

Background

The milk pregnancy test is an enzyme-linked immunoassay for the detection of pregnancy-associated glycoproteins (PAGs) in milk samples from cattle. Test confirms pregnancy status from 28 days post breeding and 60 days post calving. The test is not long lasting respectively for less than 3.5 hours it can be tested up to 184 samples. The test is negative if the PAGs are < 0.100 and positive if the values are ≥ 0.250 . For values ≥ 0.100 and < 0.250 the test is positive only in cows where the sample is taken 45 days minimal or more after the insemination otherwise they are in re-check zone. This test enables producers to identify open animals to ensure re-breeding in a timely manner to maximize milk production and profitability. In this study we aimed to evaluate and show the application of commercially available ELISA pregnancy test in milk of lactating cows. For this purpose preserved DHI samples collected for regular DHI control were tested. Samples were delivered to the laboratory mostly from northwest and east Croatia preserved and cooled at 4 to 8°C. During period from October 2017 till January 2019 total of 6528 samples were tested of which 72% were positive and 1% were in re-check zone.

Materials and methods

Table 1. Equipment used in ELISA milk pregnancy test

Equipment	Manufacturer and Model
Reader	BioSan, model HiPo MPP-96
Washer	BioSan, model 3D-IW8
Plate Shaker-Termostat	BioSan, model PST-60HL
ELISA kit	Idexx Milk Pregnancy Test



Picture 1. ELISA protocol for milk pregnancy determination

Results

Table 2. Results of repeatability and intermediate precision

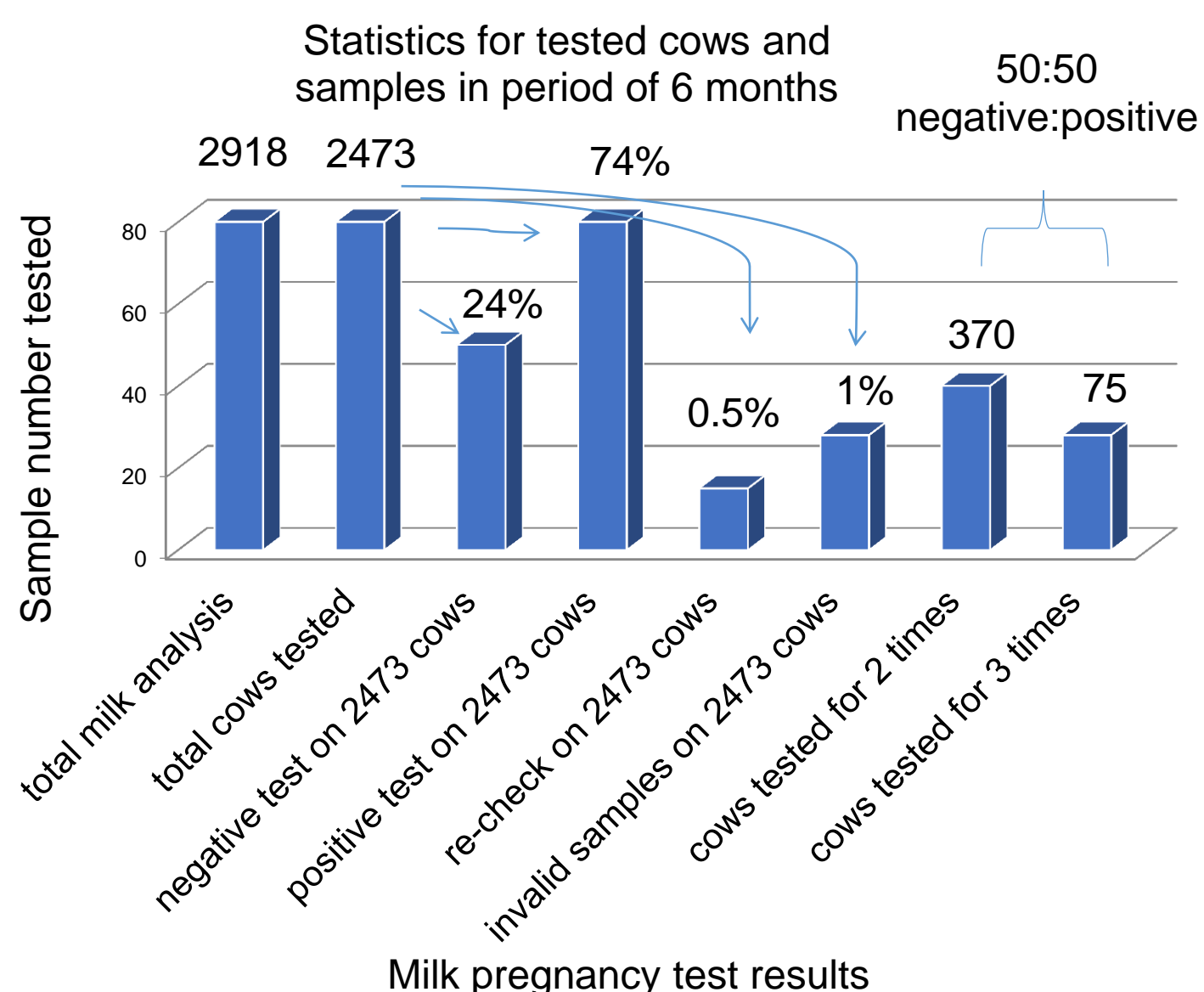
Statistics	Sample ID and S-N values (PAGs)				
	41	42	43	44	45
average	0,047	3,963	2,184	3,720	2,854
min	0,030	3,957	2,051	3,561	2,546
max	0,085	3,971	2,333	3,870	3,338
σ	0,016	0,006	0,093	0,099	0,247
RSD, %	34,072	0,151	4,258	2,655	8,650

Table 3. Method validation results

Parameter	Eligibility criteria	Validation results
Repeatability (samples)	$\sigma=0.029$, RSD 4,60 % ^a	41: $\sigma=0.003$, RSD=7,36% 42: $\sigma=0.000$, RSD=0.00% 43: $\sigma=0.035$, RSD=1.52% 44: $\sigma=0.052$, RSD=1.35% 45: $\sigma=0.095$, RSD=3.00%
Repeatability (test controls)	$\sigma=0.029$, RSD 4,60 % ^a	NC: $\sigma=0.002$, RSD=4.20% PC: $\sigma=0.066$, RSD=4.23%
Intermediate precision (samples)	$\sigma=0.034$, RSD 5.28 % ^a	41: $\sigma=0.016$, RSD=34.07% 42: $\sigma=0.006$, RSD=0.15% 43: $\sigma=0.093$, RSD=4.25% 44: $\sigma=0.099$, RSD=2.65% 45: $\sigma=0.247$, RSD=8.65%
Intermediate precision (test controls)	$\sigma=0.034$, RSD 5.28 % ^a	NC: $\sigma=0.004$, RSD=10.59% PC: $\sigma=0.095$, RSD=6.52%
Sensitivity	expected results: 41/0, 42/1, 43/1, 44/1, 45/1	41/0, 42/1, 43/1, 44/1, 45/1
Specificity	σ for S-N 0.004 ^b	σ for S-N=0.003
Accuracy	expected results: 41/0, 42/1, 43/1, 44/1, 45/1	41/0, 42/1, 43/1, 44/1, 45/1
Positive Control minus Negative Control	O.D. ≥ 0.500 ^c	range from 1.273 to 1.633
Negative Control	O.D. ≤ 0.200 ^c	range from 0.028 to 0.044
Limit of quantitation (LOQ)	information	< 0.079
Limit of detection (LOD)	information	0.001

Legend:^aValidation report, Idexx (it was taken the lowest value), ^bCertificate of analysis, Idexx, lot broj J391, ^cManufacture instructions, Idexx (criteria for daily measurement evaluation)

For total of 6528 milk analysis Laboratory have only 2918 test registered through the system for automated delivery pregnancy test results to the dairy within 24 hours which are statistically showed on graph 1.



Graph 1. Milk pregnancy test results based on 6 months period

PAG level \pm estimated measurement uncertainty of 13,19%

Conclusion

Evaluation of milk pregnancy test was based on method validation parameters. Performance of ELISA milk pregnancy test was conducted at different PAGs levels. Obtained results showed good repeatability (RSD=max to 7.36%) and intermediate precision (RSD=0.15% to 34.07%). The test was sufficiently sensitive, specific ($\sigma=0,003$) with excellent recovery. The detection limit and quantification limit were 0.001 and 0.079 respectively and estimated uncertainty was 13.19%. Using test with DHI samples it could be concluded that the test is very convenient and useful tool for achieving reproductive management programs on the farms and should be integrated as routine monitoring of pregnancy.