premier
Lyme Combo Ab

CLIA Complexity: High

REF **612096**

IVD

Rx Only

ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
Borrelia burgdorferi VlsE and OspC	IgG/IgM	Ag-coated microplate wells	96 x 01 (96)

Intended use: The Premier Lyme Combo Ab test kit is intended for the qualitative determination of IgG and/or IgM class antibodies against Borrelia burgdorferi in human serum and plasma (K^+-EDTA, Li^+-heparin) from symptomatic patients or people suspected of B. burgdorferi infection. It is used as an aid in the diagnosis of Lyme disease, in conjunction with other laboratory and clinical findings. All positive and borderline results should be supplemented by a second step testing method such as Western blot.

Summary and Explanation

Introduction: Lyme disease is a condition caused by Borrelia burgdorferi. A B. burgdorferi infection can manifest itself in the areas of dermatology, neurology, ophthalmology, rheumatology and internal medicine [1, 2, 3]. The clinical expression of borreliosis can be divided into three stages:

Stage I: The typical primary manifestation of a B. burgdorferi infection is erythema migrans (EM) (80%), a reddening of the skin which appears around the area of the tick bite and spreads in a circular manner. The erythema is accompanied by influenza-like general symptoms with fever, shaking chill, headaches and vomiting. Lymphadenopathies are observed in a few cases (lymphadenosis cutis benigna) [2, 3]. The leading clinical types of the erythema-free borreliosis (20%) are neurological, arthromyalgic, influenza-like, cardiovascular borreliosis and borreliosis with hepatitis, with regional lymphadenitis and mixtures of these [1, 2, 3].

Stage I can result in spontaneous healing or can develop into a generalised borreliosis. The transition phase is generally symptom-free. IgM antibodies against B. burgdorferi can be detected serologically in 50% to 90% of patients during stage I. Humans produce specific antibodies against the outer surface protein C (OspC) shortly after infection with B. burgdorferi [4, 5, 6]. VlsE antigen from B. burgdorferi is found to be sensitive for IgG antibody detection, whereas OspC is found to be the sensitive antigen for IgM antibody detection [7, 8, 9].

Stage II: A variety of symptoms can develop several weeks to some months after receiving the tick bite. In the foreground of these are neurological manifestations: meningitis, encephalitis, asymmetric poly- neuritis, cranial nerve pareses, Bannwarth's lymphocytic meningoradiculoneuritis. One of the most frequent neurological symptoms of borreliosis in children is the peripheral facial palsy. Usually the paresis of the facial nerve appears on one side and after several weeks on the opposite side. Also arthritis, particularly of the knee joints, and non-localised pains in the bones, joints and muscles are frequently found. Less frequently are cardiological manifestations such as myocarditis and pericarditis [1, 3]. Antibodies against B. burgdorferi can be detected in 50% to 90% of patients in stage II. In the early phase of this stage mainly IgM antibodies are found but in the late phase often only IgG antibodies occur. However, IgM antibodies can persist for a long time [3, 10]. VlsE based detection systems were found to be convenient in detecting anti-B. burgdorferi IgG antibodies [7, 8, 11].

Stage III: The typical manifestations of a B. burgdorferi infection in stage III are chronic-relapsing erosive arthritis, acrodermatitis chronica atrophicans and progressive encephalomyelitis, which can proceed in a similar way to multiple sclerosis [1, 3, 12]. Without treatment, the tertiary stage can develop over a period of years to decades after the original infection. In this stage, IgG antibody titers are significantly increased in 90% to 100% of patients, while IgM antibodies are only rarely detectable [11, 12, 13].

The Premier Lyme Combo Ab test has B. burgdorferi sensu stricto B31 US strain VlsE and OspC antigens for detecting the antibodies. Serological diagnosis should follow the principle of a two-step procedure: ELISA as a first step, which, if reactive, is followed by an immunoblot [11, 15, 16].

Antigen: Borrelia burgdorferi sensu stricto B31 US strain VlsE and OspC antigens.

Principles of the test: The test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with B. burgdorferi antigens. In the first reaction step, diluted patient samples, calibrator and controls are incubated in the wells. Anti-Borrelia burgdorferi antibodies will bind to the antigens coated in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, anti-human IgG/IgM HRP enzyme conjugate (rabbit/goat) is added to each well. The enzyme conjugate will bind to any wells that have human IgG and/or IgM binding to the B. burgdorferi antigens. The wells are washed to remove any unbound HRP enzyme conjugate and 3,3,5,5 tetramethylbenzidine (TMB) enzyme substrate is added. If the HRP enzyme is present in the well (positive reaction), the HRP enzyme will react with the TMB substrate and produce a blue color. After an additional incubation time to allow the color development, a stop solution is added which turns the blue color yellow and inhibits further color development to allow for a stable spectrophotometric reading. The test strips are placed in a microplate reader and the optical density of the color is measured. The amount of antigen specific bound antibody is proportional to the color intensity.

Materials

Contents of the test kit:

Component	Color	Format	Symbol
1. Microplate wells coated with *Borrelia burgdorferi* antigens 12 microplate strips each containing 8 individual break- off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator IgG/IgM, prepared from human serum, contains 0.09% sodium azide, pre-diluted and ready for use	red	1 x 2.0 mL	CAL
3. Positive control IgG/IgM, prepared from human serum, contains 0.09% sodium azide, pre-diluted and ready for use	blue	1 x 2.0 mL	CONTROL +
4. Negative control IgG/IgM, prepared from human serum, contains 0.09% sodium azide, pre-diluted and ready for use	green	1 x 2.0 mL	CONTROL −
5. Enzyme conjugate peroxidase-labelled anti-human IgG/IgM (goat), contains protein, buffer and non-azide preservatives, ready for use	blue	1 x 12 mL	CONJ
6. Sample buffer contains PBS, protein and 0.09% sodium azide as a preservative, ready for use	light blue	1 x 100 mL	BUF SMP
7. Wash buffer 10x concentrate, contains PBS, Tween and 0.02% sodium azide as a preservative	colorless	1 x 100 mL	BUF WASH 10X
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colorless	1 x 12 mL	SUBS
9. Stop solution 0.5 M sulphuric acid, ready for use	colorless	1 x 12 mL	SOLN STOP
10. Protective foil	---	2 pieces	FOIL
11. Quality control certificate	---	1 protocol	

Additional materials and equipment (not supplied):

- **Incubator:** Capable of maintaining temperature at +37 C ± 1 C.
- **Automatic microplate washer:** This is recommended, however, plate washing can be performed manually.
- **Plate reader:** Capable of measuring optical densities at 450 nm and a reference wavelength of between 620 nm and 650 nm.
- **Calibrated micropipettes:** For dispensing 1000, 100 and 10 µL.
- **Multichannel pipette:** Recommended for dispensing 100 µL volumes of conjugate, substrate and stop solution.
- **Glass/plastic tubes:** For sample dilution.

Warnings and Precautions

1. For in vitro diagnostic use. For use by laboratory professionals in a clinical or research laboratory setting.
2. Before starting the assay, carefully read the instructions. Use only the valid version provided with the kit.
3. Do not substitute or mix with reagents from other manufacturers.
4. Observe prudent laboratory practice and safety guidelines. Avoid eye and skin contact with specimens and reagents. In case of eye or skin contact, wash off thoroughly with plenty of clean running water. Remove and wash contaminated clothing. In case of ingestion, obtain medical attention.
5. The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be decontaminated or disposed of with proper biohazard precautions. Some of the reagents contain sodium azide in a non-declarable concentration, as an antibacterial agent. Avoid skin contact.
6. **Waste disposal:** Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. Follow local, state and federal regulations regarding handling and disposal of hazardous waste, including potentially explosive materials such as accumulated or con- centrated sodium azide.

Preparation and Stability of the Reagents

Storage and stability: The test kit has to be stored at a temperature between +2 C to +8 C. Do not freeze.
Unopened, all test kit components are stable until the indicated expiry date.
All reagents must be brought to room temperature (+18 C to +25 C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2 C to +8 C and protected from contamination, unless stated otherwise below.
The thermostat adjusted ELISA incubator must be set at **+37 C ± 1 C**.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature (+18 C to +25 C) to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bags).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2 C and +8 C for 4 months, but not longer than the indicated expiry date.

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to +37 C and mix well before diluting. The amount required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water). For example: For 1 microplate strip, 5 mL concentrate plus 45 mL water. The working strength wash buffer is stable for up to 28 days when stored at +2 C to +8 C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light  . The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue colored.

- **Stop solution:** Ready for use.

Preparation and Stability of the Patient Samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: CLSI GP44-A4 recommends the following storage conditions for samples: Samples should be stored at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at +2 C to +8 C. If the assay will not be completed within 48 hours, or if the samples will be stored beyond 48 hours, samples should be frozen at -20 C or lower. Samples should not be repeatedly frozen and thawed. Frozen samples must be mixed well after thawing and prior to testing. Diluted samples should be incubated within 8 hours. Do not use bacterially contaminated samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Sample dilution: Patient samples to be investigated are diluted **1:101** in sample buffer.
Example: Add 10 µL of sample to 1.0 mL sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

Note: The calibrator and controls are prediluted and ready for use, do not dilute them.

Procedure

Sample incubation: (1st step)

Transfer 100 µL of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the instrument manufacturer's recommendations with regard to microwell plate sealing.
Incubate **60 minutes at +37 C ± 1 C**.

Washing:

Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µL of working strength wash buffer for each wash.
Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µL of working strength wash buffer.

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Attention: Residual liquid (>10 µL) in the reagent wells after washing can interfere with the substrate and lead to false low OD values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high OD values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µL of enzyme conjugate (peroxidase-labelled anti-human IgG/IgM) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18 C to +25 C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µL of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18 C to +25 C) (protect from direct sunlight).

Stopping:

Pipette 100 µL of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the color intensity should be made at a **wave- length of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, carefully shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting Protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.

Calculation and Interpretation of Results

Quality control: The controls and calibrator included in the test kit must be used with each run. Results cannot be validated if the control values deviate from the expected values stated on the quality control certificate. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated. The positive control and negative control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay cut-off. Additional controls may be required according to guidelines or local, state, and/or federal regulations (such as 42 CFR 493.1256) or accrediting organizations. Refer to CLSI document C24-A for guidance on appropriate QC practices.

Ratio-based analysis: Results can be evaluated by calculating a ratio of the OD value of the control or patient sample over the OD value of the calibrator. Calculate the ratio according to the following formula:

Meridian Bioscience recommends interpreting results as follows:

$$\frac{\text{OD of the control or patient sample}}{\text{OD of calibrator}} = \textbf{Ratio}$$

Ratio < 0.8:	**Negative**
Ratio ≥ 0.8 to < 1.1:	**Borderline**
Ratio ≥ 1.1:	**Positive**

Determination of assay cut-off: This recommendation is based on a ROC analysis from the results of 81 reference samples obtained from the Centers for Disease Control and Prevention in Atlanta, GA.

Interpretation: A negative serological result does not exclude an infection, particularly in the early phase of an infection. All positive or borderline results should be followed up with IgM and/or IgG Western blot testing. For Lyme disease diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Limitations of the Procedure

1. This kit is used as an aid in diagnosis only. A positive result should be interpreted with clinical findings and other serological tests.
2. The results obtained from this assay are not diagnostic proof of the presence or absence of Lyme disease. Interpret test results of specimens from immunosuppressed patients with caution.
3. The binding activity of the antibodies and the activity of the enzyme used are temperature- dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature during the incubation steps, the greater will the OD values be. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, with the result that such variations will be largely compensated in the calculation of the results.
4. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect high OD values.
5. Sodium azide has been known to inhibit conjugate activity. Clean pipette tips must be used for the conjugate addition so that sodium azide is not carried over from other reagents.
6. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.

Expected Values

The range of values and positivity of different populations among different studies with the Premier Lyme Combo Ab test kit are presented below with available patient demographics.

Population	n	Sex	Age range	Ratio results			Qualitative results	
				Mean	Range	Std. dev.	Positive or borderline	%
Prospective study	418	173 men, 243 women, 2 unknown	19-76 y; 2 unknown	1.5	0.0 - 9.8	2.29	140	33.5%
Sensitivity study	100	36 men, 52 women, 12 unknown	16-80 y; 12 unknown	5.1	0.2 - 10.7	3.38	95	95.0%
CDC panel (Lyme disease)	35	unknown	unknown	4.2	0.3 - 8.5	2.79	32	80.0%
Normal endemic	98	89 men, 9 women	18-56 y	0.4	0.1 - 5.8	0.85	3	3.1%
Normal non-endemic	100	82 men, 18 women	2-76 y	0.3	0.1 - 3.8	0.40	5	5.0%

Note: It is recommended that each laboratory determine its own normal range based on the population and equipment used.

Performance Characteristics

Sensitivity study: A study, consisting of 100 clinically characterized Lyme disease specimens, was conducted at the manufacturer's site with the Premier Lyme Combo Ab test device in parallel with a C6 peptide-based ELISA as the predicate device. These specimens contain samples from early, early disseminated and late phases of the disease. The panel consisted of 36 men, 52 women and 12 unknowns. The age ranged from 16 to 80 years.

Disease Stage	n	Premier Lyme Combo Ab		C6 peptide-based ELISA	
		Positive or borderline	Sensitivity (%) 95% C.I.	Positive or borderline	Sensitivity (%) 95% C.I.
Acute (EM or culture positive, < 3 months after onset)	46	44	95.7 85.2 - 99.5%	35	76.1 61.2 - 87.4%
Convalescent (EM or culture positive, 3-12 months after onset)	30	27	90.0 73.5 - 97.9%	25	83.3 65.3 - 94.4%
Late (Lyme disease with presentations other than EM, onset unknown or > 1 year)	24	24	100.0 85.8 - 100.0%	22	91.7 73.0 - 99.0%
Total	100	95	95.0 88.7 - 98.4%	82	82.0 73.1 - 89.0%

Method comparison study: A prospective study was performed with clinical samples collected from various locations in the Northeastern US. The samples were tested with the Premier Lyme Combo Ab in parallel with a C6 peptide-based ELISA as the predicate device. The panel consisted of 173 men, 243 women and 2 unknowns with the age range from 19 to 76 years. The table below shows the results from prospective studies.

n = 418		C6 peptide-based ELISA		
		Positive	Equivocal	Negative
Premier Lyme Combo Ab	Positive	103	2	20
	Borderline	4	0	11
	Negative	2	2	274

Positive agreement:	109/113 = 96.5%	95% C.I.:	91.2 - 99.0%
Negative agreement:	274/305 = 89.8%	95% C.I.:	85.9 - 93.0%

Note: Borderline/equivocal counted as positives.

CDC panel testing: Forty (40) samples of various reactivity were acquired from the Centers for Disease Control and Prevention in Atlanta, GA and evaluated internally. Of the 40 samples, 5 samples were from normal blood donors and 35 samples were from patients diagnosed with Lyme disease (clinically characterized borreliosis stratified by disease stage). All samples were tested with the Premier Lyme Combo Ab in parallel with a C6 peptide-based ELISA as the predicate device. Note: The results of the testing are presented here as a means of conveying further information on the performance of this assay with a characterized serum panel and does not imply an endorsement of the assay by the CDC.

Agreement to clinical diagnosis:

Disease stage	n	Premier Lyme Combo Ab		C6 peptide-based ELISA	
		Positive or borderline	Agreement with clinical diagnosis	Positive or borderline	Agreement with clinical diagnosis
Normals	5	0	100.0%	0	100.0%
< 1 month	6	6	100.0%	6	100.0%
> 1 - 3 months	11	10	90.9%	10	90.9%
> 3 - 12 months	11	9	81.8%	9	81.8%
> 12 months	7	7	100.0%	7	100.0%
Total	40	32	80.0%	32	80.0%

Analytical specificity study: The levels of anti-Borrelia burgdorferi antibodies from asymptomatic populations were analyzed with the Premier Lyme Combo Ab in a panel of 98 samples from an endemic region (Pennsylvania; 89 men and 9 women; age range: 18 to 56 y) and in another panel of 100 samples from a non-endemic region (Tennessee; 82 men and 18 women; age range: 2 to 76 y). The results are shown below.

Sample type	n	Negative	Equivocal	Positive	% Equivocal + positive
Endemic	98	95	0	3	3.1%
Non-endemic	100	95	3	2	5.0%

Cross reactivity: Cross reactivity was investigated using a panel of 886 serologically characterized sera positive for antibodies against different disease conditions and the results obtained are shown in the table below.

No.	Panel	n	Premier Lyme Combo Ab	
			Negative	%
1	Anti-Treponema pallidum	88	87*	98.9
2	Anti-Adenovirus	50	50	100.0
3	Anti-Bordetella pertussis toxin	50	50	100.0
4	Anti-Bordetella FHA	50	50	100.0
5	Anti-CMV	50	50	100.0
6	Anti-EBV-CA	50	47	94.0
7	Anti-Helicobacter pylori	50	50	100.0
8	Anti-HSV-1	50	50	100.0
9	Anti-Influenza virus type A	50	50	100.0
10	Anti-Influenza virus type B	50	50	100.0
11	Anti-Measles virus	50	50	100.0
12	Anti-Mumps virus	50	50	100.0
13	Anti-Mycoplasma pneumoniae	50	50	100.0
14	Anti-Parainfluenza virus types 1-4	50	50	100.0
15	Anti-RSV	50	50	100.0
16	Anti-Parvovirus B19	35	34	97.1
17	ANA	14	14	100.0
18	Rheumatoid arthritis	17	16	94.1
19	Fibromyalgia	18	18	100.0
20	Multiple sclerosis and other neurological diseases	22	22	100.0

* 1 Borderline sample counted as positive.

Precision: Repeatability was investigated using samples with values at different concentrations. The intra-assay repeatability is based on 20 determinations and the inter-assay repeatability is based on 40 determinations performed in 20 different runs on 10 different days (with 2 runs per day and 2 replicates per run). The results are shown below.

Sample No.	Mean ratio	Within-run		Within-day		Between-days		Total	
		SD	%CV	SD*	%CV	SD	%CV	SD	%CV
1	0.1	0.006	4.1	0.00	0.0	0.009	6.6	0.01	7.8
2	0.2	0.008	3.9	0.00	0.0	0.007	3.5	0.01	5.2
3	0.5	0.022	4.2	0.00	0.0	0.031	6.0	0.04	7.3
4	0.8	0.051	6.4	0.00	0.0	0.051	6.4	0.07	9.1
5	1.1	0.029	2.5	0.00	0.0	0.058	5.2	0.06	5.8
6	1.9	0.065	3.5	0.00	0.0	0.133	7.2	0.15	8.0
7	3.0	0.103	3.4	0.00	0.0	0.223	7.4	0.25	8.2
8	3.7	0.113	3.0	0.00	0.0	0.247	6.6	0.27	7.3

* When the SD value is negative, it is set to 0 [ref.17].

Reproducibility: Reproducibility was investigated using samples with values at different concentrations, which are based on 30 determinations performed with 3 different lots with 1 run per lot and 10 replicates per run according to the package insert. Site-to-site testing was done in 48 determinations per sample performed at 3 different sites for 4 days with 2 runs per day and 2 replicates per run. The results are shown below.

Sample No.	Mean ratio	Within-run		Within-day		Between-days		Between-sites		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.6	0.056	8.7	0.014	2.1	0.014	2.1	0.020	3.0	0.030	4.6
2	0.8	0.047	6.0	0.074	9.4	0.074	9.4	0.051	6.5	0.057	7.3
3	1.0	0.099	9.8	0.017	1.7	0.017	1.7	0.094	9.3	0.070	6.9
4	5.9	0.367	6.2	0.587	9.9	0.587	9.9	0.138	2.3	0.364	6.2
5	6.4	0.268	4.2	0.769	12.0	0.769	12.0	0.227	3.5	0.421	6.6
6	7.1	0.218	3.1	0.941	13.2	0.941	13.2	0.310	4.3	0.490	6.9
7	9.2	0.373	4.0	1.136	12.3	1.136	12.3	0.307	3.3	0.605	6.5
8	10.1	0.606	6.0	1.182	11.7	1.182	11.7	0.316	3.1	0.701	6.9

Interferences: Hemolytic, lipemic and icteric samples showed no influence on the result up to a con- centration of 1000 mg/dL for hemoglobin, 2000 mg/dL for triglycerides and 40 mg/dL for bilirubin in this ELISA.

Serum/plasma comparison: The usability of plasma was investigated using sample pairs each of serum and corresponding plasma (K^+-EDTA, Li^+-heparin). Passing-Bablok regression was calculated for the comparison of serum to plasma. The regression equation is near the ideal correlation (intercept 0; slope 1.0) indicating equivalence of concentrations between serum and the corresponding plasma matrices. Coefficients of determination were found to be above 0.975 and % recovery compared to serum was in the range of 87% to 109% (serum = 100%).

	K^+-EDTA plasma	Li^+-Heparin plasma
n	20	20
Concentration range (Serum)	Ratio 0.4 - 4.3	Ratio 0.4 - 4.3
Concentration range (Plasma)	Ratio 0.4 - 4.2	Ratio 0.4 - 4.2
Regression equation (y = plasma, x = serum)	y = -0.00 + 0.97 x	y = 0.04 + 0.96 x
95% C.I. of intercept	-0.05 - 0.05	-0.03 - 0.07
95% C.I. of slope	0.92 - 1.01	0.94 - 1.00
Coefficient of determination R^2	0.9944	0.9961
Mean % recovery	97%	100%
Range of % recovery	87 - 103%	93 - 109%

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