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CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

022201

The AOAC Research Institute hereby certifies the method known as:

ZymoSnap™ ALP

manufactured by

Hygiena LLC.
941 Avenida Acaso
Camarillo, CA 93012
USA

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested* SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (February 7, 2022 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

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Scott Coates, Senior Director
Signature for AOAC Research Institute

February 10, 2022
_____
Date

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SUBMITTING COMPANY
Hygiena LLC.
941 Avenida Acaso
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METHOD NAME
ZymoSnap™ ALP

CATALOG NUMBERS
ZS-ALP-100

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APPLICABILITY OF METHOD
Target Analyte – Alkaline Phosphatase (ALP)

Matrixes – - Pasteurized Milk (4%, 2%, 1%, and 0.1% fat content), Cream (40%), Chocolate Milk (1%), Strawberry Milk, Pasteurized Goat Milk.

Performance claims - The ZymoSnap Alkaline Phosphatase test was proven effective at detecting the presence of ALP in Pasteurized Milk (4%, 2%, 1%, and 0.1% fat content), Cream (40%), Chocolate Milk (1%), Strawberry Milk, and Pasteurized Goat Milk.

REFERENCE METHODS
2400j-1 (May 2014)
2400j-3 (Oct 2019)

ORIGINAL CERTIFICATION DATE
February 7, 2022

CERTIFICATION RENEWAL RECORD
New Approval 2022.

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC® *Performance Tested*[SM] License Number, 022201 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The ZymoSnap™ ALP device measures ALP enzyme levels in milk and other milk-based products. The 5 - minute test verifies pasteurization efficiency to meet regulatory standards and demonstrates the absence of raw milk. It is a rapid bioluminogenic method that determines the level of ALP enzyme via conversion of Relative Light Units (RLUs) to milliunits per liter (mU/L) of ALP in pasteurized milk samples. ZymoSnap ALP is intended for use with pasteurized liquid milk and dairy products. It is not suitable for use with long life or ambient stable milk products.

The assay uses a single self-contained device in one simple procedure. Milk sample is added to the ZymoSnap ALP tube, the device is activated to release the detection reagent, and the activated device is incubated for 5 minutes. ALP enzymatic activity is measured in the EnSURE™ Touch Monitoring System. ZymoSnap ALP is sensitive enough to measure below regulatory levels (350mU/L of ALP). Before using ZymoSnap ALP test devices, a positive and negative control must be prepared with ZymoSnap ALP Positive Control Kit (Part No. ZS-ALP-PC) in order to establish a Pass/Fail RLU.

DISCUSSION OF THE VALIDATION STUDY (1)

ZymoSnap ALP is an easy-to-use device that produces a bioluminescent signal in the presence of alkaline phosphatase enzyme. It is intended to provide rapid results that reveal the efficiency of the pasteurization process in a wide range of dairy matrixes, giving actionable data at the point of production. To function well in this application ALP test methods need to be sensitive below the post-pasteurization regulatory limit of 350mU/L, give results that are linear with respect to the concentration of ALP in the sample, be capable of giving accurate results in a wide range of dairy sample types, and be capable of detecting different forms of ALP (bovine, caprine, microbial, etc). We believe the above validation study demonstrates that ZymoSnap ALP delivers all the performance criteria of an efficient ALP test.

The correlation of ZymoSnap ALP to 2400j-1 (May 2014) method in the pure analyte study was 0.99, with an LOD in skimmed milk of 10.93mU/L (UK data) and 16.18mU/L (US data). The broader matrix study produced very similar performance across a range of products that varied in fat content, colorings, flavoring, and milk origin (bovine and caprine). ZymoSnap ALP correlation with the 2400j-1 (May 2014) method was 0.99 across all tested matrixes, with an LOD of <50mU/L in all cases (UK LOD range 2.04-48.54mU/L, US LOD range 5.05-29.66mU/L). In the Independent laboratory study, the following matrixes tested (4%, 2%, 1% cow's milk; 40% cream; 1% chocolate milk, strawberry milk, caprine milk) passed the NCIMS criteria when compared to 2400j-1 (May 2014) and 2400j-3 (Oct 2019) methods. The 1% and 0.1% milk matrixes both fell within the NCIMS spec for some of the tested ALP levels, but only when substituting in the Q Laboratories data in place of Lab 3. This may show an issue with the calibration channel being used in Lab 3.. Across two further experiments it was shown that ZymoSnap ALP can differentiate between residual, reactive (magnesium acetate experiment), and microbial (heat lability experiment) ALP. In each case ZymoSnap ALP produced mU/L results that were in very close agreement to the reference method. The selectivity study showed that ZymoSnap ALP will not give false positive results in the presence of non-ALP analytes in a product matrix. The recovery of target analyte signal from ALP in the presence of these non-target analytes was also very good with an RSD of 13.55% across all tested non-target analytes. It should be noted here that both ZymoSnap ALP and 2400j-1 (May 2014) method gave near identical mU/L readings in this selectivity study. This suggests that the two methods were likely unaffected by the selectivity compounds, but that there was some effect on the alkaline phosphate enzyme that altered the readings from both methods by near equal amounts. This is useful to keep in mind when testing more complex dairy products that are perhaps fortified with vitamins and other flavor compounds. The robustness study has shown that ZymoSnap ALP will still produce accurate results even if the specific usage guidelines are not followed precisely. However, it is still recommended to stick closely to the kit insert guidance to achieve the most accurate and reproducible results. The consistency testing also demonstrated that results from ZymoSnap ALP will remain accurate across different manufacturing batches and across the shelf-life of the product.

Table 5: Replicate mU/L, mean mU/L, S_r, and RSD values of ZymoSnap ALP method used on 11 various milk matrixes and compared to NCIMS approved methods (2400j-1 and 2400j-3). The matrixes were spiked with pure analyte (raw cow's milk) at target contamination level (TCL). All ZymoSnap ALP mU/L values measured in an EnSURE TOUCH luminometer. A negative control is just the pasteurized matrix un-spiked. Data produced in Guildford, Surrey, United Kingdom. (1)

Whole Milk 4%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L						2400j-3 (Oct 2019) mU/L					
	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]
5000	9,104	7,643	12,153	9,633	2,301	24	8532	8895	9934	9,120	728	8	11567	12511	13386	12,488	910	7
625	1,937	1,562	1,156	1,552	391	25	1158.4	1216.8	1183.3	1,186	29	2	1576	1417	1666	1,553	126	8
400	856	821	949	875	66	8	716.7	770.5	781.9	756	35	5	928	1090	901	973	102	11
320	733	790	761	761	29	4	604	581.5	567.7	584	18	3	446	582	452	493	77	16
160	199	287	353	279	77	28	274.9	286.4	281.8	281	6	2	332	438	397	389	53	14
80	119	44	229	131	93	71	153.1	153.1	153.1	153	0	0	190	181	214	195	17	9
20	49	79	79	69	18	26	54.2	38.2	58.4	50	11	21	44	53	56	51	6	12
0	31	22	40	31	9	29	31.7	10	10	17	13	73	0	0	0	0	0	N/A
LOD	48.54 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9994			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9988		

Semi-Skimmed Milk 2%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L						2400j-3 (Oct 2019) mU/L					
	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]
5000	4,470	6,092	5,118	5,227	816	16	5,778.0	5,880.0	6,509.0	6,056	396	7	4,428	4,292	4,476	4,399	95	2
625	687	1,210	1,057	984	269	27	875.3	776.9	799.0	817	52	6	1,295	131	1,701	1,042	815	78
400	418	485	489	464	40	9	439.5	444.5	481.8	455	23	5	369	399	476	415	55	13
320	307	332	340	326	17	5	303.9	398.6	396.7	366	54	15	796	883	772	817	58	7
160	151	256	265	224	64	28	183.4	196.8	203.6	195	10	5	404	296	415	372	66	18
80	142	74	134	117	37	32	105.3	91.5	101.6	99	7	7	74	167	84	108	51	47
20	38	49	27	38	11	29	25.3	30.3	35.4	30	5	17	19	34	34	29	9	30
0	7	11	13	10	3	27	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
LOD	15.84 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9986			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9916		

Skimmed Milk 0.1%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L						2400j-3 mU/L					
	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]
5000	6,137	5,730	6,272	6,046	282	5	8,711.0	8,106.0	8,454.0	8,424	304	4	10,366	11,326	11,796	11,163	729	7
625	750	1,018	889	886	134	15	1,198.9	1,187.4	1,198.9	1,195	7	1	1,258	1,051	1,059	1,123	117	10
400	542	420	439	467	66	14	685.4	660.1	662.0	669	14	2	878	838	768	828	56	7
320	516	428	424	456	52	11	595.8	556.7	591.6	581	21	4	782	609	885	759	139	18
160	233	250	220	234	15	7	285.0	253.8	255.6	265	18	7	289	388	321	333	51	15
80	82	162	130	125	40	32	142.5	138.8	105.3	129	20	16	79	68	70	72	6	8
20	30	28	12	23	10	42	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
0	2	1	1	1	0	46	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
LOD	2.04 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9999			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9991		

Whole Goat's Milk

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L						2400j-3 (Oct 2019) mU/L					
	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]	1	2	3	Mean[b]	S_r[c]	RSD[d]
5000	10,268	11,230	11,901	11,133	821	7	8,477.0	9,447.0	8,688.0	8,871	510	6	7,951	11,295	9,694	9,647	1,673	17
625	1,386	1,369	1,272	1,343	61	5	1,032.5	1,139.6	1,222.3	1,131	95	8	1,298	1,567	1,239	1,368	175	13
400	804	882	846	844	39	5	719.0	690.5	706.1	705	14	2	1,158	1,144	1,232	1,178	47	4
320	585	604	682	624	51	8	557.6	638.5	586.1	594	41	7	557	481	574	537	50	9
160	219	277	360	286	71	25	298.3	280.9	280.9	287	10	4	283	315	252	283	32	11
80	183	105	175	154	43	28	156.8	152.6	135.2	148	11	8	83	116	125	108	22	20
20	64	39	55	53	13	24	50.1	50.1	54.2	51	2	5	0	0	0	0	0	N/A
0	22	22	22	22	0	0	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
LOD	22.18 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9999			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9987		

Skimmed Goat's Milk

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L						2400j-3 (Oct 2019) mU/L					
	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]
5000	13,499	21,550	21,550	18,866	4,648	25	24,801.0	23,082.0	23,399.0	23,761	915	4	42,226	40,026	45,057	42,436	2,522	6
625	2,984	4,042	3,886	3,637	571	16	3,188.5	3,240.0	3,162.7	3,197	39	1	4,242	4,155	4,562	4,320	214	5
400	2,429	2,606	1,952	2,329	338	15	1,946.8	2,045.7	2,146.3	2,046	100	5	2,438	2,836	2,822	2,699	226	8
320	1,681	2,090	2,035	1,936	222	11	1,706.9	1,631.5	1,597.5	1,645	56	3	2,360	2,131	2,193	2,228	118	5
160	787	880	745	804	70	9	855.5	836.2	874.8	856	19	2	1,081	1,104	1,061	1,082	22	2
80	405	375	493	425	62	15	413.7	462.9	426.6	434	26	6	464	449	345	419	65	15
20	119	89	140	116	25	22	158.6	126.4	122.3	136	20	15	78	79	98	85	11	13
0	2	1	1	1	1	43	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
LOD	2.68 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9978			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9953		

Chocolate Milk 1%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L						2400j-3 (Oct 2019) mU/L					
	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]
5000	7319	8488	7541	7,782	621	8	7,153.0	6,900.0	6,850.0	6,968	162	2	15,547	14,592	14,166	14,768	707	5
625	566	507	1147	740	354	48	749.3	809.1	827.9	795	41	5	1,855	2,077	1,691	1,874	194	10
400	353	327	327	336	15	4	505.0	497.4	522.7	508	13	3	1,087	1,144	1,140	1,124	32	3
320	242	344	489	359	124	35	418.8	415.6	425.2	420	5	1	969	908	895	924	40	4
160	225	225	216	222	5	2	201.3	220.2	229.8	217	15	7	505	453	463	474	28	6
80	80	63	88	77	13	17	116.3	110.3	110.3	112	3	3	253	174	230	219	41	19
20	71	71	37	60	20	33	10.0	10.0	34.5	18	14	78	51	45	42	46	5	10
0	0	0	11	4	7	173	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
LOD	16.94 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9994			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9991		

Strawberry Milk

TCL mU/L[a]	ZymoSnap ALP (mU/L)						2400j-1 (May 2014) mU/L						2400j-3 (Oct 2019) mU/L					
	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]
5000	15,763	14,840	19,139	16,580	2,263	14	18,135.0	14,352.0	14,720.0	15,736	2,086	13	21,139	21,042	20,116	20,766	565	3
625	2,270	2,168	2,035	2,158	118	5	1,614.0	1,818.1	2,058.3	1,830	222	12	2,553	2,828	2,904	2,762	185	7
400	1,099	1,125	1,281	1,168	98	8	1,140.1	1,083.5	1,111.6	1,112	28	3	1,590	1,824	1,704	1,706	117	7
320	966	874	1,354	1,065	255	24	938.7	973.2	976.4	963	21	2	1,422	1,466	1,451	1,446	22	2
160	566	513	513	531	31	6	471.2	452.3	468.0	464	10	2	659	642	741	681	53	8
80	261	195	308	255	57	22	254.2	259.0	263.9	259	5	2	406	448	423	426	21	5
20	76	83	50	69	18	25	72.2	69.0	78.6	73	5	7	117	104	114	112	7	6
0	17	20	26	21	5	24	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
LOD	31.07 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9999			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9999		

40% Cream Diluted 1:5

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L						2400j-3 (Oct 2019) mU/L					
	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]	1	2	3	Mean[b]	Sr[c]	RSD[d]
5000	10,546	8,708	8,379	9,211	1,167	13	8,252.0	8,394.0	8,155.0	8,267	120	1	9,423	9,678	8,528	9,210	604	7
625	858	1,183	1,026	1,022	163	16	1,009.5	982.4	988.8	994	14	1	870	1,221	1,156	1,082	187	17
400	727	753	759	746	17	2	631.6	646.3	682.2	653	26	4	748	714	855	772	74	10
320	289	552	529	457	146	32	512.1	505.7	508.0	509	3	1	612	622	641	625	15	2
160	256	263	273	264	8	3	241.3	241.3	245.5	243	2	1	290	342	365	332	38	12
80	141	138	105	128	20	16	121.8	130.1	126.0	126	4	3	170	178	191	180	11	6
20	23	30	36	30	7	22	29.4	37.7	29.4	32	5	15	47	49	51	49	2	4
0	7	3	3	4	2	43	10.0	10.0	10.0	10	0	0	0	0	0	0	0	N/A
LOD	8.18 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9999			Correlation ZALP vs 2400j-3 (Oct 2019)			0.9999		

[a] The target contamination level (TCL) of each matrix with pure analyte in the form of raw cow's milk.

[b] The mean result of 3 technical replicates per TCL.

[c] S_r calculated from 3 technical replicates per contamination level.

[d] Coefficient of variance percentage calculated from 3 technical replicates per contamination level.

Table 6: Replicate mU/L, mean mU/L, S$_r$, and RSD values of ZymoSnap ALP method used on 11 various milk matrixes and compared to NCIMS approved 2400j-1 method. The matrixes were spiked with pure analyte (raw cow's milk) at target contamination level (TCL). All ZymoSnap ALP mU/L values measured in an EnSURE TOUCH luminometer. A negative control is just the pasteurized matrix un-spiked. Data produced in Camarillo, California, United States. (1)

Cow's Milk 4%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L					
	1	2	3	Mean[b]	S$_r$[c]	RSD[d]	1	2	3	Mean[b]	S$_r$[c]	RSD[d]
5000	4,695	4,422	4,385	4,501	169	4	5,333.0	5,201.0	5,374.0	5,302.7	90.4	1.7
625	447	367	379	398	43	11	656.2	594.2	649.2	633.2	34.0	5.4
400	311	272	223	269	44	16	308.3	342.5	311.9	320.9	18.8	5.9
320	159	176	116	151	31	21	156.8	158.6	158.6	158.0	1.0	0.7
160	93	107	104	101	7	7	77.5	80.3	77.5	78.4	1.6	2.1
80	73	84	68	75	8	11	39.7	37.9	39.2	38.9	0.9	2.4
20	39	43	43	42	3	6	22.2	19.8	21.6	21.2	1.2	5.9
0	17	12	15	15	2	16	<10	<10	<10	10.0	0.0	0.0
LOD	19.69 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9993		

Cow's Milk 2%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L					
	1	2	3	Mean[b]	S$_r$[c]	RSD[d]	1	2	3	Mean[b]	S$_r$[c]	RSD[d]
5000	5,683	6,270	6,741	6,231	530	9	4,817.0	4,874.0	5,029.0	4,906.7	109.7	2.2
625	656	859	698	738	107	15	648.0	630.8	638.4	639.1	8.6	1.3
400	356	339	414	370	39	11	317.3	324.9	342.1	328.1	12.7	3.9
320	236	263	221	240	21	9	162.5	166.3	160.2	163.0	3.1	1.9
160	124	116	109	116	8	6	82.2	86.0	83.0	83.7	2.0	2.4
80	36	45	28	36	8	23	43.0	42.0	39.0	41.3	2.1	5.0
20	24	19	28	24	5	20	22.0	21.0	20.0	21.0	1.0	4.8
0	17	17	11	15	3	22	<10	<10	<10	10.0	0.0	0.0
LOD	21.50 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9999		

Cow's Milk 1%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L					
	1	2	3	Mean[b]	S$_r$[c]	RSD[d]	1	2	3	Mean[b]	S$_r$[c]	RSD[d]
5000	5,809	4,929	5,022	5,253	483	9	5,123.0	5,483.0	5,535.0	5,380.3	224.4	4.2
625	711	872	739	774	86	11	643.0	624.0	628.0	631.7	10.0	1.6
400	356	303	288	316	36	11	314.0	315.9	314.0	314.6	1.1	0.3
320	145	170	188	167	21	13	163.6	159.0	157.9	160.2	3.0	1.9
160	70	83	96	83	13	16	86.8	82.0	86.5	85.1	2.7	3.2
80	55	44	42	47	7	16	36.0	39.5	37.6	37.7	1.8	4.6
20	25	29	27	27	2	8	18.8	20.2	20.8	19.9	1.0	5.1
0	11	7	8	9	2	24	<10	<10	<10	10.0	0.0	0.0
LOD	13.37 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			0.9996		

Cow's Milk 0.1%

TCL mU/L[a]	ZymoSnap ALP mU/L						2400j-1 (May 2014) mU/L					
	1	2	3	Mean[b]	S$_r$[c]	RSD[d]	1	2	3	Mean[b]	S$_r$[c]	RSD[d]
5000	5,012	5,073	4,453	4,846	342	7	4,962.0	5,078.0	5,201.0	5,080.3	119.5	2.4
625	624	608	551	594	38	6	622.3	587.5	567.6	592.5	27.7	4.7
400	354	385	306	349	40	11	305.4	338.6	336.9	327.0	18.7	5.7
320	159	186	191	179	17	10	170.9	159.3	169.3	166.5	6.3	3.8
160	94	97	87	93	5	5	78.0	74.7	73.0	75.2	2.5	3.4
80	41	57	52	50	8	16	36.5	38.2	41.5	38.7	2.5	6.6
20	32	29	27	29	2	8	23.2	18.6	20.3	20.7	2.3	11.2
0	10	11	10	11	1	7	<10	<10	<10	10.0	0.0	0.0

LOD	12.17 mU/L						Correlation ZALP vs 2400j-1 (May 2014)			1.0000		
	40% Cream											
TCL mU/L[a]	**ZymoSnap ALP mU/L**						**2400j-1 (May 2014) mU/L**					
	1	**2**	**3**	**Mean[b]**	**S$_r$[c]**	**RSD[d]**	**1**	**2**	**3**	**Mean[b]**	**S$_r$[c]**	**RSD[d]**
5000	7,037	8,056	9,197	8,097	1,081	13	5,161.0	5,439.0	5,599.0	5,399.7	221.6	4.1
625	749	622	800	724	92	13	660.2	655.0	597.0	637.4	35.1	5.5
400	474	454	397	442	40	9	310.0	325.7	325.7	320.5	9.1	2.8
320	204	178	239	207	31	15	157.6	159.4	168.1	161.7	5.6	3.5
160	76	0	76	51	44	87	85.8	84.1	89.3	86.4	2.7	3.1
80	61	56	66	61	5	8	43.8	42.0	42.0	42.6	1.0	2.4
20	31	25	46	34	11	31	22.8	21.0	22.3	22.0	0.9	4.2
0	25	25	20	24	3	12	<10	<10	<10	10.0	0.0	0.0
LOD	**29.66 mU/L**						Correlation ZALP vs 2400j-1 (May 2014)			0.9996		
	Chocolate Milk 0.1%											
TCL mU/L[a]	**ZymoSnap ALP mU/L**						**2400j-1 (May 2014) mU/L**					
	1	**2**	**3**	**Mean[b]**	**S$_r$[c]**	**RSD[d]**	**1**	**2**	**3**	**Mean[b]**	**S$_r$[c]**	**RSD[d]**
5000	4859	3691	4704	4,418	634	14	4,762.2	4,655.3	4,833.2	4,750.2	89.6	1.9
625	611	540	431	527	91	17	587.3	586.0	612.9	595.4	15.2	2.5
400	448	639	420	502	119	24	316.6	321.7	324.8	321.0	4.1	1.3
320	180	244	286	236	53	23	179.8	169.8	157.0	168.9	11.4	6.8
160	71	39	56	55	16	29	86.1	78.5	78.4	81.0	4.4	5.5
80	39	49	25	38	12	33	43.1	43.3	42.1	42.8	0.6	1.5
20	11	7	25	14	9	66	20.7	20.8	22.8	21.4	1.2	5.5
0	11	0	14	8	7	89	<10	<10	<10	10.0	0.0	0.0
LOD	**22.93 mU/L**						Correlation ZALP vs 2400j-1 (May 2014)			0.9987		
	Strawberry Milk											
TCL mU/L[a]	**ZymoSnap ALP mU/L**						**2400j-1 (May 2014) mU/L**					
	1	**2**	**3**	**Mean[b]**	**S$_r$[c]**	**RSD[d]**	**1**	**2**	**3**	**Mean[b]**	**S$_r$[c]**	**RSD[d]**
5000	6218	6591	6510	6,440	196	3	5,202.0	5,224.0	5,316.0	5,247.3	60.5	1.2
625	795	803	870	823	41	5	669.2	608.0	615.4	630.9	33.4	5.3
400	331	315	329	325	9	3	304.0	318.0	317.0	313.0	7.8	2.5
320	193	174	158	175	17	10	159.4	161.3	170.0	163.6	5.7	3.5
160	75	77	128	93	30	32	76.0	79.7	79.7	78.5	2.1	2.7
80	24	38	58	40	17	43	42.6	43.3	44.5	43.5	1.0	2.2
20	7	5	3	5	2	37	22.2	21.3	20.3	21.3	1.0	4.5
0	3	0	0	1	2	173	<10	<10	<10	10.0	0.0	0.0
LOD	**5.05**						Correlation ZALP vs 2400j-1 (May 2014)			0.9999		

[a] The target contamination level (TCL) of each matrix with pure analyte in the form of raw cow's milk.

[b] The mean result of 3 technical replicates per TCL.

[c] S$_r$ calculated from 3 technical replicates per contamination level.

[d] Coefficient of variance percentage calculated from 3 technical replicates per contamination level.

Table 7: Independent Laboratory Study Data (1)

4% Cow's Milk

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	2728.1	3198.8	3335.0	3880.3	3471.4	347.6	3123.7	3819.0	5301.7	2144.7	4184.3	2570.0	2966.3	1586.5	1.45	No
625	331.1	325.8	397.5	455.3	392.3	73.8	318.5	466.1	631.7	374.3	464.3	416.0	418.2	92.3	-0.35	Yes
313	176.0	226.2	264.7	273.3	254.7	25.1	229.7	279.8	348.0	174.0	312.3	245.0	243.8	89.3	0.44	Yes
156	170.5	182.3	181.0	243.3	202.2	35.6	166.6	237.8	331.7	220.0	273.3	206.5	236.6	63.3	-0.97	Yes
78	95.4	122.3	121.3	149.7	131.1	15.7	115.4	146.8	155.0	71.3	198.0	51.0	106.8	72.9	1.55	No
39	59.1	78.0	69.3	85.0	77.4	8.0	69.5	85.4	91.0	47.7	128.3	59.0	78.3	46.6	-0.11	Yes
20	40.0	41.2	50.7	40.7	44.2	10.7	33.5	54.9	22.7	12.7	45.7	51.0	36.4	19.6	0.72	Yes
0	10.0	31.6	28.0	32.3	30.6	2.9	27.7	33.5	0.0	1.7	31.3	28.0	20.3	19.7		

2% Cow's Milk

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	3000.7	3358.7	3253.3	3541.0	3384.3	325.7	3058.6	3710.1	4201.7	2186.0	2479.7	4410.7	3025.4	1518.2	1.10	No
625	366.6	365.6	392.3	355.7	371.2	34.3	336.9	405.5	565.3	394.7	290.7	417.7	367.7	114.6	0.10	Yes
313	239.3	187.7	238.7	245.3	223.9	38.3	185.6	262.2	428.7	116.7	252.0	204.0	190.9	97.4	0.86	Yes
156	185.6	191.7	192.0	167.7	183.8	18.1	165.7	201.8	374.0	66.0	230.7	232.0	176.2	98.1	0.42	Yes
78	96.9	71.7	120.0	102.7	98.1	25.1	73.0	123.2	154.0	57.3	109.0	142.7	103.0	42.1	-0.19	Yes
39	54.5	54.4	74.0	60.7	63.0	14.2	48.8	77.2	82.3	76.0	80.3	72.3	76.2	26.5	-0.93	Yes
20	27.9	38.4	40.3	34.3	37.7	3.6	34.1	41.3	45.7	21.0	59.0	56.0	45.3	27.2	-2.09	No
0	27.6	29.6	30.3	21.3	27.1	5.5	21.6	32.6	3.0	18.0	40.0	42.7	33.6	16.7		

1% Cow's Milk[a]

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	2788.6	2386.9	3291.3	3647.0	3108.4	807.7	2300.7	3916.1	3490.7	2948.7	8468.0	1917.3	2785.6	857.5	0.40	Yes
625	291.9	241.6	295.3	396.3	311.1	73.9	237.2	385.0	506.7	382.3	722.0	171.5	334.4	182.4	0.32	Yes
313	230.4	260.9	252.3	294.3	269.2	22.7	246.5	291.9	352.3	328.0	653.7	129.5	281.7	115.1	0.55	Yes
156	153.9	165.7	144.7	175.3	161.9	13.9	148.0	175.8	334.3	141.3	318.7	52.7	176.1	131.3	1.02	Yes
78	83.3	89.3	78.0	110.3	92.6	15.2	77.4	107.8	127.3	72.7	196.0	43.3	81.1	38.0	0.75	Yes
39	61.4	46.9	53.0	60.7	53.5	7.0	46.5	60.5	88.0	42.7	111.7	21.0	56.9	31.6	0.48	Yes
20	21.9	26.5	27.7	26.7	26.9	2.2	24.7	29.2	31.3	13.7	55.3	8.7	20.1	23.1	3.04	No
0	21.9	10.0	16.7	18.3	15.0	4.6	10.4	19.6	4.0	14.0	25.0	4.3	7.4	5.6		

0.1% Cow's Milk[a]

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	3325.1	3232.3	3429.3	3567.3	3409.6	245.8	3163.9	3655.4	5965.7	3698.3	11994.7	3659.7	4441.2	1179.6	4.20	NO
625	327.3	371.9	349.3	390.0	370.2	40.0	330.2	410.2	519.7	544.0	1201.0	329.0	464.2	134.1	2.35	NO
313	282.9	242.9	213.0	263.0	239.6	23.4	216.2	263.0	605.7	325.3	953.3	181.7	370.9	205.2	5.61	NO
156	155.7	185.3	152.3	179.7	172.4	21.1	151.3	193.5	335.0	286.7	441.3	200.3	274.0	75.1	4.81	NO
78	83.1	89.5	84.3	102.0	91.9	10.3	81.6	102.3	144.7	148.3	315.3	109.7	134.2	23.5	4.10	NO
39	38.9	54.1	53.0	68.3	58.5	12.5	46.0	70.9	69.0	55.3	165.7	53.0	59.1	27.7	0.05	YES

TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
20	25.8	24.5	26.3	33.7	28.2	7.6	20.6	35.7	34.0	33.3	83.3	21.3	29.6	7.7	0.18	YES
0	19.2	24.2	25.0	23.0	24.2	4.4	19.8	28.6	4.7	19.0	64.0	16.5	13.0	7.5		

40% Cream

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	3939.0	2302.2	9748.7	7994.3	6681.7	3414.1	3267.6	10095.8	4470.0	11515.0	798.0	777.7	4363.6	5403.7	0.68	Yes
625	584.5	267.5	1484.0	1421.3	1057.6	596.7	461.0	1654.3	567.0	1392.7	130.7	116.3	546.6	637.0	0.86	Yes
313	439.8	212.8	2894.3	2530.7	1879.3	1267.6	611.6	3146.9	356.7	813.7	318.0	267.3	466.3	281.5	1.11	No
156	369.0	103.2	795.0	757.0	551.7	340.4	211.3	892.1	254.7	537.0	73.0	91.7	233.9	231.5	0.93	Yes
78	214.8	40.3	356.3	387.3	261.3	174.5	86.8	435.8	134.0	265.0	64.7	54.7	128.1	103.8	0.76	Yes
39	155.4	33.9	302.3	288.3	208.2	133.9	74.2	342.1	80.3	141.0	42.7	36.7	73.4	53.8	1.01	Yes
20	104.2	10.0	96.7	150.0	77.5	61.7	15.8	139.2	19.3	79.3	25.0	31.7	45.3	29.0	0.52	Yes
0	81.5	10.0	72.3	102.3	61.6	43.0	18.5	104.6	0.0	31.7	23.0	34.0	29.6	11.2		

1% Chocolate Milk

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	2912.2	3104.7	3006.7	616.3	2242.6	1222.2	1020.3	3464.8	4602.0	2861.3	4973.7	169.3	2668.1	2141.4	-0.35	Yes
625	292.8	315.0	317.3	262.7	298.3	62.3	236.1	360.6	708.0	257.0	584.7	92.7	311.4	230.8	-0.21	Yes
313	224.6	189.9	212.7	228.7	210.4	52.1	158.3	262.5	517.3	176.7	318.7	117.3	204.2	108.0	0.12	Yes
156	145.6	139.3	169.7	190.0	166.3	35.7	130.6	202.0	374.3	146.7	268.3	151.3	188.8	70.6	-0.63	Yes
78	67.5	75.5	80.0	102.7	86.1	23.1	63.0	109.2	175.3	133.0	183.3	62.0	126.1	58.3	-1.73	No
39	32.6	21.1	37.0	90.3	53.0	43.7	9.3	96.7	92.0	92.0	118.0	52.7	87.6	40.2	-0.79	Yes
20	10.0	10.0	15.0	18.0	14.3	6.0	8.4	20.3	31.7	73.0	133.7	56.3	87.7	36.3	-12.31	No
0	10.0	10.0	5.7	0.0	5.2	4.4	0.9	9.6	6.3	65.0	75.7	48.0	62.9	17.4		

Strawberry Milk

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	3066.4	2940.2	7037.3	1300.7	3759.4	2567.1	1192.3	6326.5	4512.0	4036.3	4848.0	272.0	3052.1	2325.5	0.28	Yes
625	332.0	251.6	715.3	572.7	513.2	225.0	288.2	738.2	550.0	430.3	417.7	206.3	351.4	117.2	0.72	Yes
313	234.4	198.3	444.0	174.0	272.1	131.8	140.3	403.9	445.0	97.3	453.0	72.3	207.6	198.8	0.49	Yes
156	176.6	123.6	262.7	227.3	204.5	67.2	137.4	271.7	301.3	182.7	271.3	91.0	181.7	102.0	0.34	Yes
78	95.3	60.7	155.0	130.0	115.2	50.5	64.7	165.7	187.7	120.7	212.0	81.3	138.0	60.6	-0.45	Yes
39	46.6	37.1	66.3	213.7	105.7	88.0	17.7	193.7	90.7	40.7	91.0	95.0	75.6	34.6	0.34	Yes
20	12.9	10.0	17.7	91.0	39.6	52.4	-12.8	91.9	28.7	15.7	64.3	99.0	59.7	40.0	-0.38	Yes
0	10.0	10.0	0.0	0.0	3.3	5.0	-1.7	8.3	2.3	16.0	24.7	7.3	16.0	9.0		

Caprine Milk

	Reference Methods								Hygiena Methods						Z Score	
TCL (mU/L)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Activity Range (mean mU/L - 1Sr)	Activity Range (mean mU/L + 1Sr)	Lab 1	Lab 2	Lab 3	Lab 4	Overall Mean	SR	Ref vs Hygiena	NCIMS Spec
5000	3066.4	2940.2	7037.3	1300.7	3759.4	2567.1	1192.3	6326.5	4512.0	4036.3	4848.0	272.0	3052.1	2325.5	0.28	Yes
625	332.0	251.6	715.3	572.7	513.2	225.0	288.2	738.2	550.0	430.3	417.7	206.3	351.4	117.2	0.72	Yes
313	234.4	198.3	444.0	174.0	272.1	131.8	140.3	403.9	445.0	97.3	453.0	72.3	207.6	198.8	0.49	Yes
156	176.6	123.6	262.7	227.3	204.5	67.2	137.4	271.7	301.3	182.7	271.3	91.0	181.7	102.0	0.34	Yes

78	95.3	60.7	155.0	130.0	115.2	50.5	64.7	165.7	187.7	120.7	212.0	81.3	138.0	60.6	-0.45	Yes
39	46.6	37.1	66.3	213.7	105.7	88.0	17.7	193.7	90.7	40.7	91.0	95.0	75.6	34.6	0.34	Yes
20	12.9	10.0	17.7	91.0	39.6	52.4	-12.8	91.9	28.7	15.7	64.3	99.0	59.7	40.0	-0.38	Yes
0	10.0	10.0	0.0	0.0	3.3	5.0	-1.7	8.3	2.3	16.0	24.7	7.3	16.0	9.0		

ªQ Laboratories (Laboratory 1) data used for average, Sr, and Z Score calculations in place of Laboratory 3

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