

AccuDiag™
Lyme Disease IgG
ELISA

Cat# 1428-2



Test	Lyme Disease IgG
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA : Antigen Coated Plate
Detection Range	Qualitative: Positive, Negative Control & Cut Off
Sample	10µL
Total Time	~ 70 min
Shelf Life	12-18 Months from the manufacturing date
Specificity	96.40%
Sensitivity	91.70%

TEST PRINCIPLE

The Diagnostic Automation *B. burgdorferi* ELISA test system is designed to detect IgG class antibodies to *B. burgdorferi* in human sera. Wells of plastic microwell strips are sensitized by passive absorption with *B. burgdorferi* antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG (γ chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

SPECIMEN COLLECTION AND PREPARATION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

MATERIALS AND COMPONENTS

Materials provided with the test kits

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).

1. **Plate.** 96 wells configured in twelve 1x8-well strips coated with inactivated *B. burgdorferi* B31 strain antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. **Conjugate.** Conjugated (horseradish peroxidase) goat anti-human IgG (γ chain specific). Ready to use. One, 15 mL vial with a white cap.
3. **Positive Control** (Human Serum). One, 0.35 mL vial with a red cap.
4. **Calibrator** (Human Serum). One, 0.5 mL vial with a blue cap.
5. **Negative Control** (Human Serum). One, 0.35 mL vial with a green cap.
6. **Sample Diluent.** One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate- buffered-saline, (pH 7.2 \pm 0.2). Green solution, ready to use. Note: Shake Well Before Use. (Product #: 1428-2Z). Preservative added.
7. **TMB:** One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO < 15% (w).
8. **Stop solution:** One 15 mL bottle (red cap) containing 1M H₂SO₄, 0.7MHCl. Ready to use.
9. **Wash buffer concentrate (10X):** dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 \pm 0.2.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

Materials required but not provided

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200µL.
3. Multichannel pipette capable of accurately delivering (50-200µL).
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

PROCEDURE NOTES

1. For In Vitro Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS MATERIALS**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg, and for antibodies against HCV and

HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens.

5. The sample diluent, controls, wash buffer, and conjugate contain sodium azide at a concentration of 0.1% (w/v) Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
6. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20-25°C) before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
7. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

software and reader requirements for the correct Controls/ Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. The Sample Diluent will undergo a color change confirming that the specimen has been combined with the diluent.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

A. Manual Wash Procedure:

- a. Vigorously shake out the liquid from the wells.
- b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
- c. Repeat steps a. and b. for a total of 5 washes.
- d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 + 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check

RESULTS

A. Calculations:

1. Correction Factor

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

2. Cutoff OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.

$$(CF \times \text{mean OD of Calibrator} = \text{cutoff OD value})$$

3. Index Values or OD Ratios

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:	
Mean OD of Calibrator	0.793
Correction Factor (CF)	0.25
Cut off OD	$0.793 \times 0.25 = 0.198$
Unknown Specimen OD	0.432
Specimen Index Value or OD Ratio	$0.432 / 0.198 = 2.18$

B. Interpretations:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥ 1.10

The OD ratio is interpreted and reported as follows:

- **Negative:** No detectable IgG antibody; result does not exclude *B. burgdorferi* infection. An additional sample should be tested within 4-6 weeks if early infection is suspected.
- **Equivocal:** Current recommendations state that equivocal results should be followed by supplemental Western Blot testing. (Western blot assays for antibodies to *B. burgdorferi* are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM.) This equivocal result should be reported with results from Western Blot testing. Results should not be reported until the supplemental testing is completed.
- **Positive:** IgG antibody to *B. burgdorferi* presumptively detected. Per current recommendations, the result cannot be further interpreted without supplemental Western Blot testing. (Western Blot assays for antibodies to *B. burgdorferi* are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM.) Results should not be reported until the supplemental testing is completed.

Dilute 1:2 in Sample Diluent; then, 1:21 as the procedure indicates.

Re-test Result: Ratio = 1.88 = $17.09 \text{ IU/mL} \times 2 = 34.18 \text{ IU/mL}$

PERFORMANCE CHARACTERISTICS

A. Comparative Study

The Diagnostic Automation, Inc. *B. burgdorferi* antibody IgG ELISA test system was compared to a commercially available indirect fluorescent antibody (IFA) test

procedure for detection of IgG antibodies to *B. burgdorferi*. Two hundred seven (207) serum samples were tested by the two procedures. The results of this study are summarized in Table 1 below:

		B. Burgdorferi IFA Procedure	
		Pos.	Neg.
Diagnostic Automation, Inc. <i>B. burgdorferi</i> IgG ELISA	Pos.	54	12
	Neg.	2	133
	Equivocal *	1	5

Relative Sensitivity = 96.4 % (54/56)

Relative Specificity = 91.7 5 (133/145)

Concordance = 93.0 % (187/201)

*Equivocal samples were excluded from the calculations for sensitivity, specificity, and concordance.

Table 2 shows test results obtained using a serum panel from the CDC. The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

The CDC *B. burgdorferi* Disease Serum Panel Stratified by Time After Onset

Time from onset	Pos	+ / -	Neg.	Total	% agreement with clinical diagnosis
Normals	0	0	5	5	100%
< 1 month	1	1	4	6	20%
1-2 months	2	1	5	8	28%
3-12- months	8	3	9	20	47%
> 1 year	8	0	0	8	100%
Total	19	5	23	47	57%

B. Reproducibility

To assess intra-an inter-assay variation of the test procedure seven serum samples ranging from negative to high positive were tested by the Diagnostic Automation, Inc. Lyme IgG ELISA. Eight replicates of each sample were tested on three consecutive days. The mean OD ratio and coefficient of variation (CV) were calculated for each sample. These data are shown below:

Serum #	Intra-Assay (n = 8)						Inter-Assay (n = 3)	
	Day # 1		Day # 2		Day # 3		Mean Ratio	CV
Serum # 1	1.75	8.2%	1.76	6.0%	1.50	9.6%	1.67	7.2%
Serum # 2	1.22	3.9%	1.21	6.5%	1.12	3.4%	1.18	3.8%
Serum # 3	4.25	2.8%	4.30	6.7%	3.84	2.1%	4.13	5.0%
Serum # 4	2.73	3.5%	3.14	2.1%	2.53	4.1%	2.80	9.1%
Serum # 5	2.69	2.5%	2.97	7.1%	2.64	4.3%	2.77	15.2%
Serum # 6	0.49	5.5%	0.60	5.1%	0.59	8.1%	0.56	8.9%
Serum # 7	0.35	7.4%	0.43	2.1%	0.39	8.1%	0.39	8.4%

C. Cross Reactivity

Serum specimens from patients with North American borreliosis, yaws, pinta, leptospirosis, autoimmune diseases, and syphilis may crossreact.

QUALITY CONTROL

- Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
- Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
- The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

OD Range

Negative Control	≤ 0.250
Calibrator	≥ 0.300
Positive Control	≥ 0.500



- The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9 .
 - The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25 .
 - If the above conditions are not met the test should be considered invalid and should be repeated.
- The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
 - Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
 - Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

LIMITATIONS OF PROCEDURE

- The antibody titer of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
- Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
- Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original specimen to look for seroconversion or, an IgM specific assay should be performed.
- A positive rubella IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. However, a negative test for IgG antibody in the neonate may help exclude congenital infection.

PRECAUTIONS

- Store the unopened kit between 2° and 8°C.
- Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
- Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
- Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
- TMB: Store between 2° and 8°C.
- Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.
- Sample Diluent: Store between 2° and 8°C.
- Stop Solution: Store between 2° and 25°C.

ISO 13485 ISO 9001   Diagnostic Automation/ Cortez Diagnostics, Inc. 23961 Craftsman Road, Suite E/F, Calabasas, California 91302 USA			
Date Adopted	Cat # 1428-2-FD		
2006-07-06	AccuDiag™- Lyme Disease IgG ELISA -2013		
<table border="1"> <tr> <td>EC</td> <td>REP</td> </tr> </table>	EC	REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
EC	REP		
Revision Date: 09-9-2013			