YALE-NEW HAVEN HOSPITAL	**TITLE:** Lyme IgG & IgM Line Blot: **Viramed Biotech AG – Borrelia B31 IgG and IgM ViraStripe®** **SOFT code: LYWB**		**DEPT OF LAB MEDICINE Policy and Procedure Manual** **DOCUMENT # IMM 205** **Page 1 of 14**
WRITTEN BY: Voula J. Kalmanidis Penny Smith	**EFFECTIVE DATE:** March 7, 2013	**REVISION:** NEW	**SUPERCEDES:** IMM168 Lyme Western Blot IgG & IgM MarDX

I. Intended Use

A line blot serologic assay for the detection of IgG and IgM-specific antibodies against Borrelia burgdorferi in human serum. *The Borrelia B31 IgG and IgM ViraStripe® use the strain Borrelia burgdorferi sensu stricto (American strain Borrelia burgdorferi B31, low passage, tick isolate). Each membrane strip has an integrated control system with controls for function and conjugate specificity.*

The Viramed Biotech AG Borrelia B31 IgG and IgM ViraStripe® are in vitro qualitative assays for the detection of IgG and IgM antibodies to *Borrelia burgdorferi* in human serum. These kits are intended for use in the testing of human serum samples which have been found positive or equivocal using an EIA or IFA test procedure for *B. burgdorferi* antibodies. Positive results from this line blot assay are supportive evidence of infection with *B. burgdorferi*, the causative agent for Lyme disease. The Viramed Biotech AG Borrelia B31 IgG ViraStripe® can be used anytime after onset provided the EIA or IFA are positive or equivocal.

The Viramed Biotech AG Borrelia B31 IgM ViraStripe® can be used any time after onset provided the EIA or IFA are positive or equivocal. The Viramed Biotech AG Borrelia B31 IgM ViraStripe® can be used during the acute phase (0-4 weeks of symptoms onset) of *B. burgdorferi* infection. After this early period, infected patients are usually found to be Western blot positive for IgG. A positive IgM test alone is not recommended for use in determining active disease in persons with illness of longer than one month.

II. Introduction

Borrelia burgdorferi is a spirochete that causes Lyme disease. The organism is transmitted by ticks of the genus *Ixodes*. In endemic areas, these ticks are commonly found on vegetation and animals such as deer, mice, dogs, horses, and birds.

B. burgdorferi infection shares features with other spirochetal infections (diseases caused by three genera in humans: *Treponema, Borrelia,* and *Leptospira*). Skin is the portal of entry for *B.burgdorferi* and the tick bite often causes a characteristic rash called *erythema migrans* (EM) developed around the tick bite in 60% to 80% of patients. Spirochetemia occurs early with wide spread dissemination through tissue and body fluids. Lyme disease occurs in stages, often with intervening latent periods and with different clinical manifestations.

At Yale-New Haven Hospital (YNHH) requests for Lyme testing will first be screened with the Immunetics®C6 *B. burgdorferi* (Lyme) ELISA™ Kit. All positive or equivocal ELISA results will be confirmed by IgG and IgM Line Blot testing using the Viramed Biotech AG – Borrelia B31 IgG ViraStripe® . Line Blot testing without prior ELISA screening will only be performed for another hospital that is doing their EIA in-house. If a doctor insists on having a western blot done on an EIA negative sample, after attempts at education by the attending, the line blot will be performed but an interpretive comment will be added indicating that this is contrary to guidelines and likely a false positive.

III. Principle of the assay

The Viramed Biotech AG Borrelia B31 IgG and IgM ViraStripe® are considered line blot assays. A line blot can be considered as a modified solid-phase enzyme linked immunosorbent assay. Isolated antigens are bound to a solid phase nitrocellulose support membrane. In vitro cultures of *Borrelia burgdorferi B31* spirochetes were harvested, concentrated, washed, and extracted to produce antigen fractions. Applying biotechnological purification methods purified antigens with the following molecular weights could be obtained, for IgG: 93kD, 66kD, 58kD, 45kD, 41kD, 39kD, 30kD, 28kD, 23kD, 18kD and for IgM: 41kD, 39 kD, 23kD. The purified antigens were immobilized as individual bands (lines) onto the nitrocellulose membrane. Positions of the lines are exactly defined and can be assigned to the antigen bands reliably. Function and conjugate controls are also applied to the membrane, the membrane is labeled and cut into individual line blot assay strips.

For each test to be performed, the line blot strip and diluted test serum is added to a *line blot strip well*. If specific antibodies that recognize an antigen are present, they will bind to the specific antigens on the strip. After incubation the line blot strip is washed to remove unbound antibodies.

Alkaline-phosphatase anti-human conjugate is then added to each strip and incubated. If antibody is present, the conjugate will bind to the antibody attached to the specific antigens. The strip is washed to remove unbound conjugate and the substrate solution is added. If the enzyme/antibody complex is present, the substrate will undergo a precipitation and color change. After an incubation period, the reaction is stopped and the presence of precipitated substrate is visualized at specific locations on the strip. The presence of a colored precipitation at various locations on the line blot strip is an indirect measurement of *Borrelia burgdorferi* specific antibodies in the patient specimen. Every strip has an integrated control system including function control and conjugate control. Visualized bands from the reaction are compared for intensity with a separate strip containing the Cut-off control band for evaluation. Any band found having a visual intensity equal to or greater than that of the Cut-off control band intensity is considered as a significant band.

IV. Materials Provided in Kit

Viramed Biotech AG – Borrelia B31 IgG ViraStripe®
IgG Kit 1 x 50 tests: Product-No.: V-BBSGUS

Specimen: 20 µL serum

Time for testing: 90 minutes
Storage/Stability: 12 months at 2-8 °C

Materials Provided:

1 x 50	**Membrane-Strips,** strips of nitrocellulose, striped with purified antigens *of Borrelia burgdorferi* strips *sensu stricto* B31 and with bands for assay function and conjugate specificity; ready to use. **(Product No.: V-BSGAS)**
1 x 0.33 mL	Borrelia burgdorferi B31 ViraStripe® **IgG Positive Control**, human serum, ready to use. **(Product No.: V-BBSGPK)**
1 x 0.33 mL	Borrelia burgdorferi B31 ViraStripe® **IgG Cut-off-Control**, human serum, ready to use. **(Product No.: V-BBSGCO)**
1 x 0.33 mL	Borrelia burgdorferi ViraStripe® **Negative Control**, human serum, ready to use. **(Product No.: V-BSSPNK)**
1 x 9.0 mL	**AP- Anti-Human IgG Conjugate** concentrate. **(Product No.: V-VNGKI)**
1 x 100 mL	**Sample Diluent / Wash Buffer** 10x concentrate. **(Product No.: V-UVNUWP)**
1 packet	**Sample Diluent / Wash Powder** (5g). **(Product No.: V-UVNUMP)**
1 x 90 mL	**Chromogen-/Substrate Solution,** ready to use. **(Product No.: V-UVNUCS)**
1 ea	**Instructions for Use** Viramed Biotech AG Borrelia B31 IgG ViraStripe® test kit
2 ea	**Evaluation protocol** for Viramed Biotech AG Borrelia B31 IgG ViraStripe®

Viramed Biotech AG – Borrelia B31 IgM ViraStripe®
IgM Kit 1 x 50 tests: Product-No.: V-BBSMUS

Specimen: 20 µL serum
Time for testing: 90 minutes
Storage/Stability: 12 months at 2-8 °C

Materials Provided:

1 x 50	**Borrelia B31 ViraStripe® Antigen Strips (IgM),** strips of nitrocellulose, striped with purified antigens *of Borrelia burgdorferi sensu stricto* B31 and with bands for assay function and conjugate specificity; ready to use. **(Product No.: BBSMAS)**
1 x 0.33 mL	**Borrelia B31 ViraStripe® IgM Positive Control**, human serum, ready to use. **(Product No.: V-BBSMPK)**
1 x 0.33 mL	**Borrelia B31 ViraStripe® IgM Cut off Control**, human serum, ready to use. **(Product No.: V-BBSMCO)**

1 x 0.33 mL	**Borrelia ViraStripe® IgG,A,M Negative Control**, human serum, ready to use. **(Product No.: V-BSSPNK)**
1 x 4.5 mL	**AP- Anti-Human IgM Conjugate** concentrate. **(Product No.: V-UVNMKI45) to be used as 20x concentrate.**
1 x 100 mL	**Diluent / Wash Buffer** 10x concentrate. **(Product No.: V-UVNUWP)**
1 packet	**Diluent / Wash Powder** (5g). **(Product No.: V-UVNUMP)**
1 x 90 mL	**Chromogen /Substrate Solution,** ready to use. **(Product No.: V-UVNUCS)**
1 ea	**Instructions for Use** Viramed Biotech AG Borrelia B31 IgM ViraStripe® test kit
2 ea	**Evaluation protocol** for Viramed Biotech AG Borrelia B31 IgG ViraStripe®

V. Additional Materials Required But Not Provided.

1. Clinical Laboratory Reagent Water (CLRW)
2. MT07800 20-strip tray, black (25 per bag) $75.00 MedTec, Inc.
3. Blunt tipped forceps
4. 1-20 µL Pipetman pipette with disposable tips
5. 1- 200 µL Pipetman pipette with disposable tips
6. Graduated serological pipette capable of delivering 10.0 mL.
7. Graduated serological pipette capable of delivering 5.0 mL
8. 1L Erlenmyer flask
9. 250 mL graduated cylinder
10. 50 mL disposable conical tubes

VI. Specimen Collection

The test is performed on serum only (from red top tube). Separate serum by centrifugation. Serum samples may be stored refrigerated (2-8°C) up to 5 days. If the sample cannot be tested in this period, store frozen at -20°C for up to 3 months. Repeat freezing and thawing should be avoided.

Do not perform the test on grossly hemolyzed or lipemic serum. Lipemic serum should be spun at 10,000 rpm for 20 minutes to remove contaminating lipids.

Standard Aliquot volume = 200 µL
Minimum Aliquot volume = 100 µL
Grossly Hemolyzed: Reject
Grossly Lipemic: Spin at 10,000 rpm for 20 min.

Grossly Icteric: Reject
Stability: 5 days refrigerated, 3 months at frozen (-20°C)

VII. Reagent Preparation and Storage

1. Store kits at 2-8°C. The unopened test kit is usable until date of expiration.
2. Antigen-Strips: Strips in closed bags are stable until expiration date if stored at 2 - 8°C. Close bags with unused strips tightly.
3. IgG Conjugate, **10x concentrate**: stable until expiration date if stored at 2-8°C. *Working dilution:* To be used in a single use.
4. IgM Conjugate, **20x concentrate**: Stable until expiration date if stored at 2-8°C. *Working dilution:* To be used in a single use.
5. Diluent/Wash Buffer: concentrate and powder: Stable until expiration date if stored at 2 - 8°C.
6. Buffer working dilution: 2 weeks usable if stored at 2 - 8°C. The buffer working dilution can be stored for 60 days in frozen aliquots.
7. Chromogen/Substrate Solution: Stable until expiration date if stored at 2 - 8°C.

VIII. Test Procedure

1. Follow the LIS steps below before setting up a run, refer to Soft Immunology Manual (Imm 120) for additional LIS information.
   a. Call a pending list in SoftLab by Tests LYWB and LYME.
   b. The result for LYWB is automatically entered (not verified) as "Sent for Interp". Verifying this result will send the order to SoftFlow. **Verify using Resulting Worklist or Batch Entry.**
   c. Investigate/address any ordering issues based on the Lyme ELISA result. For example, a Western Blot requested on a negative Lyme ELISA or the absence of an ELISA result Review results and make sure only POSITIVE EIAs get Western Blot. If WB is ordered without an EIA:
      i. All samples will be tested by C6 peptide EIA, with reflex to line blots if EIA is positive or indeterminate.
      ii. The lab will <u>not do line (Western) blot only</u>, unless it is for another

hospital that is doing their EIA in-house.

iii. If a doctor insists on having a blot done on an EIA negative sample, contact the Immunology Laboratory Resident to discuss the case with the ordering physician;

iv. If a Lyme blot (LYWB)only is ordered in error, do not cancel. Add the Lyme EIA and only when the EIA has been resulted, should the LYWB be canceled.

d. In SoftFlow, receive the samples by going to Specimen Receiving Worklist. Print a Flow label for each patient.

e. Refer to Procedure AutoBlot 2000 / ViraCam Operation, IMM204 for creating a worklist, Test Procedure and Reading of Strips. Scan the Flow labels to generate a tasklist . Use the worklist created in the Prepare Module of ViraCam as a tasklist for setting up the assay. Use the final report generated in the Evaluate Module of ViraCam as the results provided to the resident/attending. Affix the SoftFlow labels to this worksheet. Indicate the actual strip number and the Lyme ELISA result for each specimen on the final report.

f. Call a pending list in SoftLab by Tests LGWBR and LMWBR to identify any older interpretations that may still be pending. Follow up with resident/attending as needed to result these tests.

2. Bring all components to room temperature (20-23°C) prior to use.
3. Antigen-strips: Carefully separate the required number of strips by use of forceps. Touch the strips with forceps only at the label.
4. Diluent/Wash Buffer working dilution: To prepare buffer working dilution, dilute the **Diluent / Wash Buffer** 10x concentrate with distilled water (100 mL concentrate + 900 mL CLRW) and add the **Diluent /Wash Powder** completely and mix until dissolved. Place the buffer working dilution for 10 to 15 minutes on a magnetic stir plate.
5. Conjugate working dilution: Dilute the needed amount of **10-fold concentrate** according to Table 1 for IgG prior to the first washing step Dilute the needed amount of 20-fold concentrate according to Table 2 for IgM.
6. Chromogen /Substrate Solution: Ready to use.
7. Controls: Use 100 µL each of positive, Cutoff, and negative control undiluted per test run. Patient samples: Use 20 µL patient serum undiluted per test.

Table 1: Dilution of IgG Conjugate Working Dilution (10 fold dilution)

Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate	Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate
1	1.35 ml	+	0.15 ml	1.5 ml	26	35.10 ml	+	3.90 ml	39.0 ml
2	2.70 ml	+	0.30 ml	3.0 ml	27	36.45 ml	+	4.05 ml	40.5 ml
3	4.05 ml	+	0.45 ml	4.5 ml	28	37.80 ml	+	4.20 ml	42.0 ml
4	5.40 ml	+	0.60 ml	6.0 ml	29	39.15 ml	+	4.35 ml	43.5 ml
5	6.75 ml	+	0.75 ml	7.5 ml	30	40.50 ml	+	4.50 ml	45.0 ml
6	8.10 ml	+	0.90 ml	9.0 ml	31	41.85 ml	+	4.65 ml	46.5 ml
7	9.45 ml	+	1.05 ml	10.5 ml	32	43.20 ml	+	4.80 ml	48.0 ml
8	10.80 ml	+	1.20 ml	12.0 ml	33	44.55 ml	+	4.95 ml	49.5 ml
9	12.15 ml	+	1.35 ml	13.5 ml	34	45.90 ml	+	5.10 ml	51.0 ml
10	13.50 ml	+	1.50 ml	15.0 ml	35	47.25 ml	+	5.25 ml	52.5 ml
11	14.85 ml	+	1.65 ml	16.5 ml	36	48.60 ml	+	5.40 ml	54.0 ml
12	16.20 ml	+	1.80 ml	18.0 ml	37	49.95 ml	+	5.55 ml	55.5 ml
13	17.55 ml	+	1.95 ml	19.5 ml	38	51.30 ml	+	5.70 ml	57.0 ml
14	18.90 ml	+	2.10 ml	21.0 ml	39	52.65 ml	+	5.85 ml	58.5 ml
15	20.25 ml	+	2.25 ml	22.5 ml	40	54.00 ml	+	6.00 ml	60.0 ml
16	21.60 ml	+	2.40 ml	24.0 ml	41	55.35 ml	+	6.15 ml	61.5 ml
17	22.95 ml	+	2.55 ml	25.5 ml	42	56.70 ml	+	6.30 ml	63.0 ml
18	24.30 ml	+	2.70 ml	27.0 ml	43	58.05 ml	+	6.45 ml	64.5 ml
19	25.65 ml	+	2.85 ml	28.5 ml	44	59.40 ml	+	6.60 ml	66.0 ml
20	27.00 ml	+	3.00 ml	30.0 ml	45	60.75 ml	+	6.75 ml	67.5 ml
21	28.35 ml	+	3.15 ml	31.5 ml	46	62.10 ml	+	6.90 ml	69.0 ml
22	29.70 ml	+	3.30 ml	33.0 ml	47	63.45 ml	+	7.05 ml	70.5 ml
23	31.05 ml	+	3.45 ml	34.5 ml	48	64.80 ml	+	7.20 ml	72.0 ml
24	32.40 ml	+	3.60 ml	36.0 ml	49	66.15 ml	+	7.35 ml	73.5 ml
25	33.75 ml	+	3.75 ml	37.5 ml	50	67.50 ml	+	7.50 ml	75.0 ml

Table 2: Dilution of IgM Conjugate Working Dilution (20fold dilution)

Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate	Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate
1	1.425 ml	+	0.075 ml	1.5 ml	26	37.050 ml	+	1.950 ml	39.0 ml
2	2.850 ml	+	0.150 ml	3.0 ml	27	38.475 ml	+	2.025 ml	40.5 ml
3	4.275 ml	+	0.225 ml	4.5 ml	28	39.900 ml	+	2.100 ml	42.0 ml
4	5.700 ml	+	0.300 ml	6.0 ml	29	41.325 ml	+	2.175 ml	43.5 ml
5	7.125 ml	+	0.375 ml	7.5 ml	30	42.750 ml	+	2.250 ml	45.0 ml
6	8.550 ml	+	0.450 ml	9.0 ml	31	44.175 ml	+	2.325 ml	46.5 ml
7	9.975 ml	+	0.525 ml	10.5 ml	32	45.600 ml	+	2.400 ml	48.0 ml
8	11.400 ml	+	0.600 ml	12.0 ml	33	47.025 ml	+	2.475 ml	49.5 ml
9	12.825 ml	+	0.675 ml	13.5 ml	34	48.450 ml	+	2.550 ml	51.0 ml
10	14.250 ml	+	0.750 ml	15.0 ml	35	49.875 ml	+	2.625 ml	52.5 ml
11	15.675 ml	+	0.825 ml	16.5 ml	36	51.300 ml	+	2.700 ml	54.0 ml
12	17.100 ml	+	0.900 ml	18.0 ml	37	52.725 ml	+	2.775 ml	55.5 ml
13	18.525 ml	+	0.975 ml	19.5 ml	38	54.150 ml	+	2.850 ml	57.0 ml
14	19.950 ml	+	1.050 ml	21.0 ml	39	55.575 ml	+	2.925 ml	58.5 ml
15	21.375 ml	+	1.125 ml	22.5 ml	40	57.000 ml	+	3.000 ml	60.0 ml
16	22.800 ml	+	1.200 ml	24.0 ml	41	58.425 ml	+	3.075 ml	61.5 ml
17	24.225 ml	+	1.275 ml	25.5 ml	42	59.850 ml	+	3.150 ml	63.0 ml
18	25.650 ml	+	1.350 ml	27.0 ml	43	61.275 ml	+	3.225 ml	64.5 ml
19	27.075 ml	+	1.425 ml	28.5 ml	44	62.700 ml	+	3.300 ml	66.0 ml
20	28.500 ml	+	1.500 ml	30.0 ml	45	64.125 ml	+	3.375 ml	67.5 ml
21	29.925 ml	+	1.575 ml	31.5 ml	46	65.550 ml	+	3.450 ml	69.0 ml
22	31.350 ml	+	1.650 ml	33.0 ml	47	66.975 ml	+	3.525 ml	70.5 ml
23	32.775 ml	+	1.725 ml	34.5 ml	48	68.400 ml	+	3.600 ml	72.0 ml
24	34.200 ml	+	1.800 ml	36.0 ml	49	69.825 ml	+	3.675 ml	73.5 ml
25	35.625 ml	+	1.875 ml	37.5 ml	50	71.250 ml	+	3.750 ml	75.0 ml

IX. Quality Control

1. **Validity of the Test: Each individual test strip is valid when:** The bands for the function control and the conjugate control **are clearly visible for that strip.** The function control indicates addition of serum and all necessary reagents to the test strip. The conjugate control indicates addition of the specific conjugate. If a single test strip or strips are not valid, that individual test(s) must be repeated under exact observance of the working instructions. All valid test strips for the test run may be interpreted for results. Do not assess invalid test strips.
2. **Controls:** For the results of the assay to be considered valid, the following conditions must be met:
   Negative Control: Interpretation of the Negative Control strip must be negative.
   Cut-off Control: The **OspC** band must be clearly visible on the Cut-off control strip.
   Positive Control: Interpretation of the Positive Control strip must be positive.

X. Interpretation of Results

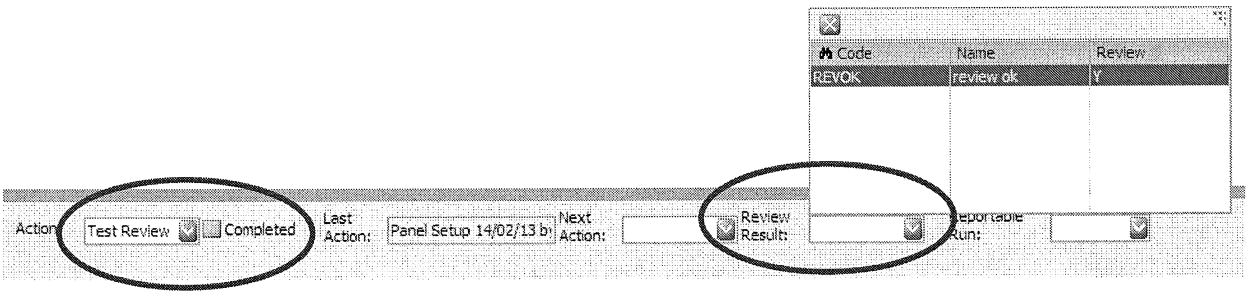
Interpretation of results:

1. **Do Not** read the results when the strips are wet. Read the results **when dry** within the same working day.
2. Results will be read using the ViraCam and ViraScan software. See Procedure IMM204.

Result	**Bands (kD)**	**Interpretation**
IgG Positive	At least five significant (+) bands from: 93, 66, 58, 45, 41, 39, 30, 28, 23, 18.	IgG-antibodies against *Borrelia species* detectable. Presumptive evidence of *B. burgdorferi* infection.
IgG Negative	No bands or less than five significant bands.	No IgG-antibodies against *Borrelia species* detectable. In case of a clinically based suspicion of an infection with *Borrelia*: check additionally for IgM-antibodies and possibly check a second sample for IgG- and IgMantibodies after 2-3 weeks.
IgM Positive	At least two significant (+) bands from: 41, 39, 23	IgM-antibodies against *Borrelia species* detectable. Presumptive evidence of *B.burgdorferi* infection.
IgM Negative	No bands or less than two significant. Bands	No IgM-antibodies against *Borrelia species* detectable. In case of a clinically based suspicion of an infection with *Borrelia*: check additionally for IgG-antibodies and possibly check a second sample for IgG- and IgMantibodies after 2-3 weeks.

XI. Reporting of Results

2. In Flow, click on Result
3. Test Review Entry
4. In Test Review Entry, enter the Flow #
5. For both IgG and IgM, select the Action: Test Review; Click the Completed Box
6. Select the Review Result: REVOK
7. Then Click Save.
8. Place the Worksheet in the Attending Box in the read out room.
9. Page the Microbiology Resident that Lyme Western Blot results are ready.



XII. Limitations of Procedure

1. Serum from normal individuals or patients with other spirochetal infections may have cross-reactive antibodies present. Cross-reactions with antigens of *Borrelia* are described in infections with *Treponema*, *Leptospira* and other bacteria. Cross-reactions are also described in cases of autoimmune diseases, MS, ALS, and Influenza.
2. Potential cross-reactivity due to circulating antibodies from infections with *Treponema phagedenis, Neisseria meningitidis, Haemophilus influenza, Yersinia enterocolitica, Campylobacter jejuni, Listeria monocytogenes, Pseudomonas aeruginosa, E. coli, Salmonella enterica* serovar *typhimurium, Shigella flexneri*, and *Legionella micdadei* have not been challenged, therefore the performance of this device is unknown if the specimen contains any of these circulating antibodies.
3. Grossly hemolyzed, lipemic, or icteric sera should not be used for testing in addition sera with elevated bilirubin, and triglycerides were not tested. Do not use heat-inactivated sera.
4. An early antibiotic therapy can suppress the development of antibodies.
5. The detection of specific antibodies for *Borrelia burgdorferi* in any given specimen can vary with assays from different manufacturers due to reagent specificity, assay methodology.
6. The Viramed Biotech AG Borrelia B31 IgG & IgM ViraStripe®kits are intended to be an aid to diagnosis only. These assays are to be performed on samples that are found to be positive or equivocal in an EIA or IFA test. Results must be used in conjunction with symptoms, patient's history, and other clinical findings.

7. These tests are not intended for the determination of immune status but are only for the detection of IgG and IgM antibodies to *Borrelia burgdorferi* B31 antigens.

XIII. Expected Values

1. IgM antibodies usually appear 2-3 weeks after beginning of the disease. The antibody titers often decrease some weeks to months after convalescence. It is also possible that antibody titers remain constant up to some years.

2. IgG-antibodies appear some weeks to months after an infection. In the early stage of the infection they often are not yet detectable. IgM should be checked in case of a suspected recent infection. In this case a second sample should be checked some time later. Patients in the 2nd or 3rd stage of the disease are usually positive for IgG-antibodies. The antibody-titers steadily decrease in convalescence.

3. The immune response and therefore the band pattern differ from patient to patient. A general rule is the increase of antibody types and the amount of specific bands with the progression of the disease.

4. An early antibiotic therapy can suppress the development of antibodies.

5. The incidence of IgG antibodies to *B. burgdorferi* antigenic proteins used in the Borrelia IgG ViraStripe® are shown in Table 3. The incident of IgM antibodies found to various *B. burgdorferi* antigenic proteins with the Viramed Biotech B31 IgM ViraStripe® are shown in Table 3. The 41kD flagellar protein is most often seen in both Lyme Borreliosis and blood donor populations. The incident of specific bands 39kD and 23kD increases in later stages of Lyme Borreliosis but is infrequent in the blood donor populations.

6. (IgG): Specimens from potential cross-reactive diseases are frequently found to have a band at the 41kD flagellar protein. At a much lower frequency the 66kD Hsp (heat shock protein) can be seen. (IgM): Specimens from potential cross-reactive diseases are frequently found to have a band at the 41kD flagellar protein. Disease sera from patients diagnosed with Ehrlichia, Babesia can have Borrelia specific bands from co-infection with Borrelia burgdorferi.

Table 3: Expected Values
IgG:

Bands in kD	93	66	58	45	41	39	30	28	23	18
Early Lyme Disease	9%	28%	17%	45%	67%	52%	5%	13%	62%	62%
Late Lyme Disease	85%	90%	73%	85%	88%	93%	80%	90%	80%	95%
Non-Endemic Blood Donors	1%	1%	2%	3%	12%	1%	0%	1%	4%	8%
Endemic Blood Donors	2%	0%	2%	0%	9%	0%	0%	2%	3%	12%

IgM:

Bands in kD	41	39	23
Early Lyme Disease	57%	17%	57%
Late Lyme Disease	38%	31%	56%
Non-Endemic Blood Donors	9%	1%	1%
Endemic Blood Donors	16%	0%	0%

See respective kits inserts for complete data

XIII. Validation

Correlation:

Correlation was performed by comparison with the current method, MarDx B. burgdorferi Strip Test System* and showed a 92.2% agreement for IgG and 84.3% for IgM.

IgG:
# of samples: 51
Agreement: 92.2 %
Positive Agreement: 81.8%
Negative Agreement: 95.0%

IgM:
# of samples: 51
Agreement: 84.3 %
Positive Agreement: 75.0%
Negative Agreement: 87.2%

*Ten of the 51 samples tested were from a serum Validation Panel for Lyme Disease provided by the CDC and showed 100% agreement with the CDC results for both IgG and IgM. The 5 Lyme disease controls are from physician-diagnosed patients with various stages (1, 2 and 3) of disease. The healthy controls are from individuals residing in Lyme disease endemic or non-endemic regions who have no prior history of physician-diagnosed Lyme disease.

Discrepant analysis:

Discrepant samples (n=11) were repeated. After repeat, the correlations were unchanged: 7/50 IgMs and 4/50 IgGs were discrepant.

Chart review was conducted, but unfortunately 6/11 samples were from draw stations with no clinical information provided. Five of 11 had charts available for review.

IgM Discrepants (n=7):
ViraMed had two false positive IgMs with primary EBV and CMV.
MarDx had one false positive IgM with psoriatic arthritis.
No charts were available for review for 4 samples.

IgG Discrepants (n=4):
Viramed had one false positive IgG with primary CMV (same patient as above)

Viramed was delayed in one true positive IgG compared to MarDx, but this had no clinical impact as the sample was IgM positive.
No charts were available for 2 samples.

The interpretive comment was revised to highlight the problem with false positive IgM results (see below).

EIA POSITIVE, IgM W. blot POSITIVE, IgG W. blot NEGATIVE

For symptoms present less than 30 days: A positive EIA with a positive IgM western blot during the first 4 weeks of disease symptoms supports a diagnosis of early Lyme disease. However, false positive Lyme serology results can be seen with many infections, including EBV, CMV, other spirochetal diseases, and endocarditis, and with autoimmune disease.

For symptoms present for more than 30 days: If this patient has been ill for over a month, a positive IgM western blot with a negative IgG western blot is most likely a false positive result and NOT indicative of Lyme disease. Serum samples from patients with disseminated or late stage Lyme disease almost always have a strong IgG response.

If Lyme disease is strongly suspected, a second sample collected 2-4 weeks after the first can be tested to document serconversion of the IgG western blot. Serological confirmation of typical erythema chronicum migrans lesions is not necessary.

Precision:

Intrarun Precision: Intra-assay performance was evaluated by testing of 2 specimens, a Negative and a Positive for both IgG and IgM 4 times on a single run. All the results were in agreement.

Interrun Precision: Inter-assay performance was evaluated by testing 2 specimens, a Negative and a Positive for both IgG and IgM, on 4 separate runs. All the results were in agreement.

Reference Range Verification:

# of specimens: 10
Reference Range Verified (Y/N): Y
Comment: The assay reference range of Negative was verified by testing 10 randomly selected specimens representing YNHH's testing population. All 10 samples were Negative for IgG and nine out of ten samples were negative for IgM. This meets YNHH acceptable criteria for normal range validation.

CAP Proficiency Results: Three CAP samples were tested, TTD-4, TTD-5 and TTD-6 from the TTD-B 2012 survey. Results for TTD-4 and TTD-5 correlated to the results for other ViraMed users. For TTD-6, the IgM results correlated. For IgG, 42.9% of ViraMed participants reported positive, 42.9% reported negative while 14.3% reported indeterminate. Our results were negative with four bands present.

Carryover: Carryover studies were performed by running a tray with alternating negative and positive controls (for IgG). No carryover was detected.

XIV. SOFT Test Codes

Test Code	Orderable	Description	IgG or IgM
LYWB	YES	Ordered individually or reflex from Lyme screen	IgG and IgM
LGWBG	NO	Refexed from verifying LYMB	IgG
LMWBG	NO	Refexed from verifying LYMB	IgM
LGWBR	NO	Component of group test LGWBG. Used for result in SoftFlow	IgG
LYGWB	NO	Component of group test LGWBG. Used for interp. in SoftFlow	IgG
LMWBR	NO	Component of group test LMWBG. Used for result in SoftFlow	IgM
LYMWB	NO	Component of group test LMWBG. Used for interp. in SoftFlow	IgM

XVI. Appendix

205-A	Lyme Line Blot Quick Reference Guide
205-B	Lyme Line Blot Quiz
205-Q	Lyme Line Blot QC Chart
204-A	AUTO Blot 2000 & ViraCam Checklist

XVII. References

1. **Borrelia B31 IgM ViraStripe** [package insert]. Viramed Biotech AG, Behringstrasse 11, D-82152 Planegg/Steinkirchen, Germany, July, 2011, REV Q
2. **Borrelia B31 IgG ViraStripe** [package insert]. Viramed Biotech AG, Behringstrasse 11, D-82152 Planegg/Steinkirchen, Germany, February, 2011, REV F

1. **Bring all reagents out to room temperature**
2. **Run SOFT pending list, locate samples and resolve any ordering discrepancies**
3. **Verify "Sent for Interp" for each sample**
4. **Receive all specimens in Soft Flow**
5. **Generate a Flow Label for each Specimen**
6. **Prepare Buffer Working Dilution and Conjugate (use Tables 1 & 2)**

Table 1: Dilution of IgG Conjugate Working Dilution (10 fold dilution)

Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate	Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate
1	1.35 ml	+	0.15 ml	1.5 ml	26	35.10 ml	+	3.90 ml	39.0 ml
2	2.70 ml	+	0.30 ml	3.0 ml	27	36.45 ml	+	4.05 ml	40.5 ml
3	4.05 ml	+	0.45 ml	4.5 ml	28	37.80 ml	+	4.20 ml	42.0 ml
4	5.40 ml	+	0.60 ml	6.0 ml	29	39.15 ml	+	4.35 ml	43.5 ml
5	6.75 ml	+	0.75 ml	7.5 ml	30	40.50 ml	+	4.50 ml	45.0 ml
6	8.10 ml	+	0.90 ml	9.0 ml	31	41.85 ml	+	4.65 ml	46.5 ml
7	9.45 ml	+	1.05 ml	10.5 ml	32	43.20 ml	+	4.80 ml	48.0 ml
8	10.80 ml	+	1.20 ml	12.0 ml	33	44.55 ml	+	4.95 ml	49.5 ml
9	12.15 ml	+	1.35 ml	13.5 ml	34	45.90 ml	+	5.10 ml	51.0 ml
10	13.50 ml	+	1.50 ml	15.0 ml	35	47.25 ml	+	5.25 ml	52.5 ml
11	14.85 ml	+	1.65 ml	16.5 ml	36	48.60 ml	+	5.40 ml	54.0 ml
12	16.20 ml	+	1.80 ml	18.0 ml	37	49.95 ml	+	5.55 ml	55.5 ml
13	17.55 ml	+	1.95 ml	19.5 ml	38	51.30 ml	+	5.70 ml	57.0 ml
14	18.90 ml	+	2.10 ml	21.0 ml	39	52.65 ml	+	5.85 ml	58.5 ml
15	20.25 ml	+	2.25 ml	22.5 ml	40	54.00 ml	+	6.00 ml	60.0 ml
16	21.60 ml	+	2.40 ml	24.0 ml	41	55.35 ml	+	6.15 ml	61.5 ml
17	22.95 ml	+	2.55 ml	25.5 ml	42	56.70 ml	+	6.30 ml	63.0 ml
18	24.30 ml	+	2.70 ml	27.0 ml	43	58.05 ml	+	6.45 ml	64.5 ml
19	25.65 ml	+	2.85 ml	28.5 ml	44	59.40 ml	+	6.60 ml	66.0 ml
20	27.00 ml	+	3.00 ml	30.0 ml	45	60.75 ml	+	6.75 ml	67.5 ml
21	28.35 ml	+	3.15 ml	31.5 ml	46	62.10 ml	+	6.90 ml	69.0 ml
22	29.70 ml	+	3.30 ml	33.0 ml	47	63.45 ml	+	7.05 ml	70.5 ml
23	31.05 ml	+	3.45 ml	34.5 ml	48	64.80 ml	+	7.20 ml	72.0 ml
24	32.40 ml	+	3.60 ml	36.0 ml	49	66.15 ml	+	7.35 ml	73.5 ml
25	33.75 ml	+	3.75 ml	37.5 ml	50	67.50 ml	+	7.50 ml	75.0 ml

Table 2: Dilution of IgM Conjugate Working Dilution (20 fold dilution)

Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate	Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate
1	1.425 ml	+	0.075 ml	1.5 ml	26	37.050 ml	+	1.950 ml	39.0 ml
2	2.850 ml	+	0.150 ml	3.0 ml	27	38.475 ml	+	2.025 ml	40.5 ml
3	4.275 ml	+	0.225 ml	4.5 ml	28	39.900 ml	+	2.100 ml	42.0 ml
4	5.700 ml	+	0.300 ml	6.0 ml	29	41.325 ml	+	2.175 ml	43.5 ml
5	7.125 ml	+	0.375 ml	7.5 ml	30	42.750 ml	+	2.250 ml	45.0 ml
6	8.550 ml	+	0.450 ml	9.0 ml	31	44.175 ml	+	2.325 ml	46.5 ml
7	9.975 ml	+	0.525 ml	10.5 ml	32	45.600 ml	+	2.400 ml	48.0 ml
8	11.400 ml	+	0.600 ml	12.0 ml	33	47.025 ml	+	2.475 ml	49.5 ml
9	12.825 ml	+	0.675 ml	13.5 ml	34	48.450 ml	+	2.550 ml	51.0 ml
10	14.250 ml	+	0.750 ml	15.0 ml	35	49.875 ml	+	2.625 ml	52.5 ml
11	15.675 ml	+	0.825 ml	16.5 ml	36	51.300 ml	+	2.700 ml	54.0 ml
12	17.100 ml	+	0.900 ml	18.0 ml	37	52.725 ml	+	2.775 ml	55.5 ml
13	18.525 ml	+	0.975 ml	19.5 ml	38	54.150 ml	+	2.850 ml	57.0 ml
14	19.950 ml	+	1.050 ml	21.0 ml	39	55.575 ml	+	2.925 ml	58.5 ml
15	21.375 ml	+	1.125 ml	22.5 ml	40	57.000 ml	+	3.000 ml	60.0 ml
16	22.800 ml	+	1.200 ml	24.0 ml	41	58.425 ml	+	3.075 ml	61.5 ml
17	24.225 ml	+	1.275 ml	25.5 ml	42	59.850 ml	+	3.150 ml	63.0 ml
18	25.650 ml	+	1.350 ml	27.0 ml	43	61.275 ml	+	3.225 ml	64.5 ml
19	27.075 ml	+	1.425 ml	28.5 ml	44	62.700 ml	+	3.300 ml	66.0 ml
20	28.500 ml	+	1.500 ml	30.0 ml	45	64.125 ml	+	3.375 ml	67.5 ml
21	29.925 ml	+	1.575 ml	31.5 ml	46	65.550 ml	+	3.450 ml	69.0 ml
22	31.350 ml	+	1.650 ml	33.0 ml	47	66.975 ml	+	3.525 ml	70.5 ml
23	32.775 ml	+	1.725 ml	34.5 ml	48	68.400 ml	+	3.600 ml	72.0 ml
24	34.200 ml	+	1.800 ml	36.0 ml	49	69.825 ml	+	3.675 ml	73.5 ml
25	35.625 ml	+	1.875 ml	37.5 ml	50	71.250 ml	+	3.750 ml	75.0 ml

7. **Turn on ViraCam and AutoBlot 2000**
8. Prepare ViraCam Worklist
    a. From the drop down menu, click on process
    b. Then click on Prepare
    c. Enter the File Name: LGMMDDYYYY or LMMMDDYYYY
    Ex. LG02062013
    (screen indicates Lot No. of Strip but this is used as run number)
    Always start with LG (IgG) or LM (IgM) for the run file name.
    d. Continue
    e. Cancel *.imp (Import from LIS)
    f. Scan label for each specimen (beginning with Negative and Positive QC)
    g. Indicate the position of the Cut-off by clicking in the Cut-off Control-Strip box
    h. Click on the > button
    i. Then click in the Patient ID field to continue entering/scanning Patient ID information
    j. To print a worklist
        i. click on Print
        ii. select desired printer (HP LaserJet Professional P 1102w)
        iii. Click on Print
        iv. Utilize this as Tasklist for pipetting specimens
    k. Click on Prepare Evaluation

9. **AutoBlot 2000:**
    a. Prepare Wash Buffer and add it to the correct bottle.
    b. Prepare the CLRW, Substrate and Conjugate bottles
    c. Attach the Tubing to the bottles and snap the pump pressure pads in place.
    d. Place the tray onto the platform for the test being run. Have the strips ready.
    e. Lock Pump Pressure Pads
    f. Begin Assay

SCREEN DISPLAY	**ACTION**
Ready For A New Test ***Yes or No***	Press YES to begin a test.
Assay Name ***01***	Select the program to be run (**VIRAG** or **VIRAM**) by using the arrow keys (< and >) to scroll through the available programs. Press ENTER to select a program and continue.
Pump Pads in Place? ***Press YES***	Press YES if the pump pressure pads are locked in place.
Prime Pumps? ***YES or NO***	Press YES to enter the PUMP Prime mode. The pumps should be primed just prior to running an assay.
Prime Wash Line? ***Prime DI Pump?*** ***Prime Conjugate Pump?*** ***Prime Substrate Pump?*** ***Prime Stop Pump?***	Press YES at each prompt to prime that particular Pump. Be sure to press YES repeatedly for each Pump until fluid is dispensed into the drip tray. Then press NO to move on to the next pump to be primed. After all lines are primed, the display will return to *Prime Pumps?* Press NO to exit the Prime Pumps Routine.
Strip Count 20	Indicate the number of strips to be read by using the arrow keys to increment through the numbers on the display.

	Press ENTER after selecting the correct number of strips.
Load Tray ***Press ENTER***	Place the tray onto the tray platform. Press ENTER to continue.
Start Assay? ***Press YES***	Press YES at the *Start Assay?* Prompt and the automatic operation begins. If the strip count needs to be reset, press NO at the *StartAssay?* prompt to return to the *Ready For a New Test* screen.
Dispense Soak (Blocking) Solution	If an automatic soak cycle was programmed for the assay, soak (blocking) solution will now be dispensed into the trays. While the soak routine is in progress, prepare the other reagents and samples. After the soak solution has been dispensed, the instrument pauses and the tray platform levels. Now add the strips to the trays. Gently tweeze the strips into the trays, being careful not to bend the strips (this may cause a strip to be picked up by the aspirate arm during the assay run). Count the number of wells needed starting from the far right. Strip number one (1) is located in the trough closest to the aspirate arm in home position (looking at the front of the unit, strip one is farthest to the right). Any empty troughs should be located to the left of the tray.
Prepare Sampl Solution	During this time prepare samples; For Negative QC, Positive QC and Cutoff use 100 µL and for patients use 20 µL; Pipette samples left to right starting with Neg. Pos, Cutoff and patients
Sampl Prepared	PRESS YES (or silence alarm)
Sampl Installed?	YES OR NO; YES begins assay; Pipet samples from right to left beginning With Negative QC, Positive QC and Cut-off; then Pipet Specimens. DO NOT pipette QC/sample directly on the strips.

AUTOBLOT 2000 will incubate, wash, add conjugate and substrate.

The entire assay takes approximately 90 minutes.

Alarm will sound at end of assay.

Remove Tray and lightly tap to remove excess CLRW.

Using an applicator stick, move strips up leaving approximately ½ inch from the end of the well. Allow strips to dry.

When strips are dry, reverse tray so that strips begin at the left hand side of the tray as this is where the ViraCam starts reading.

10. To Scan Strips using ViraCam
    a. From the drop down menu, click on process
    b. Click on Scan
    c. Enter **File Name (LGMMDDYYYY or LMMMDDYYYY)**

d. Select: Well 1 – 29 (default is 1 to 50)
e. Continue
f. A DOS screen will appear indicating that the lamp is warming up (in German); When scanning is complete, the window will disappear.

11. To Evaluate Strips using ViraCam
    a. From the drop down menu, click on process
    b. Click on Evaluate
    c. Enter File ID (will default to last File Name entered)
    d. Click on Continue
    e. Enter Band Locator; *LG or LM* for IgG or IgM, respectively
    f. Enter location of Cut-off if not displayed, this is the actual well number in which the cut-off is located.
    g. Enter Lab Name and examiner (tech)
    h. Click on Continue to evaluate/review strips
    i. Use the >> button to advance to the next sample
    j. Advance one more time after final strip, the print button becomes available
    k. Click on Print
        i. Select desired printer (HP LaserJet Professional P 1102w)
        ii. Click on Preferences
        iii. Click on icon of a "document" to change orientation to landscape
        iv. Click OK
        v. Click Print
    l. DO NOT USE the Evaluate function to review scans that have already been evaluated as clicking on Print or END will overwrite results. Use the Archive function for this purpose.
    m. Indicate Lyme ELISA result for each Specimen on this report
12. Perform Purge Tubing (and any other required maintenance) on AutoBlot 2000
    a. Empty waste and clean reagent bottles
    b. Turn off ViraCam and AutoBlot 2000
13. Test Review Entry in Soft Flow
14. Page Micro Resident and submit results for review by Resident & Attending

Document # IMM205-B
ViraMedLyme IgG & IgM Line Blot Quiz

1. The ViraStripe® IgG and IgM line blot assays are considered:
    a. Western Blot
    b. A modified solid-phase enzyme linked immunosorbent assay
    c. None of the above
    d. Both a and b

2. What are the necessary volumes of IgG Conjugate Concentrate and Diluted Working Buffer to prepare a working Conjugate for 20 samples?
    a. 27.0 mL Diluted Working Buffer + 3.0 mL Conjugate Concentrate
    b. 28.5 mL Diluted Working Buffer + 1.425 mL Conjugate Concentrate
    c. 3.0 mL Diluted Working Buffer + 27.0 mL Conjugate Concentrate
    d. 2.70 mL Diluted Working Buffer + 3.0 mL Conjugate Concentrate

3. How many bands must be present to call a blot IgM positive?

4. Can results be reported if the patient strips DO NOT show reactivity with the function control and the conjugate control?

5. How many bands must be present to call a blot IgG positive?

6. True or False: A cut-off control must be used with each run/tray?

7. Can strips from different kits be combined?
    a. Yes
    b. No
    c. Yes but only if the same lot number

8. True or False: The Viramed Biotech AG Borrelia B31 ViraStripe kits can be used any time after onset provided the EIA or IFA results are negative?

9. The AUTOBLOT 2000 instrument performs which of the following functions:

    a. Addition of test samples
    b. Incubates
    c. Washes
    d. Reagent addition
    e. All of the above
    f. None of the above
    g. Only B, C & D

10. True or False: The ViraCam requires the use of a cut off strip for every test run?

LYME Line Blot QC Doc# IMM205-Q

Date	Tech		Function Control and Conjugate Control are Visible (Y/N)	CutOff Control is Visible (Y/N)	Neg QC Interpretation = Negative (Y/N)	Pos QC Interpretation = Positive (Y/N)	Comments	Review
		IgG						
		IgM						
		IgG						
		IgM						
		IgG						
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Document Author
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March 7, 2013

Signature Approval for Annual Review
Name: Lyme IgG & IgM Line Blot: **Viramed Biotech AG – Borrelia B31 IgG and IgM ®**

Document #: IMM205

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER	Teodorico Lee	3/6/13	NEW		3/7/13
BRIAN SMITH	LAB DIRECTOR	BS	3/6/13	NEW		3/7/13