Trinity Biotech

EU Lyme + VlsE IgG Western Blot
Western Blot Test System for the Detection of IgG Antibodies to *Borrelia afzelii "PKO", Borrelia garinii, and Borrelia burgdorferi VlsE*

IVD

REF 44-2020GV

Store Kit and Components at +2 to +8°C

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
Dla innych języków

Para outras línguas
Για τις άλλες λώσσες
För andra språk
For andre språk

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INTENDED USE

The Trinity Biotech EU Lyme Western Blot is a qualitative in vitro assay for the detection of IgG antibodies in human serum against specific antigens/proteins of *Borrelia afzelii "PKO", Borrelia garinii and Borrelia burgdorferi VlsE*[9]. The EU Lyme Western Blot is intended as a confirmatory assay for use in testing human serum samples which have been previously found to be positive or equivocal using an EIA, IFA or other appropriate screening method. For Professional Use Only

SUMMARY AND PRINCIPLES

Lyme disease is a multisystem disease caused by a spirochete of the genus *Borrelia*[1,2,3]. The three major species of *Borrelia* are *burgdorferi, afzelii* and *garinii. B. burgdorferi* has been isolated in the USA and Europe while *B. afzelii* and *B. garinii* appear to be exclusive to Europe and Japan.

The organism is transmitted through an arthropod vector from an animal reservoir. In Europe, the arthropod vector is the *Ixodes ricinus* tick. In the USA, three species of ticks have been identified as vectors, *Ixodes dammini, Ixodes scapularis*, and *Ixodes pacificus.*

Lyme disease has been documented in Europe since early in the 20th century. In the USA, Lyme disease was first documented in a 1975 epidemic among children in Old Lyme, Connecticut[4]. These children exhibited arthritic symptoms which Steere et al. recognized to be a separate clinical disease state. Lyme symptoms may be nonspecific and may be confused with juvenile rheumatoid arthritis, lupus erythematosis, multiple sclerosis, rheumatic fever, Reiters Syndrome, myocarditis and viral meningitis.

Animal reservoirs include deer, wild mice, birds, raccoons, horses, dogs, and cats. Ticks are commonly found on vegetation in endemic areas especially in wooded areas common to the infected animals.

Historically, the incidence of Lyme disease in humans coincides with the tick season from April through September.

Lyme Disease has multiple clinical manifestations, some of which are specific to the stage of the disease. Steere has proposed three major stages: primary or early , neurologic and arthritic.

Primary:

The symptoms of Early Lyme Disease include a characteristic red lesion on or near the site of the tick bite[5] (AKA Erythema Migrans,"EM"), arthritic symptoms, a "flu like" fever, headaches, dizziness, stiff neck, fatigue, general malaise, muscular aches and pains, abdominal pain, and irregular heartbeat, myalgia, arthralgia and lymphadenopathy.
The later stages can resemble a variety of diseases.

Neurological, cardiac and musculoskeletal stage:

Generally, symptoms in these areas may appear weeks to months following the initial infection. This stage is characterized by symptoms of dizziness, weakness, irregular heartbeat, meningitis, inflamed nerve roots, facial palsy, loss of memory, poor motor coordination and somnolence.

Arthritic Stage:
This stage is characterized by swelling and inflammation of the joints. These arthritic attacks may be localized or systemic and may be recurrent.

DIAGNOSIS

Serologic methods are among the most common techniques used in the laboratory to establish a diagnosis for Lyme Disease. Screening assays are useful in establishing a presumptive diagnosis of Lyme disease. These assays are generally inexpensive, easy to perform, and suited for screening large numbers of patients. These include enzyme immunoassay (EIA), indirect immunofluorescence (IFA), and indirect hemagglutination (IHA). EIA is considered to be more sensitive, easier to perform and less subjective than either IFA or IHA[6,7,8].

Confirmatory assays are supplemental assays used to confirm screening assays. The most popular confirmatory assay is the Western Blot. The sensitivity of Western Blots is greater than or equal to that of screening assays. The specificity of the Western Blot is greater than that of the screening assays. The increased sensitivity and specificity is mainly due to the Western Blot's ability to differentiate antibodies produced in response to individual *Borrelia* antigens/proteins.

Other methods used to establish a diagnosis for Lyme disease include Polymerase Chain Reaction (PCR) and Direct Culture of the Spirochete.

PCR is primarily used to detect spirochetes in Synovial fluid, Cerebrospinal Fluid, and Tissue samples. PCR directly identifies, through the use of specific probes, the spirochete DNA instead of measuring the host's response to it. PCR amplifies the organisms DNA and may be able to detect as few as one to five organisms. The test is considered rapid when compared to culture, however, it is very tedious and expensive when compared to EIA or Western Blot assays. PCR is considered to be highly specific but the sensitivity may be as low as 60%.

Culture of the Spirochete from Tissue biopsies, Cerebrospinal Fluid or Synovial Fluid is recommended only in early Lyme disease and is virtually useless in late Lyme disease. Culture of this fastidious organism requires the use of specialized growth media, a specialized growth environment and may take from 6 to 8 weeks to grow. Although commercial growth media is available, the procedures for specimen processing, media inoculation and prevention of contamination are not standardized. In addition, supplemental growth additives may be necessary depending on the individual species of spirochete. For these reasons, culture is rarely performed.

TEST PRINCIPLES

The Trinity Biotech EU Lyme Western Blot is a second generation Western Blot technique which utilizes antigens of *Borrelia afzelii* "PKO" which are separated during Polyacrylamide Gel Electrophoresis (PAGE). Subsequently, the resolved proteins are transferred to nitrocellulose by a second electrophoresis. Also present on each strip are the purified "Osp C" of *Borrelia garinii*, the VlsE protein of *Borrelia burgdorferi*, a Serum Addition Control and a Conjugate Addition Control. During the assay, individual test strips are incubated with diluted patient serum. After incubation, the strips are washed and incubated with anti-human immunoglobulin conjugated to alkaline Phosphatase. Unbound conjugated anti-human antibodies are removed by additional washes. Antibodies bound to the antigen on the strip are visualized by incubation with a substrate.

REAGENTS

Description	Active Ingredient	Amount
EU Lyme + VlsE Strips 44-2018V	Nitrocellulose strips bound with B. afzelii, B. garini and B burgforferi antigens	40 strips
Negative Control 44-2021	Ready to use human serum, Negative for Anti-Borrelia & IgG Antiboridies with stabilizers	100 µL
Weakly Reactive Control 44- 2041	Ready to use human serum +/- Indicator for Anti-borrelia IgG antibodies with stabilizers	100 µL
Positive Control 44-2023	Ready to use human serum, Positive for Anti-borrelia IgG with stabilizers	250 µL
10X Conjugate 44-2055G	Anti-Human (Goat) IgG Alkaline Phosphotase Conjugate with stabilizers	9 mL
Substrate 44-2017	Ready to use, 5- bromo-4- chloro- 3 indolyl- phosphate nitro blue tetrazolium developing solution	90 mL
Sample Diluent Wash Solution (10 X) 40-2019	TRIS buffered Saline < 0.1% Na Azide	100 mL
Sample Diluent Milk Powder 40-2030	White powder milk proteins	5 g
Blot Banding Template	Blot banding template * guide only	1

ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

A) *Platform rocker. Capable of rocking at a minimum platform angle of 7° ± 2° at 35-40 cycles per minute. Note: The use of a rotary platform is not recommended and may adversely affect the results and reproducibility of this assay.*
B) Blunt tipped forceps.
C) Pipettes of various sizes (calibrated precision micropipettes and serological pipettes).
D) Disposible pipette tips.
E) 100 mL and 1.0 L graduated cylinders.
F) Laboratory timer.
G) Repeating dispenser capable of dispensing 2.0 mL.
H) Clean glass or plastic containers for dispensing Sample Diluent / Wash Solution and for preparing and dispensing Conjugate.
I) Deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable.
J) Disposable latex gloves or an acceptable equivalent.
K) Analytical Balance or Scale.

MATERIALS PROVIDED

Product#	Description	Quantity
44-2018V	EU Lyme + VlsE Western Blot Strips	40 strips
44-2023	EU Lyme WB IgG Positive Control	250 uL
44-2041	EU Lyme WB IgG Weakly Reactive Control	100 uL
44-2021	EU Lyme WB Negative Control	100 uL
44-2055G	EU Lyme Anti-Human IgG Alkaline Phosphatase Conjugate	9ml
44-2017	Alkaline Phosphatase Developing Solution	90 mL
40-2019	10X Sample Diluent/ Wash Solution	100 mL
40-2030	Sample Diluent/ Wash Powder	5 g
	Western Blot Band Reading Guide	1 ea
	Kit record sheet	1 ea
99-1113	Disposable White Strip incubation trays	4

WARNINGS AND PRECAUTIONS

A) HANDLE SAMPLES, STRIPS, NEGATIVE AND POSITIVE CONTROLS AS IF THEY WERE CAPABLE OF TRANSMITTING INFECTION. Only serum which has been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, Hepatitis-C virus antibodies and Hepatitis-B surface antigen are used as control sera. Nevertheless, since there is no method that can assure that human blood derivatives will not transmit infectious agents, these components must be handled as potentially infectious.

B) Do not pipette by mouth.

C) Wear disposable gloves (latex or equivalent) during the entire process. Discard the gloves with the rest of the biohazardous material. Wash your hands well after working with these reagents.

D) Clean up any splashes or spills with a solution of 10% sodium hypochlorite (domestic bleach) after running the assay. Do not use bleach in or around the area where the assay is being run. Contaminated material must be discarded as biohazardous.

E) Treat all reagents and materials that come in contact with potentially infectious samples as biohazardous waste. Solid biohazardous waste material should be incinerated or autoclaved. Liquid waste must be disinfected by mixing with an equal volume of 1 % sodium hypochlorite (10% liquid domestic bleach) and remaining in contact for at least 60 minutes.

F) Do not ingest or allow the reagents or components of this kit to come in contact with your skin or mucous membranes.

G) Some of the reagents in this kit contain sodium azide as a preservative. Sodium azide may react with drains or pipes of copper or lead to produce metallic azides that are highly explosive. Flush drains with water thoroughly after disposing of fluids containing sodium azide.

H) Do not use metal instruments with the substrate.

I) Samples contaminated with bacteria or fungi may cause dark stains on the strips that may make them unreadable. In addition, lipemic or hemolysed specimens may also cause dark background or spots/blotches to appear on the strips making them unreadable.

J) Use only distilled or deionized water of good quality to dilute reagents.

K) Do not mix components from different lots of kits or from kit to kit within a lot.

L) Do not use the kit after the expiry date printed on the kit box and component vials.

M) Prevent microbial contamination of reagents. Employ an aseptic procedure for opening and taking out aliquots from the original vials. Store the reagents under refrigeration (2-8°C) when not in use.

N) Do not interchange the caps of the vials. This could cause cross contamination of the reagents.

O) Do not remove the NITROCELLULOSE STRIPS from their tube until immediately before they are to be used. To prevent condensation inside the tube containing the strips, allow it to reach room temperature before opening (approximately 30 minutes). Close the tube immediately after removing the strips which are going to be used. The strips should be held in a dry dark place at 2-8ºC. Condensation in the tube generally does not cause any problems, however, excessive moisture in the tube over long periods of time may lead to contamination of the strips by bacteria or fungi.

P) Do not use the reagents and other material in the kit until they have reached room temperature (18-25°C, approximately 30 minutes).

Q) Do not cut the strips. Narrower strips could cause false interpretations because they may turnover in the incubation tray or artifacts may be produced in the reaction zone that might be confused with positive bands or could prevent the recognition of positive bands.

R) Changes in the physical aspect of the reagents may indicate instability or deterioration.

S) We do not recommend the re-use of disposable incubation trays. If re-usable trays are used they should be soaked in 10% sodium hypochlorite (domestic bleach) for a minimum of six hours after use and then thoroughly rinsed out with distilled or deionized water.

The safety data sheet is available upon request.

WARNING
Some components of this kit contain < 0.1% sodium azide.
EUH031: Contact with acid liberates toxic gas.
H302: Harmful if swallowed.
H317: May cause an allergic skin reaction.
H335: May cause respiratory irritation.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330: If swallowed, rinse mouth.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

COLLECTION OF SAMPLES

A) The Trinity Biotech EU Lyme Western Blot kit can be used with serum or plasma. Serum is preferred when available. The reliability of the results is unknown when contaminated, lipemic or hemolyzed samples are used.

B) Do not inactivate samples by heating as this may degrade serum proteins that may cause increased background on the strips. Heat inactivated sera cannot be used for IgM antibody determinations.

C) Samples may be held under refrigeration (2-8°C) for up to a week before analysis. Otherwise they should be frozen at <-20°C.

D) Avoid repeated freezing and thawing of samples.

E) Mix all samples well. If necessary, centrifuge samples before analysis to remove particulates.

F) If specimens are to be shipped, they should be packed in compliance with Government Regulations covering the transportation of etiologic agents. Specimens may be shipped refrigerated (2-8° C) or frozen (< -20° C).

STORAGE AND STABILITY

A) Store kit at +2 to +8°C.

B) Bring all required components to room temperature prior to use (+18 to +25°C).

C) Check all reagents for expiration date. Do not use reagents beyond their expiration date.

D) Diluted conjugate must be used on the day it is prepared.

E) Diluted Sample Diluent / Wash Buffer Solution (1X) is stable for up to 14 days when stored at + 2 to + 8°C.

REAGENT PREPARATION

A) EU Lyme + VlsE WB Strips: Ready to use strips packed in a reseable plastic vial. Place any remaining strips back in the vial and reseal cap tightly.

B) EU Lyme WB IgG Negative Control - Ready to use negative serum does not require any prior dilution before assay.

C) EU Lyme WB IgG Weakly Reactive Control - Ready to use +/- indicating serum does not require any prior dilution before assay.

D) EU Lyme WB IgG Positive Control - Ready to use positive serum does not require any prior dilution before assay.

E) Developing Color Solution: Ready to use color developing solution.

F) Preparation of 1X Sample Diluent/Wash Solution:

Note: 10X Sample Diluent/Wash Solution may contain undissolved salts once removed from refrigeration. Allow 10X diluent to reach room temperature and shake the bottle to dissolve any salts. Do not dispense the reagent until all of the salts are dissolved.

1. Dilute: 100 mL of 10X Sample Diluent/Wash Solution with 900 mL of distilled (or deionized) water in a clean bottle.

2. Add the content of item # 40-2030 Sample Diluent Milk Powder to the 1X Sample Diluent/Wash Solution. Mix until all powder dissolves.

REAGENT PREPARATION (continued)

G) Alternative 1X Sample Diluent/Wash Milk Solution:

Below is a guide for alternative 1X Sample Diluent/Wash Milk Solution to be prepared based on the number of WB strips to be tested.

Note:, Stable for 14 days when stored unused 1X Sample Diluent/Wash Solution Milk is stored at *2 – 8 ° C* .

Table 1: 1X Sample DILUENT/WASH MILK SOLUITON guide based on number of test strips.

Test Strips	Sample Dil./Wash Solution (10X)	Distilled Deionized Water	Sample Dil. Milk Powder	Working Sample Dil./Wash Soln. (1X)
5	9 mL	81 mL	0.45 g	90 mL
10	18 mL	162 mL	0.90 g	180 mL
15	27 mL	243 mL	1.35 g	270 mL
20	36 mL	324 mL	1.80 g	360 mL
25	45 mL	405 mL	2.25 g	450 mL
30	54 mL	486 mL	2.70 g	540 mL
35	63 mL	567 mL	3.15 g	630 mL
40	72 mL	648 mL	3.60 g	720 mL

H) EU Lyme IgG Alkaline Phosphatase Conjugate: Dilute 1 part of the EU Lyme 10X Goat Anti-Human IgG Conjugate with 9 parts of the 1X Sample Diluent/Wash Milk Solution. Mix well.

Note: Diluted conjugate is stable for day of use only and cannot be stored for future use. Discard any excess.

I) Alternative Conjugate required: 2.0 mL of the diluted conjugate is required for each specimen or control tested. Do not prepare more conjugate than necessary. See table 2 guide below for amount of conjugate to be made based on number of WB strips to be assayed

Table 2 Guide: Based on number of test strips

Test Strips	10X IgG Conjugate	1X Dil./Wash Milk Solution
5	1 mL	9 mL
10	2 mL	18 mL
15	3 mL	27 mL
20	4 mL	36 mL
25	5 mL	45 mL
30	6 mL	54 mL
35	7 mL	63 mL
40	8 mL	72 mL

Notes: Do not over dilute 1X IgG conjugate solution.

PROCEDURAL NOTES

A) All procedural steps should be followed as written. Failure to do so may result in aberrant test results.

B) Record the kit lot number and expiration date on the record sheet for future reference.

C) Do not mix components from different lot numbers or use expired components and/or kits. Each individual vial of strips is considered a separate lot number. Do not mix strips from different vials.

D) Use strips from each kit in consecutive, numerical order, and always use forceps when handling the strips.

E) The reproducibility and outcome of the Western Blot assay depends greatly on the proper incubation and washing of the strips. The use of a rocker or rocking platform which rocks at 35-40 cycles per minute at an angle of 7° ± 2° ensures that the strips will be properly exposed to the diluted patient sera and conjugated antibodies and will be washed properly. Position the tray so that the reagents mix lengthwise along the strip. Do not use a rotating platform to incubate this assay.

F) Fluid in the channels of the incubation trays must be decanted or aspirated completely prior to the addition of subsequent reagents.

G) Keep the tray level when adding reagents and specimens to the channels of the incubation tray. If necessary carefully tip the tray toward you to position the strips at the end of the channel to avoid dispensing reagents, sera and controls directly on the strip.

H) Add Sample Diluent / Wash Solution, Serum, Conjugate, and Substrate to the ends of the channels of the incubation trays and not directly on the strips. Adding reagents and serum directly on top of the strips may cause adverse reactions.

I) Exercise extreme care when decanting and adding reagents. This will prevent cross contamination between the channels of the incubation trays.

J) Temperature, water quality, and rocker frequency play a significant role in determining the substrate incubation time. Substrate incubation times may vary between 4 and 12 minutes. Substrate timing should never be performed at a set time but should reflect the time at which the Weakly Reactive Control just becomes visible.

ASSAY PROCEDURE

A) All reagents must be brought to 20- 25°C before performing the test procedure.

B) Carefully remove the required number of strips from the vial with blunt forceps. Place the strips in numberical order into the channels of the incubation tray.

One strip per channel.

DO NOT TOUCH STRIPS WITH YOUR HANDS, ALWAYS USE FORCEPS.

C) For each control or sample to be tested, fill a channel with exactly 2.0 mL 1X Sample Diluent/Wash Solution.

NOTE: Add 1X Sample Diluent/Wash Solution slowly and directly to the space above the strip number. Visually check to make sure strip is completely wet and not partially floating on top of the buffer.

D) Allow strips to soak for a minimum of 5 minutes while rocking on an appropriate platform rocker.

E) Remove the incubation tray from the platform rocker and dispense:
1. 80 µL of positive control to channel #1 slightly above the strip number.
2. 20 µL of the weakly reactive control to channel #2 slightly above the trip number.
3. 20 µL of negative control to channel #3 slightly above the strip number.
4. 20 uL of each sample to appropriately marked channels slightly above each strip numbers.

NOTE: Do not dispense any sera directly onto the strip. It may be necessary to carefully tip the tray toward you allowing strips to move slightly towards the end of the each channel to avoid dispensing sera directly onto the strips.

F) Place incubation tray back onto the rocking platform and rock for 30 minutes with all samples and controls.

G) Carefully remove incubation tray from rocker and decant all solution by carefully tipping the incubation tray.

H) Add 2.0 mL of 1X Sample Diluent/Wash Solution to each channel containing each strip and incubate for 5 minutes while rocking on the platform rocker.

I) Decant all solution and repeat this process (2) additional times to ensure thorough rinsing of the unbound specimen from the strips. Do not use squeeze bottle to wash strips.

J) After last decant, pipette 2.0 mL of Diluted 1X IgG Conjugate onto each channel containing a strip.

K) Place the incubation tray onto the platform rocker and incubate by rocking for 15 minutes.

L) Remove incubation tray from the platform, decant all solution by carefully tipping the incubation tray.

M) Add 2.0 mL of 1X Sample Diluent/Wash Solution to each channel containing a strip and incubate for 5 minutes while rocking on the platform rocker.

N) Decant all solution and repeat this process (2) additional times to ensure thorough rinsing of the unbound conjugate from the membrane strips. Do not use squeeze bottle to wash strips.

O) After last decant, Add 2.0 mL of distilled or deionized water to each channel containing a strip. Allow strips to rock for (1) minute.

P) Decant all distilled or deionized water from the incubation tray.

ASSAY PROCEDURE (CONTINUED)

Q) Add 2.0 mL of Substrate developing solution to each strip in the incubation tray.

Place the incubation tray on the platform rocker. Carefully monitor and examine the Weakly Reactive Control strip development during this incubation period. Proceed to next step as soon as the 41kD band becomes visible this *should occur between 4 - 12 minutes of incubation.*

NOTE:
1. Do Not Use A Fixed Time For The Color Development Step.
2. Very Important Not To Walk Away From Platform Rocker During This Point.

The Weakly Reactive Control will produce a faint band if properly developed.

R) Remove the incubation tray from the platform rocker after incubation.

S) Decant all solution by carefully tipping the incubation tray.

T) Add 2.0 mL of distilled or deionized water to each channel of the incubation tray.

U) Rock the incubation tray by hand 3 - 4 times. Repeat this step two (2) additional times.

V) Remove the strips from the channels while wet (using blunt forceps) and place wet strips onto paper towels and allow to air dry.

NOTE: Do not expose the membrane to direct lighting for extended periods. This will cause fading of the color.

W) Record Kit Lot number and Expiration date to Result Log (provided in kit) and mount strips in consecutive, numerical order and interpret results.

Note: Never handle strips by hand. Always use forceps when handling the strips

QUALITY CONTROL

A) The Weakly Reactive IgG Control and Negative Control must be included in each run. The IgG Positive Control needs only to be run once per vial of strips.

B) The IgG Positive Control is supplied to assist in the location of significant bands on patient strips. It must show reactivity at the following band locations: Serum Addition Control, Conjugate Addition Control, B. garinii Osp. C, B. burgdorferi VlsE[9], 100kD, 41kD, 39kD, 22kD Osp "C", and 17kD. Other bands may be present but are not required.

C) The IgG Positive Control may be compared to illustrations or images of reacted strips found in the kit insert in order to identify the relative positions of the bands.

D) The Weakly Reactive IgG Control must show reactivity with the Serum and Conjugate Controls and show a weak band at the 41kD position. Other bands may be present but should not be used as the reading standard.

E) The Negative Control should not show any significant bands. Any non-significant bands should be weakly visible if present. It must show reactivity with the Serum and Conjugate Controls.

F) If the Positive, Weakly Reactive, and Negative Controls do not perform as required or do not show reactivity with both the Serum Addition and the Conjugate Addition Control bands the run is considered invalid and all results may not be reported.

G) All Patient strips run must show reactivity with both the Serum Addition and the Conjugate Addition Control bands. If there is no reactivity with either or both of these bands, the patient results cannot be reported.

INTERPRETATION OF RESULTS

The relative intensity of the bands is scored as follows:

Intensity of Band	Relative Reactivity
Absent	Negative
•Less than the intensity of the 41kD Band on the Weakly Reactive Contro	Negative
•Equal to or greater than the intensity of the 41kD band on the Weakly Reactive Control.	Positive

Carefully compare the intensity of bands detected on patient strips with the intensity of the 41kD band on the Weakly Reactive control. The importance of the Weakly Reactive Control is two fold. It provides a reading standard by which patient bands can be graded and it provides an indication of the assay reproducibility. Bands which have intensities equal to or greater that the Weakly Reactive Control will be reproducible from kit to kit and lot to lot. Bands which have intensities less than that of the Weakly Reactive Control should be considered negative and are not reportable.

Modified MiQ 12 2000 Interpretive Criteria

Interpretive Criteria for Europe excluding FDR Germany

IgG Positive +	IgG Indeterminate +/-	IgG Negative -	Unreadable
3 of the following bands: p14 p17 22kD - OspC p30 p39 p43 p58 p100 *B. garinii* -OspC, *B. burgdorferi* VlsE[9].	2 of the following bands: p14 p17 22kD - OspC p30 p39 p43 p58 p100 *B. garinii* -OspC, *B. burgdorferi* VlsE[9].	< 2 of the following bands p14 p17 22kD - OspC p30 p39 p43 p58 p100 *B. garinii* -OspC *B. burgdorferi* VlsE[9].	Strips which exhibit dark staining, spots, streaks or blotches which hinder interpretation.

Interpretive Criteria (for FDR Germany)

IgG Positive +	IgG Indeterminate +/-	IgG Negative -	Unreadable
2 of the following bands: p14 p17 22kD - OspC p30 p39 p43 p58 p100 *B. garinii* -OspC, *B. burgdorferi* VlsE[9].	1 of the following bands: p14 p17 22kD - OspC p30 p39 p43 p58 p100 *B. garinii* -OspC, *B. burgdorferi* VlsE[9].	Zero of the following bands p14 p17 22kD - OspC p30 p39 p43 p58 p100 *B. garinii* -OspC *B. burgdorferi* VlsE[9].	Strips which exhibit dark staining, spots, streaks or blotches which hinder interpretation.

Note: Proceed to next step as soon as you see a faint band on the weakly reactive control. Do not use a fixed time

Western Blot Band Reading Guide

A) Attach the developed Positive and Weakly Reactive Control Strips to the Band Template Reading Guide using glue or tape.

B) Align the strips using the black alignment mark on the numbered label.

C) Refer to the Positive Control Strip for the Location of significant bands.

D) Refer to the Weakly Reactive Control for the location of the 41kD band.

E) Align the Serum and Conjugate Control bands from the Positive Control with each patient strip and interpret the patient bands.

F) The location of the Bands on individual strips may shift up or down slightly as compared to the Positive Control. This shifting is common to all Western Blots.

G) Individual band intensity is essential to interpretation. All patient bands should be compared to the Weakly Reactive 41kD band for intensity.

LIMITATIONS OF THE PROCEDURE

A) Perform the assay in strict accordance with the enclosed instructions.

B) The Trinity Biotech EU Lyme Western Blot is optimal when used on clear fresh human serum. Plasma may be used if it is fresh, clear and free from hemolysis.

C) Individuals with Positive Western Blots for antibodies to *B. afzelii/garinii/burgdorferi* should be referred for medical evaluation that may include additional testing. The diagnosis of Lyme Disease must include careful clinical evaluation and should not be based upon the detection of antibodies to *B. afzelii/garinii/burgdorferi* alone.

D) If a specimen repeatedly yields unreadable strips and symptoms persist, a fresh specimen should be collected and tested in 2-4 weeks.

E) A negative interpretation does not exclude the possibility of infection with *B. afzelii/garinii/burgdorferi*.

F) Sera from individuals with other spirochetal diseases such as syphilis, yaws, pinta, leptospirosis, relapsing fever and periodontal disease may give rise to false positive results. Individuals with connective tissue autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosis, and individuals with anti-nuclear antibodies may also give false positive results. Individuals with bacterial and viral infections such as Rockey Mountain Spotted Fever, Epstein-Barr Virus and Cytomegalovirus may have antibodies which will cross-react with *Borrelia afzelii/garinii/burgdorferi.*

G) The Trinity Biotech EU-Lyme Western Blot should not be used to determine the success or failure of antibiotic therapy.

H) Western Blot testing should not be performed as a screening procedure.

I) A positive EU Lyme IgG Western Blot result only indicates probable immunologic exposure, however, the band intensity or identity has not been correlated with active infection.

J) When testing specimens from patients during early Lyme disease, IgM tests are generally sensitive within the first 2 months after onset of symptoms. A suitable IgG Western Blot test can be used at any time after onset but is most sensitive during the later stages of the disease.

K) Studies have demonstrated that antibiotic therapy may or may not affect the seroconversion from IgM to IgG during the course of the disease.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

An evaluation of 115 well characterized clinically defined Lyme patients was performed in Europe.

The results are as follows:

Sensitivity:

Disease State	Number	IgG Positive	Percent %
Erythema Migrans	33	30	90.9%
Early Disseminated	34	32	94.1%
Neuroborreliosis	19	18	94.7%
Arthritis /Acrodermatitis	15	14	93.3%

Specificity:

	Number	Negative	Percent %
Non-Lyme / Normals	177	175	98.9%

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Consult Instructions for Use

REF — Product Number

LOT — Lot Number

IVD — *In Vitro* Diagnostic Medical

Use By

Caution, consult accompanying documents

Temperature limitation

Manufacturer

WARNING

CONTROL - Negative Control

CONTROL + Positive Control

CONTROL WR — Weakly Reactive Control

CONJ — Conjugate

SUBS — WB Color Developer

DIL 10X — 10X Sample Diluent/Wash Solution

DIL PDR — Sample Diluent/Wash Powder

STRPS — Western Blot Strips

EC REP Trinity Biotech plc.
IDA Business Park
Bray, County Wicklow, Ireland
Phone: +353-1-276-9800
FAX: +353-1-276-9888
Web: www.trinitybiotech.com

Manufacturer
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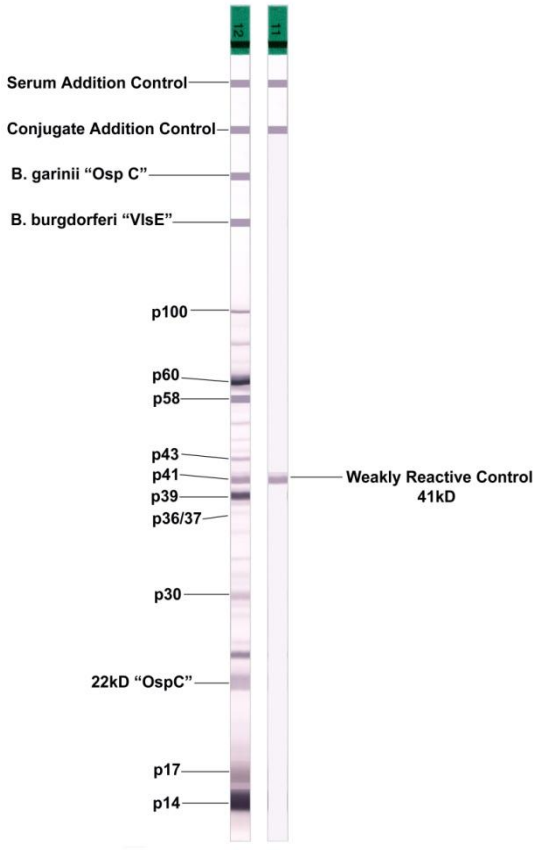
Phone: 800-375-3424
FAX: 760-929-0124

44-2020GV-29-Rev. 8
10/2018

EU Lyme + VlsE IgG Western Blot Interpretive Criteria



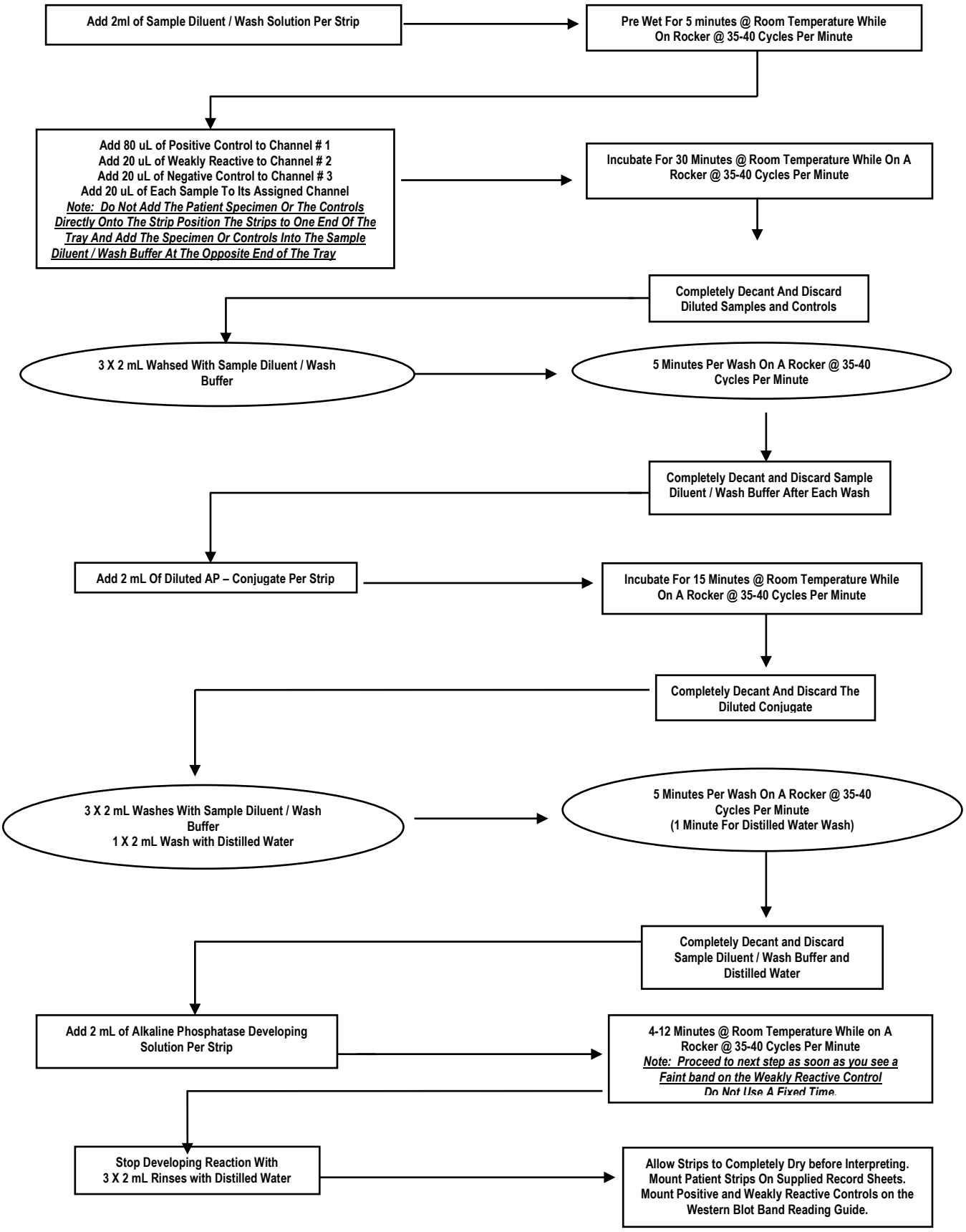
SAC= Serum Addition Control
CAC= Conjugate Addition Control
WRC = Weakly Reactive Control

Modified MiQ 12 2000 interpretive Criteria for Europe excluding FDR Germany
IgG positive = = Any 3 of the following bands : p14, p17, 22kD OspC, p30, p39, p43, p58, p100, *B. garinii* OspC,*B. burgdorferi* VlsE.
IgG Indeterminate = Any 2 of the following bands: p14, p17, 22kD OspC, p30, p39, p43, p58, p100, *B. garinii* OspC, *B. burgdorferi* VlsE.
IgG Negative = < 2 of the following bands: p14, p17, 22kD OspC, p30, p39, p43, p58, p100, *B. garinii* OspC, *B. burgdorferi* VlsE.

Modified MiQ 12 2000 interpretive Criteria for FDR Germany
IgG positive = = Any 2 of the following bands : p14, p17, 22kD OspC, p30, p39, p43, p58, p100, *B. garinii* OspC,*B. burgdorferi* VlsE.
IgG Indeterminate = Any 1 of the following bands: p14, p17, 22kD OspC, p30, p39, p43, p58, p100, *B. garinii* OspC, *B. burgdorferi* VlsE.
IgG Negative = Zero (0) of the following bands: p14, p17, 22kD OspC, p30, p39, p43, p58, p100, *B. garinii* OspC, *B. burgdorferi* VlsE.

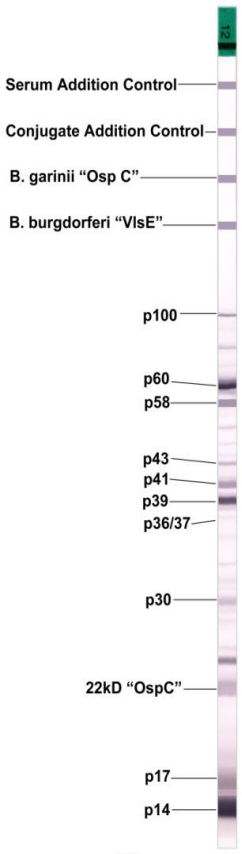
.

EU Lyme Abbreviated Procedure



Western Blot Test System
Troubleshooting Guide

Problem (Error)	Possible Cause	Solution
No visible Weakly Reactive Control band	✕Did not add Weakly Reactive Control. ✕Did not add conjugate. ✕Added incorrect dilution of conjugate. Conjugate too dilute. ✕Stopped assay before Weakly Reactive Control was visible. ✕Added incorrect conjugate.	✕Repeat assay. Make sure to add correct amounts of controls and patient sera. ✕Repeat assay. Make sure to add conjugate. ✕Repeat assay. Add conjugate at 1:10 dilution. ✕Repeat assay. Stop reaction when Weakly Reactive Control is just visible. ✕Repeat assay. Add correct conjugate.
Weakly Reactive Control band seen within 1 minute of adding substrate	✕Added more than 20 uL of Weakly Reactive Control. ✕Added incorrect dilution of conjugate. ✕Added less than 2 mL of wash at serum incubation stage. ✕Weakly reactive may be contaminated.	✕Repeat assay. Add 20 uL of weakly reactive control for a dilution of 1:100 per 2 mL of wash. ✕Repeat assay. Add conjugate at correct dilution of 1:10. ✕Repeat assay. Add correct amount of diluent/wash solution. 2 mL l is required per strip. ✕Repeat assay using a new vial of Weakly Reactive Control. Make sure to change pipette tip.
Missing bands on the Positive Control strip	✕Added incorrect conjugate. ✕Added less than 80 uL of Positive Control. ✕Added incorrect dilution of conjugate.	✕Repeat assay. Add IgG conjugate to IgG assay and IgM conjugate to IgM assay. ✕Repeat assay. Add 80 uL of Positive Control for a dilution of 1:25 per 2 mL of wash. ✕Repeat assay. Add conjugate at correct dilution of 1:10.
Missing Serum Control band	✕Did not add serum to incubation tray.	✕Repeat assay. Add serum at correct dilution of 1:100.
Missing Conjugate Control band	✕Did not add conjugate to incubation tray.	✕Repeat assay. Add conjugate at correct dilution of 1:10.
False positive test results	✕Assay allowed to incubate too long in serum, conjugate or substrate stage. ✕Added more than 20 uL of patient sera.	✕Repeat assay. Serum incubation stage should be 30 minutes. Conjugate incubation stage should be 15 minutes. Substrate 4-12 minutes. Stop reaction as soon as weakly reactive band is visible. ✕Repeat assay. Add correct amount of sera. 20 uL per 2 mL of diluent/wash for a dilution of 1:100.
Dark purple background on strips	✕ Omitted washing step. ✕Omitted washing with water before substrate addition. ✕Incomplete washing of strips. ✕Could be specimen related if only observed on some strips. (plasma may cause blotchy, purple strips)	✕Repeat assay. Wash 3 times with 2 mL ml of Diluent/Wash for 5 minutes after serum and conjugate steps. ✕Repeat assay. Make sure to wash with water (2 mL for 1 minute) before substrate addition to remove all wash solution. ✕Repeat assay. Correct rocker angle and/or speed setting. Angle=7° ; rocker speed = 35-40 cycles per min. ✕Repeat assay. If problem persists it may be specimen related. Use fresh serum if possible, mix well. Avoid using plasma.
Smeared bands	✕Incorrect rocker speed setting and/or angle. ✕Using incorrect type of rocker.	✕Repeat assay. If problem persists, rocker speed and/or angle may be incorrect. Angle should be 7°. Speed should be between 35-40 cycles per min. ✕Repeat assay. Rocker should be a platform rocker.

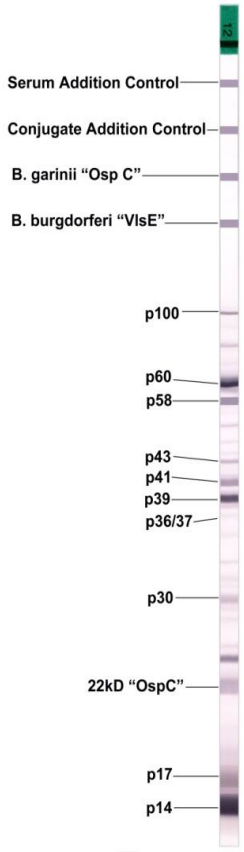


1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

N. strip# ****Interpretation / Result**

*č. proužku	Č. vzorku	SAC	CAC	*B. Garinii* Osp C	B. b. VlsE	p100	P58	P43	p41	p39	p30	22kD Osp C	p17	p14	**Interpretace / Výsledek

SAC= Serum Addition Control
CAC= Conjugate Addition Control



21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40

N. strip# ****Interpretation / Result**

*č. proužku	Č. vzorku	SAC	CAC	*B. Garinii* Osp C	B. b. VlsE	p100	P58	P43	p41	p39	p30	22kD Osp C	p17	p14	**Interpretace / Výsledek

SAC= Serum Addition Control
CAC= Conjugate Addition Control