Technical File - Assay Characteristics

17-OH Progesterone

IVD

CE

REF **EIA-1292**

Σ **96 wells**

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Content:

1 INTENDED USE

The **DRG 17-OH Progesterone ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of 17-α -OH Progesterone in serum and plasma (EDTA, lithium heparin or citrate plasma).

2 SUMMARY AND EXPLANATION

The steroid 17-α-Hydroxyprogesterone (17-α-OHP) is produced by both the adrenal cortex and gonads. Even though 17-α-OHP has relatively low progestational activity, it is of intense clinical interest because it is the immediate precursor to 11-desoxycortisol (Cpd-S). Because Cpd-S is produced by 21-hydroxylation of 17-α-OHP, measurement of 17-α-OHP is a useful indirect indicator of 21-hydroxylase activity. In congenital 21-hydroxylase deficiency, the most common variety of congenital adrenal hyperplasia (CAH), 17-α-OHP is secreted in abundant excess. It is moderately elevated in the 11-β-hydroxylase deficiency as well. Measurement of 17-α-OHP is therefore valuable in the initial diagnosis of CAH.

Clinical Physiology:

Adult non-pregnant women:

In adult non-pregnant women in the childbearing age group, 17-α-OHP concentrations vary over the menstrual cycle with luteal phase concentrations being higher than follicular phase concentrations. This is because 17-α-OHP is secreted parallel with progesterone from maturing follicles or from the corpus luteum. There is also a diurnal variation of 17-α-OHP concentrations.

This rhythm is parallel with adrenal cortisol secretion such that maximum 17-α-OHP concentrations are measured in samples obtained between midnight and 8:00 am.

Adult males:

There is little information available on the systematic variability of 17-α-OHP concentration in adult males.

Pregnant women and newborn children:

The steroid 17-α-OHP is produced in large amounts by the fetus and the adrenals. It is secreted in abundance into both the fetal and maternal circulation. The maternal concentrations of 17-α-OHP increase very sharply after 32 weeks gestational age to about 4-fold above basal concentrations at term.

Clinical Applications:

Congenital adrenal hyperplasia:

The principal application of the 17-α-OHP ELISA is in the diagnosis of CAH in newborns with ambiguous genitalia and in virilized adolescent girls. Since 17-α-OHP is the immediate precursor to 11-desoxycortisol, basal 17-α-OHP concentrations are sharply elevated in patients with 21-hydroxylase deficiency and to a lesser degree in patients with 11-hydroxylase deficiency.

Because 17-α-OHP concentrations are so markedly elevated in newborns and adolescent girls afflicted with CAH, a single basal measurement is all that is normally required to make the diagnosis.

Late onset adrenal hyperplasia:

More recently, 17-α-OHP concentrations have been utilized in the evaluation of androgenized women where late onset of 21-hydroxylase deficiency is suspected. This condition is clinically very subtle and since the presentation is the same as classical polycystic ovarian disease, basal plasma 17-α-OHP concentrations, unlike classical congenital adrenal hyperplasia, are normal. The diagnosis is made by administration of an ACTH stimulation test.

Other applications:

Measurement of 17-α-OHP concentrations is also utilized in evaluation of both men and women with acne vulgaris, male pattern baldness and in some subtle forms of infertility. Experiences with these applications are very limited.

2.1 Literature

1. Chrousos GP et al. Late onset 21-hydroxylase deficiency mimicking idiopathic hirsutism or polycystic ovarian disease. Annals Intern Med. 1982; 96,143-148.
2. Choi J-H, and Yoo H-W. Management issues of congenital adrenal hyperplasia during transition from pediatric to adult care. Korean J. Pediatr. 2017; 60(2), 31-37.
3. Eisenhoefer G et al. Reference intervals for plasma concentrations of adrenal steroids measured by LC-MS/MS: Impact of gender, age, oral contraceptives, body mass index and blood pressure status. Clin. Chim Acta. 2017; 470, 115-124.
4. Honour JW. 17-Hydroxyprogesterone in children, adolescents and adults. Annals of Clinical Biochemistry, 2014; 51(4), 424-440.
5. Hughes IA, Riad-Fahmy D, and Griffith K. Plasma 17OH-progesterone concentrations in newborn infants. Archives of Disease in Childhood. 1979; 54, 347-349.
6. Ishii T et al. Guidelines for diagnosis and treatment of 21-hydroxylase deficiency (2014 revision). Clin. Pediatr. Endocrinol. 2015; 24(3), 77-105.
7. Kang MJ et al. Relationships of basal level of serum 17-hydroxyprogesterone with that of serum androstenedione and their stimulated responses to a low dose of ACTH in young adult patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J. Korean Med. Sci. 2011; 26(11), 1454-60.
8. Maas KH et al. Relationship between 17-Hydroxyprogesterone responses to human chorionic gonadotropin and markers of ovarian follicle morphology in women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 2015; 100(1), 293-300.

3 REQUIREMENTS FOR ASSAY DEVELOPMENT

The following documents were used:

va-7.3.0	Design and Development
aa-7.3.6.0	Verification of Development
aa-7.3.6.0_Anlage II	Amendment Development Verification (Sandwich)
aa-7.3.7.0	Development Validation
aa-7.3.7.0_Anlage II	Amendment Development Validation (Sandwich)
aa-7.3.3.0	Requirements for manual assays
aa-7.3.2.0	Development phases and milestones (new development)
aa-7.3.2.2	Phases of Test Development and Milestones
fb-7.3.6.0.1	Verification Plan
fb-7.3.7.0.1	Validation Plan
fb-7.3.10.0	Design History File Manual Assays
xx-fb-904	Device Master Record
fb-7.3.7.0.3	Technical File Manual Assays
xxx-fb-8.6.0.0.2	Risk Management Report

4 PRINCIPLE OF THE TEST

The DRG 17-OH Progesterone ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **principle of competitive binding.**

The microtiter wells are coated with a polyclonal (rabbit) antibody directed towards antigenic sites of the 17-OH Progesterone molecule.

Samples are pre-incubated in the coated wells.

During the second incubation, 17-α-OHP in the added sample competes with the added enzyme conjugate, which is 17-α-OHP conjugated to horseradish peroxidase, for binding to the coated antibody.

After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of colour is inversely proportional to the concentration of the analyte in the sample.

A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5 REAGENTS PROVIDED

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with anti 17-α-OHP antibody (polyclonal).

2. ***Standard (Standard 0-6),*** 7 vials, 1 mL, ready to use;
   Concentrations: 0; 0.15; 0.5; 1.5; 3; 7.5; 20 ng/mL
   0; 0.45; 1.5; 4.5; 9.1; 22.7; 60.6 nmol/L
   Conversion: ng/mL x 3.03 = nmol/L
   The standards are calibrated against the following reference material: Certified Reference Material Cerilliant H-085
   Contain non-mercury preservative.

3. ***Control Low & High,*** 2 vials, 1 mL each, ready to use;
   For control values and ranges please refer to vial label or to the Certificate of Analysis.
   Contain non-mercury preservative.

4. ***Enzyme Conjugate***, 1 vial, 25 mL, ready to use,
   17-α-OHP antigen conjugated with horseradish peroxidase;
   Contains non-mercury preservative.

5. ***Substrate Solution***, 1 vial, 25 mL, ready to use,
   Tetramethylbenzidine (TMB).

6. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

7. ***Wash Solution***, 1 vial, 30 mL (40X concentrated)
   See “Reagent Preparation“.

Note: Additional *Standard 0* for dilution is available upon request.

5.1 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Wash Solution

Add distilled water to the 40X concentrated Wash Solution.

Dilute 30 mL of concentrated Wash Solution with 1170 mL distilled water to a final volume of 1200 mL.

The diluted Wash Solution is stable for 1 week at room temperature.

6 SPECIMEN COLLECTION/PREPARATION

6.1 Sample Volume

25 µL of sample volume is needed for one determination.

6.2 Sample Type

Serum or plasma (EDTA, lithium heparin or citrate plasma) can be used in this assay.
Note: Samples containing sodium azide should not be used in the assay.

In general, it should be avoided to use hemolytic, icteric or lipaemic specimens. For further information refer to chapter Matrix Interference.

6.3 Sample Storage

Specimens should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to performing the assay.
Specimens stored for a longer time (up to 12 months) should be frozen only once at -20 °C prior to the assay.
Thawed samples should be inverted several times prior to testing.

6.4 Sample Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and re-assayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL sample + 90 µL *Standard 0* (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

7 ASSAY SPECIFICATIONS

7.1 Verification (Evaluation of Assay Characteristics)

7.1.1 Test Procedure (VI-1)

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each ***Standard, Control*** and **sample** with new disposable tips into appropriate wells.
3. Incubate for **5 minutes** at room temperature.
4. Dispense **200 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **60 minutes** at room temperature.
6. Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **200 µL** of ***Substrate Solution*** to each well.
8. Incubate for **30 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
10. Measure the optical density of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

7.1.2 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Density
Standard 0 (0 ng/mL)	2.15
Standard 1 (0.15 ng/mL)	1.77
Standard 2 (0.5 ng/mL)	1.28
Standard 3 (1.5 ng/mL)	0.77
Standard 4 (3.0 ng/mL)	0.49
Standard 5 (7.5 ng/mL)	0.25
Standard 6 (20 ng/mL)	0.12

7.1.3 Specificity (VI-2)

Introduction:

Relevant substances (should be related structurally or biochemically to the ELISA antigen) were added at physiological concentrations to the Standard Matrix. The mean bias (in ng/mL and %) was calculated for each substance.

Materials and Methods:

One batch (22K120) was used throughout the study. Two controls are used.

3 samples in 4 different concentrations of the substances are used for this study:

Results:

Substance	Conc. Range (ng/mL)	Mean Bias (ng/mL)	Mean Bias %
17-Benzoate Estradiol	2 - 2000	0.05	<0.01
17-Cypionate Estradiol	2 - 2000	<0.01	<0.01
17-Valerate Estradiol	2 - 2000	<0.01	<0.01
Aldosterone	2 - 2000	0.06	<0.01
Androstenedione	2 - 2000	<0.01	<0.01
Corticosterone	2 - 200	<0.01	<0.01
Cortisol	2 - 200	<0.01	<0.01
Cortisone	2 - 200	<0.01	<0.01
DHEA	2 - 2000	<0.01	<0.01
DHEA-S(a)	2 - 2000	<0.01	<0.01
Estradiol	2 - 2000	<0.01	<0.01
Estriol	2 - 2000	<0.01	<0.01
Estrone	2 - 2000	<0.01	<0.01
Progesterone	2 - 20	0.05	1.31
Testosterone	2 - 2000	<0.01	<0.01

Conclusion:

No substantial cross-reactivity of the assay to structurally related substances is detected.

Progesterone shows positive bias (>1 ng/mL) at concentrations ≥ 200 ng/mL.

Corticosterone and Cortisol show positive bias (>1 ng/mL) at concentrations ≥ 2000 ng/mL.

7.1.4 Analytical Sensitivity (VI-3a)

Introduction:

The Standard 0 solution was determined with 20 replicates. The analytical sensitivity of the DRG ELISA was calculated by subtracting 2 standard deviations from the mean OD of 20 replicate analyses of the Zero Standard (S0). The OD value of "mean - 2xSD" must be quantified with the standard curve.

Materials and Methods:

One batch (22K060) was used throughout the study. Two controls are used (in duplicate).

Results:

Mean OD	2.430		
SD	0.046	**Analytical sensitivity The OD corresponds to the following concentration:**	
2 x SD	0.092		
Mean OD - 2 x SD	**2.338**	**0.013**	ng/mL
n	20.00		

Conclusion:

The Analytical Sensitivity is 0.013 ng/mL.

7.1.5 Limit of Blank (LoB) (VI-3b)

Introduction:

The Standard 0 solution was determined with 20 replicates. The sensitivity is calculated on the basis of CLSI EP17-A2.

Materials and Methods:

One batch (22K060) was used throughout the study. Two controls are used (in duplicate).

Results:

Mean OD	1.716		
SD	0.048	**Limit of blank The OD corresponds to the following concentration:**	
1.645 x SD	0.079		
Mean OD - 1.645 x SD	**1.637**	**0.006**	ng/mL
n	20		

Conclusion:

The Limit of Blank (LoB) is 0.006 ng/mL.

7.1.6 Sensitivity (LoD, LoQ) (VI-3c)

Introduction:

The sensitivity was determined on the basis of CLSI EP17-A2.

Materials and Methods:

One batch (22K060) was used throughout the study. Standard 1 (S1) of the DRG ELISA kit was diluted with *S0* according to the IFU to get 3 samples which have the concentrations of 75%, 50%, and 25% of S1. Every sample was measured in the run with 20 replicates. The sensitivity was calculated on the basis of CLSI EP17-A2

Results:

LoD = LoB + 1.645 x (SD low concentration sample)	Limit of Detection:	**0.042**	**ng/mL**
LoQ = concentration that results in a CV = 20%	Limit of Quantitation:	**0.156**	**ng/mL**

Conclusion:

The Limit of Detection (LoD) is 0.042 ng/mL.

The Limit of Quantification (LoQ) is 0.156 ng/mL, which is still below the 5% percentile of the normal range.

7.1.6.a Assay Dynamic range

The range of the assay is between 0.042 - 20 ng/mL.

7.1.7 Traceability (VI-5a)

Introduction:

To identify an internationally approved Reference Method or Reference Material, DRG performed a search in the database of the Joint Committee on Traceability in Laboratory Medicine (JCTLM), a division of Bureau International des Poids et Mesures (BIPM).

This method was standardized against the certified reference material 17-oH-Progesterone (H-085; Lot: FN06191406; Cerilliant)

Materials and Methods:

One batch (22K100) was used throughout the study. A stock solution with defined concentration of reference material was prepared as recommended in specification sheet of the reference material. The stock solution was diluted with *S0* to get 3 reference samples with high, medium and low concentrations covering the measuring range of DRG ELISA. The 3 samples were measured in 6 replications within one run.

Traceability Chain:

An overview over the procedure to confirm traceability to the SI-unit ng/mL is shown in Figure 1. The 17-α-OHP result in ng/mL, the DRG 17-OH Progesterone measurement method and the kit standards were traced to SI-Unit ng/mL by measurement of the certified reference material H-085.

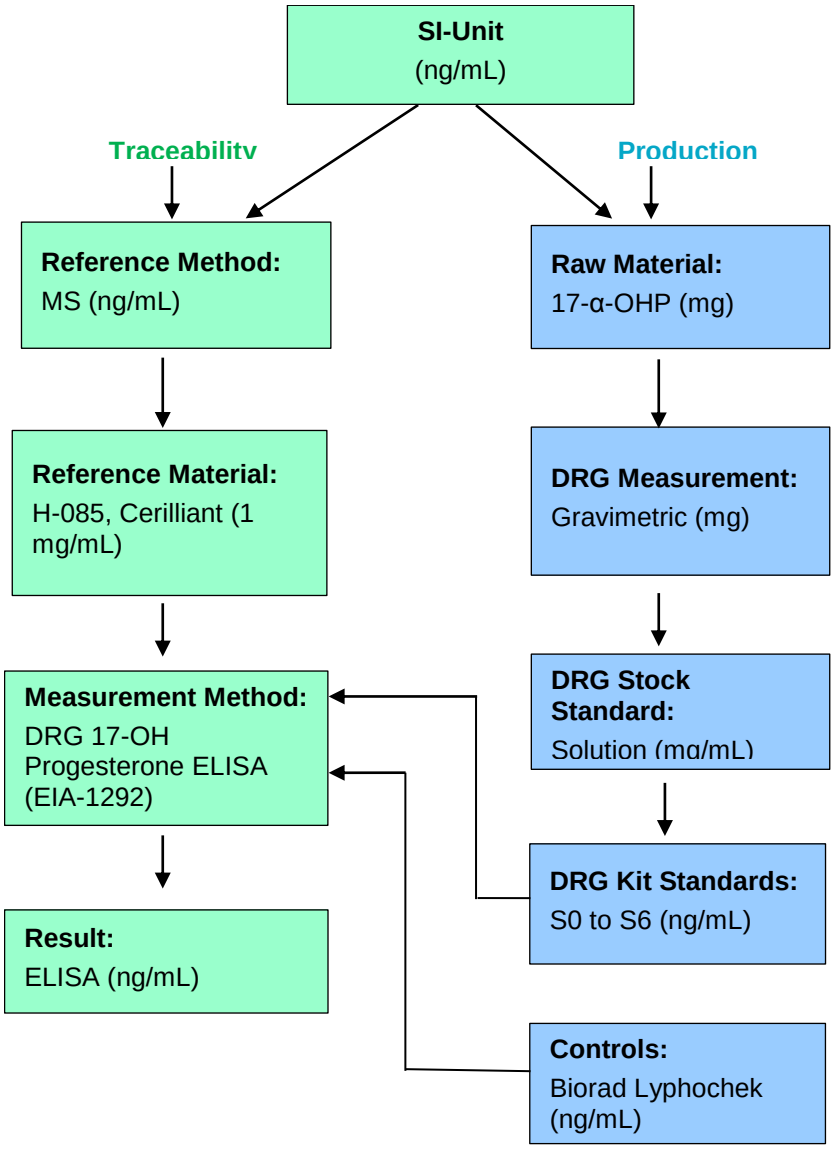


Figure 1: Traceability Chain

Results:

The results for the traceability und uncertainty of standards are shown in the following tables.

	Expected Conc.	Measured Conc.	Acceptance Range (±20%)		Difference RMV (%)	Status
	ng/mL	ng/mL	from	to		
Sample 1	12.50	12.07	10.00	15.00	-0.43	**passed**
		13.14			0.64	
		15.10			2.60	
		11.60			-0.90	
		12.32			-0.18	
		12.86			0.36	
Mean	**ng/mL**	12.85				
Standard Deviation	**ng/mL**	1.23				
Uncertainty	**as CV%**	9.6				
Bias	**ng/mL**	-0.35				

	Expected Conc.	Measured Conc.	Acceptance Range (±20%)		Difference RMV (%)	Status
	ng/mL	ng/mL	from	to		
Sample 2	5.25	4.33	4.20	6.30	-0.93	**passed**
		4.67			-0.58	
		4.55			-0.70	
		4.71			-0.54	
		3.77			-1.48	
		4.45			-0.80	
Mean	**ng/mL**	4.41				
Standard Deviation	**ng/mL**	0.35				
Uncertainty	**as CV%**	7.8				
Bias	**ng/mL**	0.84				

	Expected Conc.	Measured Conc.	Acceptance Range (±20%)		Difference RMV (%)	Status
	ng/mL	ng/mL	from	to		
Sample 3	0.78	0.67	0.63	0.94	-0.11	**passed**
		0.96			0.18	
		0.72			-0.06	
		0.65			-0.13	
		0.78			-0.01	
		0.72			-0.06	
Mean	**ng/mL**	0.75				
Standard Deviation	**ng/mL**	0.11				
Uncertainty	**as CV%**	15.1				
Bias	**ng/mL**	0.03				

Conclusion:

The DRG Kit Standards are calibrated in the range of the certified reference material H-085. The difference from the expected value is below ±20%. The uncertainty of the reference material was found as 9.6%, 7.8%, and 15.1% for the concentrations of 12.5 ng/mL, 5.25 ng/mL, and 0.78 ng/mL.

7.1.8 Uncertainty of Controls (VI-5b)

Introduction:

Objective of the study is to demonstrate uncertainty of Controls of DRG 17-OH Progesterone Kit.

Materials and Methods:

Three lots (22K060, 22K070, 22K100) of DRG 17-OH Progesterone Kit [EIA-1292] were used to determine the 17-OH Progesterone concentrations in two Controls. 2 replicates per lot were run for each Control. The assay was performed according to the IFU.

Results:

The results for the uncertainty of Controls are shown in Table 1 and Table 2.

Lot	ng/mL
22KL060	0.59
	0.63
22KL070	0.55
	0.61
22KL100	0.44
	0.48
Mean (ng/mL)	**0.55**
Standard Deviation (ng/mL)	**0.08**
Uncertainty (as CV %)	**13.8**

Table 1: Results of Control Low and calculation of uncertainty

Lot	ng/mL
22KL060	6.18
	6.34
22KL070	7.12
	7.40
22KL100	8.45
	8.57
Mean (ng/mL)	**7.34**
Standard Deviation (ng/mL)	**1.01**
Uncertainty (as CV %)	**13.8**

Table 2: Results of Control High and calculation of uncertainty

Conclusion:

The uncertainties found for the Control Low and Control High are found 13.8% and 13.8%, respectively.

7.1.9 Intra Assay Precision (VI-6)

Introduction:

The precision study was designed according to ICH guideline.

Materials and Methods:

The assay was performed according to the IFU. The Intra-assay precision was determined with 4 patient samples covering the complete measuring range in 1 run with 10 replicates. CV was calculated as mean CV of 10 replicates.

Results:

Sample	n	Mean (ng/mL)	CV (%)	Status
Sample 1; Li-Heparin plasma	10	0.23	**4.6**	**passed**
Sample 2; Serum	10	2.10	**4.0**	**passed**
Sample 3; Serum	10	7.74	**3.0**	**passed**
Sample 4; Citrate plasma	10	12.11	**4.2**	**passed**

Conclusion:

All Precision data are below the acceptance limit of 10 %.

7.1.10 Inter Assay Precision (VI-7)

Introduction:

The precision study was designed according to ICH guideline.

Materials and Methods:

The assay was performed according to the IFU. Inter-Assay precision was determined for 4 patient samples covering the measuring range in 3 independent runs on 3 days with 10 determinations. CV was calculated from 30 determinations.

Results:

Sample	n	Mean (ng/mL)	CV (%)	Status
Sample 1; Li-Heparin plasma	30	0.21	**8.7**	**passed**
Sample 2; Serum	30	1.99	**6.3**	**passed**
Sample 3; Serum	30	7.41	**6.3**	**passed**
Sample 4; Citrate plasma	30	11.53	**6.2**	**passed**

Conclusion:

Precision data for all samples are below the acceptance limit of 15 %.

7.1.11 Spiked Recovery (VI-8)

Introduction:

Recovery of the DRG ELISA was determined by adding increasing amounts of the analyte to 4 different patient samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) and the standards (18.6, 15.25, 12.50, 7.60 ng/mL) were assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

Materials and Methods:

One batch (22K060) was used throughout the study. The assay was performed according to the IFU.

Results:

Sample Type		Li-Heparin plasma	Citrate plasma	EDTA plasma	Serum
Concentration (ng/mL)		0.17	0.46	0.72	1.92
Average Recovery (%)		97.9	96.0	95.7	93.8
Range of Recovery (%)	from	92.6	89.2	93.3	90.2
	to	103.8	103.2	98.6	96.5
Status Recovery (100 ± 15%)		**passed**	**passed**	**passed**	**passed**

Conclusion:

All recovery data are within the acceptance range of 85 % - 115 %.

7.1.12 Linearity of Dilutions (VI-9)

Introduction:

4 samples containing different amounts of analyte were serially diluted with *S0*. The percentage recovery was calculated by comparing the expected and measured values for the analyte. All Recovery data must be found within the acceptance range of 85-115 %.

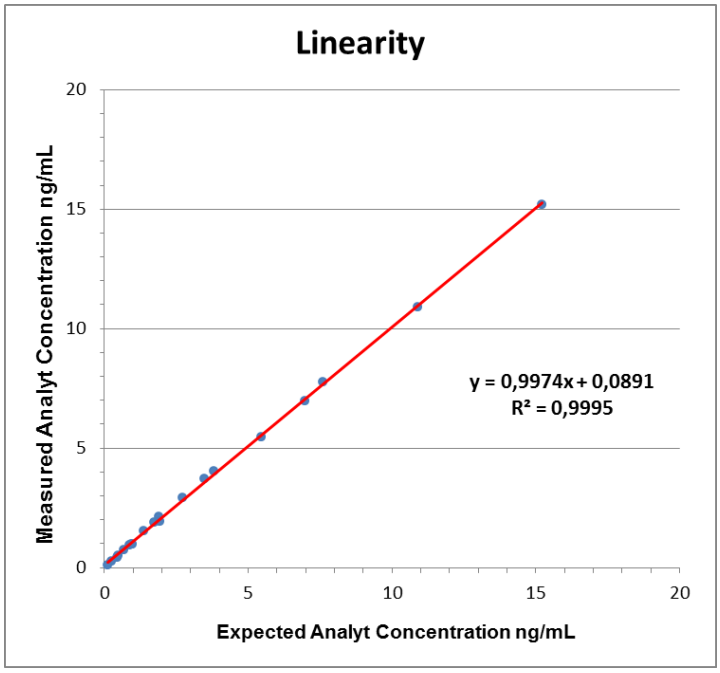
Materials and Methods:

One batch (22K060) was used throughout the study. The assay was performed according to the IFU.

4 samples containing different amounts of analyte were serially diluted with *S0* 1:2 to 1:16 and assayed according to the IFU. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

Results:

Sample		Citrate plasma	Serum	EDTA plasma	Li-Heparin plasma
Concentration (ng/mL)		1.31	6.99	10.90	15.20
Average Recovery (%)		104.1	104.0	108.0	105.7
Range of Recovery (%)	from	93.9	92.2	100.0	102.4
	to	111.4	108.8	113.8	111.1
Status Linearity (100 ± 15 %)		**passed**	**passed**	**passed**	**passed**



Conclusion:

All linearity data are within the acceptance range of 85 % - 115 %.

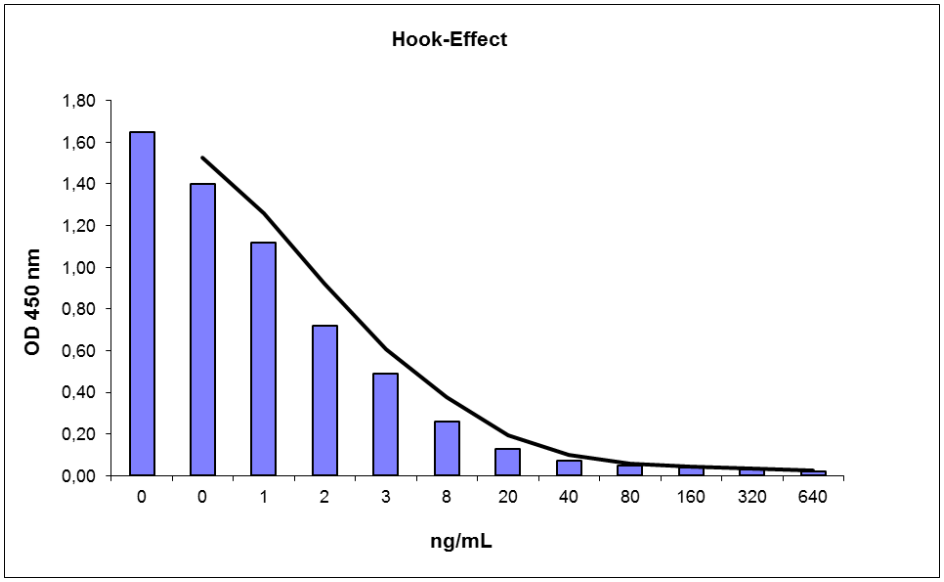
7.1.13 High Dose Hook Effect (VI-10)

Introduction:

Increasing concentrations of analyte were spiked to a sample. Hook effects are observed when concentrations decrease with increasing amounts of added analyte.

Results:

"High Dose Hook Effect" is not detected up to 640 ng/mL.



Conclusion:

"High Dose Hook Effect" is not detected in the range between 0 - 640 ng/mL for this assay.

7.1.14 Matrix Interference (VI 11a)

Introduction:

Increasing amounts of bilirubin, hemoglobin, and triglycerides (1:10 dilution of 10-fold stock solutions) were added to 4 patient samples. After determination of the analyte concentrations in the Bilirubin-, Hemoglobin-, and Triglyceride-spiked samples, the results were compared with the acceptance ranges.

Materials and Methods:

One batch (22K060) was used throughout the study. The assay was performed according to the IFU.

Bilirubin stock solution:

5 mg/mL Stock Solution of bilirubin is prepared by dissolving 50 mg bilirubin in 10 mL PBS. This solution is diluted in 2-folds with PBS up to 1:8 to get 3 further stock solutions of bilirubin with concentrations of 2.5 mg/mL, 1.25 mg/mL and 0.625 mg/mL. Each stock solution is diluted with each sample 1:10 to prepare the samples containing defined concentrations of bilirubin.

Hemoglobin stock solution:

40 mg/mL stock solution of hemoglobin is prepared by dissolving 400 mg hemoglobin in 10 mL PBS. This solution is diluted with PBS up to 1:4 to get 2 further stock solutions of hemoglobin with concentrations of 20 mg/mL and 10 mg/mL. Each stock solution is diluted with each sample 1:10 to prepare the samples containing defined concentrations of hemoglobin.

Triglyceride stock solution:

Triglyceride is an oily liquid which leads to the formation of two phases by mixing with PBS. Therefore, prepared triglyceride stock solution cannot be diluted correctly to get the further concentrations. In that case, each triglyceride stock solutions with concentrations of 4.5 mg/mL, 18.8 mg/mL, 37.5 mg/mL and 75 mg/mL are prepared separately by adding 225 µL, 940 µL, 1875 µL and 3500 µL triglyceride solution (200 mg/mL) into 9.775 mL, 9.060 mL, 8.125 mL and 6.5 mL PBS. Each stock solution is diluted with each sample 1:10 to prepare the samples containing defined concentrations of triglyceride.

Results:

Hemoglobin (up to 4 mg/mL), bilirubin (up to 0.5 mg/mL) and triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Conclusion:

In general, haemolytic, icteric or lipaemic samples should be avoided, but can be tolerated up to at least 4 mg/mL hemoglobin, 0.5 mg/mL bilirubin, and 7.5 mg/mL triglycerides.

7.1.15 Drug Interference (VI 11b)

Introduction:

Interfering substances were added to 3 patient samples in a ratio which yield to a concentration of physiological range, 10-fold lower and 10-fold higher. The same amount of dilution buffer in which the interfering substances were dissolved was also added to the non-spiked patient samples to get the same matrix conditions as in the spiked samples.

Materials and Methods:

One batch (22K120) was used throughout the study. The assay was performed according to the IFU.

Non-spiked and spiked samples were assayed and analyte concentrations of the samples were calculated from the standard curve. The measured concentration of non-spiked samples is subtracted from the mean concentration of spiked samples. The mean bias was calculated for each substance.

Results

The following drugs were tested:

Substance	Concentration	Mean Bias
	ng/mL	ng/mL
Coumestrol	5 - 500	-0.34
Daidzein	5 - 500	0.59
Ethisterone	23.4 - 2340	-0.48
Fulvestrant	5 - 500	-0.11
Genistein	5 - 500	0.28
Levonorgestrel	0.2 - 20	0.27
Mifepristone	23.4 - 2340	-0.53
Prednisolone	0.2 - 20	-0.12
Prednisone	0.2 - 20	-0.59
Secoisolariciresinol	5 - 500	0.23

Conclusions:

Daidzein will increase the measured 17-OH Progesterone concentration in a sample on average by more than 0.5 ng/mL. Mifepristone and Prednisolone will decrease the measured 17-OH Progesterone concentration in a sample on average by more than 0.5 ng/mL. All other tested substances will not change the 17-OH Progesterone concentration by more than ±0.5 ng/mL.

7.1.16 Sample Collection (VI-12a)

Introduction:

To analyze the influence of the sample collection procedure on the outcome of the DRG ELISA, 10 blood samples of a patient group were taken using EDTA, Lithium-Heparin, and Citrate as an additive for each sample and compared with serum from the same patients.
If more than 20% difference to the reference values is noted, a warning must be included in the IFU.

Materials and Methods:

One batch (22K060) was used throughout the study. The assay was performed according to the IFU.

Results:

	% recovery in comparison to serum samples			Mean serum concentration
Samples	**Li-Heparin**	**EDTA**	**Citrate**	**ng/mL**
1	95.21	96.21	94.21	0.50
2	95.45	96.34	100.65	1.23
3	98.87	101.31	100.26	0.38
4	80.38	101.65	80.97	0.85
5	108.38	120.42	104.19	0.38
6	89.30	99.22	73.89	0.38
7	76.58	93.29	82.41	1.03
8	87.65	88.63	105.13	0.82
9	87.50	90.83	87.50	0.60
10	96.10	96.29	98.89	1.62
Mean	**91.54**	**98.42**	**92.81**	
Median	**92.25**	**96.31**	**96.55**	
Acceptance	**passed**	**passed**	**passed**	

Conclusion:

Serum and Plasma (EDTA, Li-Heparin, and Citrate) can be used for this assay.

7.1.17 Temperature Interference (VI 13)

Introduction:

The influence of increased or decreased temperature on the results of the DRG ELISA was analyzed.

Materials and Methods:

The influence of temperature was determined by measuring 4 samples using the temperature as prescribed in the instructions for use (room temperature (20 °C - 24 °C), 2 °C - 8 °C, and 40 °C. If a condition differs more than 20 % from room temperature values, a warning must be included in the IFU.

Results:

Standards / Controls / Samples	Room temperature (20 °C - 24 °C)		40 °C			2 °C - 8 °C			Acceptance Range 100+/-20 %	
	OD	Conc. ng/mL	OD	Conc. ng/mL	Status	OD	Conc. ng/mL	Status	Min	Max
S0	2.43	0.00	3.18	0.16		1.34	0.00			
S1	1.96	0.16	3.19	0.14		1.01	0.16			
S2	1.50	0.49	3.05	0.35		0.74	0.47			
S3	0.96	1.41	2.07	1.66	**See below**	0.42	1.50	**See below**	ODmax > 1.2	
S4	0.58	3.32	1.50	2.92		0.25	3.22			
S5	0.33	7.44	0.85	6.60		0.13	7.56			
S6	0.15	19.70	0.45	32.10		0.06	17.77			
Control Low	1.55	0.44	3.17	0.16	**failed**	0.73	0.49	**passed**	0.35	0.53
Control High	0.39	5.97	1.10	4.53	**failed**	0.14	6.71	**passed**	4.78	7.17
Sample 1	1.97	0.19	3.20	0.18	**passed**	0.94	0.22	**passed**	0.15	0.23
Sample 2	1.59	0.41	3.00	0.41	**passed**	0.74	0.47	**passed**	0.33	0.49
Sample 3	0.33	7.45	0.82	7.00	**passed**	0.14	6.74	**passed**	5.96	8.94
Sample 4	0.24	10.80	0.60	12.02	**passed**	0.09	11.05	**passed**	8.64	12.96

Conclusion:

Increased (40 °C) temperatures will not influence the results of the 17-OH Progesterone ELISA, but will increase the concentration of controls.

Decreased (2 °C - 8 °C) temperatures will not influence the results of the 17-OH Progesterone ELISA.

Therefore, the temperatures recommended in the IFU should be used to perform the ELISA.

7.1.18 Volume Interference (VI 14)

Introduction:

The influence of increased or decreased pipetting volumes on the results of the DRG ELISA was analyzed.

Materials and Methods:

The influence of pipetting volumes was determined by measuring 4 samples using the pipetting volumes as prescribed in the instructions for use (IFU), with 20 % increased volume, and with 20 % decreased volume. If a condition differs more than 20 % from recommended volumes, a warning must be included in the IFU.

Volumes (µL)	Standards, Controls, Samples	Enzyme Conjugate	Substrate Solution	Stop Solution
Volumes as prescribed	25	200	200	100
20 % increased volumes	30	240	240	120
20 % decreased volumes	20	160	160	80

Results:

Standards / Controls / Samples	Volumes as prescribed in the IFU		20 % increased volumes			20 % decreased volumes			Acceptance Range 100+/-20 %	
	OD	Conc.	OD	Conc.	Status	OD	Conc.	Status	Min	Max
		ng/mL		ng/mL			ng/mL			
S0	2.07	0.00	2.45	0.00		2.05	0.00			
S1	1.67	0.15	1.97	0.15		1.71	0.15			
S2	1.18	0.52	1.44	0.50		1.33	0.50			
S3	0.72	1.44	0.87	1.46	**passed**	0.87	1.45	**passed**	ODmax > 1.2	
S4	0.45	3.06	0.54	3.12		0.57	3.08			
S5	0.22	7.60	0.28	7.66		0.30	7.87			
S6	0.10	19.51	0.14	19.62		0.15	18.58			
Control Low	1.34	0.40	1.53	0.42	**passed**	1.43	0.39	**passed**	0.32	0.47
Control High	0.28	6.61	0.37	5.37	**passed**	0.38	5.53	**passed**	5.29	7.94
Sample 1	1.51	0.23	1.82	0.22	**passed**	1.57	0.25	**passed**	0.19	0.28
Sample 2	1.26	0.43	1.46	0.48	**passed**	1.37	0.46	**passed**	0.35	0.52
Sample 3	1.02	0.73	1.18	0.81	**passed**	1.14	0.78	**passed**	0.59	0.88
Sample 4	0.64	1.78	0.75	1.86	**passed**	0.77	1.83	**passed**	1.42	2.14

Conclusion:

Increased (by 20 %) volumes will not influence the results of the 17-OH Progesterone ELISA.

Decreased (by 20 %) volumes will not influence the results of the 17-OH Progesterone ELISA.

Nevertheless, the volumes recommended in the IFU should be used to perform the ELISA.

7.1.19 Time Interference (VI 15)

Introduction:

The influence of increased or decreased incubation times on the results of the DRG ELISA was analyzed.

Materials and Methods:

The influence of incubation times was determined by measuring 4 samples using the incubation times as prescribed in the instructions for use (IFU), with 20 % increased incubation times, and with 20 % decreased incubation times. If a condition differs more than 20 % from recommended incubation times, a warning must be included in the IFU.

Times (min)	Enzyme Conjugate	Substrate Solution
Times as prescribed	60	30
20 % increased times	72	36
20 % decreased times	48	24

Results:

Standards / Controls / Samples	Times as prescribed in the IFU		20 % increased times			20 % decreased times			Acceptance Range 100+/-20 %	
	OD	Conc.	OD	Conc.	Status	OD	Conc.	Status	Min	Max
		ng/mL		ng/mL			ng/mL			
S0	2.51	0.00	2.66	0.00		2.04	0.00		ODmax > 1.2	
S1	2.01	0.15	2.13	0.15		1.64	0.16			
S2	1.47	0.50	1.57	0.50		1.26	0.47			
S3	0.91	1.45	0.96	1.47		0.76	1.51			
S4	0.56	3.14	0.61	3.09		0.49	3.08			
S5	0.3	7.50	0.32	7.57		0.24	7.72			
S6	0.14	19.27	0.15	19.21		0.12	18.39			
Control Low	1.56	0.42	1.64	0.44	**passed**	1.25	0.49	**passed**	0.34	0.51
Control High	0.34	6.39	0.37	6.24	**passed**	0.30	6.11	**passed**	5.11	7.66
Sample 1	1.26	0.74	1.31	0.79	**passed**	0.99	0.88	**passed**	0.59	0.89
Sample 2	0.77	1.92	0.79	2.05	**passed**	0.62	2.10	**passed**	1.54	2.30
Sample 3	0.30	7.61	0.31	7.66	**passed**	0.25	7.70	**passed**	6.09	9.13
Sample 4	0.21	12.13	0.22	11.79	**passed**	0.18	11.09	**passed**	9.70	14.56

Conclusion:

Increased (by 20 %) incubation times will not influence the results of the 17-OH Progesterone ELISA.

Decreased (by 20 %) incubation times will not influence the results of the 17-OH Progesterone ELISA.

Nevertheless, the incubation times recommended in the IFU should be used to perform the ELISA.

7.1.20 Influence of shaking (VI 16)

Introduction:

The influence of shaking on the results of the DRG ELISA was analyzed.

Materials and Methods:

The DRG ELISA was performed with shaking of all incubation steps besides substrate incubation. Results were compared with performance without shaking. If both values differ by more than 20 % in OD values or concentration values, a recommendation must be included in the assay procedure description of the IFU.

Results:

Standards / Controls / Samples	Without shaking		With shaking		Acceptance range 100 +/- 20%		Status
	OD	Conc.	OD	Conc.	% of OD without shaking	% of concentration without shaking	100 % ± 20 %
		ng/mL		ng/mL			
S0	2.70	0.00	2.58	0.00	95.56	-	**passed**
S1	2.28	0.16	2.17	0.15	95.18	93.75	**passed**
S2	1.79	0.48	1.64	0.50	91.62	104.17	**passed**
S3	1.09	1.51	1.02	1.47	93.58	97.35	**passed**
S4	0.70	3.10	0.65	3.08	92.86	99.35	**passed**
S5	0.36	7.27	0.33	7.49	91.67	103.03	**passed**
S6	0.16	19.97	0.15	19.62	93.75	98.25	**passed**
Control Low	1.64	0.62	1.69	0.46	102.68	73.95	**failed**
Control High	0.42	6.09	0.41	5.69	98.57	93.45	**passed**
Sample 1	2.25	0.18	2.18	0.15	96.76	83.62	**passed**
Sample 2	1.92	0.38	1.74	0.42	90.63	109.47	**passed**
Sample 3	1.57	0.70	1.40	0.77	88.80	110.27	**passed**
Sample 4	0.95	1.92	0.93	1.75	97.37	90.98	**passed**

Conclusion:

The assay must be performed **without** shaking.

7.1.21 Sample Stability (VI-17) (adapted from TF version 2.0, since data are not kit-specific)

Introduction:

4 fresh patient samples were analyzed at day 0. Thereafter, samples were divided into 3 aliquots. Aliquot 1 was stored at 4 °C, aliquot 2 and 3 at -20 °C. After 1, 2, 3, 4 and 7 days, aliquot 1 and 2 were measured according to the IFU. Aliquot 2 was frozen again after every measurement. At day 7, aliquot 3 was also measured according to the IFU.

The recoveries should not deviate more than 15 % from the concentrations at day 0.

Materials and Methods:

One batch (22K106-4) was used throughout the study. The assay was performed according to the IFU. Each run included a standard curve and 2 Controls. All samples and standards were measured in duplicate.

Results:

Day 0	Fresh Sample	
	Sample type	Conc. Mean
		ng/mL
Sample 1	Serum	0.65
Sample 2	EDTA plasma	0.63
Sample 3	Heparin plasma	0.67
Sample 4	Citrate plasma	0.68

Day 7	Aliquot 1 stored at 4 °C		
	Conc. Mean	Recovery	Status
	ng/mL	%	100 ± 20%
Sample 1	0.72	110.6	passed
Sample 2	0.70	110.5	passed
Sample 3	0.73	107.9	passed
Sample 4	0.71	104.0	passed

Day 7	Aliquot 2 stored at -20 °C, multiple freeze-thaw-cycles		
	Conc. Mean	Recovery	Status
	ng/mL	%	100 ± 20%
Sample 1	0.71	109.1	passed
Sample 2	0.68	107.8	passed
Sample 3	0.70	103.6	passed
Sample 4	0.64	93.4	passed

Day 7	Aliquot 3 stored at -20 °C		
	Conc. Mean	Recovery	Status
	ng/mL	%	100 ± 20%
Sample 1	0.71	109.8	passed
Sample 2	0.70	110.3	passed
Sample 3	0.69	102.4	passed
Sample 4	0.67	98.4	passed

Conclusion:

Samples can be stored for 7 days at -20 °C or 7 days at 4 °C, without affecting the measured concentrations.
The results were within the specifications (differences < 15%).
In addition, the samples can be subjected to 5 freeze-thaw cycles without affecting the measured concentrations.

7.1.22 Stress Test (VI-18a)

Introduction:

A complete DRG ELISA kit was stored at 40 °C for 7 days and subsequently used for the determination of analyte concentrations in 4 samples (internal controls or samples).

The results were compared with values for the controls or samples obtained with a kit of the same lot that had been stored at 2 °C - 8 °C as recommended. Samples are stored at -20 °C.

If the kit passed all acceptance criteria after 7 days at 40 °C, another test will be performed after 14 days at 40 °C.

If the kit passed all acceptance criteria after 14 days at 40 °C, another test will be performed after 28 days at 40 °C.

Materials and Methods:

One batch (22K070) was used throughout the study. The assay was performed according to the IFU.

Each run included a standard curve and 2 Controls. All samples and standards were measured in duplicate.

Results:

Standards / Controls / Samples	storage at 2 °C - 8 °C		28 days storage at 40 °C			Acceptance range 100 +/- 20%	
	OD	Conc. ng/mL	OD	Conc. ng/mL	Status	Min	Max
S0	2.01	0.00	1.59	0.01	passed	ODmax > 1.2	
S1	1.72	0.13	1.42	0.13			
S2	1.21	0.53	1.01	0.52			
S3	0.71	1.51	0.57	1.53			
S4	0.47	2.79	0.37	2.79			
S5	0.24	7.40	0.20	6.91			
S6	0.12	24.10	0.10	33.01			
Control Low	1.33	0.40	1.11	0.40	passed	0.36	0.64
Control High	0.30	5.50	0.23	5.63	passed	4.04	8.40
Sample 1	1.69	0.15	1.33	0.20	passed	0.12	0.23
Sample 2	1.34	0.40	1.05	0.47	passed	0.32	0.48
Sample 3 (1:2 diluted sample)	0.26	6.66	0.22	5.98	passed	5.33	7.99
Sample 4 (1:2 diluted sample)	0.19	10.90	0.16	10.28	passed	8.72	13.08

Conclusion:

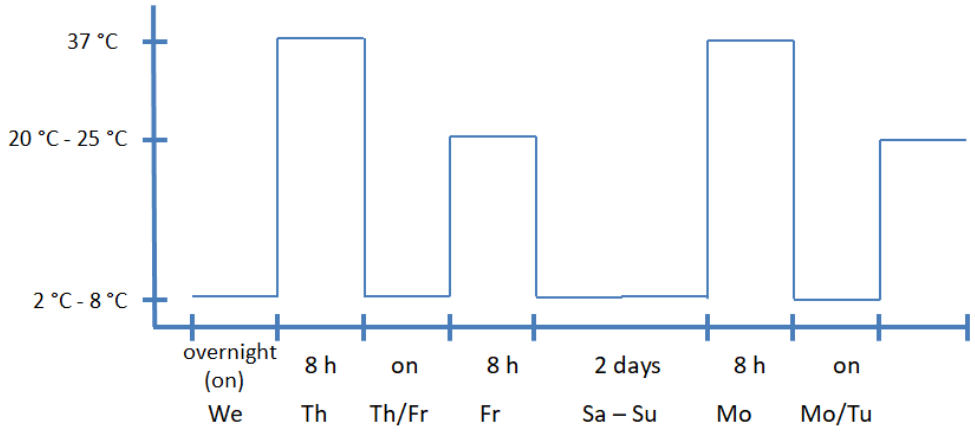
28 days exposure to 40 °C does not affect the results obtained for the two controls and 4 samples.

7.1.23 Transport Stability

Introduction:

All components of DRG ELISA kit were stored at 2 °C - 8 °C as recommended in IFU.

Two complete kits from the same lot are then successively exposed to the following temperatures; 8 h at 37 °C, overnight (8 h to 16 h) at 2 °C - 8 °C, 8 h at RT (20 °C - 25 °C), overnight at 2 °C - 8 °C, 8 h at 37 °C, and overnight at 2 °C - 8 °C. The procedure is summarized in the graph below.



The components of kit # 1 are then equilibrated at RT for at least 30 minutes and subsequently used for the determination of analyte concentrations in 2 internal controls, 4 samples and 2 diluted samples. Two of four samples which were used in this evaluation were diluted 1:2 in *S0* to get the 2 diluted samples.

The results were compared with values for the controls or samples obtained with a kit of the same lot that had been stored at 2 °C - 8 °C as recommended. Samples are stored at -20 °C.

After exposition to the different temperatures, kit # 2 is the stored at 2 °C - 8 °C for 12 months. Then the determination of analyte concentrations is repeated with this kit as described above.

Materials and Methods:

One batch (22K120) was used throughout the study. The assay was performed according to the IFU.

Each run included a standard curve and 2 Controls. All samples and standards were measured in duplicate.

Results Kit # 1:

Standards / Controls / Samples	storage at 2 °C - 8 °C		test directly after transport simulation			Acceptance range 100 +/- 20%	
	OD	Conc. ng/mL	OD	Conc. ng/mL	Status	Min	Max
0.00	1.90	0.00	1.67	0.00	passed	ODmax > 1.2	
0.15	1.59	0.15	1.43	0.15			
0.50	1.18	0.50	1.09	0.50			
1.50	0.70	1.53	0.68	1.50			
3.00	0.47	2.94	0.46	2.96			
7.50	0.26	7.16	0.25	8.01			
20	0.12	22.05	0.15	20.73			
Control Low	1.19	0.49	1.12	0.45	passed	0.31	0.64
Control High	0.28	6.46	0.26	7.61	passed	4.04	8.40
Sample 1	1.48	0.22	1.34	0.22	passed	0.18	0.26
Sample 2	1.20	0.48	1.13	0.44	passed	0.38	0.57
Sample 3	0.96	0.82	0.94	0.75	passed	0.66	0.99
Sample 4	0.63	1.83	0.61	1.81	passed	1.46	2.19

Results Kit # 2:

Conclusion:

The kit is stable directly after transport simulation.
Stability test is ongoing (12 months at 2 °C - 8 °C after transport simulation).

7.1.24 Real Time Stability (VI-19)

Introduction:

The stability study was designed according to DIN EN ISO 23640. 3 different production lots are used to analyze the real time stability (bench life) of DRG ELISA kit.

Materials and Methods:

Three batches (22K060, 22K070, 22K100) were used throughout the study. The assay was performed according to the IFU. Kits of each lot were used to estimate the analyte concentrations of 4 samples and 2 controls after 3, 6, 9, 12, and 15 months. When the results for 4 samples and 2 controls after 15 months are in the acceptance range, the same measurement will be also performed after 18 months. Kits are stored at 2 °C - 8 °C and samples at -20 °C.

Results:

This study started in June 2020 and is still in progress.

7.1.25 Stability of opened kits (VI-20)

Introduction:

The stability study was designed according to DIN EN ISO 23640.
A complete DRG ELISA Kit and 6 samples (internal controls or samples) were measured at day 0 (all vials must be opened and used). Thereafter, all vials are closed again and reevaluated after 4 weeks and 8 weeks storage at 2 °C - 8 °C. Samples are stored at -20 °C. The recovery for the samples should be found between 80-120%.

Materials and Methods:

One batch (22K060) was used throughout the study. The assay was performed according to the IFU.

Results:

Standards / Controls / Samples	day 0		4 weeks storage at 2 °C - 8 °C			8 weeks storage at 2 °C - 8 °C			Acceptance Range 100+/-20%	
	OD	Conc.	OD	Conc.	Status	OD	Conc.	Status	Min	Max
		ng/mL		ng/mL			ng/mL			
S0	2.39	0.00	2.45	0		2.28	0			
S1	1.99	0.15	2.00	0.16		1.89	0.14			
S2	1.50	0.50	1.51	0.49		1.38	0.51			
S3	0.93	1.47	0.91	1.47	**passed**	0.83	1.53	**passed**	ODmax > 1.2	
S4	0.59	3.06	0.56	3.15		0.57	2.86			
S5	0.29	7.81	0.29	7.60		0.30	7.56			
S6	0.14	19.00	0.13	18.95		0.16	21.06			
Control Low	1.53	0.47	1.50	0.49	**passed**	1.49	0.40	**passed**	0.31	0.64
Control High	0.37	5.77	0.32	6.48	**passed**	0.35	6.01	**passed**	4.04	8.40
Sample 1	1.92	0.19	1.95	0.182	**passed**	1.81	0.18	**passed**	0.15	0.23
Sample 2	1.51	0.50	1.54	0.457	**passed**	1.47	0.42	**passed**	0.40	0.60
Sample 3 (1:2 diluted sample)	0.50	3.92	0.49	3.786	**passed**	0.51	3.45	**passed**	3.14	4.70
Sample 4 (1:2 diluted sample)	0.361	5.99	0.363	5.604	**passed**	0.364	5.67	**passed**	4.79	7.19

Conclusion:

Opened kit stability could be demonstrated for all samples for 8 weeks.

7.2 Validation

7.2.1 Method Comparison (VII-1) (Concordance Study)

Introduction:

39 patient samples covering the whole measuring range of the DRG ELISA kit were quantified, and results were compared to those obtained by the reference method (DRG Instruments, 17-OH Progesterone, EIA-1292; Lot 22K040-2; **current version**). The correlation of both data sets is expressed by the correlation coefficient.

Materials and Methods:

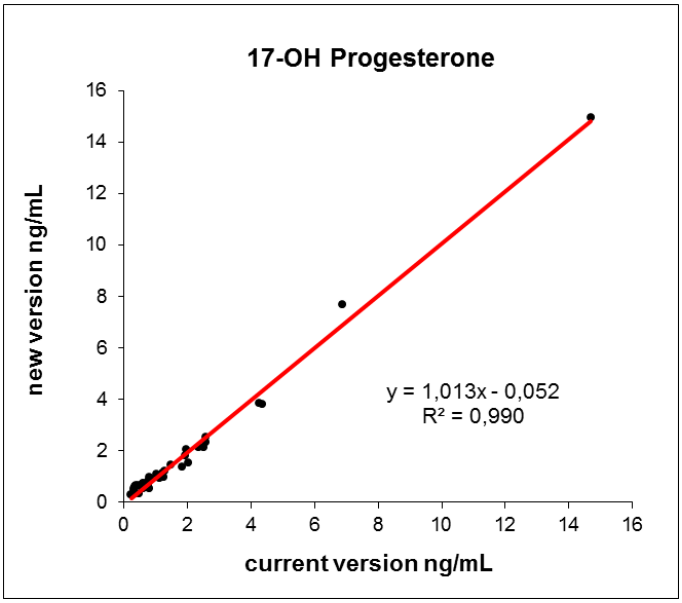
One batch (22K070) **(new version)** was used throughout the study. The assay was performed according to the IFU.

Results:

A comparison of the **new version after design change** of DRG 17-OH Progesterone EIA-1292 (y) and Reference Method (**current version** DRG 17-OH Progesterone EIA-1292) (x) using clinical samples gave the following correlation:

n = 39

r = 0.995



Conclusion:

The data of the new version of the 17-OH Progesterone ELISA perfectly correlate with the data of the current version.

In consequence, it can be expected that the new version after design change will also correlate well with the Beckman RIA (see comparison below with the current version).

Furthermore, it can be expected that the new version after design change will give the same normal ranges for clinical samples as the current version. Therefore, the normal ranges as determined by the current assay version will be adapted to the new version and included in the new IFU.

7.2.2 Method Comparison (VIII-1)

Introduction:

33 patient samples covering the whole range of the DRG ELISA kit were quantified, and results were compared to those obtained by the Beckman 17-OH-Progesterone RIA. The correlation of both data sets is expressed by the correlation coefficient.

Materials and Methods:

One batch was used throughout the study. The assay was performed according to the IFU.

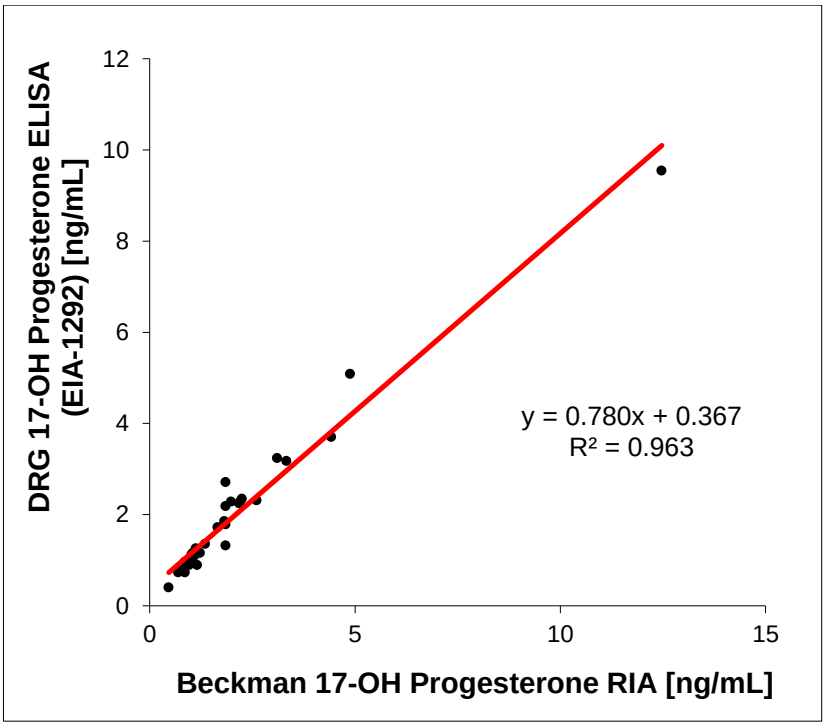
Results:

A comparison of **current version** of DRG 17-OH Progesterone ELISA EIA-1292 (y) and Beckman 17-OH Progesterone RIA (x) using clinical samples gave the following correlation:

n = 33

r = 0.981

y = 0.780x + 0.367



Conclusion:

The DRG ELISA data perfectly correlate with the data of the reference method.

7.2.3 Inter Lot (VII-2)

Introduction:

The inter-Lot variation of the DRG ELISA was determined by 6 measurements of 4 samples with 3 different kit lots. The Inter Lot CV% should be found below 15%.

Materials and Methods:

Three batches (22K060, 22K070, 22K100) were used throughout the study. The assays were performed according to the IFU.

Results:

Sample	Mean Conc. ng/mL Lot 1	Mean Conc. ng/mL Lot 2	Mean Conc. ng/mL Lot 3	Mean Conc.ng/mL	Inter Lot CV (%)	n	Status (<15%)
Sample 1	0.321	0.270	0.317	0.303	**9.4**	18	**passed**
Sample 2	0.849	0.853	0.889	0.864	**2.5**	18	**passed**
Sample 3	1.797	1.848	2.005	1.883	**5.7**	18	**passed**
Sample 4	7.093	6.815	7.757	7.221	**6.7**	18	**passed**

Conclusion:

Precision of repeated measurement of all samples was below the acceptance limit of 15 %.

7.2.4 Normal Values (VII-3)

Introduction:

The Normal Values of the DRG ELISA were determined by measuring the values individuals. It is strongly recommended that each laboratory should determine its own normal and abnormal values. The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

As shown by the Concordance Study (7.2.1), it can be expected that the new version after design change will give the same normal ranges for clinical samples as the current version. Therefore, the normal ranges as determined by the current assay version (see data below) will be adapted to the new version and included in the new IFU.

Results of the current assay version (see Technical File version 2.0).

Materials and Methods:

Different batches were used throughout the study. The assays were performed according to the IFU.

Results:

In a study conducted with newborns and children, using the DRG 17-OH Progesterone ELISA, the following values are observed.

	n		Range (min-max) (ng/mL)	Mean (ng/mL)	Median (ng/mL)	2.5th – 97.5th Percentile (ng/mL)
Newborns (boys and girls)	26	1st month after birth	0.0 – 17.3	7.2	6.7	1.0 – 17.0
	43	2nd month after birth	0.32 – 13.7	4.9	4.6	1.6 – 9.8
	21	3rd month after birth	0.06 – 4.2	2.3	2.3	0.5 – 4.1
	12	4th month after birth	0.2 – 4.6	2.1	2.3	0.2 – 4.3

	n	Age (years)	Range (min-max) (ng/mL)	Mean (ng/mL)	Median (ng/mL)	2.5th – 97.5th Percentile (ng/mL)
Children	75	1 – 10	0.03 – 2.85	1.04	0.88	0.08 – 2.58
Adolescent	3	11 – 14	0.06 – 1.38	0.65	0.50	0.07 – 1.34
	10	15 – 18	0.41 – 2.35	1.24	1.26	0.42 – 2.26

In a study conducted with apparently healthy donors, using the DRG 17-OH Progesterone ELISA, the following values were observed.

Adult females	Follicular phase	0.1 – 0.8 ng/mL
	Luteal phase	0.6 – 2.3 ng/mL
	Ovulation	0.3 – 1.4 ng/mL
	Post ACTH	< 3.2 ng/mL
	Third Trimester	2.0 – 12.0 ng/mL
	Postmenopausal	0.13 – 0.51 ng/mL
Adult males		0.5 – 2.1 ng/mL

Conclusion:

The normal range was established and corresponds well to data obtained from the literature. The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.