Alkaline phosphatase detection via chemiluminescence in 45 seconds using a one step assay

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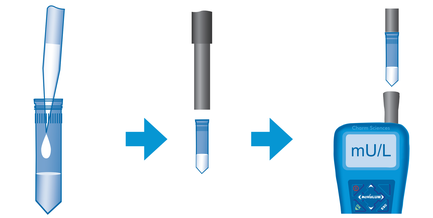
Introduction:

Alkaline phosphatase is a heat sensitive enzyme in raw milk used as a thermal marker for pasteurization. The FDA and ISO approved methods for detecting phosphatase utilize fluorometric or chemiluminescent substrates that are photo-activated in the presence of this enzyme (1, 2). US and EU regulations have been made more sensitive than older colorimetric methods and specify that pasteurized dairy products contain less than 350 mU/L alkaline phosphatase (3, 4). The purpose of this work was to compare a newly developed chemiluminescent method, Fast Alkaline Phosphatase (FAP), to the reference methods.

Method:

FAP method involves adding 100ul sample to a pre-dispensed reagent vial. The vial is mixed, attached to an adapter and inserted into a portable luminometer, NovaLUM, that monitors ambient temperature. At 45 seconds, the chemiluminescent signal of the sample is read for 5 seconds and converted into mU/L enzyme units (Figure 1) adjusted for temperature. Cream uses a 90 second incubation time. Chocolate milk requires centrifugation, 1200g for 3 minutes, prior to testing and also uses a 90 second incubation time. A linear calibration is programmed into the luminometer using two tests of negative milk and two tests with a 350 mU/L calibrator. In the comparison study, 1 minute 95°C lab pasteurized products (except sheep 45 min. 63°C) were spiked with 0.002-0.5% same-species-raw-milk (goat 0.02%-5.0%) and tested by the FAP and by the reference fluorometric and chemiluminescent methods, Fluorophos, Advanced Instruments and Paslite, Charm Sciences.

Figure 1



Results and Discussion:

Table 1 shows the FAP method compared to the chemiluminescent method. Table 2 shows FAP compared to the fluorometric method. The CVs of the mean values were typically 10-15% which is consistent with chemiluminescent detection. Table 3 are normalized data and show that across the range of spike levels, the mU/L means of the FAP were typically within 15-20 % of the reference methods with a slight positive bias at the spike levels greater than 350 mU/L and slight negative bias at the lower levels less than 350 mU/L. This trend is in all matrices with the exception of chocolate, data not shown, which had a 20% more negative bias at all levels. Cream results of the FAP method are consistent with the chemiluminescent method which also has a positive bias relative to the fluorometric method. With sheep milk the FAP was more in agreement with the fluorometric method at concentrations less than 350 mU/L. At the spike levels 0.0313% and 0.0156% raw milk, levels that bracket the action level of 350 mU/L, the differences are generally within the expected calibration variances and repeatability (RSVr ~ 10%) of the approved methods. This indicates the FAP is a sensitive alternative measure of alkaline phosphatase in dairy products. The FAP is a convenient, rapid and portable testing system for pasteurization effectiveness that provides dairies another option for meeting new more sensitive pasteurization regulations. Additional third party laboratory testing of the FAP method is in process.

References:

1. International Organization for Standardization (ISO), 11816-1 IDF 155-1; 1997 (E)
2. International Organization for Standardization (ISO), 22160 IDF 209; 2007 (E)
3. US Department of Health and Human Services, (2005) Public Health Service Food and Drug Administration, Publication 229, Pasteurized Milk Ordinance, Appendix N
4. Commission Regulation (EC) No. 1664/2006, Official J of the European Union

Table 1 - Comparison of average (N=3) mU/L determinations using Fast Alkaline Phosphatase and chemiluminescent methods when testing raw milk spiked dairy drinks

	Whole cow milk		Skim Milk		Light Cream		Sheep Milk		Goat Milk*	
Raw % level*	FAP	Chemi	FAP	Chemi	FAP	Chemi	FAP	Chemi	FAP	Chemi
0.5	5494	5915	4568	4915	6804	5998	7082	5829	7832	7936
0.0625	867	715	649	648	853	852	1025	864	1109	1090
0.0313	418	371	336	337	436	411	506	467	592	518
0.0156	223	218	159	169	192	213	312	226	294	303
0.0078	109	110	81	86	82	108	160	104	153	156
0.0039	59	59	37	47	27	55	92	43	78	74
0.002	29	29	14	22	8	23	49	18	36	39
0	8	0	0	0	0	1	0	0	0	0
350 cal	363	342	319	320	370	376	361	335	391	356
Pos Control	403	421	339	421	531	430	458	420	478	482

* for goat milk, multiply % spike level by 10

Table 2 - Comparison of average (N=3) mU/L determinations Fast Alkaline Phosphatase and fluorometric methods testing raw milk spiked dairy drinks

	Whole cow milk		Skim Milk		Light Cream		Sheep Milk		Goat Milk*	
Raw % level*	FAP	Fluor	FAP	Fluor	FAP	Fluor	FAP	Fluor	FAP	Fluor
0.5	5494	5328	4568	4701	6804	4501	7082	5539	7832	7073
0.0625	867	743	649	596	853	581	1025	791	1109	978
0.0313	418	381	336	307	436	321	506	436	592	529
0.0156	223	200	159	140	192	171	312	257	294	268
0.0078	109	106	81	67	82	92	160	160	153	153
0.0039	59	58	37	29	27	54	92	115	78	87
0.002	29	34	14	11	8	30	49	94	36	56
0	8	<10	0	<10	0	12	0	63	0	19.3
350 cal	363	357	319	293	370	299	361	360	391	310
Pos Control	403	435	339	386	531	340	458	445	478	424

* for goat milk, multiply % spike level by 10

Table 3 - Normalized data, FAP mean divided by chemiluminescent and fluorometric mean values

	Whole cow milk		Skim Milk		Light Cream		Sheep Milk		Goat Milk*	
Raw % level*	Chemi	Fluor	Chemi	Fluor	Chemi	Fluor	Chemi	Fluor	Chemi	Fluor
0.5	0.93	1.03	0.93	0.97	1.13	1.51	1.21	1.28	0.99	1.11
0.0625	1.21	1.17	1.00	1.09	1.00	1.47	1.19	1.30	1.02	1.13
0.0313	1.13	1.10	1.00	1.09	1.06	1.36	1.08	1.16	1.14	1.12
0.0156	1.02	1.12	0.94	1.14	0.90	1.13	1.38	1.21	0.97	1.10
0.0078	0.99	1.03	0.94	1.21	0.76	0.89	1.54	1.00	0.98	1.00
0.0039	1.00	1.02	0.79	1.28	0.49	0.50	2.14	0.80	1.05	0.90
0.002	1.00	0.85	0.64	1.27	0.37	0.28	2.72	0.52	0.92	0.64
350 cal	1.06	1.02	1.00	1.09	0.98	1.24	1.08	1.00	1.10	1.26
Pos Control	0.96	0.93	0.81	0.88	1.23	1.56	1.09	1.03	0.99	1.13