APPLICATION NOTE

Validation of Two Steroid Hormone ELISA Kits with Ovine Blood Samples

Cécile Bienboire-Frosini[1], Camille Chabaud[1], Rosaria Esposito[2], Morgan Mathieu[2]
[1]IRSEA, Apt, France; [2]Enzo Life Sciences, Lausen, Switzerland

PROGESTERONE ELISA KIT (ADI-900-011 / ADI-901-011)
TESTOSTERONE ELISA KIT (ADI-900-065 / ADI-901-065)

HIGHLIGHTS

- Enzo's Progesterone and Testosterone ELISA Kits were tested with ewe and ram plasma, respectively.
- Precision, accuracy, parallelism and linearity under dilution were evaluated.
- These kits are able to recognize and bind in a specific manner the hormones present in the samples, without significant interference caused by the biological matrix.

INTRODUCTION

The Phérosynthèse research and development laboratory was created in 1995, specializing in chemical communication within the living world. In 2010, Phérosynthèse became the Research Institute in Semiochemistry and Applied Ethology (IRSEA). By identifying chemical signals that play a role in the life of animals, the development of new therapeutic and zootechnical tools that respect man, animals, and the environment is possible. Dr. Cécile Bienboire-Frosini is the head of the Department of Physiological and Behavioral Mechanisms of Adaptation and is in charge of several transversal projects on chemical communication and the mechanisms of action of semiochemicals. To conduct her research projects, her team needs to adapt and validate commercial immunoassays for the study of the physiology of IRSEA's species of interest.

Progesterone is the major female sex hormone. This steroid is responsible for reproductive activities. It is secreted in large amounts by the corpus luteum and in small quantities directly by the adrenal glands (Aufrere & Benson, 1976; Bardin *et al.*, 1983; Greenspan & Forsham, 1986). Most of the progesterone circulating in the blood is bound to serum proteins such as albumin, while a small portion roams free (Dunn *et al.*, 1981). Testosterone is the main androgen secreted by Leydig cells in testes and effects both primary and secondary sexual development such as muscle mass and sex drive (Imperato-McGinley *et al.*, 1974; Wilson *et al.*, 1981). It circulates in plasma predominately bound to proteins such as albumin or sex hormone-binding globulin (SHBG) (Griffen & Wilson, 1992).

In sheep, reproduction is seasonal and breeding season ends as daylight increases. An increase in frequency of LH pulses and consequently ovulation can, however, be triggered by introducing a male into a flock of ewes in anoestrous. This phenomenon, caused by male pheromones, is called "ram effect" and it is used to manipulate sheep reproduction during the anoestrous season (Rosa *et al.*, 2002; Delgadillo *et al.*, 2009; Fabre-Nys *et al.*, 2016). The ram effect is studied by the researchers at IRSEA (Pageat *et al.*, 2014; Asproni *et al.*, 2017). In this context, rams' secretions are used to stimulate pre-ovulatory LH peaks in ewes. The levels of progesterone in ewes' plasma need to be measured to evaluate the anoestrous state. In parallel, testosterone levels in ram plasma can be evaluated in order to investigate its role in pheromones emission.

The aim of the study reported here is to validate two commercially available kits for the quantitative measurement of progesterone and testosterone levels in sheep plasma. The manufacturer has already established several performance criteria, such as the sensitivity, the linearity, the precision, the cross-reactivity and the accuracy. However, because of the different biological matrix (sheep plasma), it is necessary to perform a partial validation to test the intra-assay precision (repeatability), the accuracy as well as the linearity and parallelism (Andreasson *et al.*, 2015).

MATERIALS AND METHODS

Study Animals

Adult ewes and rams were used in this study (sp. *Ovis aries*). The animals were from IRSEA breeding facilities. Housing, husbandry, and use of animals described in this document were carried out following the French and European legislation and in compliance with the principles of replacement, reduction, and refinement. All procedures were performed with approval from the Ethics Committee C2EA125, in concordance with French and European legislation.

Blood Sampling

Blood samples were collected from the jugular vein of animals in Lithium Heparin tubes (Vacuette® Greiner Bio-One, ref. 454084). Samples were then centrifuged at 4° C at 1800 x g for 12 minutes. Plasma samples were pipetted, aliquoted, and stored at -20° C until further use.

Progesterone ELISA Kit

Progesterone levels in ewe plasma was measured using Enzo's Progesterone ELISA kit (ADI-900-011) following the manufacturer's instructions. This kit has been used in previous studies assessing progesterone levels in plasma samples from a variety of animal species including cow (Pothmann *et al.*, 2015), fish (Kidd *et al.*, 2010), horse (Siemieniuch *et al.*, 2016), and mouse (Ziegler-Waldkirch *et al.*, 2018). The kit's sensitivity is 8.57 pg/mL with an assay range between 15.62 and 500 pg/mL. Sensitivity was calculated by determining the average optical density bound for twenty zero standard replicates, and comparing to the average optical density for twenty wells run with the Standard 6 (15.62 pg/mL). The detection limit was determined as the concentration of Progesterone measured at two standard deviations from the zero along the standard curve. Intra-assay precision at low, medium and high concentrations is 7.6, 5.4, and 4.9% respectively. It was determined by assaying 12 replicates of samples containing low, medium and high concentrations of Progesterone. Inter-assay precision at low, medium and high concentrations is 6.8, 8.3, and 2.7%, respectively. It was determined by measuring samples with low, medium and high concentrations of Progesterone in eight assays.

Testosterone ELISA Kit

Testosterone levels in ram plasma was measured using Enzo's Testosterone ELISA kit (ADI-900-065) following the manufacturer's instructions. This kit has been used in previous studies assaying testosterone levels in plasma samples from a variety of species such as bird (Whittaker *et al.*, 2017), fish (Singh *et al.* 2015), lizard (Seddon *et al.*, 2016), horse (Lemasson *et al.*, 2015), mouse (Sil *et al.*, 2008), rat (Abdulai-Saiku & Vyas, 2017), and rabbit (Traish *et al.*, 2003). The sensitivity of this kit is 5.67 pg/mL with an assay range between 7.81 and 2000 pg/mL. Sensitivity was calculated by determining the average optical density bound for 20 zero standards, and comparing to the average optical density for 20 wells run with Standard 5 (7.81 pg/mL). The detection limit was determined as the concentration of Testosterone measured at two standard deviations from the zero along the standard curve. Intra-assay precision at low, medium and high concentration is 10.8, 10.0, and 7.8%, respectively. It was determined by measuring eight replicates with low, medium and high concentrations of Testosterone. Inter-assay precision at low, medium and high concentrations is 14.6, 11.3, and 9.3%, respectively. It was determined by measuring samples with low, medium and high concentrations of Testosterone in eight assays.

Progesterone and Testosterone Concentration
In order to select the appropriate samples, their concentrations were previously established by the Lyon National Veterinary School using the radioimmunoassay (RIA) method.

Sample dilution in Assay Buffer was required to remove matrix interference and obtain concentration values within the kits' standard range. The dilution was 1 in 25 to measure progesterone in ewe plasma and 1 in 50 to detect testosterone in ram plasma. Diluted samples were run in triplicates.

Activated Carbon Adsorption Treatment
Activated carbon adsorption treatment can be performed on plasma samples in order to deplete steroid hormones. This procedure allows the end user to obtain stripped plasma, a biological matrix identical to the matrix used in the assays, with an undetectable concentration of the analyte. This has been used to determine the accuracy of the kits.

Stripped plasma was obtained as previously described (Green & Leake, 1987; Sarkar *et al.*, 2008). Briefly, a solution of activated charcoal at 25 g/L (Sigma) and dextran T-70 at 2.5 g/L (Sigma) in PBS was mixed overnight at 4° C, in order to obtain a dextran-coated charcoal (DCC) solution. A volume of DCC equivalent to the volume of plasma was centrifuged in glass tubes at 2000 rpm for 15 minutes. The supernatant was removed and substituted by an equivalent volume of plasma. The tubes were vortexed for at least one minute and then incubated for 12 hours at 4° C. After a new centrifugation step at 3500 rpm for 20 minutes at 4° C, the supernatant was filtered using a 0.45 µm syringe filter (Dutscher) to obtain a dextran-coated charcoal-treated (DCCT) solution.

Assessment of Validation Criteria
According to the EMEA Guideline on bioanalytical method validation (2011), commercial kits need to be revalidated when the biological matrix is changed to ensure that the sample analysis is performed accurately and precisely. For the validation procedure with sheep plasma samples, a partial validation was performed and the following parameters were checked: precision, accuracy, parallelism, and linearity under dilution. Acceptance criteria were set in accordance with the EMEA Guideline on bioanalytical method validation (2011).

RESULTS

Precision
The precision of an immunoassay indicates its reproducibility when distinct measurements are performed with the same sample under specific and identical conditions. Precision can be defined as the "the closeness of agreement between a series of measurements obtained under the prescribed conditions". It is measured as a coefficient of variation (%CV) from the mean value. The intra-assay precision of Enzo's Progesterone and Testosterone ELISA kits was determined by measuring several samples in replicates at different levels of Progesterone and Testosterone concentrations in order to cover the entirety of the standard curve.

The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample, which can be quantified reliably, with an acceptable accuracy and precision. The lowest calibration standard is deemed to be the LLOQ. The LLOQ is 15.62 pg/mL for Enzo's Progesterone ELISA Kit and 7.81 pg/mL for Enzo's Testosterone ELISA kit. The LLOQ should not be confused with the limit of detection or sensitivity, which is outside the standard range and represents the lowest concentration distinguishable from the background (8.57 pg/mL and 5.67 pg/mL for the Progesterone and Testosterone ELISA kits respectively). Conversely, the highest calibration standard is referred to as the upper limit of quantification (ULOQ). The ULOQ is 500 pg/mL for Enzo's Progesterone ELISA Kit is and 2000 pg/mL for Enzo's Testosterone ELISA kit.

For the precision to be accepted, %CV must not exceed 20%. For concentrations comprised in an interval of 1-3 times the LLOQ, %CV must not exceed 25%. Precision profiles for the two kits have been traced, plotting the %CV against progesterone or testosterone concentration (**Fig. 1A and 1B**). None of the samples was found to have a %CV above the acceptance criteria. The precision of Progesterone and Testosterone ELISA kits was therefore validated in the low/medium range to quantify the concentration of these two hormones in sheep plasma.

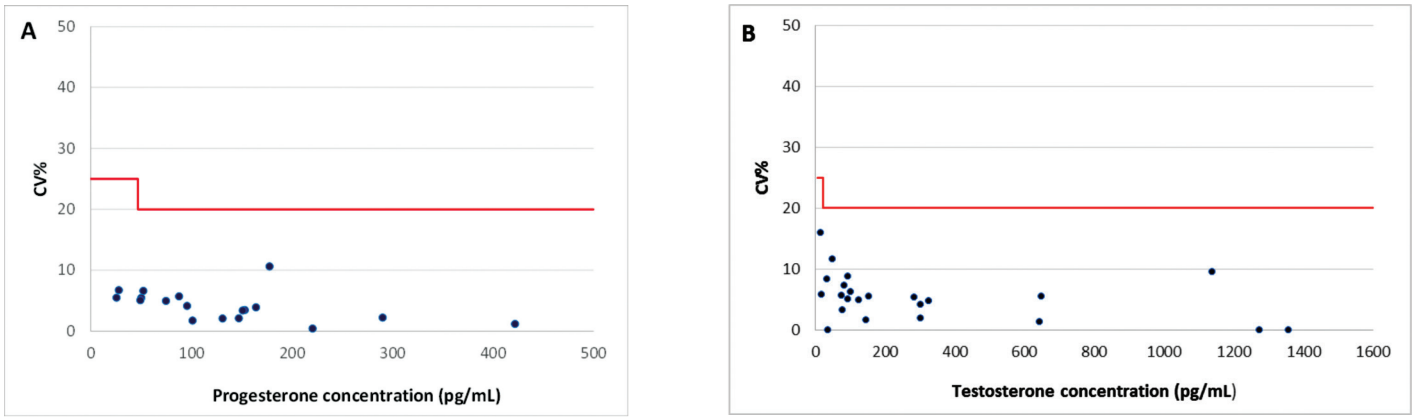


Figure 1. Precision profile of Enzo's Progesterone (A) and Testosterone (B) ELISA kits, with ewe and ram plasma, respectively.

ACCURACY

The accuracy of an ELISA can be defined as "the closeness of the determined value to the value which is accepted either as a conventional true value or an accepted true value." This test is performed by adding a known amount of the hormone standard (spike) into the natural test sample matrix. The percentage of recovery between the expected concentration and the measured value is then calculated. The difference between the actual percentage of recovery and the ideal value of 100% represents the bias. For the accuracy to be accepted, the bias cannot be higher than ±20%, except for the LLOQ where it cannot exceed ±25%. A minimum of five measurements per concentration and a minimum of three different concentrations within the assay range is recommended.

In this study two sets of spiked samples were obtained. In the first case a known standard concentration was added to three samples previously used in the study of precision (SP1, SP2, and SP3). In the second case, three quality control (QC) samples were obtained by adding determined volume of the standards to the stripped plasma (see Materials and Methods). The QC samples need to cover the entire range of the kit, with a low concentration included within three times the lower limit of quantification (QC1), a medium concentration in the middle of the standard curve (QC2), and a high concentration close to the upper limit of the standard curve (QC3). The concentrations chosen for progesterone were 20, 80, and 300 pg/mL, respectively. The concentrations chosen for testosterone were 20, 100, and 1000 pg/mL, respectively.

As for the Progesterone ELISA kit, only one sample (QC1) was found to have a bias above the acceptance criteria **(Table 1)**. However, since at least 67% of the samples (4/6) gave an error within the limits of acceptance, the accuracy of the kit could be validated. When testing the Testosterone ELISA kit, all the bias values were acceptable **(Table 2)**.

The total error expresses the combination of random error and systematic error. It is the sum of the %CV (random error) from the precision measurement and the bias (systematic error) from the accuracy estimation. It must not exceed 30% of the true value, even at LLOQ. As indicated in **tables 1 and 2**, all the samples tested in this study, except one (QC1 for the Progesterone ELISA kit), gave acceptable values. The accuracy of Enzo's Progesterone and Testosterone ELISA kits was, therefore, validated for the determination of the concentration of the two hormones in sheep plasma.

PROGESTERONE ELISA KIT												
Precision				Accuracy								
	Mean sample Conc. (pg/mL)	Intra-batch precision (%CV)	Precision acceptability Criteria	Supplem. level (pg/mL)	Expected Conc. (pg/mL)	Measured Conc. (pg/mL) (n=3)	Intra-batch precision (%CV)	Recovery (%)	± Bias (%)	Precision Acceptance Criteria	Total error (%)	Acceptability Criteria
SP1	52,23	6,6	≤20%	300	348,32	421,908	1,17	121	21	≤20%	22	<30%
SP2	74,87	4,96	≤20%	80	153,37	151,254	3,38	99	1	≤20%	4	<30%
SP3	152,23	3,45	≤20%	20	172,46	164,403	3,91	95	5	≤20%	9	<30%
QC1	≈0	N.A.	N.A.	20	20	25,686	5,47	128	28	≤25%	33	<30%
QC2	≈0	N.A.	N.A.	80	80	87,85	5,7	110	10	≤20%	16	<30%
QC3	≈0	N.A.	N.A.	300	300	290,453	2,23	97	3	≤20%	5	<30%

Table 1. Validation criteria of precision and accuracy for Enzo's Progesterone ELISA kit in ewe plasma. SP1, SP2, and SP3: spiking recovery test results. QC1, QC2, and QC3: quality control test results. *: 1/25 working dilution.

TESTOSTERONE ELISA KIT												
Precision				Accuracy								
	Mean sample Conc. (pg/mL)	Intra-batch precision (%CV)	Precision acceptability Criteria	Supplem. level (pg/mL)	Expected Conc. (pg/mL)	Measured Conc. (pg/mL) (n=3)	Intra-batch precision (%CV)	Recovery (%)	± Bias (%)	Precision Acceptance Criteria	Total error (%)	Acceptability Criteria
SP1	93,34	8,81	≤20%	50	86,005	84,831	7,29	99	1	≤20%	8	<30%
SP2	102,36	6,19	≤20%	5	92,33	93,568	4,99	101	1	≤20%	6	<30%
SP3	302,08	4,08	≤20%	1	271,91	285,54	5,26	105	5	≤20%	10	<30%
QC1**	≈26,973	N.A.	N.A.	20	46,962	48,572	11,6	103	3	≤20%	15	<30%
QC2**	≈26,973	N.A.	N.A.	100	126,919	126,494	4,81	100	0	≤20%	5	<30%
QC3**	≈26,973	N.A.	N.A.	1000	1,026,434	1,138,549	9,5	111	11	≤20%	21	<30%

Table 2. Validation criteria of precision and accuracy for Enzo's Testosterone ELISA kit in ram plasma. SP1, SP2, and SP3: spiking recovery test results. QC1, QC2, and QC3: quality control test results. *: 1/50 working dilution. **: a sample with an undetectable testosterone concentration was not available for this study. Therefore, a sample with a low testosterone concentration (26.973 pg/mL) was used as the starting point for the supplementation.

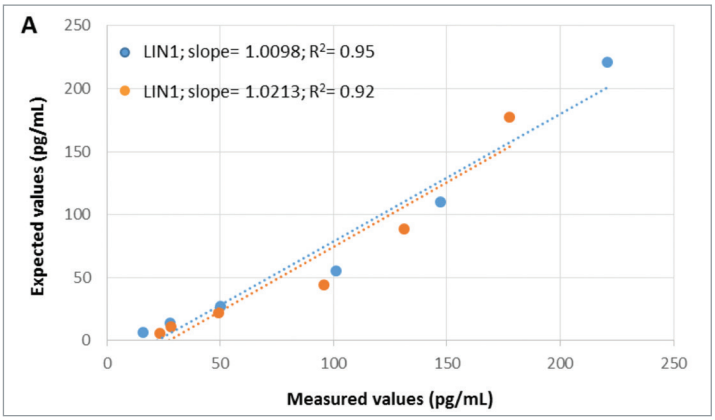
Linearity and Parallelism

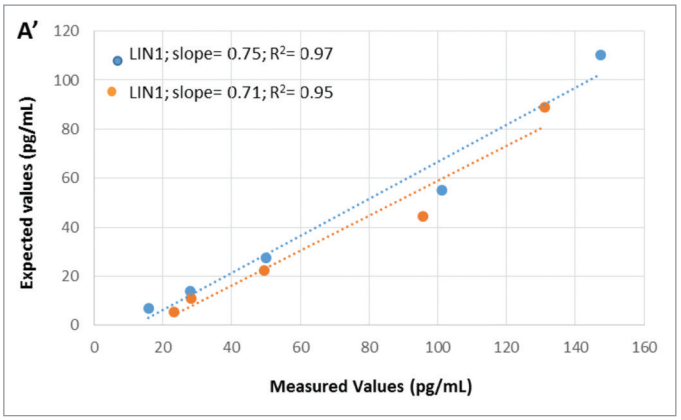
The study of linearity is meant to assess the selectivity of the assay. Selectivity of an ELISA is its ability to measure and differentiate unequivocally the analyte of interest in the presence of other components habitually present in the biological matrix, such as degraded proteins, impurities, metabolites, and other constituents of the matrix. Selectivity issues may arise from matrix interference unrelated to the analyte of interest and to a lesser extent, substances that are physicochemically-similar to the analyte (e.g. cross-reactants).

Matrix interference can affect an ELISA assay either positively (i.e. components from the matrix causing background or binding to the antibody in an unspecific manner) or negatively (i.e. components from the matrix preventing binding of the analyte). For the study of linearity, the biolog-

ical matrix is spiked at a concentration falling close to the top of the curve (i.e. greater than ULOQ), to demonstrate that a sample with a spiked concentration above the ULOQ can be diluted within the working range and still give a reliable result. Serial dilutions of the analyte are prepared and the relationship between expected and measured values is investigated. In this study, serial 1:2 dilutions of the analyte were obtained starting from highly concentrated samples (based on RIA measures). For each kit, dilutional linearity was demonstrated by representing measured values against expected theoretical values, for two samples (LIN1 and LIN2).

For the Progesterone ELISA kit, the curves obtained with the linearity test had a slope and a R2 value close to 1 **(Fig. 2A)**. If the values for the highest concentration were excluded (expected value = measured value), the slope of the curves was close to 0.7, thus suggesting a potential overestimation of samples concentration **(Fig. 2A')**. However, as discussed later, this observation was not confirmed by the accuracy results. **Figure 2B** shows the results of the parallelism study.





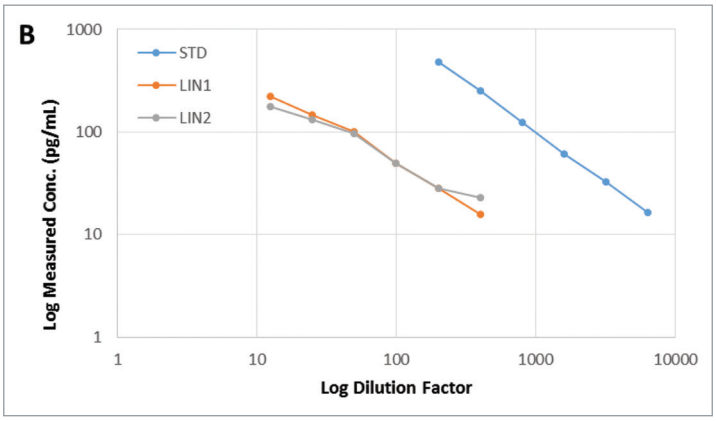


Figure 2. Study of linearity (A, A') and parallelism (B) using Enzo's Progesterone ELISA kit with ewe plasma.

The calculation of recovery percentages between expected values and measured values gave results beyond the acceptance range **(Table 3)**, the closest to the acceptation range being at the working dilution 1 in 25.

LINEARITY TEST USING PROGESTERONE ELISA KIT						
Dilution	**12,5**	**25**	**50**	**100**	**200**	**400**
Recovery LIN1	/	**134%**	183%	182%	203%	230%
Recovery LIN2	/	**147%**	215%	222%	253%	417%

Table 3: Percentage of recovery when using Enzo's Progesterone ELISA kit with diluted ewe plasma.

For the Testosterone ELISA kit, regression lines showed acceptable linearity under dilution with a slope of 1. Correlation coefficients (i.e. R^2 values) were also significant with a value close to 1, thus indicating the absence of any scattering around the regression line **(Fig. 3A)**. Selectivity of the assay can be further analyzed by conducting a study of parallelism and comparing serially diluted samples with the standard curve. The different dilution factors chosen for the study are shown in **Figure 3B** as a function of testosterone concentrations.

The dilution curves were parallel to the ones obtained by diluting the standard, thus indicating the absence of the matrix interference and the ability of the two kits to recognize specifically the hormones present in the samples in a similar way as the standards.

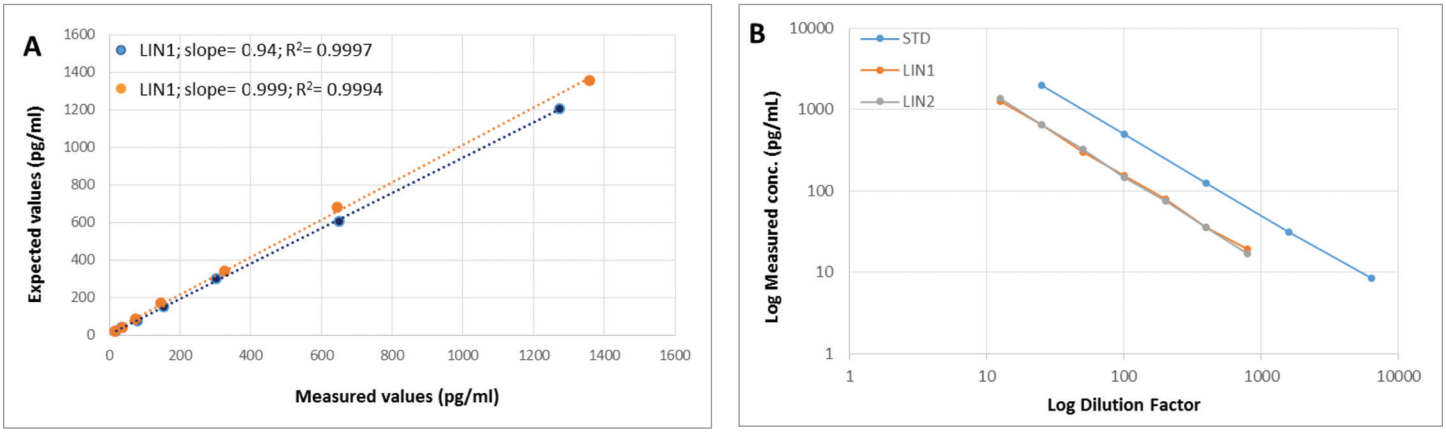


Figure 3. Study of linearity (A) and parallelism (B) using Enzo's Testosterone ELISA kit with ram plasma.

The calculation of recovery percentages between expected values and measured values demonstrated the correlation of the results even when the dilution factor is important **(Table 4)**. Furthermore, the recovery percentage at working dilution (1:50) is correct and within the accepted range (i.e. between 80 and 120%).

LINEARITY TEST USING TESTOSTERONE ELISA KIT							
Dilution	**12,5**	**25**	**50**	**100**	**200**	**400**	**800**
Recovery LIN1	105%	**108%**	**100%**	102%	104%	94%	101%
Recovery LIN2	/	**95%**	**96%**	86%	88%	85%	79%

Table 4: Percentage of recovery when using Enzo's Testosterone ELISA kit with diluted ram plasma.

CONCLUSION

Enzo's Progesterone and Testosterone ELISA kits have been validated by the manufacturer for the measurement of these hormones in a variety of biological fluids including plasma, serum, and feces. For use with sheep plasma, a partial validation had to be undertaken and a number of performance criteria had to be checked including precision, accuracy, linearity, and parallelism. The last two parameters, in particular, allow the end user to assess both the selectivity of the assay and the matrix effect.

The possible risk of overestimation of progesterone concentrations in ewe plasma suggested by the linearity study was not confirmed by the accuracy results, as assessed with two different approaches (QC and SPI samples based on supplementation charge of progesterone of stripped plasma and native samples, respectively). Therefore, all the criteria investigated in this study were considered as satisfactory, as evidenced by the results highlighted in this application note. Enzo's Progesterone ELISA kit was chosen to assay progesterone in ewe plasma with a 1 in 25 dilution. Enzo's Testosterone ELISA kit was chosen to assay testosterone in ram plasma with a 1 in 50 dilution.

References

1. Abdulai-Saiku & Vyas (2017) Loss of predator aversion in female rats after Toxoplasma gondii infection is not dependent on ovarian steroids. Brain Behav. Immun. 65, 95.
2. Andreasson *et al.* (2015) A practical guide to immunoassay method validation. Front. Neurol. 6, 1.
3. Asproni *et al.* (2017) Topography of c-Fos expression in the brain of ewes exposed to ram anteorbital gland secretion, Poster presented at NeuroFrance 2017, Bordeaux.
4. Aufrere & Benson (1976) Progesterone: an overview and recent advances. J. Pharm. Sci. 65, 783.
5. Bardin *et al.* (1983) Progesterone and Progestins. NY: Raven Press.
6. Delgadillo *et al.* (2009) The 'male Effect' in sheep and goats - revisiting the dogmas. Behav. Brain Res. 200, 304.
7. Dunn *et al.* (1981) Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J. Clin. Endocrinol. Metab. 53, 58.
8. European Medicines Agency (2011) Guideline on bioanalytical method validation.
9. Fabre-Nys *et al.* (2016) The "Ram Effect": A "non-classical" mechanism for inducing LH surges in sheep. PLoS One. 11, e0158530.
10. Green & Leake, (1987) Steroid hormones: a practical approach, IRL Press, pp. 213-214.
11. Greenspan & Forsham (1986) Basic & Clinical Endocrinology 2nd Edition. Norwalk, CN: Appleton Century-Crofts, East.
12. Griffen & Wilson (1992) Williams Textbook of Endocrinology 8th Ed. W. B. Saunders. 799-852.
13. Imperato-McGinley *et al.* (1974) Steroid 5alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism. Science. 186, 1213.
14. Kidd *et al.* (2010) Measuring multiple hormones from a single water sample using enzyme immunoassays. Gen. Comp. Endocrinol. 165, 277.
15. Lemasson *et al.* (2015) Mares prefer the voices of highly fertile stallions. PLoS One 10, e0118468.
16. Pageat *et al.* (2014) The ante orbital gland secretions of the ram increases the LH pulsatility in anoestrus ewes, in: Meet. Int. Soc. Chem. Ecol. Chem. Signals Vertebr. Gr., Urbana-Champaign, p. 121.
17. Pothmann *et al.* (2015) The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. Theriogenology. 83, 1249.
18. Rosa *et al.* (2002) The 'ram effect' as a way of modifying the reproductive activity in the ewe. Small Ruminant Res. 45, 1.
19. Sarkar *et al.* (2008) Application of sensitive enzyme immunoassay for determination of testosterone in blood plasma of yaks (Poephagus grunniens L.). General and Comparative Endocrinology. 158, 240-244.
20. Seddon *et al.* (2016) Phenotypic correlates of melanization in two Sceloporus occidentalis (Phrynosomatidae) populations: Behavior, androgens, stress reactivity, and ectoparasites. Physiol. Behav. 163, 70.
21. Siemieniuch *et al.* (2016) Type of inflammation differentially affects expression of interleukin 1β and 6, tumor necrosis factor-α and toll-like receptors in subclinical endometritis in mares. PLoS One. 11, e0154934.
22. Sil *et al.* (2008) Cadmium induced testicular pathophysiology: prophylactic role of taurine. Reprod. Toxicol. 26, 282.
23. Singh *et al.* (2015) A CYP19-based sex determination and monosex production in aquaculture species Oreochromis niloticus L. and a cyprinid Cyprinus carpio. L. Fish. Aquac. J. 6, 1.
24. Traish *et al.* (2003) Effects of medical or surgical castration on erectile function in an animal model. J. Androl. 24, 381.
25. Wilson *et al.* (1981) The hormonal control of sexual development. Science. 211, 1278.
26. Whittaker *et al.* (2017) Songbird chemical signals reflect uropygial gland androgen sensitivity and predict aggression: implications for the role of the periphery in chemosignaling. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 204, 5.
27. Ziegler-Waldkirch *et al.* (2018) Environmental enrichment reverses Aβ pathology during pregnancy in a mouse model of Alzheimer's disease. Acta. Neuropathol. Commun. 6, 44.

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Global Headquarters
ENZO LIFE SCIENCES, INC.
10 Executive Blvd.
Farmingdale, NY 11735
Ph: 800.942.0430
Fax: 631.694.7501
info-usa@enzolifesciences.com

European Sales Office
ENZO LIFE SCIENCES (ELS) AG
Industriestrasse 17
CH-4415 Lausen,
Switzerland
Ph: +41 61 926 8989
Fax: +41 61 926 8979
info-eu@enzolifesciences.com

Belgium, The Netherlands & Luxembourg
Enzo Life Sciences BVBA
Avenue Louise 65/Box 11
1050 Bruxelles
Belgium
Ph: +32 3 466 0420
Fax: +32 3 808 7033
info-be@enzolifesciences.com

France
Enzo Life Sciences (ELS) AG
Branch Office Lyon
13, avenue Albert Einstein,
F-69100 Villeurbanne,
France
Ph: +33 472 440 655
Fax: +33 481 680 254
info-fr@enzolifesciences.com

Germany
Enzo Life Sciences GmbH
Basler Strasse 57a
DE-79540 Lörrach
Germany
Ph: +49 7621 5500 526
Fax: +49 7621 5500 527
info-de@enzolifesciences.com

UK & Ireland
Enzo Life Sciences (UK) Ltd.
1 Colleton Crescent
Exeter EX2 4DG
Ph: 0845 601 1488
(UK customers)
Ph: +44 1392 825900
Fax: +44 1392 825910
info-uk@enzolifesciences.com

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