3.3. Validations

3.3.1. Campden BRI

Objective: Side-by-side results comparison of ZymoSnap ALP Fluorophos.

Summary
Cow's milk, sheep's milk and light cream were obtained by Campden BRI. Each sample was re-pasteurized and spiked with raw bovine milk at 0.01%, 0.05%, 0.15%, 0.3% and 0.5%. Blank (negative) controls, i.e. re-pasteurized samples without raw milk were used as base line. The samples were analyzed using the Fluorophos system (Advanced Instruments) and ZymoSnap ALP with five replicates for each concentration of raw milk. The data gathered with both ALP tests suggested that both systems produced highly accurate and reliable results. Correlation analysis revealed that the results were comparable.

Campden BRI
Campden BRI is the largest membership based food and drink research centre in the world. It undertakes R&D for the many industries associated with agriculture, food and drink manufacture, distribution, retailing and food service: in essence those industries which together make up the agri-food chain. Services span from microbiological, chemical, physical and sensory analytical testing services for products to operational support for companies, research contracts and repository for publications such as industrial guidelines and training provider. Campden BRI is ISO9001 certified and the technical services are UKAS (UK Accreditation Service) 17025 accredited. Therefore, validations carried out at their facilities by their staff are trusted and seen as approval and add considerable credibility to a product and its manufacturer.

Tests and Instruments under Evaluation

ZymoSnap ALP
ZymoSnap ALP is a new detection method for quantifying ALP activity in short shelf-life liquid milk samples to verify the effectiveness of the pasteurization process. It uses novel liquid stable reagents to link ALP activity to a bioluminogenic signal that provides highly sensitive and reproducible results in 10 minutes.

Fluorophos
Fluorophos is a fluorometric test that measures ALP activity by a kinetic read method. It is the current industry standard and the technology has changed little since its first introduction. As specified, the reaction was run in the Advanced Instruments FLM200.

Experimental Setup
Bovine milk, sheep milk and light cream were laboratory pasteurized and treated with 0.01%, 0.05%, 0.15%, 0.3% and 0.5% raw bovine milk. Negative controls as well as positive controls were prepared for each dairy type by reconstituting ZymoSnap ALP positive control as specified in the instructions. Five replicates of each dilution were examined with both ALP tests. Ten positive controls were tested in triplicate.

Results

Section 1 – Bovine Milk

Both tests delivered accurate data across all raw milk concentrations (figure 1 A and B). Statistical analysis showed a very close correlation between the data and the recorded ALP concentration (see table 1). Correlation analysis of ZymoSnap ALP vs Fluorophos indicated that the data produced by either method on the samples were equivalent (Figure 1C). The R^2 value was calculated as 0.991 (see table 1).

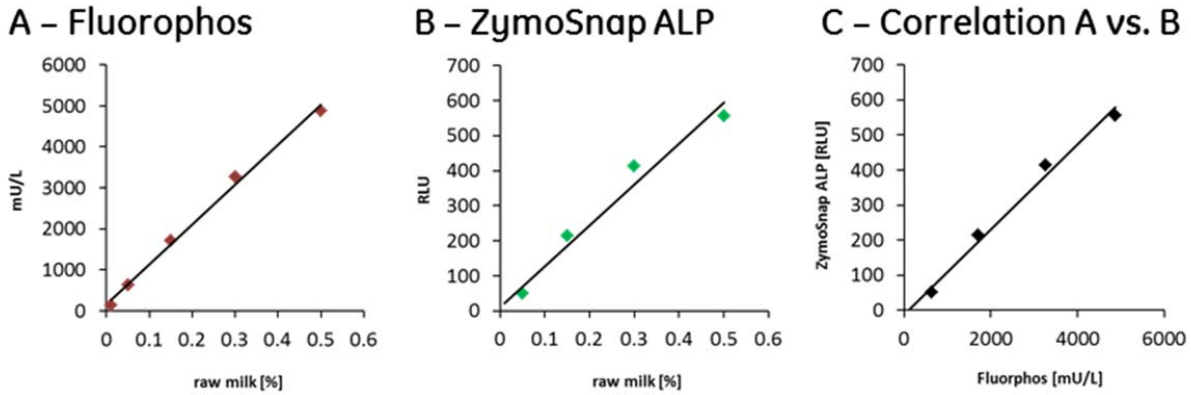

Figure 1. Regression analysis of bovine milk samples. Panel A shows Fluorophos data, panel B ZymoSnap ALP. Panel C shows the data from Fluorophos and ZymoSnap ALP plotted as x and y-axes respectively. The R2 values for all three graphs are shown in table 1.

Section 2 – Ovine Milk

Both ZymoSnap ALP and Fluorophos accurately measured raw milk in the sheep milk (figure 1 A and B). The data points close to the line of best fit highlight the close correlation between the ALP concentration in the milk and the obtained data. Direct comparison of ZymoSnap ALP with Fluorophos readings by correlation analysis indicated that the data produced by either method on the samples were equivalent (Figure 2C). The R^2 values were calculated as 0.996 (see table 1).

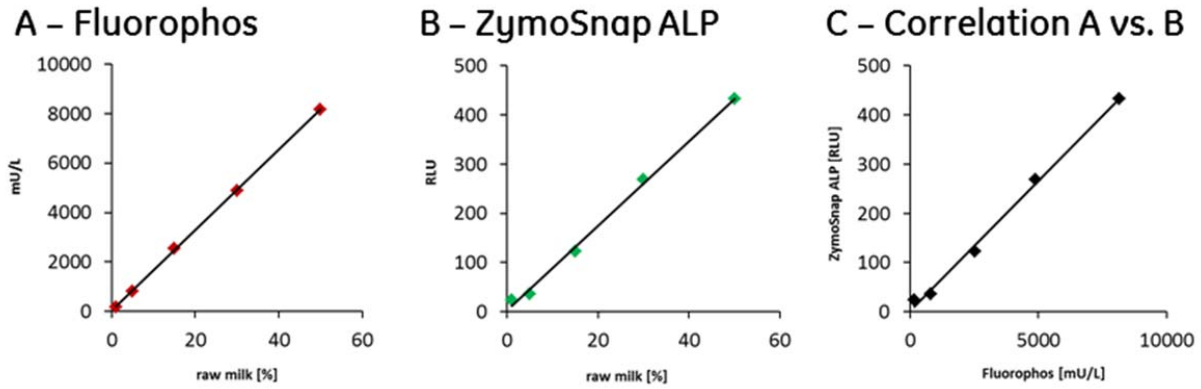

Figure 2. Regression analysis of bovine milk samples. Panel A shows Fluorophos data, panel B ZymoSnap ALP. Panel C shows the data from Fluorophos and ZymoSnap ALP plotted as x and y-axes respectively. The R2 values for all three graphs are shown in table 1.

Section 3 – Light Cream

ZymoSnap ALP and Fluorophos accurately detected raw milk in the cream (figure 1 A and B). Line-of-best-fit analysis showed close correlation between the data and the ALP present in the milk. Direct comparison of ZymoSnap ALP with Fluorophos readings by correlation analysis indicated equivalence of the methods with R^2 values close to 1 at 0.998 (Figure 3C and see table 1).

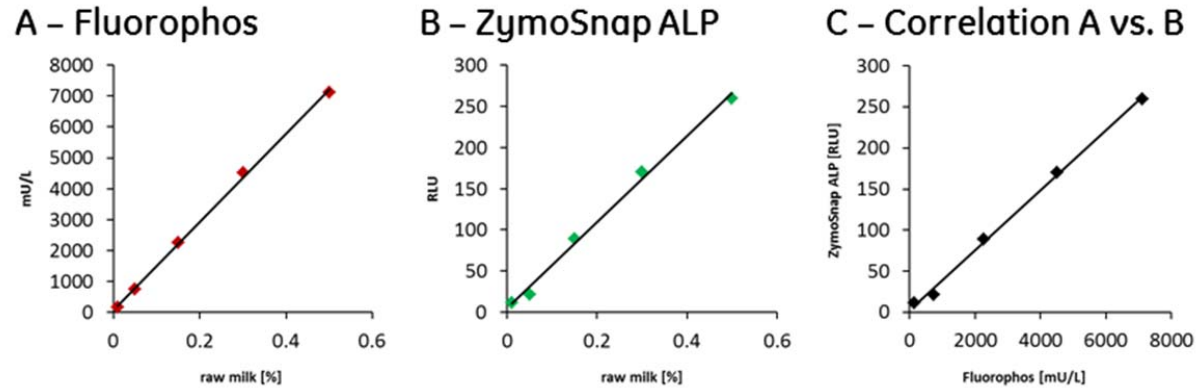

Figure 3. Linearity analysis by line of best fit of UK milk samples at 0.1%, 1.0%, 2.0% and 4.0%. The critical level at 350 mU/L is shown in red. The limit of detection for each milk type (fat content) is shown in green.

Section 4 - Data Summary

Table 1. Data analysis of dairy samples

raw milk concentration

Bovine Milk	ZymoSnap ALP	Fluorophos	ZymoSnap ALP vs Fluorophos
R^2	0.972	0.995	0.991
LOD 0.01% [mU/L]	97.3	134.4	
mean RLU at 0 mU/L	139 ± 3.6		
mean RLU at 350 mU/L	169 ± 9.9		

Sheep Milk	ZymoSnap ALP	Fluorophos	ZymoSnap ALP vs Fluorophos
R^2	0.997	0.999	0.996
LOD 0.01% [mU/L]	209	166	
mean RLU at 0 mU/L	85 ± 8.7		
mean RLU at 350 mU/L	129 ± 14.3		

Light Cream	ZymoSnap ALP	Fluorophos	ZymoSnap ALP vs Fluorophos
R^2	0.995	0.999	0.998
LOD 0.01% [mU/L]	317.2	195.9	
mean RLU at 0 mU/L	62 ± 6.0		
mean RLU at 350 mU/L	82 ± 8.9		

Conclusions

- Consistent results: High R^2 values calculated in the linearity analysis revealed that the readings were consistent with both Fluorophos and ZymoSnap ALP.
- Equal results: Correlation analysis between ZymoSnap ALP and Fluorophos
- Equal sensitivity: LOD values of ZymoSnap ALP and Fluorophos are similar.
- Precision: The observed error in the ALP measured by both tests was at or below 10%.
- Conclusion: ZymoSnap ALP shows excellent linearity, sensitivity and precision. The results obtained with the Hygiena test are equivalent to those obtained with the industry standard, Fluorophos.