4	14.9	45.9
350	325	45.0	13.8	126.1	55.9	17.2	156.6
500	451	34.1	7.6	95.4	69.5	15.4	194.7

Table 3. ALP levels (mU/L) determined by 15 laboratories in goat whole milk spiked at 4 levels

	Spike level, mU/L									
	Negative		50		100		350		500	
Lab	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	8	7	54	47	139	123	453	409	542	526
2	3	4	36	32	53	75	296	338	437	431
3	0	0	37	34	87	88	349	343	534	484
4	0	3	41	55	104	113	420	411	576	577
5	0	0	27	31	101	92	326	296	456	437
6	14	17	57	51	111[a]	168[a]	325	356	475	561
7	2	4	20	30	60	57	241	217	328[b]	321[b]
8	2	3	39	31	91	87	351	333	501	476
9	1	0	26	33	108	100	360	317	524	518
10	27	21	47	54	102	110	364	333	495	507
11	2	9	40	42	92	94	327	319	493	488
12	2	1	35	45	92	99	373	359	495	521
13	4	2	38	36	79	82	280	302	415	442
14	8	5	39	40	90	92	321	365	528	499
15	0	0	34	32	74	101	364	351	532	466

[a] Cochran outlier.
[b] Grubbs straggler.

Trial Design and Sample Delivery

Samples were prepared and sent on the same day. Each participant was sent duplicates of each ALP level, scrambled with blind coded numbers. Samples were sent in coolers refrigerated with enough block ice to last 7 days. Because the laboratories were inexperienced in using the chemiluminescence method, they received luminometer equipment and prestudy practice samples before the study. Five laboratories in the United States, 3 in the United Kingdom, 2 in Israel, and 1 each in Canada, Ireland, France, Australia, and New Zealand participated. The refrigerated samples were received by all laboratories within 5 days in good condition. The samples were stored under refrigeration and analyzed within 1 month of receipt. Cow milk samples preserved with antibiotic cocktail had previously been shown to have stable ALP readings for months at 4 C and 2 weeks at 37 C in preliminary experiments.

Experimental

The procedure followed was the 2005 revised draft of the international standard IDF-22160/IDF-209. Identical negative samples were supplied for use in each laboratory to prepare the calibrators and calibrate the luminometer. All laboratories used the negative sample that was supplied for calibration, and they received identical samples for testing.

Data and Statistical Analysis

A single analysis of each sample was performed by the same technician. Sample results and any observations were reported by fax and e-mail. Data were decoded and collated

Table 4. Precision data calculated for the determination of ALP in goat whole milk

Target ALP level, mU/L	Average ALP level, mU/L	s_r	RSD_r, %	r	s_R	RSD_R, %	R
0	5	2.0	41.1	5.7	6.8	136.7	19.0
50	39	4.8	12.4	13.5	9.3	24.1	26.1
100	92	8.1	8.8	22.8	19.0	20.6	53.1
350	340	20.2	5.9	56.6	49.5	14.6	138.6
500	486	24.5	5.0	68.6	61.4	12.6	171.9

Table 5. ALP levels (mU/L) determined by 12 laboratories in sheep whole milk spiked at 4 levels

	Spike level, mU/L									
	Negative		50		100		350		500	
Lab.	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	0	0	34	29	85	52	398	436	532	498
2	0	0	50	43	106	99	406	354	495	495
3	0	0	16	26	46	49	278	233	394	386
4	—	—	—	—	—	—	—	—	—	—
5	0	0	67	55	101	84	413	342	514	584
6	0	0	25	33	65	71	273	282	398	397
7	—	—	—	—	—	—	—	—	—	—
8	4	3	75	64	96	113	374	421	563	553
9	8[a]	8[a]	70	71	101	111	400	461	661	616
10	—	—	—	—	—	—	—	—	—	—
11	0	0	35	39	67	77	308	281	468	395
12	0	0	66	49	84	105	349	368	487	461
13	0	0	31	19	63	59	272	292	451	398
14	0	0	29	23	57	53	256	218	399	391
15	0	0	40	42	94	92	319	308	446	420

[a] Grubbs straggler.

for statistical analysis by following ISO 5725-2 (12). This consisted of calculating the mean value for each duplicate pair and the differences between the duplicates. The Cochran outlier test was applied to exclude outlying pair differences, and the Grubbs individual and pair outlier test was applied to exclude outlying means. After outlier exclusion, calculations were repeated until there were no further outliers. Outliers were excluded from further statistical analysis, but stragglers were included. Precision values [standard deviation for repeatability (S_r), relative standard deviation for repeatability (RSD_r), repeatability (r), standard deviation for reproducibility (S_R), relative standard deviations for reproducibility (RSD_R), and reproducibility (R)] were calculated for each test material from the remaining data.

Results and Discussion

Refrigerated samples were chosen for study. Prior prestudy and 2004 collaborative studies used freeze-dried rehydrated samples that may have introduced additional rehydration variation to precision values. Samples were microbiologically preserved with antibiotic cocktail shown not to interfere with ALP determination. This preservation extended the shelf-life of samples to accommodate multiple shipping days, customs clearance, and the laboratory testing schedule. The ALP level of 350 mU/L represented the public health action level of the U.S. PMO and that proposed for the EU (7, 10). The level of 500 mU/L level was selected for historical purposes for comparison with other ALP method studies (4, 11). The 100 mU/L level

Table 6. Precision data calculated for the determination of ALP in sheep whole milk

Target ALP level, mU/L	Average ALP level, mU/L	s_r	RSD_r, %	r	s_R	RSD_R, %	R
0	1	0.2	21.3	0.6	2.4	254.5	6.8
50	43	6.4	15.0	18.0	18.3	42.7	51.3
100	80	10.1	12.5	28.2	21.9	27.2	61.2
350	335	29.1	8.7	81.4	69.4	20.7	194.2
500	475	27.2	5.7	76.3	80.3	16.9	225.0

Table 7. ALP levels (mU/L) determined by 15 laboratories in buffalo whole milk spiked at 4 levels

	Spike level, mU/L									
	Negative		50		100		350		500	
Lab.	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	0	1	50	51	90	85	382	335	604	465
2	0	1	54	60	93	89	352	340	619	556
3	0	0	46	46	97	85	364	337	576[a]	805[a]
4	0	0	47	50	101	100	332	318	561	586
5	4	4	51	41	96	78	298	254	468	474
6	1	1	53	39	104	85	372	332	531	485
7	0	0	33	37	72	74	278	254	464	440
8	3	2	55	57	102	93	388	369	609	525
9	0	0	49	57	84	82	330	321	449	460
10	0	0	44	50	113	102	361	354	497	530
11	1	0	65	56	104	99	367	348	582	578
12	0	0	45	44	69	92	335	318	500	544
13	0	1	44	49	88	85	330	324	527	513
14	0	0	39	39	81	76	284	266	434	416
15	3	1	48	53	92	88	343	325	571	490

[a] Cochran straggler.

represented the investigational level for dairy drinks in proposed EU legislation, and 50 mU/L was half that level.

The data from the interlaboratory study are shown in Tables 1–19. Tables 1, 3, 5, 7, 9, 11, and 13 show the data for each sample tested in each laboratory for cow milk, goat milk, sheep milk, buffalo milk, cow skim milk, 2% chocolate (cow) milk, and 20% fat cream (cow), respectively. Stragglers were included in the statistical analysis, and outlier data were excluded from the precision calculations. Tables 2, 4, 6, 8, 10, 12, and 14 show the calculated precision data for each matrix. Laboratory 4 was not able to participate in the second round of testing, and thus 14 laboratories reported cream, skim milk, and chocolate drink data. Laboratories 4 and 10 reported clumped and viscous sheep milk samples and could not test this matrix. Laboratory 7 also reported the poor quality of the sheep milk sample and outlier negative ALP values for all but the highest spiking levels. Laboratories 5, 6, 8, and 13 reported clumped sheep milk, but they performed the determinations and reported statistically valid data.

The mean values determined for the samples and the overall mean value for each spiking level are shown in Table 15. The overall mean value for the 350 mU/L target spiking level was 323 mU/L, and the overall mean value for the 500 mU/L target spiking level was 457 mU/L. Tables 16 and 17 summarize the RSD_r and RSD_R values (S_r and S_R values for the 5 mU/L level) by matrix. The overall mean RSD_r values for the 323 and 457 mU/L levels were between 7 and 8%. It is not clear why the RSD_r for the 323 mU/L level in cow milk was higher than the RSD_r for the 457 mU/L level in cow milk; however, this does not appear to be a matrix-related effect, because the RSD_r values for the highest and the lower ALP levels in cow milk are similar to the

Table 8. Precision data calculated for the determination of ALP in buffalo whole milk

Target ALP level, mU/L	Average ALP level, mU/L	s_r	RSD_r, %	r	s_R	RSD_R, %	R
0	1	0.5	71.4	1.5	1.2	161.8	3.5
50	48	4.4	9.2	12.5	7.3	15.1	20.5
100	90	7.5	8.3	21.0	10.7	11.9	30.0
350	330	17.5	5.3	49.1	36.0	10.9	100.7
500	529	56.7	10.7	158.7	77.7	14.7	217.6

Table 9. ALP levels (mU/L) determined by 14 laboratories in cow skim milk spiked at 4 levels

	Spike level, mU/L									
	Negative		50		100		350		500	
Lab	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	1	0	60	56	121	115	313[a]	413[a]	473	391
2	23[a]	18[a]	186[a]	46[a]	85[a]	141[a]	263	248	340	351
3	0	2	53	52	91	93	276	270	328	342
5	1	3	44	48	86	85	306	292	322	341
6	0	0	36	39	89	77	249	239	331	316
7	0	0	56	56	102	99	284	249	371	356
8	1	2	60	60	105	113	329	317	402	394
9	1	0	41	57	102	94	288	258	242[b]	365[b]
10	0	0	42	41	89	80	332	298	413	403
11	0	0	56	60	105	115	312	296	388	426
12	0	0	57	53	86	91	277	267	384	391
13	0	0	54	51	102	106	286	277	358	335
14	0	1	64	59	121	130	313	343	396	406
15	4	0	55	66	101	108	306	320	383	433

[a] Cochran outlier or Grubbs outlier.
[b] Cochran straggler.

corresponding overall mean RSD_r values. The overall mean RSD_R values for the 2 highest ALP levels were about 15%. Higher RSD_r values were determined for the chocolate and sheep milk, which could be matrix effects because they are above the mean values calculated for most ALP levels. The higher RSD_R value for cow milk is a result of the elevated RSD_r value previously discussed.

The overall mean RSD_r of 10.5% and the overall mean RSD_R of 18.1% for the 100 mU/L level are greater than the corresponding values for the higher ALP levels. This trend is consistent with results reported for other methods (11). Similarly, the overall mean RSD_r increased to 12.6%, and the overall mean RSD_R increased to 24.6% for an ALP level of 51 mU/L. Again, higher values were obtained for the sheep and chocolate milk matrixes, suggesting a matrix effect. In the case of the chocolate milk, this effect could be due to the inherent properties of the dairy matrix, but in the case of the sheep milk, it may be due to an anomaly caused by observed quality issues related to the study sample and may overstate the precision values for sheep milk. The overall mean values for r and R decrease by 3% if the mean values for sheep and chocolate milk are excluded in the calculations.

For negative samples, standard deviations (SDs) for repeatability and reproducibility in Tables 16 and 17 are shown as absolute values rather than %RSD values. This was done because as the mU/L value for ALP approaches zero, the expression of precision as %RSD becomes erroneous. The overall mean ALP level for negative samples was 4.7 mU/L, and the mean S_R value was 4.9. This indicates that the method limit of detection (LOD) is approximately 20 mU/L (mean + 3SD), and the method limit of quantitation (LOQ) is about 55 mU/L (mean + 10SD), where SD = the standard deviation. Results for the negative sample of cow milk were erratic in different laboratories, and results for several samples were above

Table 10. Precision data calculated for the determination of ALP in cow skim milk

Target ALP level, mU/L	Average ALP level, mU/L	s_r	RSD_r, %	r	s_R	RSD_R, %	R
0	1	1.0	168.6	2.9	1.1	172.6	3.0
50	53	4.3	8.2	12.1	8.1	15.3	22.7
100	100	5.1	5.1	14.3	13.8	13.8	38.6
350	288	14.6	5.1	40.8	28.9	10.0	81.0
500	371	31.5	8.5	88.2	45.6	12.3	127.8

Table 11. ALP levels (mU/L) determined by 14 laboratories in 2% fat flavored (chocolate) cow milk spiked at 4 levels

	Spike level, mU/L									
	Negative		50		100		350		500	
Lab	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	9	0	69	77	132	122	367	332	579	462
2	63[a]	0[a]	51[b]	100[b]	131	203	269	268	436	369
3	0	0	27	49	97	112	314	338	427	455
5	1	0	89	56	132	133	295	295	209	260
6	0	0	38	33	132	63	172[a]	328[a]	273[b]	479[b]
7	1	10	52	57	114	114	302	280	397	394
8	0	0	27	31	92	105	294	249	413	431
9	0	0	76	—[c]	135	190	447	376	579	537
10	0	5	47	43	100	102	310	320	469	379
11	4	11	56	58	125	131	374	347	497	470
12	5	10	50	48	104	103	289	280	323	364
13	0	1	38	38	93	102	266	253	391	348
14	0	5	43	52	81	67	249	292	364	391
15	0	0	48	57	127	123	289	286	434	392

[a] Cochran outlier.
[b] Cochran straggler.
[c] — = No value reported because of laboratory accident.

the method LOD. The mean was also significantly higher than the mean values obtained for other negative samples from the other matrixes. Plausible explanations include differences in sample handling, laboratory effects, or a contamination of the negative samples of cow milk that occurred when aliquots of the sample were taken. The mean ALP level and the S_R may be overstated because of this anomaly, but there is no evidence to exclude the determined values.

The absolute difference between 2 single test results, obtained by using the method to analyze identical test materials within laboratories using the same equipment and in different laboratories with different analysts using different equipment, is expected to be 5%. The absolute values and the %RSD values reported in Tables 16 and 17 are similar and consistent with the results reported for the fluorometric EPAS method for ALP determination at similar study levels (11). The repeatability of the determination indicates suitable uncertainty for detection at the 100 and 350 mU/L levels because the values are generally less than half the determined value. The overall r and R values for the method may be overstated by 3% because of the influence of matrix effects in the analysis of chocolate and sheep milks.

Conclusions

The results of the interlaboratory study provide useful applied information for incorporation into the ISO-22160/IDF-209 standard for ALP determination using

Table 12. Precision data calculated for the determination of ALP in 2% fat flavored (chocolate) cow milk

Target APL level, mU/L	Average ALP level, mU/L	s_r	RSD_r, %	r	s_R	RSD_R, %	R
0	2	3.3	139.6	9.3	3.7	157.2	10.5
50	53	12.8	24.2	36.0	18.3	34.5	51.2
100	117	22.2	19.0	62.2	30.2	25.9	84.5
350	307	21.7	7.1	60.9	47.0	15.3	131.5
500	412	53.8	13.1	150.6	87.2	21.2	244.3

Table 13. ALP levels (mU/L) determined by 14 laboratories in 20% fat cream (cow) spiked at 4 levels

	Spike level, mU/L									
	Negative		50		100		350		500	
Lab	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	5	7	72	68	118	115	359	347	479	503
2	0[a]	16[a]	53	66	100	127	373	344	509	478
3	1	0	65	60	106	115	393	385	433[a]	682[a]
5	4	4	65	65	105	110	347	359	439	476
6	19	15	76	56	123	104	317	260	444	438
7	3	8	41	41	79	81	223	253	326	327
8	7	3	59	62	106	101	337	363	461	475
9	5	5	70	62	123	119	326	334	593	569
10	0	0	55	51	98	99	317	310	453	457
11	7	4	89[a]	5[a]	138	118	351	365	564	523
12	10	7	58	58	108	111	325	341	456	427
13	7	9	61	54	120	106	326	327	471	450
14	3	4	57	65	108	109	333	343	454	471
15	12	13	79	75	131	127	391	377	534	564

[a] Cochran outlier.

the chemiluminescence EPAS method. Both the fluorescence and chemiluminescence EPAS methods have similar repeatability and reproducibility values in analyses of whole milk of multiple species that are applicable to lower ALP threshold levels for public safety that have been adopted in the United States and are being proposed in the EU. Additional lower control thresholds are feasible at 50 and 100 mU/L.

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Table 14. Precision data calculated for the determination of ALP in 20% fat cream (cow)

Target ALP level, mU/L	Average ALP level, mU/L	s_r	RSD_r, %	r	s_R	RSD_R, %	R
0	6	1.8	29.2	5.1	4.8	76.4	13.3
50	61	5.6	9.2	15.8	9.5	15.5	26.6
100	111	8.2	7.4	22.9	13.6	12.2	38.0
350	337	15.7	4.7	43.9	40.2	11.9	112.5
500	475	17.4	3.7	48.8	64.1	13.5	179.4

Table 15. Mean ALP levels (mU/L) determined in samples spiked at 4 levels

		Spike level, mU/L			
Matrix	Negative	50	100	350	500
Cow milk	17	58	110	325	451
Goat milk	5	39	92	340	486
Sheep milk	1	43	80	335	475
Buffalo milk	1	48	90	330	529
Skim milk (cow)	1	53	100	288	371
Cream, 20% fat	6	61	111	337	475
Flavored milk	2	53	117	307	412
Mean	4.7	50.7	100.0	323.2	456.8

Table 16. Intralaboratory repeatability reported as the RSD_r value for each matrix at each spiking level (except for the 5 mU/L level[a])

	RSD_r, % at				
Matrix	5 mU/L (S_r)	51 mU/L	100 mU/L	323 mU/L	457 mU/L
Cow milk	5.1	9.8	12.3	13.8	7.6
Goat milk	2.0	12.4	8.8	5.9	5.0
Sheep milk	0.2	15.0	12.5	8.7	5.7
Buffalo milk	0.5	9.2	8.3	5.3	10.7
Skim milk (cow)	1.0	8.2	5.1	5.1	8.5
Cream, 20% fat	1.8	9.2	7.4	4.7	3.7
Flavored milk	3.3	24.2	19.0	7.1	13.1
Mean	2.0	12.6	10.5	7.2	7.8

[a] Reported as S_r values.

Table 17. Interlaboratory reproducibility reported as the RSD_R value for each matrix at each spiking level (except for the 5 mU/L level[a])

	RSD_R, % at				
Matrix	5 mU/L (S_r)	51 mU/L	100 mU/L	323 mU/L	457 mU/L
Cow milk	14.6	24.8	14.9	17.2	15.4
Goat milk	6.8	24.1	20.6	14.6	12.6
Sheep milk	2.4	42.7	27.2	20.7	16.9
Buffalo milk	1.2	15.1	11.9	10.9	14.7
Skim milk (cow)	1.1	15.3	13.8	10.0	12.3
Cream, 20% fat	4.8	15.5	12.2	11.9	13.5
Flavored milk	3.7	34.5	25.9	15.3	21.2
Mean	4.9	24.6	18.1	14.4	15.2

[a] Reported as S_R values.

Table 18. Absolute repeatability ranges (r) of the chemiluminescence method for the matrixes studied

	ALP target level, mU/L				
Matrix	Negative	50	100	350	500
Cow milk	14.1	16.0	37.8	126.1	95.4
Goat milk	5.7	13.5	22.8	56.6	68.6
Sheep milk	0.6	18.0	28.2	81.4	76.3
Buffalo milk	1.5	12.5	21.0	49.1	158.7
Skim milk (cow)	2.9	12.1	14.3	40.8	88.2
Cream, 20% fat	5.1	15.8	22.9	43.9	48.8
Flavored milk	9.3	36.0	62.2	60.9	150.6
Mean	5.6	17.7	29.9	65.5	98.1

Table 19. Absolute reproducibility ranges (R) of the chemiluminescence method for the matrixes studied

	ALP target level, mU/L				
Matrix	Negative	50	100	350	500
Cow milk	40.8	40.3	45.9	156.6	194.7
Goat milk	19.0	26.1	53.1	138.6	171.9
Sheep milk	6.8	51.3	61.2	194.2	225.0
Buffalo milk	3.5	20.5	30.0	100.7	217.6
Skim milk (cow)	3.0	22.7	38.6	81.0	127.8
Cream, 20% fat	13.3	26.6	38.0	112.5	179.4
Flavored milk	10.5	51.2	84.5	131.5	244.3
Mean	13.8	34.1	50.2	130.7	194.4

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