



IVD Application

ARTG No : 279792

Class 2 Status: Approved

Application Change history

Application Progress Date

Date received: 31/08/2016

Review Information

Review flag: Returned from applicant review

Auto review required: No

Device Product Characteristics

Does this application include any IVDs that are:

- Class 3 and intended for detecting the presence of, or exposure to, a sexually transmitted agent
- For managing and monitoring the treatment of infections diagnosed using Class 4 IVD
- To be supplied for use in a national disease screening program
- Non-assay specific quality control material that is intended for monitoring a Class 4 IVD
- To be supplied under the Pharmaceutical Benefits Scheme
- Intended for point-of-care testing
- Intended for self-testing? No

Does this application include a system or procedure pack? No

Is the device software or does it incorporate software?

Application Summary

Application ID: DV-2016-IVA-12008-1

Submission ID: DA-2016-04821-1

Sponsor's own reference: Borrelia ViraStripe IgM and IgG test kits

Application for: Medical Device - IVD

Will you be applying for listing of this product or procedure in the Medicare Benefit Schedule (MBS)? ☐ Yes ☒ No

Will you be applying for listing of this product on the Prosthesis List? ☐ Yes ☒ No

Will you be applying for listing of this product on the Co-dependent or hybrid technology application list? ☐ Yes ☒ No

Sponsor name:	Australian Rickettsial Reference Laboratory Foundation Ltd
Sponsor ID:	64750
Agent name:	
Contact details:	s22
Contact email:	s22 @barwonhealth.org.au
Is this application supported by EU MDR/IVDR certification?	

Manufacturer Information

Manufacturer's evidence:	DV-2016-MC-12098-1 :VIRAMED BIOTECH AG- manufacturer evidence submission Goto
Manufacturer name:	Viramed Biotech AG (Germany)[64749]
Certificate issued under:	CMDCAS ISO 13485 Certification
Conformity assessment procedure:	ISO 13485 Manufacturing and Design
Assessment body:	DQS Medizinprodukte GmbH [0297]
GMDN code:	Bacterial infectious disease IVDs[CT353]
GMDN description:	IVDs that are intended to be used in testing to provide information about infection with or exposure to one or a number of bacterial diseases.
Intended purpose:	The intended use of Borrelia ViraStripe IgM and IgG test kits are for the confirmation of Lyme disease. It is generally used as a secondary test to support the findings of a Lyme immunofluorescence assay or enzyme-immunoassay. It is used for the qualitative detection of IgM and IgG antibodies against specific Borrelia antigens in human serum. The testing is performed in the diagnostic laboratory environment. Limitations include possible cross-reaction with other spirochetes.

Device Category Terms

Attached Documentation

-  Product brochure/ catalogue - 2550_Borrelia_ViraStripe_IgG_AL_en.pdf
-  CMDCAS ISO 13485 Certificate (IVDs) - Viramed Biotech AG_Certificate ISO 13485_2003 under CMDCAS_expiry date 2019_06_01_englisch.pdf
-  Product brochure/ catalogue - 2551_Borrelia_ViraStripe_IgM_AL_en.pdf
-  Product brochure/ catalogue - 2550_Borrelia_ViraStripe_IgG_AL_en_RevA_AU (1).pdf
-  Product brochure/ catalogue - 2551_Borrelia_ViraStripe_IgM_AL_en_RevB_AU.pdf

History

31/08/2016 11:02:24 AM Approved.

Review Completed - Accepted, 31/08/2016)

Record		Date	
Fee:	980	Date Paid:	17/08/2016
		Date Decision:	31/08/2016

Start Dates		Finish Dates		Working Days
Application Received	31/08/2016	Payment Received	17/08/2016	0
Payment Received	17/08/2016	Application Decision	31/08/2016	0
Total Working Days				0



IVD Application

ARTG No : 205351

Class 2 Status: Approved

Application Change history

Application Progress Date

Date received: 21/01/2013

Review Information

Review flag:

Auto review required: No

Device Product Characteristics

Does this application include any IVDs that are:

- Class 3 and intended for detecting the presence of, or exposure to, a sexually transmitted agent
- For managing and monitoring the treatment of infections diagnosed using Class 4 IVD
- To be supplied for use in a national disease screening program
- Non-assay specific quality control material that is intended for monitoring a Class 4 IVD
- To be supplied under the Pharmaceutical Benefits Scheme
- Intended for point-of-care testing
- Intended for self-testing? No

Does this application include a system or procedure pack? No

Is the device software or does it incorporate software?

Application Summary

Application ID: DV-2013-IVA-01001-1

Submission ID: DA-2013-00481-1

Sponsor's own reference: Novatec CT 353 class 2 bacterial

Application for: Medical Device - IVD

Will you be applying for listing of this product or procedure in the Medicare Benefit Schedule (MBS)? ☐ Yes ☒ No

Will you be applying for listing of this product on the Prosthesis List? ☐ Yes ☒ No

Will you be applying for listing of this product on the Co-dependent or hybrid technology application list? ☐ Yes ☒ No

Sponsor name:	Immuno Pty Ltd
Sponsor ID:	12706
Agent name:	
Contact details:	s22
Contact email:	s22@immuno.com.au
Is this application supported by EU MDR/IVDR certification?	

Manufacturer Information

Manufacturer's evidence:	DV-2012-MC-17681-1 :Novatec CMDCAS Goto
Manufacturer name:	Gold Standard Diagnostics Frankfurt GmbH (Germany)[44194]
Certificate issued under:	CMDCAS ISO 13485 Certification
Conformity assessment procedure:	ISO 13485 Manufacturing and Design
Assessment body:	DQS GmbH Deutsche Gesellschaft zur Zertifizierung von Managementsystemen
GMDN code:	Bacterial infectious disease IVDs[CT353]
GMDN description:	IVDs that are intended to be used in testing to provide information about infection with or exposure to one or a number of bacterial diseases.
Intended purpose:	IVDs that are intended to be used in testing to provide information about infection with or exposure to one or a number of bacterial diseases.

Device Category Terms

Device category 1:	In vitro diagnostic devices
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Attached Documentation

History

25/01/2013 3:09:45 PM Approved.

Review Completed - Accepted, 25/01/2013)

Record	Date
Fee:	890
Date Paid:	22/01/2013
Date Decision:	24/01/2013

Start Dates	Finish Dates	Working Days
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Application Received	21/01/2013	Payment Received	22/01/2013	1
Payment Received	22/01/2013	Application Decision	24/01/2013	3
Total Working Days				4



IVD Application

ARTG No : 208811

Class 2 Status: Approved

Application Change history

Application Progress Date

Date received: 24/04/2013

Review Information

Review flag:

Auto review required: No

Device Product Characteristics

Does this application include any IVDs that are:

- Class 3 and intended for detecting the presence of, or exposure to, a sexually transmitted agent
- For managing and monitoring the treatment of infections diagnosed using Class 4 IVD
- To be supplied for use in a national disease screening program
- Non-assay specific quality control material that is intended for monitoring a Class 4 IVD
- To be supplied under the Pharmaceutical Benefits Scheme
- Intended for point-of-care testing
- Intended for self-testing? No

Does this application include a system or procedure pack? No

Is the device software or does it incorporate software?

Application Summary

Application ID: DV-2013-IVA-01255-1

Submission ID: DA-2013-02497-1

Sponsor's own reference: Bacterial infectious disease IVDs

Application for: Medical Device - IVD

Will you be applying for listing of this product or procedure in the Medicare Benefit Schedule (MBS)? ☐ Yes ☒ No

Will you be applying for listing of this product on the Prosthesis List? ☐ Yes ☒ No

Will you be applying for listing of this product on the Co-dependent or hybrid technology application list? ☐ Yes ☒ No

Sponsor name:	DiaSorin Australia Pty Ltd		
Sponsor ID:	54278		
Agent name:			
Contact details:	s22		
Contact email:	s22@diasorin.com.au		
Is this application supported by EU MDR/IVDR certification?			

Manufacturer Information

Manufacturer's evidence:	DV-2011-MC-07060-3 :DiaSorin Italy G-MED ISO 13485 CMDCAS Goto		
Manufacturer name:	DiaSorin Italia SpA (Italy)[8004]		
Certificate issued under:	CMDCAS ISO 13485 Certification		
Conformity assessment procedure:	ISO 13485 Manufacturing and Design		
Assessment body:	Laboratoire National de metrologie et dEssais (LNE)		
GMDN code:	Bacterial infectious disease IVDs[CT353]		
GMDN description:	IVDs that are intended to be used in testing to provide information about infection with or exposure to one or a number of bacterial diseases.		
Intended purpose:	IVDs that are intended to be used in testing to provide information about infection with or exposure to one or a number of bacterial diseases		

Device Category Terms

Device category 1:	In vitro diagnostic devices
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Attached Documentation

History

29/04/2013 3:07:16 PM Approved.

Review Completed - Accepted, 29/04/2013)

Record	Date		
Fee:	890	Date Paid:	26/04/2013
		Date Decision:	29/04/2013

Start Dates	Finish Dates	Working Days
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Application Received	24/04/2013	Payment Received	26/04/2013	2
Payment Received	26/04/2013	Application Decision	29/04/2013	3
Total Working Days				5



IVD Application

ARTG No : 176268

Class 2 Status: Approved

Application Change history

Application Progress Date

Date received: 17/09/2010

Review Information

Review flag:

Auto review required: No

Device Product Characteristics

Does this application include any IVDs that are:

- Class 3 and intended for detecting the presence of, or exposure to, a sexually transmitted agent
- For managing and monitoring the treatment of infections diagnosed using Class 4 IVD
- To be supplied for use in a national disease screening program
- Non-assay specific quality control material that is intended for monitoring a Class 4 IVD
- To be supplied under the Pharmaceutical Benefits Scheme
- Intended for point-of-care testing
- Intended for self-testing? No

Does this application include a system or procedure pack? No

Is the device software or does it incorporate software?

Application Summary

Application ID: DV-2010-IVA-13504-3

Submission ID: DA-2010-05365-7

Sponsor's own reference: Bacterial infectious disease IVD

Application for: Medical Device - IVD

Will you be applying for listing of this product or procedure in the Medicare Benefit Schedule (MBS)? ☐ Yes ☐ No

Will you be applying for listing of this product on the Prosthesis List? ☐ Yes ☐ No

Will you be applying for listing of this product on the Co-dependent or hybrid technology application list? ☐ Yes ☐ No

Sponsor name:	ESL Biosciences (Australia) Pty Ltd
Sponsor ID:	40631
Agent name:	
Contact details:	s22
Contact email:	s22 @eslbioscience.com
Is this application supported by EU MDR/IVDR certification?	

Manufacturer Information

Manufacturer's evidence:	DV-2010-MC-09586-3 :ISO 13485 Goto
Manufacturer name:	EUROIMMUN Medizinische Labordiagnostika AG (Germany)[32218]
Certificate issued under:	Other ISO 13485 Certification
Conformity assessment procedure:	ISO 13485
Assessment body:	TUV Rheinland LGA Products GmbH [0197]
GMDN code:	Bacterial infectious disease IVDs[CT353]
GMDN description:	IVDs that are intended to be used in testing to provide information about infection with or exposure to one or a number of bacterial diseases.
Intended purpose:	IVDs that are intended to be used to determine (qualitative and or quantitative) information about infection with or exposure to one or a number of bacterial diseases in a clinical specimen. Each IVD is intended to be used by a medical laboratory scientist. Techniques used include: ELISA, Indirect Immunofluorescence, immune blots or western blot

Device Category Terms

Device category 1:	In vitro diagnostic devices
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Attached Documentation

History

1/10/2010 11:00:28 AM Approved.

Review Completed - Accepted, 1/10/2010)

Record	Date
Fee:	810
Date Paid:	20/09/2010
Date Decision:	01/10/2010

Start Dates		Finish Dates		Working Days
Application Received	17/09/2010	Payment Received	20/09/2010	1
Payment Received	20/09/2010	Application Decision	01/10/2010	10
Total Working Days				11



Borrelia VirStripe® IgG Test Kit

Stripe-Immunoblot for the qualitative detection of **IgG** antibodies against specific **Borrelia species** antigens in human serum or cerebrospinal fluid. For using the **Borrelia VirStripe® IgG Testkit** with cerebrospinal fluid (CSF) as sample material the corresponding, separately available instruction for use has to be considered.

The Borrelia VirStripe® IgG Testkit is an **immunoblot** based on an enzyme-immunoassay in the line/strip format, carrying highly purified specific native antigens from *Borrelia afzelii* (Pko) and *Borrelia burgdorferi sensu stricto* as well as recombinant VlsE. The Borrelia VirStripe® IgG Testkit is manufactured according to the guidelines **98/79/EG** and **DIN 58967-40**.

The Borrelia VirStripe® IgG Testkit fulfils the quality standards of the microbiological-infectiological guidelines ("**MiQ**" **12/2000**), the general DIN recommendation (**DIN 58967-40**) and can be evaluated according to "**MiQ**" **12/2000** (18) and **DIN 58969-44** (5) criteria. These guidelines postulate a two-band criterion for a positive result and describe special requirements for the detection of antibodies against *Borrelia burgdorferi*.

Principle of the assay

During the serum incubation step *Borrelia* specific IgG antibodies bind to the immobilised antigens on the test strip. During the conjugate reaction, AP-conjugate binds to the antigen-antibody complex. The alkaline phosphatase converts the chromogen/substrate and thus, stains the antigen-antibody complex on the test strip purple. The washing procedures following serum, conjugate and chromogen/substrate incubation steps remove unbound antibodies and reagents.

The green separation line divides the test strip into a control section and an analytical section. The control section contains the **negative control band**, the **serum control**, **three conjugate controls** (IgG, IgA, IgM) and the **cut off control**.

The test strip code for Borrelia VirStripe® IgG test strips is **BG**. Test strips are numbered from **01** to **50**. The analytical section contains the *Borrelia* specific antigens.

Order No.:	V-BSSGOK	Order No.:	V-BSSGDK (Deca Kit)
Kit size:	1x 50 test strips	Kit size:	10x 50 test strips
Specimen:	20 µl serum	Specimen:	20 µl serum
Time for testing:	approx. 90 minutes	Time for testing:	approx. 90 minutes

Materials provided

1x or 10x 50 test strips	Borrelia VirStripe® Antigen Strips (IgG) Test strips including a control section and <i>Borrelia</i> specific antigens in the analytical section, ready to use	(Prod. No.: V-BSSGAS)
1x or 10x 9 ml	ViraStripe® / ViraBlot® AP-Anti-Human IgG Conjugate Concentrate, goat	(Order No.: V-UVNGKI)
1x or 10x 100 ml	ViraStripe® / ViraBlot® Diluent / Wash Buffer 10x concentrate	(Order No.: V-UVNUWP)
1x or 10x 5 g	ViraStripe® / ViraBlot® Diluent / Wash Powder	(Order No.: V-UVNUMP)
1x or 10x 90 ml	ViraStripe® / ViraBlot® Chromogen / Substrate Solution Ready to use	(Order No.: V-UVNUCS)
1 or 10 copies	Evaluation Protocol for Borrelia VirStripe® IgG Testkit	

Additionally available

330 µl	Borrelia VirStripe® IgG Positive Control Human, ready to use	(Order No.: V-BSSGPK)
330 µl	Borrelia VirStripe® IgG,A,M Negative Control Human, ready to use	(Order No.: V-BSSPNK)
50 copies	Borrelia VirStripe® IgG evaluation protocols for automated interpretation with ViraScan® software	(Order No.: V-BSSGEP)

Preparation of reagents and patient samples

Bring all reagents to room temperature (20-25°C) prior to use. Information about stability can be found on page 5.

Diluent / Wash Buffer	Dilute Diluent / Wash Buffer Concentrate 1:10 with distilled or deionised water (100 ml concentrate + 900 ml water). Add Diluent / Wash Powder completely and stir well until all powder is dissolved. If needed, place onto a magnetic stirrer for 10-15 minutes. The pH value should be around pH 7.5 at 20°C.
Working Dilution:	
Antigen Strips:	Carefully separate the required number of test strips by use of forceps at the label and place the test strips in the prepared incubation tray (see assay procedure, step 2). Use test strips directly after removing from packing. Do not touch test strips by hand. Return unused test strips directly into the original packing, seal well and store at 2-8°C.
Patient samples:	Use 20 µl patient serum undiluted per test strip.
Controls:	Use 100 µl of Positive Control or 100 µl of Negative Control undiluted per test strip respectively.
Conjugate Working Dilution:	Prepare Conjugate Concentrate 1:10 with Diluent / Wash Buffer Working Dilution (see table 1). Prepare freshly prior to each test run. Do not store for further use.
Chromogen / Substrate Solution:	Ready to use.

Borrelia ViraStripe® IgG Test Kit

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Preparation of Conjugate Working Dilution IgG

Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume	Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume
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 1: 1:10 dilution of conjugate concentrate with Diluent / Wash Buffer Working Dilution

Assay procedure

1. Rinse incubation tray channels once with 1.5 ml Diluent / Wash Buffer Working Dilution, decant the liquid
2. Place the needed amount of test strips into the incubation tray - one test strip per channel
3. Add 1.5 ml Diluent / Wash Buffer Working Dilution and incubate by rocking for 5 minutes at room temperature (RT)
4. Add 20 µl of each patient serum or 100 µl of each control
5. Incubate by rocking for 30 minutes at RT
6. Decant the liquid
7. 3 x washing:
 - add 1.5 ml Diluent / Wash Buffer Working Dilution
 - incubate by rocking for 5 minutes at RT
 - decant the liquid
8. Add 1.5 ml fresh Conjugate Working Dilution
9. Incubate by rocking for 15 minutes at RT
10. Decant the liquid
11. 3 x washing as in step 7
12. Add 1.5 ml distilled or deionised water and incubate by rocking for 1 minute at RT
13. Decant the liquid
14. Add 1.5 ml Chromogen / Substrate Solution
15. Incubate by rocking at RT
Borrelia ViraStripe® IgG: approx. 5 to 15 minutes
16. Stop the reaction by decanting the liquid
17. Wash 3 x with 1.5 ml distilled or deionised water
18. Dry test strips for interpretation

Mark the trays with water-resistant pen. Rinsing removes dust particles.

For each patient serum and each control, carefully separate one test strip by use of forceps at the label and place them into the incubation tray channels. **The side showing the green separation line and the label must face up.**

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min. Avoid spilling of liquid. **Do not decant the liquid after incubation.**

Add patient sera and controls directly onto the labelled end of the test strips while the 2D rocker is running or make sure to tilt the incubation tray after adding each serum.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. **Test strips adhere to the incubation tray when liquid is decanted.**

Wash on the 2D rocker. Carefully tap the incubation tray on absorbent paper to remove the remaining liquid.

Make sure the test strips are completely covered with Conjugate Working Dilution

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Wash on the 2D rocker.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Make sure the test strips are completely covered with liquid.

Stop the reaction as soon as the cut off control becomes visible. **The Cut off control is located in the test strip control section. Caution:** Prolonged incubation causes background staining.

Carefully tap the incubation tray on absorbent paper.

Wash without incubation time.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. Place wet test strips with forceps on unbleached absorbent paper and allow to air dry before interpretation.

Borrelia ViraStripe® IgG Test Kit

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Assay interpretation

1. Evaluation protocol: Record data on the evaluation protocol. Glue the test strips on the evaluation protocol. Place the green separation line of the test strips exactly onto the separation line printed on the evaluation protocol.
2. Validity of test strips: A test strip is considered as valid if the following bands are visible:
 - The **serum control**.
 - The **conjugate control** of the conjugate class being used. If more than one of the three conjugate controls becomes visible, the strongest band must indicate the appropriate conjugate class.
 - The **cut off control**.

and if the following band is **not** visible:

 - The **negative control band**.

Do not assess invalid test strips!
3. Assignment of antigen bands: The green separation line of the test strips indicates position and orientation for the assignment of bands with the bandlocator on the evaluation protocol. Assign bands and record results according to 4.
4. Assessment of bands: According to quality laboratory guidelines, the use of a cut off control for each run is recommended (16). **The cut off control of the Borrelia ViraStripe® IgG is located in the control section on each test strip.** The intensity of the cut off control indicates the threshold of which bands are being assessed:
 A band is considered as **distinct** if its intensity is **equal** to or **higher** than the intensity of the cut off control. Mark bands with **X** in the evaluation protocol appropriately.
 A band is not assessed if it is **not present** or if its intensity is **lower** than the intensity of the cut off control.
5. Interpretation of patient bands: Patient bands have to be considered as symptoms of the disease. A final clinical diagnosis should always be made considering anamnesis, clinical manifestations and laboratory data (24).
 “If the pattern of reactive bands meets the specific conditions, the result is positive, i.e. the positive result of an EIA or another test of the first step is confirmed. If, despite the presence of specific diagnostic bands, the criteria for a positive result are not fulfilled, the result is considered equivocal. In such a case a follow-up control may be recommended.” (18)
 Bands of the following antigens are considered as **highly specific** for Borrelia species:
p83, p58, p43, p39, p30, OspC, p21, Osp17/DbpA, p14 and VlsE.

IgG Interpretation criteria

General note: Distinct bands must have a minimum intensity ($\geq$ cut off), which has to be determined by the cut off control. The cut off control is located in the control section of each test strip.

Identified bands	Result	Interpretation
At least two distinct bands out of: p83, p58, p43, p39, p30, OspC, p21, Osp17 or DbpA, p14, VlsE	Positive	Specific antibodies against Borrelia species detectable. An infection with Borrelia species is probable.
One distinct VlsE band	Equivocal	Specific antibodies against VlsE detectable. An infection with Borrelia species is possible. If a recent infection is suspected, check additionally for IgM antibodies. If possible test a second sample for IgM and IgG specific antibodies after 2-3 weeks.
One or no distinct bands (Exception: singular VlsE band)	Negative	No specific antibodies against Borrelia species detectable. If an infection is suspected, check a second sample for IgM and IgG specific antibodies after 2-3 weeks.

IgG test strip

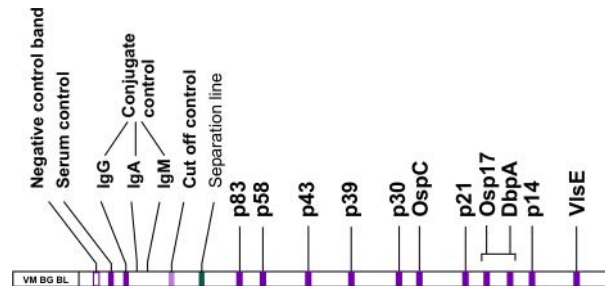


Figure 1: Schematic drawing of a Borrelia ViraStripe® IgG test strip in full scale.

Nomenclature and description of Borrelia species bands from literature

Band nomenclature:	Antigen:	Comments:
VisE	Specific VisE (Variable major protein (VMP) like sequence <u>E</u> xpressed)	Antibodies against VisE are described as specific. IgG antibodies are already being developed at an early stage and remain until the late stage of the disease (9).
p83/100	Highly specific protein of membrane vesicles on the cell surface	Generally these antibodies appear 6-12 weeks after infection. They are typical for stage III, but might also appear in stages I and II (1,4,14,15).
p58	Specific	Protein not well characterised, separated from 60 kD (Hsp60), appears often in stage III (6).
p43	Specific	Still little characterised. Mainly described for stage III.
p39	Highly specific BmpA (Borrelia <u>m</u> embrane protein <u>A</u>)	In many patients antibodies against p39 are already detectable in the early stage of the disease (1,15).
p30	Specific	At present protein not well characterised (6).
OspC	Highly specific OspC (Outer <u>s</u> urface protein <u>C</u>)	At least 13 different immuno-distinct types of OspC are known (1,8,12,13,21,22).
p21	Specific	Still little characterised (3).
Osp17 / DbpA	Specific Osp17 (Outer <u>s</u> urface protein <u>17</u>) / DbpA (Decorin binding protein <u>A</u>)	Binding to decorin on the host cell. Antibodies against Osp17/DbpA are described as specific. The presence of IgG antibodies is associated among others with Arthritis and Neuroborreliosis. Species specific (9,10,19).
p14	Specific	Protein from Borrelia afzelii is characterised as specifically immunogenic (7).

Diagnostic significance of antibodies against Borrelia species

- IgG antibodies** are produced for the first time several weeks to months after infection and are often not detectable in early stages of infection (22). In suspicion of a recent infection, IgM antibodies should be checked and a second sample should be analysed later. Patients in the 2nd or 3rd stage of the disease are usually positive for IgG antibodies. Antibody titers decrease gradually during convalescence (22).
- IgM antibodies** usually appear 2-3 weeks after onset of the disease for the first time (22). Antibody titers often decline several weeks to months after convalescence. But they may also persist up to several years (7,11,20).
- IgA antibodies** are detectable at an early stage of borreliosis in many patients, in some cases earlier than IgM antibodies.
- The immune response and consequently the band pattern differs from patient to patient. As a general rule: The number of

antibody types and therefore the number of specific bands is increasing with progression of the disease (1).

- An early antibiotic therapy can suppress the development of antibodies (17).

- Medication and immunoglobulin therapy can cause unspecific antibody reactions (24).

- Cross reactivities to Borrelia antigens are described for infections with Treponema, Leptospira and other bacteria with flagella (2,15,22). An acute EBV infection can cause a polyclonal stimulation of Borrelia antibodies (22). If IgM antibodies against OspC or p41 are detected without clinical symptoms for borreliosis an EBV infection needs to be tested for. Cross reactivities in cases of autoimmune diseases, MS, ALS, Influenza and Syphilis are described as well.

Borrelia ViraStripe® IgG Testkit

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IgG performance data

Sensitivity:

156 sera from patients with the Lyme borreliosis manifestations Erythema migrans, Erythema chronicum migrans, multiple Erythema migrans, Acrodermatitis chronica atrophicans, Lyme arthritis and neuroborreliosis were assessed to determine the sensitivity of the Borrelia ViraStripe® IgG Testkit.

Stage of Lyme borreliosis	Borrelia ViraStripe® IgG, % (n)	Borrelia ViraStripe® IgG / IgM, % (n)
Erythema migrans (n= 29)	48% (14)	79% (23)
Multiple Erythema migrans (n= 13)	85% (11)	85% (11)
Erythema chronicum migrans (n= 27)	89% (24)	96% (26)
Neuroborreliosis (n= 24)	92% (22)	96% (23)
Acrodermatitis chronica atrophicans (n= 34)	97% (33)	97% (33)
Lyme arthritis (n= 29)	100% (29)	100% (29)

Specificity:

To determine the specificity of the Borrelia ViraStripe® IgG Testkit 129 sera from blood donors showing a negative result with a reference test (Borrelia „MiQ“ + VlsE ViraBlot® IgM and IgG Testkit) were assessed.

Collective	Borrelia ViraStripe® IgG, % (n)	Borrelia ViraStripe® IgG / IgM, % (n)
Blood donors (n= 129)	99% (128)	97% (125)

Warnings and precautions

1. All human serum components were tested for HCV, HIV1,2 antibodies and HBs antigens and found to be negative. Nevertheless, all human kit components as well as the patient samples should be considered as potentially infectious and handled according to safety precautions. While working with potentially infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be taken into account. This also applies to storage and disposal of chemicals and reagents being used.

2. While working with hazardous or toxic substances/ biological agents precautions have to be applied following national biosafety regulations. In general, biological and chemical agents should be handled according to „Good Laboratory Practice (GLP)“ guidelines. Precautions among others are:

- Do not pipette by mouth.
- Wear disposable gloves and safety glasses while working.
- Do not eat, drink or smoke in the working area.

3. The chromogen/substrate solution contains BCIP and NBT. Avoid contact with skin and mucous membranes. In case of contact with skin and eyes wash immediately with large quantities of water.

4. Samples and all potentially contaminated materials must be decontaminated using validated laboratory techniques, e.g. by autoclaving 20 minutes at 121°C under humid conditions. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite. Incubate 30 min for complete disinfection.

5. Please refer to material safety data sheets for detailed information on potential risks, first aid guidelines, accidental release measures, handling and storage recommendations, personal protective equipment, directions for disposal and indications to toxicology.

Storage and stability of reagents

1. **Test strips:** In closed bags stable until the expiration date if stored at 2-8°C.

2. **Conjugate Concentrate:** Stable until the expiration date if stored at 2-8°C.

3. **Conjugate Working Dilution:** Prepare freshly prior to each run. Do not store for further use.

4. **Diluent / Wash Buffer Concentrate, 10x:** Stable until the expiration date if stored at 2-8°C.

5. **Diluent / Wash Buffer Working Dilution:** Stable for 2 weeks if stored at 2-8°C. For longer storage, aliquot and freeze at -20°C.

6. **Diluent / Wash Powder:** Stable until the expiration date if stored at 2-8°C.

7. **Chromogen / Substrate Solution:** Stable until the expiration date if stored at 2-8°C. Avoid exposure to light!

Specimen indications

1. The **Borrelia ViraStripe® IgG Testkit** must be used with human serum or cerebrospinal fluid.

2. Only clear, non-hemolysed, non-microbially contaminated specimens must be used.

3. Using icteric, lipemic, hemolytic and/or heat-inactivated serum may lead to false results.

4. Normally, human serum can be stored up to 5 days at 2-8°C. Specimens may be stored at -20°C (or below) for long term storage.

5. Prior test processing, specimens should have reached room temperature. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.

6. Avoid multiple freeze and thaw cycles.

Borrelia ViraStripe® IgG Testkit

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Limitation of the procedure

1. To ensure reliable results, follow carefully the Instruction for Use and "Good Laboratory Practice".
2. A positive result is based on elevated specific antibody titers and should be considered as a symptom. The correlation to a disease is only conditionally possible.
3. A negative result does not exclude a contact with the pathogen or the presence of a disease.
4. Adequately trained personnel only should perform the assay procedure.

5. The detection of specific antibodies can vary within different assays from different manufacturers and can lead to different results due to different sensitivity, specificity and assay methodologies.
6. Test strips showing a high background level should not be interpreted, especially if band intensities are lighter than the background level.
7. *In vitro* diagnostics must not be used beyond expiration date as reliable results may not be possible.
8. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false results.

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Symbols used

	Manufacturer		Order Number
	Refer to Instructions for Use		Use by / Expiration Date
	<i>In-Vitro</i> Diagnostic Medical Device		Temperature Limitation (Storage)
	Lot Number		Positive Serum Control
	Sufficient for 50 Tests		Negative Serum Control
	Room Temperature in °C		Control
	Person in Charge		Date
	Serum Number		Chromogen/Substrate Incubation Time in Minutes
	Evaluation Protocol		Protocol Number



Borrelia ViraStripe® IgM Test Kit

Stripe-Immunoblot for the qualitative detection of **IgM** antibodies against specific **Borrelia species** antigens in human serum or cerebrospinal fluid. For using the **Borrelia ViraStripe® IgM Test Kit** with cerebrospinal fluid (CSF) as sample material the corresponding, separately available instruction for use has to be considered.

The Borrelia ViraStripe® IgM Test Kit is an **immunoblot** based on an enzyme-immunoassay in the line/strip format, carrying highly purified specific native antigens from *Borrelia afzelii* (Pko) and *Borrelia burgdorferi sensu stricto* as well as recombinant VlsE. The Borrelia ViraStripe® IgM Test Kit is manufactured according to the guidelines **98/79/EG** and **DIN 58967-40**.

The Borrelia ViraStripe® IgM Test Kit fulfils the quality standards of the microbiological-infectiological guidelines ("MiQ" 12/2000), the general DIN recommendation (**DIN 58967-40**) and can be evaluated according to "MiQ" 12/2000 (18) and **DIN 58969-44** (5) criteria. These guidelines postulate a one-band criterion for a positive result and describe special requirements for the detection of antibodies against *Borrelia burgdorferi*.

Principle of the assay

During the serum incubation step *Borrelia* specific IgM antibodies bind to the immobilised antigens on the test strip. During the conjugate reaction, AP-conjugate binds to the antigen-antibody complex. The alkaline phosphatase converts the chromogen/substrate and thus, stains the antigen-antibody complex on the test strip purple. The washing procedures following serum, conjugate and chromogen/substrate incubation steps remove unbound antibodies and reagents.

The green separation line divides the test strip into a control section and an analytical section. The control section contains the **negative control band**, the **serum control**, **three conjugate controls** (IgG, IgA, IgM) and the **cut off control**.

The test strip code for Borrelia ViraStripe® IgM test strips is **BM**. Test strips are numbered from **01** to **50**. The analytical section contains the *Borrelia* specific antigens.

Order No.:	V-BSSMOK	Order No.:	V-BSSMDK (Deca Kit)
Kit size:	1x 50 test strips	Kit size:	10x 50 test strips
Specimen:	20 µl serum	Specimen:	20 µl serum
Time for testing:	approx. 90 minutes	Time for testing:	approx. 90 minutes

Materials provided

1x or 10x 50 test strips	Borrelia ViraStripe® Antigen Strips (IgM) Test strips including a control section and <i>Borrelia</i> specific antigens in the analytical section, ready to use	(Prod. No.: V-BSSMAS)
1x or 10x 9 ml	ViraStripe® / ViraBlot® AP-Anti-Human IgM Conjugate Concentrate, goat	(Order No.: V-UVNMKI)
1x or 10x 100 ml	ViraStripe® / ViraBlot® Diluent / Wash Buffer 10x concentrate	(Order No.: V-UVNUWP)
1x or 10x 5 g	ViraStripe® / ViraBlot® Diluent / Wash Powder	(Order No.: V-UVNUMP)
1x or 10x 90 ml	ViraStripe® / ViraBlot® Chromogen / Substrate Solution Ready to use	(Order No.: V-UVNUCS)
1 or 10 copies	Evaluation Protocol for Borrelia ViraStripe® IgM Test Kit	

Additionally available

330 µl	Borrelia ViraStripe® IgM Positive Control Human, ready to use	(Order No.: V-BSSMPK)
330 µl	Borrelia ViraStripe® IgG,A,M Negative Control Human, ready to use	(Order No.: V-BSSPNK)
50 copies	Borrelia ViraStripe® IgM evaluation protocols for automated interpretation with ViraScan® software	(Order No.: V-BSSMEP)

Preparation of reagents and patient samples

Bring all reagents to room temperature (20-25°C) prior to use. Information about stability can be found on page 5.

Diluent / Wash Buffer Working Dilution: Dilute **Diluent / Wash Buffer Concentrate 1:10** with distilled or deionised water (100 ml concentrate + 900 ml water). Add Diluent / Wash Powder completely and stir well until all powder is dissolved. If needed, place onto a magnetic stirrer for 10-15 minutes. The pH value should be around pH 7.5 at 20°C.

Antigen Strips: Carefully separate the required number of test strips by use of **forceps** at the **label** and place the test strips in the prepared incubation tray (see assay procedure, step 2). Use test strips directly after removing from packing. Do not touch test strips by hand. Return unused test strips directly into the original packing, seal well and store at 2-8°C.

Patient samples: Use **20 µl patient serum** undiluted per test strip.

Controls: Use **100 µl** of **Positive Control** or **100 µl** of **Negative Control** undiluted per test strip respectively.

Conjugate Working Dilution: Prepare **Conjugate Concentrate 1:10** with Diluent / Wash Buffer Working Dilution (see table 1). Prepare freshly prior to each test run. Do not store for further use.

Chromogen / Substrate Solution: Ready to use.

Borrelia ViraStripe® IgM Test Kit

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Preparation of Conjugate Working Dilution IgM

Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume	Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume
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 1: 1:10 dilution of conjugate concentrate with Diluent / Wash Buffer Working Dilution

Assay procedure

1. Rinse incubation tray channels once with 1.5 ml Diluent / Wash Buffer Working Dilution, decant the liquid
2. Place the needed amount of test strips into the incubation tray - one test strip per channel
3. Add 1.5 ml Diluent / Wash Buffer Working Dilution and incubate by rocking for 5 minutes at room temperature (RT)
4. Add 20 µl of each patient serum or 100 µl of each control
5. Incubate by rocking for 30 minutes at RT
6. Decant the liquid
7. 3 x washing:
 - add 1.5 ml Diluent / Wash Buffer Working Dilution
 - incubate by rocking for 5 minutes at RT
 - decant the liquid
8. Add 1.5 ml fresh Conjugate Working Dilution
9. Incubate by rocking for 15 minutes at RT
10. Decant the liquid
11. 3 x washing as in step 7
12. Add 1.5 ml distilled or deionised water and incubate by rocking for 1 minute at RT
13. Decant the liquid
14. Add 1.5 ml Chromogen / Substrate Solution
15. Incubate by rocking at RT
Borrelia ViraStripe® IgM: approx. 5 to 15 minutes
16. Stop the reaction by decanting the liquid
17. Wash 3 x with 1.5 ml distilled or deionised water
18. Dry test strips for interpretation

Mark the trays with water-resistant pen. Rinsing removes dust particles.

For each patient serum and each control, carefully separate one test strip by use of forceps at the label and place them into the incubation tray channels. **The side showing the green separation line and the label must face up.**

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min. Avoid spilling of liquid. **Do not decant the liquid after incubation.**

Add patient sera and controls directly onto the labelled end of the test strips while the 2D rocker is running or make sure to tilt the incubation tray after adding each serum.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. **Test strips adhere to the incubation tray when liquid is decanted.**

Wash on the 2D rocker. Carefully tap the incubation tray on absorbent paper to remove the remaining liquid.

Make sure the test strips are completely covered with Conjugate Working Dilution

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Wash on the 2D rocker.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Make sure the test strips are completely covered with liquid.

Stop the reaction as soon as the cut off control becomes visible. **The Cut off control is located in the test strip control section. Caution:** Prolonged incubation causes background staining.

Carefully tap the incubation tray on absorbent paper.

Wash without incubation time.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. Place wet test strips with forceps on unbleached absorbent paper and allow to air dry before interpretation.

Assay interpretation

1. Evaluation protocol: Record data on the evaluation protocol. Glue the test strips on the evaluation protocol. Place the green separation line of the test strips exactly onto the separation line printed on the evaluation protocol.
2. Validity of test strips: A test strip is considered as valid if the following bands are visible:
 - The **serum control**.
 - The **conjugate control** of the conjugate class being used. If more than one of the three conjugate controls becomes visible, the strongest band must indicate the appropriate conjugate class.
 - The **cut off control**.

and if the following band is **not** visible:

 - The **negative control band**.

Do not assess invalid test strips!
3. Assignment of antigen bands: The green separation line of the test strips indicates position and orientation for the assignment of bands with the bandlocator on the evaluation protocol. Assign bands and record results according to 4.
4. Assessment of bands: According to quality laboratory guidelines, the use of a cut off control for each run is recommended (16). **The cut off control of the Borrelia ViraStripe® IgM is located in the control section on each test strip.** The intensity of the cut off control indicates the threshold of which bands are being assessed:
 A band is considered as **distinct** if its intensity is **equal** to or **higher** than the intensity of the cut off control. Mark bands with **X** in the evaluation protocol appropriately.
 A band is not assessed if it is **not present** or if its intensity is **lower** than the intensity of the cut off control.
5. Interpretation of patient bands: Patient bands have to be considered as symptoms of the disease. A final clinical diagnosis should always be made considering anamnesis, clinical manifestations and laboratory data (24).
 “If the pattern of reactive bands meets the specific conditions, the result is positive, i.e. the positive result of an EIA or another test of the first step is confirmed. If, despite the presence of specific diagnostic bands, the criteria for a positive result are not fulfilled, the result is considered equivocal. In such a case a follow-up control may be recommended.” (18)
 Bands of the following antigens are considered as **highly specific** for Borrelia species:
p41 (limited specificity), **p39**, **OspC**, **Osp17** and **VisE**

IgM Interpretation criteria

General note: Distinct bands must have a minimum intensity ($\geq$ cut off), which has to be determined by the cut off control. The cut off control is located in the control section of each test strip.

Identified bands	Result	Interpretation
At least one distinct band out of: p39, OspC, Osp17, VisE or p41 higher than cut off	Positive	Specific antibodies against Borrelia species detectable. An infection with Borrelia species is probable.
No distinct bands or p41 equal to cut off	Negative	No specific antibodies against Borrelia species detectable. If an infection is suspected, check a second sample for IgM and IgG specific antibodies after 2-3 weeks.

IgM test strip

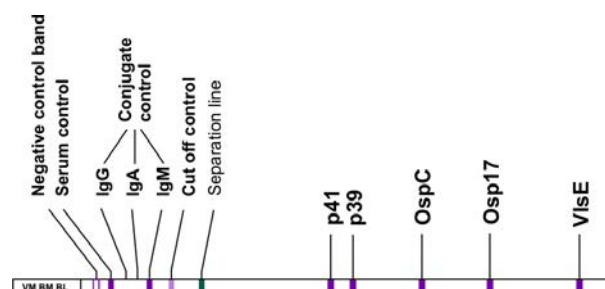


Figure 1: Schematic drawing of a Borrelia ViraStripe® IgM test strip in full scale.

Nomenclature and description of Borrelia species bands from literature

Band nomenclature:	Antigen:	Comments:
VlsE	Specific VlsE (Variable major protein (VMP) like sequence <u>E</u> xpressed)	Antibodies against VlsE are described as specific. IgG antibodies are already being developed at an early stage and remain until the late stage of the disease. IgM antibodies against VlsE could appear as well in early stages but their serologic detection is less frequent (9).
p41	Limited specificity Flagellum protein	Cross-reactivities with other spirochetes and other flagellum carrying bacteria are described (2,15,22,23).
p39	Highly specific BmpA (<u>B</u> orrelia <u>m</u> embrane protein <u>A</u>)	In many patients antibodies against p39 are already detectable in the early stage of the disease (1,15).
OspC	Highly specific OspC (<u>O</u> uter <u>s</u> urface protein <u>C</u>)	At least 13 different immuno-distinct types of OspC are known. IgM antibodies against OspC are often the first Borrelia specific antibodies detectable in patients and may appear before IgM antibodies against p41 (1,8,12,13,21,22).
Osp17	Specific Osp17 (<u>O</u> uter <u>s</u> urface protein <u>17</u>)	Binding to decorin on the host cell. Antibodies against Osp17 are described as specific. The presence of IgG antibodies is associated among others with Arthritis and Neuroborreliosis. Species specific (9,10,19).

Diagnostic significance of antibodies against Borrelia species

- IgG antibodies** are produced for the first time several weeks to months after infection and are often not detectable in early stages of infection (22). In suspicion of a recent infection, IgM antibodies should be checked and a second sample should be analysed later. Patients in the 2nd or 3rd stage of the disease are usually positive for IgG antibodies. Antibody titers decrease gradually during convalescence (22).
- IgM antibodies** usually appear 2-3 weeks after onset of the disease for the first time (22). Antibody titers often decline several weeks to months after convalescence. But they may also persist up to several years (7,11,20).
- IgA antibodies** are detectable at an early stage of borreliosis in many patients, in some cases earlier than IgM antibodies.
- The immune response and consequently the band pattern differs from patient to patient. As a general rule: The number of

- antibody types and therefore the number of specific bands is increasing with progression of the disease (1).
- An early antibiotic therapy can suppress the development of antibodies (17).
 - Medication and immunoglobulin therapy can cause unspecific antibody reactions (24).
 - Cross reactivities to Borrelia antigens are described for infections with Treponema, Leptospira and other bacteria with flagella (2,15,22). An acute EBV infection can cause a polyclonal stimulation of Borrelia antibodies (22). If IgM antibodies against OspC or p41 are detected without clinical symptoms for borreliosis an EBV infection needs to be tested for. Cross reactivities in cases of autoimmune diseases, MS, ALS, Influenza and Syphilis are described as well.

IgM performance data

Sensitivity:

156 sera from patients with the Lyme borreliosis manifestations Erythema migrans, Erythema chronicum migrans, multiple Erythema migrans, Acrodermatitis chronica atrophicans, Lyme arthritis and neuroborreliosis were assessed to determine the sensitivity of the Borrelia VirStripe® IgM Test Kit.

Stage of Lyme borreliosis	Borrelia VirStripe® IgM, % (n)	Borrelia VirStripe® IgG / IgM, % (n)
Erythema migrans (n= 29)	69% (20)	79% (23)
Multiple Erythema migrans (n= 13)	77% (10)	85% (11)
Erythema chronicum migrans (n= 27)	52% (14)	96% (26)
Neuroborreliosis (n= 24)	75% (18)	96% (23)
Acrodermatitis chronica atrophicans (n= 34)	53% (18)	97% (33)
Lyme arthritis (n= 29)	66% (19)	100% (29)

Borrelia ViraStripe® IgM Test Kit

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Specificity:

To determine the specificity of the Borrelia ViraStripe® IgM Test Kit 129 sera from blood donors showing a negative result with a reference test (Borrelia „MiQ“ + VlsE ViraBlot® IgM and IgG Test Kit) were assessed.

Collective	Borrelia ViraStripe® IgM, % (n)	Borrelia ViraStripe® IgG / IgM, % (n)
Blood donors (n= 129)	98% (126)	97% (125)

Warnings and precautions

1. All human serum components were tested for HCV, HIV1,2 antibodies and HBs antigens and found to be negative. Nevertheless, all human kit components as well as the patient samples should be considered as potentially infectious and handled according to safety precautions. While working with potentially infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be taken into account. This also applies to storage and disposal of chemicals and reagents being used.
2. While working with hazardous or toxic substances/ biological agents precautions have to be applied following national biosafety regulations. In general, biological and chemical agents should be handled according to „Good Laboratory Practice (GLP)“ guidelines. Precautions among others are:
 - Do not pipette by mouth.
 - Wear disposable gloves and safety glasses while working.
 - Do not eat, drink or smoke in the working area.

3. The chromogen/substrate solution contains BCIP and NBT. Avoid contact with skin and mucous membranes. In case of contact with skin and eyes wash immediately with large quantities of water.
4. Samples and all potentially contaminated materials must be decontaminated using validated laboratory techniques, e.g. by autoclaving 20 minutes at 121°C under humid conditions. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite. Incubate 30 min for complete disinfection.
5. Please refer to material safety data sheets for detailed information on potential risks, first aid guidelines, accidental release measures, handling and storage recommendations, personal protective equipment, directions for disposal and indications to toxicology.

Storage and stability of reagents

1. **Test strips:** In closed bags stable until the expiration date if stored at 2-8°C.
2. **Conjugate Concentrate:** Stable until the expiration date if stored at 2-8°C.
3. **Conjugate Working Dilution:** Prepare freshly prior to each run. Do not store for further use.
4. **Diluent / Wash Buffer Concentrate, 10x:** Stable until the expiration date if stored at 2-8°C.

5. **Diluent / Wash Buffer Working Dilution:** Stable for 2 weeks if stored at 2-8°C. For longer storage, aliquot and freeze at -20°C.
6. **Diluent / Wash Powder:** Stable until the expiration date if stored at 2-8°C.
7. **Chromogen / Substrate Solution:** Stable until the expiration date if stored at 2-8°C. Avoid exposure to light!

Specimen indications

1. The **Borrelia ViraStripe® IgM Test Kit** must be used with human serum or cerebrospinal fluid.
2. Only clear, non-hemolysed, non-microbially contaminated specimens must be used.
3. Using icteric, lipemic, hemolytic and/or heat-inactivated serum may lead to false results.

4. Normally, human serum can be stored up to 5 days at 2-8°C. Specimens may be stored at -20°C (or below) for long term storage.
5. Prior test processing, specimens should have reached room temperature. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.
6. Avoid multiple freeze and thaw cycles.

Limitation of the procedure

1. To ensure reliable results, follow carefully the Instruction for Use and "Good Laboratory Practice".
2. A positive result is based on elevated specific antibody titers and should be considered as a symptom. The correlation to a disease is only conditionally possible.
3. A negative result does not exclude a contact with the pathogen or the presence of a disease.
4. Adequately trained personnel only should perform the assay procedure.

5. The detection of specific antibodies can vary within different assays from different manufacturers and can lead to different results due to different sensitivity, specificity and assay methodologies.
6. Test strips showing a high background level should not be interpreted, especially if band intensities are lighter than the background level.
7. **In vitro** diagnostics must not be used beyond expiration date as reliable results may not be possible.
8. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false results.

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Symbols used

	Manufacturer		Order Number
	Refer to Instructions for Use		Use by / Expiration Date
	<i>In-Vitro</i> Diagnostic Medical Device		Temperature Limitation (Storage)
	Lot Number		Positive Serum Control
	Sufficient for 50 Tests		Negative Serum Control
	Room Temperature in °C		Control
	Person in Charge		Date
	Serum Number		Chromogen/Substrate Incubation Time in Minutes
	Evaluation Protocol		Protocol Number

Borrelia VirStripe® IgM Test Kit



Stripe-Immunoblot for the qualitative detection of **IgM** antibodies against specific **Borrelia species** antigens in human.

The Borrelia VirStripe® IgM Test Kit is an **immunoblot** based on an enzyme-immunoassay in the line/strip format, carrying highly purified specific native antigens from *Borrelia afzelii* (Pko) and *Borrelia burgdorferi sensu stricto* as well as recombinant VlsE. The Borrelia VirStripe® IgM Test Kit is manufactured according to the guidelines **98/79/EG** and **DIN 58967-40**.

The Borrelia VirStripe® IgM Test Kit fulfils the quality standards of the microbiological-infectiological guidelines ("MiQ" **12/2000**), the general DIN recommendation (**DIN 58967-40**) and can be evaluated according to "MiQ" **12/2000** (18) and **DIN 58969-44** (5) criteria. These guidelines postulate a one-band criterion for a positive result and describe special requirements for the detection of antibodies against *Borrelia burgdorferi*.

Principle of the assay

During the serum incubation step *Borrelia* specific IgM antibodies bind to the immobilised antigens on the test strip. During the conjugate reaction, AP-conjugate binds to the antigen-antibody complex. The alkaline phosphatase converts the chromogen/substrate and thus, stains the antigen-antibody complex on the test strip purple. The washing procedures following serum, conjugate and chromogen/substrate incubation steps remove unbound antibodies and reagents.

The green separation line divides the test strip into a control section and an analytical section. The control section contains the **negative control band**, the **serum control**, **three conjugate controls** (IgG, IgA, IgM) and the **cut off control**.

The test strip code for Borrelia VirStripe® IgM test strips is **BM**. Test strips are numbered from **01** to **50**. The analytical section contains the *Borrelia* specific antigens.

Order No.:	V-BSSMOK	Order No.:	V-BSSMDK (Deca Kit)
Kit size:	1x 50 test strips	Kit size:	10x 50 test strips
Specimen:	20 µl serum	Specimen:	20 µl serum
Time for testing:	approx. 90 minutes	Time for testing:	approx. 90 minutes

Materials provided

1x or 10x 50 test strips	Borrelia VirStripe® Antigen Strips (IgM) Test strips including a control section and <i>Borrelia</i> specific antigens in the analytical section, ready to use	(Prod. No.: V-BSSMAS)
1x or 10x 9 ml	ViraStripe® / ViraBlot® AP-Anti-Human IgM Conjugate Concentrate , goat	(Order No.: V-UVNMKI)
1x or 10x 100 ml	ViraStripe® / ViraBlot® Diluent / Wash Buffer 10x concentrate	(Order No.: V-UVNUWP)
1x or 10x 5 g	ViraStripe® / ViraBlot® Diluent / Wash Powder	(Order No.: V-UVNUPM)
1x or 10x 90 ml	ViraStripe® / ViraBlot® Chromogen / Substrate Solution Ready to use	(Order No.: V-UVNUCS)
1 or 10 copies	Evaluation Protocol for Borrelia VirStripe® IgM Test Kit	

Additionally available

330 µl	Borrelia VirStripe® IgM Positive Control Human, ready to use	(Order No.: V-BSSMPK)
330 µl	Borrelia VirStripe® IgG,A,M Negative Control Human, ready to use	(Order No.: V-BSSPNK)
50 copies	Borrelia VirStripe® IgM evaluation protocols for automated interpretation with ViraScan® software	(Order No.: V-BSSMEP)

Preparation of reagents and patient samples

Bring all reagents to room temperature (20-25°C) prior to use. Information about stability can be found on page 5.

Diluent / Wash Buffer Working Dilution: Dilute **Diluent / Wash Buffer Concentrate 1:10** with distilled or deionised water (100 ml concentrate + 900 ml water). Add Diluent / Wash Powder completely and stir well until all powder is dissolved. If needed, place onto a magnetic stirrer for 10-15 minutes. The pH value should be around pH 7.5 at 20°C.

Antigen Strips: Carefully separate the required number of test strips by use of **forceps** at the **label** and place the test strips in the prepared incubation tray (see assay procedure, step 2). Use test strips directly after removing from packing. Do not touch test strips by hand. Return unused test strips directly into the original packing, seal well and store at 2-8°C.

Patient samples: Use **20 µl patient serum** undiluted per test strip.

Controls: Use **100 µl of Positive Control** or **100 µl of Negative Control** undiluted per test strip respectively.

Conjugate Working Dilution: Prepare **Conjugate Concentrate 1:10** with Diluent / Wash Buffer Working Dilution (see table 1). Prepare freshly prior to each test run. Do not store for further use.

Chromogen / Substrate Solution: Ready to use.

Borrelia ViraStripe® IgM Test Kit

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Preparation of Conjugate Working Dilution IgM

Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume	Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume
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 1: 1:10 dilution of conjugate concentrate with Diluent / Wash Buffer Working Dilution

Assay procedure

1. Rinse incubation tray channels once with 1.5 ml Diluent / Wash Buffer Working Dilution, decant the liquid
2. Place the needed amount of test strips into the incubation tray - one test strip per channel
3. Add 1.5 ml Diluent / Wash Buffer Working Dilution and incubate by rocking for 5 minutes at room temperature (RT)
4. Add 20 µl of each patient serum or 100 µl of each control
5. Incubate by rocking for 30 minutes at RT
6. Decant the liquid
7. 3 x washing:
 - add 1.5 ml Diluent / Wash Buffer Working Dilution
 - incubate by rocking for 5 minutes at RT
 - decant the liquid
8. Add 1.5 ml fresh Conjugate Working Dilution
9. Incubate by rocking for 15 minutes at RT
10. Decant the liquid
11. 3 x washing as in step 7
12. Add 1.5 ml distilled or deionised water and incubate by rocking for 1 minute at RT
13. Decant the liquid
14. Add 1.5 ml Chromogen / Substrate Solution
15. Incubate by rocking at RT

Borrelia ViraStripe® IgM: approx. 5 to 15 minutes
16. Stop the reaction by decanting the liquid
17. Wash 3 x with 1.5 ml distilled or deionised water
18. Dry test strips for interpretation

Mark the trays with water-resistant pen. Rinsing removes dust particles.

For each patient serum and each control, carefully separate one test strip by use of forceps at the label and place them into the incubation tray channels. **The side showing the green separation line and the label must face up.**

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min. Avoid spilling of liquid. **Do not decant the liquid after incubation.**

Add patient sera and controls directly onto the labelled end of the test strips while the 2D rocker is running or make sure to tilt the incubation tray after adding each serum.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. **Test strips adhere to the incubation tray when liquid is decanted.**

Wash on the 2D rocker. Carefully tap the incubation tray on absorbent paper to remove the remaining liquid.

Make sure the test strips are completely covered with Conjugate Working Dilution

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Wash on the 2D rocker.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Make sure the test strips are completely covered with liquid.

Stop the reaction as soon as the cut off control becomes visible. **The Cut off control is located in the test strip control section. Caution:** Prolonged incubation causes background staining.

Carefully tap the incubation tray on absorbent paper.

Wash without incubation time.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. Place wet test strips with forceps on unbleached absorbent paper and allow to air dry before interpretation.

Assay interpretation

1. Evaluation protocol: Record data on the evaluation protocol. Glue the test strips on the evaluation protocol. Place the green separation line of the test strips exactly onto the separation line printed on the evaluation protocol.
2. Validity of test strips: A test strip is considered as valid if the following bands are visible:
 - The **serum control**.
 - The **conjugate control** of the conjugate class being used. If more than one of the three conjugate controls becomes visible, the strongest band must indicate the appropriate conjugate class.
 - The **cut off control**.

and if the following band is **not** visible:

 - The **negative control band**.

Do not assess invalid test strips!
3. Assignment of antigen bands: The green separation line of the test strips indicates position and orientation for the assignment of bands with the bandlocator on the evaluation protocol. Assign bands and record results according to 4.
4. Assessment of bands: According to quality laboratory guidelines, the use of a cut off control for each run is recommended (16). **The cut off control of the Borrelia ViraStripe® IgM is located in the control section on each test strip.** The intensity of the cut off control indicates the threshold of which bands are being assessed:
 A band is considered as **distinct** if its intensity is **equal** to or **higher** than the intensity of the cut off control. Mark bands with **X** in the evaluation protocol appropriately.
 A band is not assessed if it is **not present** or if its intensity is **lower** than the intensity of the cut off control.
5. Interpretation of patient bands: Patient bands have to be considered as symptoms of the disease. A final clinical diagnosis should always be made considering anamnesis, clinical manifestations and laboratory data (24).
 "If the pattern of reactive bands meets the specific conditions, the result is positive, i.e. the positive result of an EIA or another test of the first step is confirmed. If, despite the presence of specific diagnostic bands, the criteria for a positive result are not fulfilled, the result is considered equivocal. In such a case a follow-up control may be recommended." (18)
 Bands of the following antigens are considered as **highly specific** for Borrelia species: **p41** (limited specificity), **p39**, **OspC**, **Osp17** and **VisE**

IgM Interpretation criteria

General note: Distinct bands must have a minimum intensity ($\geq$ cut off), which has to be determined by the cut off control. The cut off control is located in the control section of each test strip.

Identified bands	Result	Interpretation
At least one distinct band out of: p39, OspC, Osp17, VisE or p41 higher than cut off	Positive	Specific antibodies against Borrelia species detectable. An infection with Borrelia species is probable.
No distinct bands or p41 equal to cut off	Negative	No specific antibodies against Borrelia species detectable. If an infection is suspected, check a second sample for IgM and IgG specific antibodies after 2-3 weeks.

Rheumatoid factor can affect the reactivity of antigen bands in IgM tests. In the case of unclear band constellation use RF-Absorbent (ViraSorb, 5 ml, Order No.: CB003) for IgM analysis.

IgM test strip

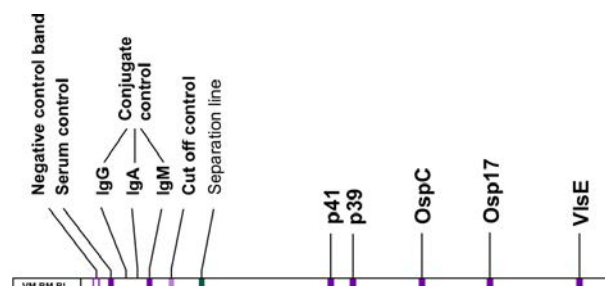


Figure 1: Schematic drawing of a Borrelia ViraStripe® IgM test strip in full scale.

Nomenclature and description of Borrelia species bands from literature

Band nomenclature:	Antigen:	Comments:
VlsE	Specific VlsE (<u>V</u> ariable major protein (VMP) <u>l</u> ike sequence <u>E</u> xpressed)	Antibodies against VlsE are described as specific. IgG antibodies are already being developed at an early stage and remain until the late stage of the disease. IgM antibodies against VlsE could appear as well in early stages but their serologic detection is less frequent (9).
p41	Limited specificity Flagellum protein	Cross-reactivities with other spirochetes and other flagellum carrying bacteria are described (2,15,22,23).
p39	Highly specific BmpA (<u>B</u> orrelia <u>m</u> embrane protein <u>A</u>)	In many patients antibodies against p39 are already detectable in the early stage of the disease (1,15).
OspC	Highly specific OspC (<u>O</u> uter <u>s</u> urface protein <u>C</u>)	At least 13 different immuno-distinct types of OspC are known. IgM antibodies against OspC are often the first Borrelia specific antibodies detectable in patients and may appear before IgM antibodies against p41 (1,8,12,13,21,22).
Osp17	Specific Osp17 (<u>O</u> uter <u>s</u> urface protein <u>17</u>)	Binding to decorin on the host cell. Antibodies against Osp17 are described as specific. The presence of IgG antibodies is associated among others with Arthritis and Neuroborreliosis. Species specific (9,10,19).

Diagnostic significance of antibodies against Borrelia species

- IgG antibodies** are produced for the first time several weeks to months after infection and are often not detectable in early stages of infection (22). In suspicion of a recent infection, IgM antibodies should be checked and a second sample should be analysed later. Patients in the 2nd or 3rd stage of the disease are usually positive for IgG antibodies. Antibody titers decrease gradually during convalescence (22).
- IgM antibodies** usually appear 2-3 weeks after onset of the disease for the first time (22). Antibody titers often decline several weeks to months after convalescence. But they may also persist up to several years (7,11,20).
- IgA antibodies** are detectable at an early stage of borreliosis in many patients, in some cases earlier than IgM antibodies.
- The immune response and consequently the band pattern differs from patient to patient. As a general rule: The number of

- antibody types and therefore the number of specific bands is increasing with progression of the disease (1).
- An early antibiotic therapy can suppress the development of antibodies (17).
 - Medication and immunoglobulin therapy can cause unspecific antibody reactions (24).
 - Cross reactivities to Borrelia antigens are described for infections with Treponema, Leptospira and other bacteria with flagella (2,15,22). An acute EBV infection can cause a polyclonal stimulation of Borrelia antibodies (22). If IgM antibodies against OspC or p41 are detected without clinical symptoms for borreliosis an EBV infection needs to be tested for. Cross reactivities in cases of autoimmune diseases, MS, ALS, Influenza and Syphilis are described as well.

IgM performance data

Sensitivity:

156 sera from patients with the Lyme borreliosis manifestations Erythema migrans, Erythema chronicum migrans, multiple Erythematosa migratoria, Acrodermatitis chronica atrophicans, Lyme arthritis and neuroborreliosis were assessed to determine the sensitivity of the Borrelia ViraStripe® IgM Test Kit.

Stage of Lyme borreliosis	Borrelia ViraStripe® IgM, % (n)	Borrelia ViraStripe® IgG / IgM, % (n)
Erythema migrans (n= 29)	69% (20)	79% (23)
Multiple Erythematosa migratoria (n= 13)	77% (10)	85% (11)
Erythema chronicum migrans (n= 27)	52% (14)	96% (26)
Neuroborreliosis (n= 24)	75% (18)	96% (23)
Acrodermatitis chronica atrophicans (n= 34)	53% (18)	97% (33)
Lyme arthritis (n= 29)	66% (19)	100% (29)

Borrelia ViraStripe® IgM Test Kit

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Specificity:

To determine the specificity of the Borrelia ViraStripe® IgM Test Kit 129 sera from blood donors showing a negative result with a reference test (Borrelia „MiQ“ + VlsE ViraBlot® IgM and IgG Test Kit) were assessed.

Collective	Borrelia ViraStripe® IgM, % (n)	Borrelia ViraStripe® IgG / IgM, % (n)
Blood donors (n= 129)	98% (126)	97% (125)

Warnings and precautions

1. All human serum components were tested for HCV, HIV1,2 antibodies and HBs antigens and found to be negative. Nevertheless, all human kit components as well as the patient samples should be considered as potentially infectious and handled according to safety precautions. While working with potentially infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be taken into account. This also applies to storage and disposal of chemicals and reagents being used.

2. While working with hazardous or toxic substances/ biological agents precautions have to be applied following national biosafety regulations. In general, biological and chemical agents should be handled according to „Good Laboratory Practice (GLP)“ guidelines. Precautions among others are:

- Do not pipette by mouth.
- Wear disposable gloves and safety glasses while working.
- Do not eat, drink or smoke in the working area.

3. The chromogen/substrate solution contains BCIP and NBT. Avoid contact with skin and mucous membranes. In case of contact with skin and eyes wash immediately with large quantities of water.

4. Samples and all potentially contaminated materials must be decontaminated using validated laboratory techniques, e.g. by autoclaving 20 minutes at 121°C under humid conditions. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite. Incubate 30 min for complete disinfection.

5. Please refer to material safety data sheets for detailed information on potential risks, first aid guidelines, accidental release measures, handling and storage recommendations, personal protective equipment, directions for disposal and indications to toxicology.

Storage and stability of reagents

1. **Test strips:** In closed bags stable until the expiration date if stored at 2-8°C.

2. **Conjugate Concentrate:** Stable until the expiration date if stored at 2-8°C.

3. **Conjugate Working Dilution:** Prepare freshly prior to each run. Do not store for further use.

4. **Diluent / Wash Buffer Concentrate, 10x:** Stable until the expiration date if stored at 2-8°C.

5. **Diluent / Wash Buffer Working Dilution:** Stable for 2 weeks if stored at 2-8°C. For longer storage, aliquot and freeze at -20°C.

6. **Diluent / Wash Powder:** Stable until the expiration date if stored at 2-8°C.

7. **Chromogen / Substrate Solution:** Stable until the expiration date if stored at 2-8°C. Avoid exposure to light!

Specimen indications

1. The **Borrelia ViraStripe® IgM Test Kit** must be used with human serum.

2. Only clear, non-hemolysed, non-microbially contaminated specimens must be used.

3. Using icteric, lipemic, hemolytic and/or heat-inactivated serum may lead to false results.

4. Normally, human serum can be stored up to 5 days at 2-8°C. Specimens may be stored at -20°C (or below) for long term storage.

5. Prior test processing, specimens should have reached room temperature. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.

6. Avoid multiple freeze and thaw cycles.

Limitation of the procedure

1. To ensure reliable results, follow carefully the Instruction for Use and "Good Laboratory Practice".

2. A positive result is based on elevated specific antibody titers and should be considered as a symptom. The correlation to a disease is only conditionally possible.

3. A negative result does not exclude a contact with the pathogen or the presence of a disease.

4. Adequately trained personnel only should perform the assay procedure.

5. The detection of specific antibodies can vary within different assays from different manufacturers and can lead to different results due to different sensitivity, specificity and assay methodologies.

6. Test strips showing a high background level should not be interpreted, especially if band intensities are lighter than the background level.

7. **In vitro** diagnostics must not be used beyond expiration date as reliable results may not be possible.

8. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false results.

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Symbols used

	Manufacturer		Order Number
	Refer to Instructions for Use		Use by / Expiration Date
	<i>In-Vitro</i> Diagnostic Medical Device		Temperature Limitation (Storage)
	Test Kit Lot Number		Positive Serum Control
	Sufficient for 50 Tests		Negative Serum Control
	Room Temperature in °C		Control
	User		Date
	Serum Number		Chromogen/Substrate Incubation Time in Minutes
	Evaluation Protocol		Protocol Number



Borrelia ViraStripe® IgG Test Kit

Stripe-Immunoblot for the qualitative detection of **IgG** antibodies against specific **Borrelia species** antigens in human.

The Borrelia ViraStripe® IgG Testkit is an **immunoblot** based on an enzyme-immunoassay in the line/stripe format, carrying highly purified specific native antigens from *Borrelia afzelii* (Pko) and *Borrelia burgdorferi sensu stricto* as well as recombinant VlsE. The Borrelia ViraStripe® IgG Testkit is manufactured according to the guidelines **98/79/EG** and **DIN 58967-40**.

The Borrelia ViraStripe® IgG Testkit fulfils the quality standards of the microbiological-infectiological guidelines ("**MiQ**" **12/2000**), the general DIN recommendation (**DIN 58967-40**) and can be evaluated according to "**MiQ**" **12/2000** (18) and **DIN 58969-44** (5) criteria. These guidelines postulate a two-band criterion for a positive result and describe special requirements for the detection of antibodies against *Borrelia burgdorferi*.

Principle of the assay

During the serum incubation step *Borrelia* specific IgG antibodies bind to the immobilised antigens on the test strip. During the conjugate reaction, AP-conjugate binds to the antigen-antibody complex. The alkaline phosphatase converts the chromogen/substrate and thus, stains the antigen-antibody complex on the test strip purple. The washing procedures following serum, conjugate and chromogen/substrate incubation steps remove unbound antibodies and reagents.

The green separation line divides the test strip into a control section and an analytical section. The control section contains the **negative control band**, the **serum control**, **three conjugate controls** (IgG, IgA, IgM) and the **cut off control**.

The test strip code for Borrelia ViraStripe® IgG test strips is **BG**. Test strips are numbered from **01** to **50**. The analytical section contains the *Borrelia* specific antigens.

Order No.:	V-BSSGOK	Order No.:	V-BSSGDK (Deca Kit)
Kit size:	1x 50 test strips	Kit size:	10x 50 test strips
Specimen:	20 µl serum	Specimen:	20 µl serum
Time for testing:	approx. 90 minutes	Time for testing:	approx. 90 minutes

Materials provided

1x or 10x 50 test strips	Borrelia ViraStripe® Antigen Strips (IgG) Test strips including a control section and <i>Borrelia</i> specific antigens in the analytical section, ready to use	(Prod. No.: V-BSSGAS)
1x or 10x 9 ml	ViraStripe® / ViraBlot® AP-Anti-Human IgG Conjugate Concentrate , goat	(Order No.: V-UVNGKI)
1x or 10x 100 ml	ViraStripe® / ViraBlot® Diluent / Wash Buffer 10x concentrate	(Order No.: V-UVNUWP)
1x or 10x 5 g	ViraStripe® / ViraBlot® Diluent / Wash Powder	(Order No.: V-UVNUPM)
1x or 10x 90 ml	ViraStripe® / ViraBlot® Chromogen / Substrate Solution Ready to use	(Order No.: V-UVNUCS)
1 or 10 copies	Evaluation Protocol for Borrelia ViraStripe® IgG Testkit	

Additionally available

330 µl	Borrelia ViraStripe® IgG Positive Control Human, ready to use	(Order No.: V-BSSGPK)
330 µl	Borrelia ViraStripe® IgG,A,M Negative Control Human, ready to use	(Order No.: V-BSSPNK)
50 copies	Borrelia ViraStripe® IgG evaluation protocols for automated interpretation with ViraScan® software	(Order No.: V-BSSGEP)

Preparation of reagents and patient samples

Bring all reagents to room temperature (20-25°C) prior to use. Information about stability can be found on page 5.

Diluent / Wash Buffer Working Dilution: Dilute **Diluent / Wash Buffer Concentrate 1:10** with distilled or deionised water (100 ml concentrate + 900 ml water). Add Diluent / Wash Powder completely and stir well until all powder is dissolved. If needed, place onto a magnetic stirrer for 10-15 minutes. The pH value should be around pH 7.5 at 20°C.

Antigen Strips: Carefully separate the required number of test strips by use of **forceps** at the **label** and place the test strips in the prepared incubation tray (see assay procedure, step 2). Use test strips directly after removing from packing. Do not touch test strips by hand. Return unused test strips directly into the original packing, seal well and store at 2-8°C.

Patient samples: Use **20 µl patient serum** undiluted per test strip.

Controls: Use **100 µl of Positive Control** or **100 µl of Negative Control** undiluted per test strip respectively.

Conjugate Working Dilution: Prepare **Conjugate Concentrate 1:10** with Diluent / Wash Buffer Working Dilution (see table 1). Prepare freshly prior to each test run. Do not store for further use.

Chromogen / Substrate Solution: Ready to use.

Borrelia ViraStripe® IgG Test Kit

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Preparation of Conjugate Working Dilution IgG

Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume	Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume
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 1: 1:10 dilution of conjugate concentrate with Diluent / Wash Buffer Working Dilution

Assay procedure

1. Rinse incubation tray channels once with 1.5 ml Diluent / Wash Buffer Working Dilution, decant the liquid
2. Place the needed amount of test strips into the incubation tray - one test strip per channel
3. Add 1.5 ml Diluent / Wash Buffer Working Dilution and incubate by rocking for 5 minutes at room temperature (RT)
4. Add 20 µl of each patient serum or 100 µl of each control
5. Incubate by rocking for 30 minutes at RT
6. Decant the liquid
7. 3 x washing:
 - add 1.5 ml Diluent / Wash Buffer Working Dilution
 - incubate by rocking for 5 minutes at RT
 - decant the liquid
8. Add 1.5 ml fresh Conjugate Working Dilution
9. Incubate by rocking for 15 minutes at RT
10. Decant the liquid
11. 3 x washing as in step 7
12. Add 1.5 ml distilled or deionised water and incubate by rocking for 1 minute at RT
13. Decant the liquid
14. Add 1.5 ml Chromogen / Substrate Solution
15. Incubate by rocking at RT
Borrelia ViraStripe® IgG: approx. 5 to 15 minutes
16. Stop the reaction by decanting the liquid
17. Wash 3 x with 1.5 ml distilled or deionised water
18. Dry test strips for interpretation

Mark the trays with water-resistant pen. Rinsing removes dust particles.

For each patient serum and each control, carefully separate one test strip by use of forceps at the label and place them into the incubation tray channels. **The side showing the green separation line and the label must face up.**

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min. Avoid spilling of liquid. **Do not decant the liquid after incubation.**

Add patient sera and controls directly onto the labelled end of the test strips while the 2D rocker is running or make sure to tilt the incubation tray after adding each serum.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. **Test strips adhere to the incubation tray when liquid is decanted.**

Wash on the 2D rocker. Carefully tap the incubation tray on absorbent paper to remove the remaining liquid.

Make sure the test strips are completely covered with Conjugate Working Dilution

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Wash on the 2D rocker.

Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.

Carefully tap the incubation tray on absorbent paper.

Make sure the test strips are completely covered with liquid.

Stop the reaction as soon as the cut off control becomes visible. **The Cut off control is located in the test strip control section. Caution:** Prolonged incubation causes background staining.

Carefully tap the incubation tray on absorbent paper.

Wash without incubation time.

Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. Place wet test strips with forceps on unbleached absorbent paper and allow to air dry before interpretation.

Borrelia ViraStripe® IgG Test Kit

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Assay interpretation

1. Evaluation protocol: Record data on the evaluation protocol. Glue the test strips on the evaluation protocol. Place the green separation line of the test strips exactly onto the separation line printed on the evaluation protocol.
2. Validity of test strips: A test strip is considered as valid if the following bands are visible:
 - The **serum control**.
 - The **conjugate control** of the conjugate class being used. If more than one of the three conjugate controls becomes visible, the strongest band must indicate the appropriate conjugate class.
 - The **cut off control**.

and if the following band is **not** visible:

 - The **negative control band**.

Do not assess invalid test strips!
3. Assignment of antigen bands: The green separation line of the test strips indicates position and orientation for the assignment of bands with the bandlocator on the evaluation protocol. Assign bands and record results according to 4.
4. Assessment of bands: According to quality laboratory guidelines, the use of a cut off control for each run is recommended (16). **The cut off control of the Borrelia ViraStripe® IgG is located in the control section on each test strip.** The intensity of the cut off control indicates the threshold of which bands are being assessed:
 A band is considered as **distinct** if its intensity is **equal** to or **higher** than the intensity of the cut off control. Mark bands with **X** in the evaluation protocol appropriately.
 A band is not assessed if it is **not present** or if its intensity is **lower** than the intensity of the cut off control.
5. Interpretation of patient bands: Patient bands have to be considered as symptoms of the disease. A final clinical diagnosis should always be made considering anamnesis, clinical manifestations and laboratory data (24).
 “If the pattern of reactive bands meets the specific conditions, the result is positive, i.e. the positive result of an EIA or another test of the first step is confirmed. If, despite the presence of specific diagnostic bands, the criteria for a positive result are not fulfilled, the result is considered equivocal. In such a case a follow-up control may be recommended.” (18)
 Bands of the following antigens are considered as **highly specific** for Borrelia species:
p83, p58, p43, p39, p30, OspC, p21, Osp17/DbpA, p14 and VlsE.

IgG Interpretation criteria

General note: Distinct bands must have a minimum intensity ($\geq$ cut off), which has to be determined by the cut off control. The cut off control is located in the control section of each test strip.

Identified bands	Result	Interpretation
At least two distinct bands out of: p83, p58, p43, p39, p30, OspC, p21, Osp17 or DbpA, p14, VlsE	Positive	Specific antibodies against Borrelia species detectable. An infection with Borrelia species is probable.
One distinct VlsE band	Equivocal	Specific antibodies against VlsE detectable. An infection with Borrelia species is possible. If a recent infection is suspected, check additionally for IgM antibodies. If possible test a second sample for IgM and IgG specific antibodies after 2-3 weeks.
One or no distinct bands (Exception: singular VlsE band)	Negative	No specific antibodies against Borrelia species detectable. If an infection is suspected, check a second sample for IgM and IgG specific antibodies after 2-3 weeks.

IgG test strip

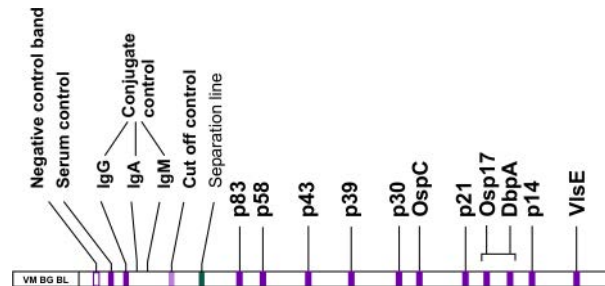


Figure 1: Schematic drawing of a Borrelia ViraStripe® IgG test strip in full scale.

Nomenclature and description of *Borrelia* species bands from literature

Band nomenclature:	Antigen:	Comments:
VisE	Specific VisE (<u>V</u> ariable major protein (VMP) <u>l</u> ike <u>s</u> equences <u>E</u> xpressed)	Antibodies against VisE are described as specific. IgG antibodies are already being developed at an early stage and remain until the late stage of the disease (9).
p83/100	Highly specific protein of membrane vesicles on the cell surface	Generally these antibodies appear 6-12 weeks after infection. They are typical for stage III, but might also appear in stages I and II (1,4,14,15).
p58	Specific	Protein not well characterised, separated from 60 kD (Hsp60), appears often in stage III (6).
p43	Specific	Still little characterised. Mainly described for stage III.
p39	Highly specific BmpA (<u>B</u> orrelia <u>m</u> embrane protein <u>A</u>)	In many patients antibodies against p39 are already detectable in the early stage of the disease (1,15).
p30	Specific	At present protein not well characterised (6).
OspC	Highly specific OspC (<u>O</u> uter <u>s</u> urface protein <u>C</u>)	At least 13 different immuno-distinct types of OspC are known (1,8,12,13,21,22).
p21	Specific	Still little characterised (3).
Osp17 / DbpA	Specific Osp17 (<u>O</u> uter surface protein <u>17</u>) / DbpA (<u>D</u> ecorin binding protein <u>A</u>)	Binding to decorin on the host cell. Antibodies against Osp17/DbpA are described as specific. The presence of IgG antibodies is associated among others with Arthritis and Neuroborreliosis. Species specific (9,10,19).
p14	Specific	Protein from Borrelia afzelii is characterised as specifically immunogenic (7).

Diagnostic significance of antibodies against *Borrelia* species

1. **IgG antibodies** are produced for the first time several weeks to months after infection and are often not detectable in early stages of infection (22). In suspicion of a recent infection, IgM antibodies should be checked and a second sample should be analysed later. Patients in the 2nd or 3rd stage of the disease are usually positive for IgG antibodies. Antibody titers decrease gradually during convalescence (22).
2. **IgM antibodies** usually appear 2-3 weeks after onset of the disease for the first time (22). Antibody titers often decline several weeks to months after convalescence. But they may also persist up to several years (7,11,20).
3. **IgA antibodies** are detectable at an early stage of borreliosis in many patients, in some cases earlier than IgM antibodies.
4. The immune response and consequently the band pattern differs from patient to patient. As a general rule: The number of antibody types and therefore the number of specific bands is increasing with progression of the disease (1).
5. An early antibiotic therapy can suppress the development of antibodies (17).
6. Medication and immunoglobulin therapy can cause unspecific antibody reactions (24).
7. Cross reactivities to *Borrelia* antigens are described for infections with *Treponema*, *Leptospira* and other bacteria with flagella (2,15,22). An acute EBV infection can cause a polyclonal stimulation of *Borrelia* antibodies (22). If IgM antibodies against OspC or p41 are detected without clinical symptoms for borreliosis an EBV infection needs to be tested for. Cross reactivities in cases of autoimmune diseases, MS, ALS, Influenza and Syphilis are described as well.

Borrelia ViraStripe® IgG Testkit

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IgG performance data

Sensitivity:

156 sera from patients with the Lyme borreliosis manifestations Erythema migrans, Erythema chronicum migrans, multiple Erythema migrans, Acrodermatitis chronica atrophicans, Lyme arthritis and neuroborreliosis were assessed to determine the sensitivity of the Borrelia ViraStripe® IgG Testkit.

Stage of Lyme borreliosis	Borrelia ViraStripe® IgG, % (n)	Borrelia ViraStripe® IgG / IgM, % (n)
Erythema migrans (n= 29)	48% (14)	79% (23)
Multiple Erythema migrans (n= 13)	85% (11)	85% (11)
Erythema chronicum migrans (n= 27)	89% (24)	96% (26)
Neuroborreliosis (n= 24)	92% (22)	96% (23)
Acrodermatitis chronica atrophicans (n= 34)	97% (33)	97% (33)
Lyme arthritis (n= 29)	100% (29)	100% (29)

Specificity:

To determine the specificity of the Borrelia ViraStripe® IgG Testkit 129 sera from blood donors showing a negative result with a reference test (Borrelia „MiQ“ + VlsE ViraBlot® IgM and IgG Testkit) were assessed.

Collective	Borrelia ViraStripe® IgG, % (n)	Borrelia ViraStripe® IgG / IgM, % (n)
Blood donors (n= 129)	99% (128)	97% (125)

Warnings and precautions

1. All human serum components were tested for HCV, HIV1,2 antibodies and HBs antigens and found to be negative. Nevertheless, all human kit components as well as the patient samples should be considered as potentially infectious and handled according to safety precautions. While working with potentially infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be taken into account. This also applies to storage and disposal of chemicals and reagents being used.

2. While working with hazardous or toxic substances/ biological agents precautions have to be applied following national biosafety regulations. In general, biological and chemical agents should be handled according to „Good Laboratory Practice (GLP)“ guidelines. Precautions among others are:

- Do not pipette by mouth.
- Wear disposable gloves and safety glasses while working.
- Do not eat, drink or smoke in the working area.

3. The chromogen/substrate solution contains BCIP and NBT. Avoid contact with skin and mucous membranes. In case of contact with skin and eyes wash immediately with large quantities of water.

4. Samples and all potentially contaminated materials must be decontaminated using validated laboratory techniques, e.g. by autoclaving 20 minutes at 121°C under humid conditions. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite. Incubate 30 min for complete disinfection.

5. Please refer to material safety data sheets for detailed information on potential risks, first aid guidelines, accidental release measures, handling and storage recommendations, personal protective equipment, directions for disposal and indications to toxicology.

Storage and stability of reagents

1. **Test strips:** In closed bags stable until the expiration date if stored at 2-8°C.

2. **Conjugate Concentrate:** Stable until the expiration date if stored at 2-8°C.

3. **Conjugate Working Dilution:** Prepare freshly prior to each run. Do not store for further use.

4. **Diluent / Wash Buffer Concentrate, 10x:** Stable until the expiration date if stored at 2-8°C.

5. **Diluent / Wash Buffer Working Dilution:** Stable for 2 weeks if stored at 2-8°C. For longer storage, aliquot and freeze at -20°C.

6. **Diluent / Wash Powder:** Stable until the expiration date if stored at 2-8°C.

7. **Chromogen / Substrate Solution:** Stable until the expiration date if stored at 2-8°C. Avoid exposure to light!

Specimen indications

1. The **Borrelia ViraStripe® IgG Testkit** must be used with human serum.

2. Only clear, non-hemolysed, non-microbially contaminated specimens must be used.

3. Using icteric, lipemic, hemolytic and/or heat-inactivated serum may lead to false results.

4. Normally, human serum can be stored up to 5 days at 2-8°C. Specimens may be stored at -20°C (or below) for long term storage.

5. Prior test processing, specimens should have reached room temperature. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.

6. Avoid multiple freeze and thaw cycles.

Borrelia ViraStripe® IgG Testkit

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Limitation of the procedure

1. To ensure reliable results, follow carefully the Instruction for Use and "Good Laboratory Practice".
2. A positive result is based on elevated specific antibody titers and should be considered as a symptom. The correlation to a disease is only conditionally possible.
3. A negative result does not exclude a contact with the pathogen or the presence of a disease.
4. Adequately trained personnel only should perform the assay procedure.
5. The detection of specific antibodies can vary within different assays from different manufacturers and can lead to different results due to different sensitivity, specificity and assay methodologies.
6. Test strips showing a high background level should not be interpreted, especially if band intensities are lighter than the background level.
7. *In vitro* diagnostics must not be used beyond expiration date as reliable results may not be possible.
8. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false results.

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Symbols used

	Manufacturer		Order Number
	Refer to Instructions for Use		Use by / Expiration Date
	<i>In-Vitro</i> Diagnostic Medical Device		Temperature Limitation (Storage)
	Test Kit Lot Number		Positive Serum Control
	Sufficient for 50 Tests		Negative Serum Control
	Room Temperature in °C		Control
	User		Date
	Serum Number		Chromogen/Substrate Incubation Time in Minutes
	Evaluation Protocol		Protocol Number