Crocodile miniWorkstation

Sension Progesterone Milk ELISA using the Crocodile miniWorkstation

Introduction:

The determination of the progesterone level in dairy cattle is a valuable tool for efficient herd management. During the bovine heat cycle the level of progesterone changes significantly. On day 0 of the oestrus very low progesterone levels can be detected due to an inactive corpus luteum. The corpus luteum develops from an ovarian follicle during the luteal phase leading to production of progesterone and therefore increasing progesterone levels. These high levels even increase further in case of pregnancy after day 21 or drop again indicating the beginning of a new cycle. Consequently the determination of the progesterone level in milk can help in maintaining an accurate time management of artificial insemination of cows.
The Sension Progesterone Milk ELISA is a competitive immunoassay for the determination of progesterone levels directly from raw milk samples.

Materials:

1. Sension Testkit Progesterone in milk. Source: Minitüb GmbH, Hauptstrasse 41, 84184 Tiefenbach-Germany.
2. Crocodile miniWorkstation by Titertek-Berthold.
3. Raw milk samples from local farms.

Results:

An example of a typical standard curve (progesterone standards in a concentration from 0 to 20 ng/ml) is shown in Figure 1.

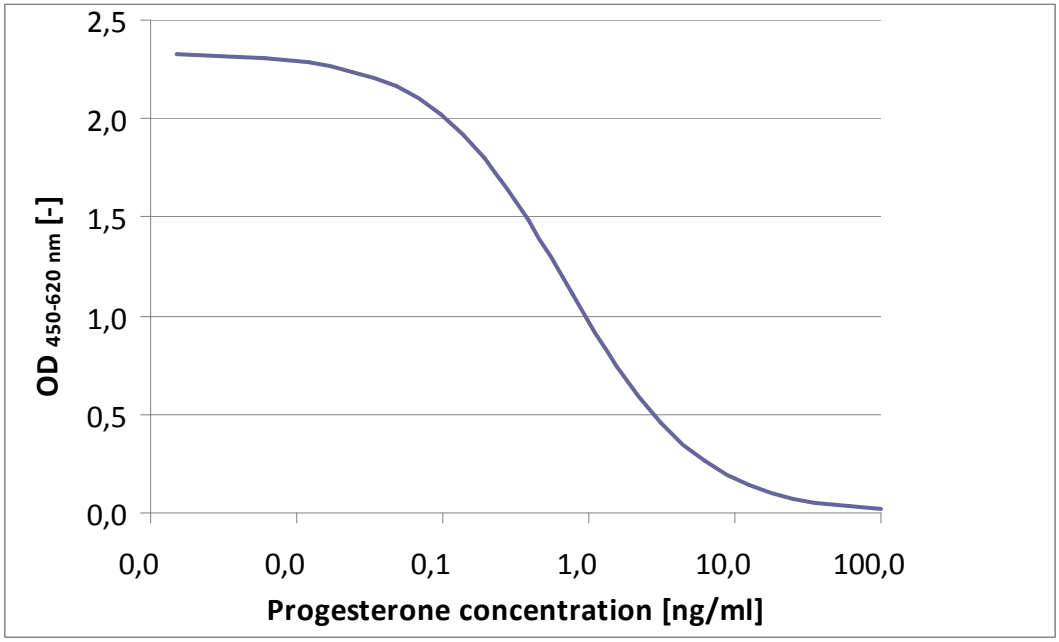

Figure 1. example of a typical standard curve

The range of the assay is between 0 – 20 ng/ml with an analytical sensitivity of 0,03 ng/ml.

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Precision:

The intra- and also the inter-assay were performed to determine the precision of the progesterone ELISA. The variability of three milk samples within the assay is shown in Table 1. The variability of three milk samples between the assays is shown in Table 2.

Table 1: Intra-assay variation

	sample 1	sample 2	sample 3
n	16	16	16
mean [ng/ml]	10,82	5,46	0,67
SD	0,33	0,33	0,08
CV	3%	6%	12%

Table 2: Inter-assay variation

	sample 1	sample 2	sample 3
n	8	8	8
mean [ng/ml]	9,53	4,49	0,60
SD	0,7	0,22	0,10
CV	7%	5%	16%

The coefficient of variation (CV) is defined as the ratio of the standard deviation (SD) to the mean and should be < 20%.

Repeatability:

To determine the repeatability within one plate a sample was measured 96 fold (Table 3). The SD and the CV allow the comparison of the variability/repeatability of one plate/assay (Table 4).
For the progesterone ELISA the CV of the $OD_{450\text{-}620\ nm}$ values should be < 10%.

Table 3: measured $OD_{450\text{-}620\ nm}$ values for one sample (96 fold)

$OD_{450\text{-}620nm}$	1	2	3	4	5	6	7	8	9	10	11	12
A	1,025	1,138	1,057	1,025	1,012	0,975	1,015	0,948	1,031	1,038	1,069	1,121
B	1,026	1,054	0,972	1,002	1,014	0,938	0,972	0,908	0,995	0,943	0,957	0,972
C	1,030	0,979	0,938	0,883	0,934	0,912	0,913	0,876	0,992	0,948	0,937	1,059
D	1,060	1,029	0,987	0,948	0,962	0,891	1,012	0,929	0,882	0,933	0,982	0,998
E	1,050	0,968	0,983	1,008	0,948	0,919	0,975	0,925	0,970	0,936	0,998	1,001
F	1,120	0,964	0,960	0,932	1,015	0,891	0,957	0,983	0,919	0,957	1,018	0,988
G	1,083	0,986	0,984	0,952	0,981	0,985	0,967	0,960	0,959	0,948	0,982	1,036
H	1,148	1,092	1,148	1,038	1,069	1,089	1,078	1,063	1,082	1,044	1,095	1,055

Table 4: SD and CV for one sample (96 fold)

mean OD 450-620 nm [-]	0,994
SD	0,06
CV	6,2%

Recovery:

UHT milk with a fat content of 0,1% has been spiked by adding a solution with a certain progesterone concentration (different levels). The recovery in % was calculated by the ratio of the expected and the measured progesterone concentration.

Table 5: Recovery after spiking UHT milk with a progesterone solution (different levels)

Expected P4-concentration [ng/ml]	Measured P4-concentration [ng/ml]	Recovery
15,39	14,17	92%
7,89	7,99	101%
3,89	4,02	103%
0,94	1,02	109%

Linearity:

Three different milk samples were serial diluted in UHT milk with a fat content of 0,1%. In UHT milk a progesterone concentration of 0,9 ng/ml was determined. The recovery in % was calculated by the ratio of the expected and the measured progesterone concentration.

Table 6: Linearity of three different milk samples

	Dilution	Expected P4-concentration [ng/ml]	Measured P4-concentration [ng/ml]	Recovery
sample 1	none		5,46	
	1:2	3,18	3,58	113%
	1:4	2,24	2,52	112%
	1:8	1,71	1,67	98%
	1:16	1,29	1,50	116%
	1:32	1,20	1,18	98%
sample 2	none		9,53	
	1:2	5,22	5,86	112%
	1:4	3,38	4,33	128%
	1:8	2,61	2,81	108%
	1:16	1,86	2,07	111%
	1:32	1,48	1,26	85%
	1:64	1,08	1,23	114%
sample 3	none		2,83	
	1:2	1,87	1,98	106%
	1:4	1,44	1,49	103%
	1:8	1,19	1,16	97%
	1:16	1,03	1,00	98%

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Progesterone progression in pregnant cows:

The progesterone concentration in blood serum was measured during a cycle. The progression of the progesterone levels is shown in Figure 2.

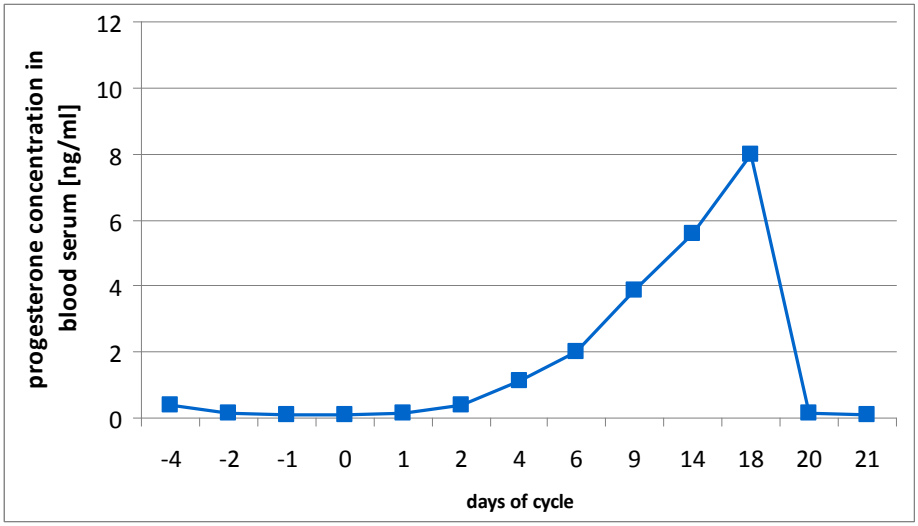

Figure 2. Concentration of progesterone during one cycle

Summary/Conclusion:

The Sension Progesterone Milk ELISA allows the determination of the progesterone concentration of up to 84 raw milk samples in less than 40 minutes without the need of sample preparation. Using the automated ELISA miniWorkstation Crocodile it is only necessary to manually apply 6 standard solutions and the raw milk samples to be analyzed and the whole analysis procedure is performed automatically by the instrument. Consequently the progesterone assay on the miniWorkstation Crocodile is an ideal tool for the reproduction management of cattle, especially when applied on-site.

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Assay Program:

#	Step Name	Description
1	**Prime Conjugate**	**Dispensing** Volume 850ul Inlet 1 Label "Conjugate" Method: Priming Well Count: 1
2	**Prime wash**	**Washing** Method: Prime Dispenser Wash Solution Inlet: 1 Cycles: 3 Volume: 1000ul Delay: 1s Wait: 100ms Dispenser Depth: 1300 (Plate Offset: 0) Aspiration Depth: 1300 (Plate Offset: 0) Well Count: 48
3	**Prime Substrate**	**Dispensing** Volume 850ul Inlet 3 Label "Substrate" Method: Priming Well Count: 1
4	**Prime Stop**	**Dispensing** Volume 850ul Inlet 4 Label "Stop" Method: Priming Well Count: 1
5	**Dispense Conjugate**	**Dispensing** Volume 120ul Inlet 1 Label "Conjugate" Method: Standard Well Count: 48
6	**Incubate**	**Incubation** Incubator On Temperature: 37 °C Duration: 00:20:00
7	**Wash1**	**Washing*** Method: Soak Wash Wash Solution Inlet: 1 Cycles: 3 Volume: 300ul Delay: 1s Wait: 100ms Dispenser Depth: 994 (Plate Offset: -152) Aspiration Depth: 2824 (Plate Offset: -95) Sweep: 1mm @ 5mm/s Well Count: 48
8	**Dispense Substrate**	**Dispensing** Volume 100ul Inlet 3 Label "Substrate" Method: Standard Well Count: 48
9	**Incubate**	**Incubation** Incubator On Temperature: 37 °C Duration: 00:10:00
10	**Dispense Stop**	**Dispensing** Volume 50ul Inlet 4 Label "Stop" Method: Standard Well Count: 48
11	**Shake1**	**Shaking** for 00:00:10 at Shaker Position with 1mm Amplitude at 10Hz
12	**Measure**	**Reading** Reference Measurement Filter 1: 450nm (Pos:2) Filter 2: 620nm (Pos:4) Well Count: 48

*Plate Offsets and Aspiration Depths are related to the instrument used for the present validation and might need to be optimized for other instrument units.