From: s22
To: s22
Cc: PGC Quality; s22
Subject: FW: DIR 90606 - ARTG 205351 - s41JA Requirement for Information for a DIR letter [SEC=OFFICIAL]
Date: Friday, 16 February 2024 1:52:43 PM
Attachments: image001.png
image002.png
image003.gif
image004.png
image005.png
GSD Frankfurt Statement ticket_ 47516-signed.pdf
BORG0040-139 141 144_07022024_47516.pdf
certificate_2023 Labcode 6115.pdf
Labquality_Borrelia burgdorferi_Client Report_202311.pdf
BORG0040-2022-07-18_Ka-ab Lot137 (1).pdf

REMINDER: Think before you click! This email originated from outside our organisation. Only click links or open attachments if you recognise the sender and know the content is safe.

Good Afternoon s22

Please find attached manufacturers response as listed below:

- Response letter
- Retention sample test data
- External QA program certificate
- External QA program data
- IFU

Kind Regards

s22
s22
Paragon Care Group Australia Pty Ltd | 1/17 Rodborough Rd, Frenchs Forest, NSW 2086
M_s22 s22 E_s22 @paragoncare.com.au W_ paragoncare.com.au

Email-Signature-BECOVIDSAFE-Branded-PCG_940x165_v1

At Paragon we work flexibly, so whilst it suits me to email you now, I do not expect a response outside of your normal working hours.

Please consider the environment before printing this email.

This email and any attachments are intended only for the use of the individual or entity to which it is addressed and may contain proprietary information that is privileged, confidential or copyright. You are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited without the authority of the sender. If you have received this e-mail message in error or are not the intended recipient, please delete and destroy all copies and notify us immediately by return mail. Any loss or damage incurred as a result of prohibited use is not the sender's responsibility. E-mail communications are not guaranteed as secure or error free, the sender accepts no liability for any damage caused by this email or its attachments due to viruses, interference, interception, corruption or unauthorised access. The sender's entire liability is limited to resending this email.

From: s22 @health.gov.au>
Sent: Friday, February 2, 2024 10:47 AM

To: s22 @paragoncare.com.au>
Subject: DIR 90606 - ARTG 205351 - s41JA Requirement for Information for a DIR letter [SEC=OFFICIAL]

Dear s22

Good morning!

Please find attached the S41JA letter.

Your response is due 16 February 2024.

Kind regards,
s22

s22
Medical Devices Investigator – Devices Post Market Monitoring Section
Medical Devices Surveillance Branch

Medical Devices and Product Quality Division | Health Products Regulation Group
Australian Government, Department of Health and Aged Care
T: s22 | E: s22 @health.gov.au
Location: Melbourne, Victoria 3000
PO Box 100, Woden ACT 2606, Australia

The Department of Health and Aged Care acknowledges First Nations peoples as the Traditional Owners of Country throughout Australia, and their continuing connection to land, sea and community. We pay our respects to them and their cultures, and to all Elders both past and present.

Important: *This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error, please notify the author immediately and delete all copies of this transmission.*

"Important: This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error please notify the author immediately and delete all copies of this transmission."

FOI 26-3320 - Document 1

Re: Complaints Regarding Novalisa Borrelia burgdorferi IgG ELISA - False Negative Results
Ref. no.: E23-275158

Dear Therapeutic Goods Administration personnel,

We acknowledge receipt of your communication regarding the complaint about our Novalisa Borrelia burgdorferi IgG ELISA kit, with the product number BORG0040, manufactured by Gold Standard Diagnostics Frankfurt GmbH (formally: NovaTec Immundiagnostica GmbH). As part of our commitment to transparency and product integrity, we would like to address the concerns outlined in your communication.

Firstly, we clearly outline in the instruction for use (IFU) in section 8.3, that the "*Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data*".

Regarding the specific issues raised in your complaint, due to the lack of sufficient and critical information, such as lot number, the quantity and types of affected samples, and potential variables including but not limited to any possible mishandling by end-users, conducting a thorough investigation is challenging. Despite these limitations, we have proactively tested the retained kits from all lots currently in circulation with valid expiry dates. Our internal testing has confirmed the validity and reliability of these kits. Furthermore, we confirm that we have not received any similar complaints concerning this specific kit. We also wish to highlight our active participation in external quality control testing programs, where we have consistently met the required standards, as evidenced by the attached internal testing data and external quality testing certificates.

In light of the aforementioned factors, we consider this event to be a non-critical incident, particularly as it pertains to a single user and given the validation of our internal and external testing procedures, we see no valid re

Without further data or collaboratio are una to conduct a comprehensive investig ovid cific support for their complaint. We enco th the kit, who can offer direct assista urth complaint to us.

We remain committed to ensuring cy, and reliability of appreciate your diligence in bringing ns t n. Should you further clarification or assistance, ple hesi dire

Thank you for your cooperation and u rstan

Sincerely,

s22

Gold Standard Diagnostics Frankfurt GmbH
Waldstrasse 23 A6, 63128 Dietzenbach, Germany. Geschäftsführer/ Managing Director: s22
Registergericht/ Court of Registration: Offenbach, HRB 12095. USt-IdNr./ VAT No.: DE813273237. Deutsche Bank AG SWIFT: DEUTDEDBFRA - IBAN: DE20 5007 0024 0010 6120 00.
Tel: +49 6074 23698-0 | Fax: +49 6074 23698-900 | Email: info.frankfurt@eu.goldstandarddiagnostics.com | Website: clinical.goldstandarddiagnostics.com

Customer	Paragon Care Diagnostics
Complaint	false negative result
Product name	Borrelia burgdorferi IgG
Product code	BORG0040

NovaTec data

s47

Validation criteria

s47

Interpretation of the assay results

Negative	< 9 NTU
Grey zone	9 - 11 NTU
Positive	> 11 NTU

FOI 26-3320 - Document 1

LABQUALITY

CERTIFICATE

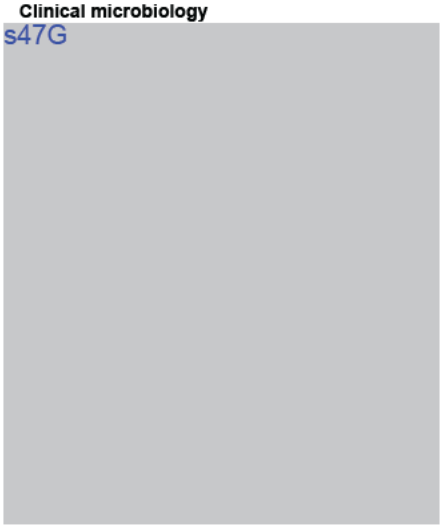
2023

Gold Standard Diag. Frankfurt GmbH

has participated in the External Quality Assessment Schemes organized by Labquality

Clinical microbiology

s47G



Issued 31.12.2023

This certificate is valid 12 months from the date issued.

*) EQA scheme is accredited according to ISO/IEC 17043 standard.

Please see participant´s report for each EQA round for performance data.

s22

EQAS CERTIFICATE LABQUALITY

ISO 9001:2015

FINAS Finnish Accreditation Service PT02 (EN ISO/IEC 17043)

s47G

FOI 26-3320 - Document 1

s47G

FOI 26-3320 - Document 1

s47G

FOI 26-3320 - Document 1

s47G

FOI 26-3320 - Document 1

s47G

FOI 26-3320 - Document 1

s47G

FOI 26-3320 - Document 1

s47G

FOI 26-3320 - Document 1

FOI 26-3320 - Document 1

Report Info

PARTICIPANTS

Altogether 113 laboratories from 19 countries participated in this EQA round.

REPORT INFO

The results are divided into groups according to the method stated by the laboratory and presented in laboratory-specific tables. Accepted results are marked with green color and laboratory's own result with a black radio button ◉. In the scoring report you will find summaries of overall success rate and sample specific success rates (%). Sample specific interpretations are shown in pie diagrams as percentages and the total interpretation and methodic counts in the tables. If you have not reported any results you will get a note: "You have not responded in time, only global report is available."

For information on report interpretation and performance evaluation, please see the "EQAS Interpretation guidelines" in LabScala User instructions. In case you have any questions regarding the reports, please contact the EQA Coordinator.

SCORING

The round is scored based on test results and clinical interpretations when 60% or more of the participants report the expected result and when at least three results are reported.

The following general rules are applied:

Correct/expected test result 2/2 points
False/deviating test result 0/2 points
Correct/expected clinical interpretation 4/4 points
False/deviating clinical interpretation 0/4 points

The performance of the laboratory is assessed by the Own success rate (%). The target is 100%. The examination-specific scores obtained by the laboratory in the round are converted to Own success rate per sample (scores/maximum scores*100). The Laboratory's Own success rate is the average of the sample success rates.

The success rate for the entire round (AVR success rate) is calculated from the total number of scores given to the results per sample (all scores/maximum scores*100). The AVG success rate of the entire round is the average of the sample success rates. The difference in the Laboratory's Own success rate (%) to the corresponding numbers for the entire round is shown in the table.

Copyright © Labquality Oy

NovaTec Immundiagnostica GmbH

NovaLisa®

Borrelia burgdorferi IgG

ELISA

CE

Only for in-vitro diagnostic use

English 2
Deutsch 7
Français 12
Italiano 17
Español 22
Português 27
Bibliography / Literatur / Bibliographie / Bibliografia / Bibliografía / Bibliografia 34
Abbreviations / Abkürzungen / Abréviations / Abbreviazioni / Abreviaciónes / Abreviaturas 34
Symbols Key / Symbolschlüssel / Explication des Symboles / Legenda / Símbolos / Tabela de símbolos 35
Summary of Test Procedure / Kurzanleitung Testdurchführung / Résumé de la procedure de test / Schema della procedura / Resumen de la técnica / Resumo do Procedimento de Teste 36

Product Number: BORG0040 (96 Determinations)

ENGLISH

1. INTRODUCTION

Spirochetes are motile bacteria with a periplasmatic axial filament. All pathogenic species belong to the family Treponemataceae, which includes the three genera: Treponema, Borrelia, and Leptospira. The Treponemae are extremely long, flexible, filamentous cells that are usually held in a characteristic spiral, or coiled-spring shape. Borreliae are the largest Treponemataceae with very coarse and irregular spirals. Borrelia burgdorferi, the causative agent of Lyme disease, is transmitted mainly by ticks but probably also by other blood-sucking insects. Habitats are the wooded, humid and temperate regions of North America, Europe, North Africa, Australia and Japan; foresters, farmers, and anybody entering infested forests may be affected.

The degree of contamination of ticks amounts to 3-60% dependent on seasonal and regional differences; up to 30% of the population may be infected (about 1500 cases annually in USA, several hundred in Europe).

Species	Disease	Symptoms (e.g.)	Transmission route
Borrelia burgdorferi	Lyme diseases	**Multisystemic illness** **Early manifestations:** Erythema migrans; flu-like symptoms like fever, myalgias and headaches; carditis; meningitis **Late manifestations:** Chronic neurological, dermatological and / or cardiological symptoms; Acrodermatitis chronica atrophicans; Herxheimer reaction; Lyme-Arthritis	Transmission by tick bite Europe: Ixodes ricinus USA: Ixodes dammini

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Borrelia burgdorferi IgG ELISA is intended for the qualitative determination of IgG class antibodies against Borrelia burgdorferi in human serum, plasma (citrate, heparin) and CSF (cerebrospinal fluid).
For the determination of intrathecally produced IgG antibodies a separate instruction for use for CSF (BORL0040) can be obtained at NovaTec.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with recombinant Borrelia burgdorferi antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with recombinant Borrelia burgdorferi antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum, plasma (citrate, heparin) and CSF (cerebrospinal fluid) samples with this assay. For CSF please use the instruction for use BORL0040. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems, we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.517	4.00
#2	24	1.790	4.23
#3	24	2.339	9.04
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	37.2	4.17
#2	12	45.3	9.28
#3	12	1.9	9.94

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 95.48% (95% confidence interval: 90.92% - 98.17%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 98.39% (95% confidence interval: 91.34% - 99.96%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

By the use of recombinant antigens, cross reactions with antibodies against the following pathogens can be excluded, as best as possible:

- Treponema pallidum
- Leptospira
- Borrelia recurrentis

A lues-infection should be excluded, because antibodies against p41i could appear.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: BORG0040 Borrelia burgdorferi IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Spirochäten sind spiralig gekrümmte, im Vergleich zu ihrem Durchmesser (0,1-3µm) unproportional lange (bis 250 µm) gram-negative Bakterien.

Alle pathogenen Spezies gehören zur Familie der Treponemataceae, zu denen folgende Gattungen zählen: Treponema, Borrelia und Leptospira. Die Treponemen sind schlanke, biegsame Bakterien gewöhnlich in charakteristischer Spiralform. Borrelien sind zarte (0,2-0,5µm), relativ lange (bis 250 µm) Spirochäten, mit 3-10 ungleichmäßigen Windungen.

Borrelia burgdorferi, der Verursacher der Borreliose-Erkrankung (Lyme-Erkrankung), wird hauptsächlich durch Zecken übertragen, möglicherweise auch durch andere blutsaugende Insekten. Hauptvektor in Europa ist die Schildzecke Ixodes ricinus, in den USA Ixodes dammini.

Außer B.burgdorferi verursachen auch B. garinii und B. afzelii Lyme-Borreliosen. Die Erkrankung verläuft klassischerweise in drei Stadien. Sie wurde 1975 in der Kleinstadt Lyme im US-Bundesstaat Connecticut beschrieben. Burgdorfer et al. konnten 1983 den Erreger erstmals isolieren.

Die Lyme-Borreliose ist eine weit verbreitete Krankheit. Eine echte Prophylaxe existiert nicht. Ca. 10-20 % der Zecken in Europa sind mit Borrelia burgdorferi durchseucht. Die Erkrankungen an Borreliose sind endemisch und treten z.B. in Süddeutschland, entlang von Flussläufen und im Einzugsgebiet großer Städte gehäuft auf. Den natürlichen Lebensraum stellen vor allem bewaldete, feucht-warme Regionen Nordamerikas, Europas, Nordafrikas, Australiens und Japans dar.

Saisonale Gipfel akuter Erkrankungen sind die Monate Juni bis September, weitgehend erkrankungsfreie Monate Januar bis März.

Bevorzugter Personenkreis sind Waldarbeiter, Förster, Jäger und in der Landwirtschaft tätige Personen.

Bedingt durch die Vielzahl möglicher uncharakteristischer Symptome (neurologische, dermatologische, kardiale, rheumatische Manifestationen möglich) ist die Diagnose der Lyme-Borreliose häufig schwer und erfolgt erst relativ spät.

Nur die frühzeitige Diagnosestellung jedoch ermöglicht eine effiziente Kontrolle der Erkrankung mittels Antibiotika, in der chronischen Phase sind die Borrelien nahezu unangreifbar.

Spezies	Erkrankung	Symptome (z.B.)	Infektionsweg
Borrelia burgdorferi	Lyme-Borreliose	**Multisystemerkrankung** **Frühmanifestation:** Erythema migrans; grippeähnliche Symptome wie Fieber, Myalgien und Kopfschmerzen; Karditis; Meningitis **Spätmanifestationen:** Chronische neurologische, dermatologische und/oder kardiologische Symptome; Akrodermatitis chronica atrophicans; Herxheimer Reaktion; Lyme arthritis	Übertragung durch Zeckenstich. Europa: Ixodes ricinus USA: Ixodes dammini

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie
- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Borrelia burgdorferi IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Borrelia burgdorferi in humanem Serum, Plasma (Citrat, Heparin) und Liquor bestimmt.
Für die Bestimmung intrathekal produzierter IgG Antikörper kann bei NovaTec eine separate Arbeitsanleitung (BORL0040) angefordert werden.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit rekombinanten Borrelia burgdorferi Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgG-Probenverdünnungspuffer:** 1 Flasche mit 100 mL Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; gelb gefärbt; gebrauchsfertig; weiße Verschlusskappe; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Stopplösung:** 1 Flasche mit 15 mL Schwefelsäure, 0,2 mol/L; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 mL eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weißer Verschlusskappen.
- **Konjugat:** 1 Flasche mit 20 mL Peroxidase-konjugierten Antikörpern gegen humanes IgG in Phosphatpuffer (10 mM); blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 mL 3,3`,5,5`-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe.
- **Positivkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Cut-off Kontrolle:** 1 Fläschchen mit 3 mL Kontrolle; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Negativkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37 °C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Mikropipetten (10 - 1000 µL)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit rekombinanten Borrelia burgdorferi Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 mL Waschpuffer + 190 mL destilliertes Wasser.
Der verdünnte Puffer ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum, Plasma- (Citrat, Heparin) und Liquor proben verwendet werden. Für Liquor ist die Arbeitsanleitung BORL0040 zu verwenden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70…-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden!
Hitzeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgG-Probenverdünnungspuffer verdünnen, z. B. 10 µL Probe und 1 mL IgG-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

Arbeitsanleitung vor Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei auf bis zu fünf und das Volumen des Waschpuffers von 300 µL auf 350 µL zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µL Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ± 1 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µL Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µL Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µL TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µL Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.1. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Damit ein Testlauf als valide betrachtet werden kann, muss diese Gebrauchsanweisung strikt befolgt werden, und die folgenden Kriterien müssen erfüllt sein:

- **Substrat-Leerwert**: Extinktionswert **< 0,100**
- **Negativkontrolle**: Extinktionswert **< 0,200 und < Cut-off**
- **Cut-off Kontrolle**: Extinktionswert **0,150 – 1,300**
- **Positivkontrolle**: Extinktionswert **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86 : 2 = 0,43

Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe x 10}}{\text{Cut-off}} = \text{[NovaTec Einheiten = NTU]}$$

Beispiel: $\frac{1{,}591 \text{ x } 10}{0{,}43} = 37 \text{ NTU}$

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als **negativ**.
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

9.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	**n**	**Mittelwert (E)**	**Vk (%)**
#1	24	0,517	4,00
#2	24	1,790	4,23
#3	24	2,339	9,04
Interassay	**n**	**Mittelwert (NTU)**	**Vk (%)**
#1	12	37,2	4,17
#2	12	45,3	9,28
#3	12	1,9	9,94

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 95,48% (95% Konfidenzintervall: 90,92% - 98,17%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 98,39% (95% Konfidenzintervall: 91,34% - 99,96%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/mL Hämoglobin, 5 mg/mL Triglyceride und 0,5 mg/mL Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Durch Verwendung rekombinanter Antigene werden Kreuzreaktionen mit Antikörpern gegen folgende Erreger weitestgehend ausgeschlossen:

- Treponema pallidum
- Leptospirap
- Borrelia recurrentis

Eine Syphilis-Infektion sollte ausgeschlossen werden, da vereinzelt Antikörper gegen p41i auftreten können.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung sind strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für qualifiziertes Personal bestimmt, das den Standards der Guten Laborpraxis (GLP) folgt.
- Zur weiteren internen Qualitätskontrolle sollte jedes Labor zusätzlich bekannte Proben verwenden.

12.1. Sicherheitshinweis für Reagenzien, die Gefahrstoffe enthalten

Die Reagenzien können CMIT/MIT (3:1) oder MIT enthalten (siehe 4.1)
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H317	Kann allergische Hautreaktionen verursachen.
P261	Einatmen von Aerosol vermeiden.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P333+P313	Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen.
P362+P364	Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen.

Weitere Informationen können dem Sicherheitsdatenblatt entnommen werden.

12.2. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: BORG0040 Borrelia burgdorferi IgG ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

Les spirochètes sont des bactéries mobiles avec un filament axial périplasmatique. Toutes les espèces pathogènes appartiennent à la famille Treponemataceae, qui comprend les trois genres: Treponema, Borrelia et Leptospira. Les Treponemae sont des cellules extrêmement longues, flexibles et filamenteuses qui sont habituellement maintenues dans une spirale caractéristique ou en forme de ressort enroulé. Borreliae est la plus grande des Treponemataceae aux spirales très grossières et ir
régulières. Borrelia burgdorferi, l'agent causal de la maladie de Lyme, est transmise principalement par des tiques, mais probablement aussi par d'autres insectes suceurs de sang. Les habitats sont les régions boisées, humides et tempérées d'Amérique du Nord, d'Europe, d'Afrique du Nord, d'Australie et du Japon; Les forestiers, les agriculteurs et toute personne entrant dans les forêts infestées peuvent être affectées.
Le degré de contamination des tiques s'élève de 3 à 60% selon les différences saisonnières et régionales; Jusqu'à 30% de la population peut être infectée (environ 1500 cas annuellement aux États-Unis, plusieurs centaines en Europe).

Espèce	La maladie	Symptômes (p.ex.)	Modes de transmission
Borrelia burgdorferi	Maladie de Lyme ou borréliose de Lyme	**Maladie multisystémique** **Symptômes précoces**: érythéme migrant; symptômes semblables à ceux de la grippe comme la fièvre, les myalgies et les maux de tête; Carditis; méningite **Symptômes tardives**: Symptômes chroniques neurologiques, dermatologiques et / ou cardiaques; Acrodermatitis chronica atrophicans; Réaction d'Herxheimer; Lyme-Arthritis	Transmission par des morsures de tiques Europe: Ixodes ricinus Etats-Unis: Ixodes dammini

L'infection ou la présence d'un agent pathogène peut être identifiée par:

- Microscopie
- PCR
- Sérologie: p.ex. ELISA

2. INDICATION D'UTILISATION

La trousse Borrelia burgdorferi IgG ELISA est prévue pour la détection qualitative des anticorps IgG anti-Borrelia burgdorferi dans le sérum humain, plasma (citrate, héparine) et le LCR (liquide céphalo-rachidien).
Pour la détection d'anticorps IgG produits par voie intrathectique, une notice séparée pour l'utilisation de LCR (BORL0040) peut être obtenue sur demande.

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur le la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).
Plaques de Microtitrage sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.
L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un photomètre de Plaques de Microtitrage ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Plaque de Microtitrage:** 12 barrettes de 8 puits sécables revêtus d'antigène recombinants de la Borrelia burgdorferi; en sachets d'aluminium refermables.
- **Tampon de Dilution d'Échantillon IgG:** 1 flacon contenant 100 mL de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH 7,2 ± 0,2; prêt à l'emploi; couleur jaune; bouchon blanc; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solution d'Arrêt:** 1 flacon contenant 15 mL d'acide sulfurique, 0,2 mol/L; prêt à l'emploi; bouchonrouge.
- **Tampon de Lavage (concentré x 20):** 1 flacon contenant 50 mL d'un tampon phosphaté (0,2 M) concentré 20 fois (pH 7,2 ± 0,2) pour laver les puits; bouchon blanc.
- **Conjugué:** 1 flacon contenant 20 mL d'anticorps IgG anti-humaines conjuguées à peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur bleue, bouchon noir.
- **Solution de Substrat TMB:** 1 flacon contenant 15 mL de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1 %; prêt à l'emploi; bouchon jaune.
- **Contrôle Positif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon rouge; ≤ 0,02% (v/v) MIT.
- **Contrôle Cut-off:** 1 flacon contenant 3 mL contrôle; prêt à l'emploi; couleur jaune; bouchon vert; ≤ 0,02% (v/v) MIT.
- **Contrôle Négatif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon bleu; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Pour les mentions de danger et les conseils de prudence voir chapitre 12.1
Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Photomètre de Plaque de Microtitrage ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des Plaques de Microtitrage
- Pipettes pour utilisation entre 10 et 1000 µL
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8 °C.

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20 ... 25 °C) et les mélanger avant de commencer le test!

6.1. Plaque de Microtitrage

Les barrettes sécables sont revêtues d'antigènes recombinants des Borrelia burgdorferi. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

6.2. Tampon de Lavage (conc. x 20)

Diluer le Tampon de Lavage 1+19; par exemple 10 mL du Tampon de Lavage + 190 mL d'eau distillée. Le Tampon de Lavage diluée est stable pendant 5 jours à la température ambiante (20...25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de Substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humains de sérum, plasma (citrate, héparine) et LCS pour ce test. Les instructions d'utilisation BORL0040 doit être utilisé pour le LCS. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70…-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation.
L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec Tampon de Dilution d'Échantillon IgG. Diluer 10 µL d'échantillon avec 1 mL de Tampon de Dilution d'Échantillon IgG dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume de la Tampon de Lavage de 300 à 350 µL. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µL de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µL de Tampon de Lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape!

   Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats.
5. Pipeter 100 µL du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20…25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µL de la Solution de Substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20…25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µL de la Solution d'Arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la Solution de Substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la Solution d'Arrêt.

8.1. Mesure

Réglez le Photomètre de Plaque de Microtitrage ELISA **à zéro** en utilisant **le Blanc substrat.**
Si - pour des raisons techniques - le Photomètre de Plaque de Microtitrage ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits **à 450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.
Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance**.

9. RESULTATS

9.1. Critères de validation

Pour qu'une série d'analyses soit considérée comme valide, ces instructions d'utilisation doivent être strictement suivies, et les critères suivants doivent être respectés:

- **Blanc Substrat :** Valeur d'absorbance **< 0,100**
- **Contrôle Négatif**: Valeur d'absorbance **< 0,200 et < Cut-off**
- **Contrôle Cut-off:** Valeur d'absorbance **0,150 – 1,300**
- **Contrôle Positif :** Valeur d'absorbance **> contrôle Cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du contrôle Cut-off.

Exemple: 0,44 DO contrôle Cut-off + 0,42 DO contrôle Cut-off = 0,86 : 2 = 0,43
Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

$$\frac{\text{Valeur (moyenne) d'absorbance de l' échantillon} \times 10}{\text{Cut-off}} = \text{[unités NovaTec = NTU]}$$

Exemple: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé **négatif**.
Negatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.
Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limité dans le cas des patients immunodéprimés et des nouveaux-nés.		

9.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec une faible titre d'IgG : → suggère une infection très récente ou aigüe Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire de l'anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.

Pour plus d'informations sur les performances du test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	**n**	**moyenne (E)**	**CV (%)**
#1	24	0,517	4,00
#2	24	1,790	4,23
#3	24	2,339	9,04
Inter-essai	**n**	**moyenne (NTU)**	**CV (%)**
#1	12	37,2	4,17
#2	12	45,3	9,28
#3	12	1,9	9,94

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 95,48% (95% intervallo di confidenza: 90,92% - 98,17%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 98,39% (95% intervallo di confidenza: 91,34% - 99,96%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/mL de hémoglobine, 5 mg/mL de triglycérides et 0,5 mg/mL de bilirubine.

10.5. Réaction croisée

Par l'utilisation d'antigènes recombinants, la réactivité croisée avec des anticorps contre les agents pathogènes suivants peuvent être exclus de la meilleure façon possible:

- Tréponèmes pallidum
- des Leptospires
- autres espèces de *Borrelia*

La syphilis infection devraient être exclus, pas que peuvent apparaître des anticorps p41i.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongelation répétés de l`échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- La procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les Plaque de Microtitrage provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- L'ELISA est uniquement conçu pour le personnel qualifié suivant les normes de bonnes pratiques de laboratoire (Good Laboratory Practice, GLP).
- Pour un contrôle de qualité interne plus poussé, chaque laboratoire doit en outre utiliser des échantillons connus.

12.1. Note de sécurité pour les réactifs contenant des substances dangereuses

Les réactifs peuvent contenir du CMIT/MIT (3 :1) ou du MIT (voir chapitre 4.1)
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attenttion	H317	Peut provoquer une allergie cutanée.
	P261	Éviter de respirer les aérosols.
	P280	Porter des gants de protection/ des vêtements de protection.
	P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
	P333+P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
	P362+P364	Enlever les vêtements contaminés et les laver avant réutilisation.

De plus amples informations peuvent être trouvées dans la fiche de données de sécurité.

12.2. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence: BORG0040 Borrelia burgdorferi IgG ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

Le Spirochete appartengono alla famiglia dei Treponomataceae che comprende le specie Treponema, Borrelia e Leptospira. Hanno una lunghezza di 250µm, sono gram negativi con un diametro di 0,1-3 µm. La Borrelia burgdorferi é l'agente eziologico del morbo di Lyme. Viene trasmessa soprattutto dalle zecche Ixodes dammini e Ixodes pacificus. Nel 1975 è stata scoperta per la prima volta a Lyme USA. Burgdorfer et al. hanno isolato per la prima volta il batterio nel 1983. Il morbo di Lyme è una malattia molto diffusa nel mondo. Non esiste una vera profilassi. Il 10-20 % circa delle zecche europee sono contaminate. I focolai naturali sono boschi umidi e caldi in Europa, Nord America, Africa, Australia e Giappone. La distribuzione stagionale della malattia individua un picco costantemente presente in tarda primavera-inizio estate (Giugno-Luglio), mentre i sintomi più tardivi si concentrano in autunno-inizio inverno. A causa dei sintomi poco specifici la diagnosi è difficile e viene fatta spesso troppo tardi. La terapia con antibiotici è efficace soltanto dopo una diagnosi precoce, nella fase cronica la cura diventa molto difficile.

Specie	Malattia	Sintomi (p.es.)	Via di trasmissione
Borrelia burgdorferi	Malattia di Lyme (borreliosi)	**Malattia multisistemica** **Manifestazioni precoci**: eritema migrante; sintomi simil-influenzali, come febbre, mialgie e mal di testa; pancardite; meningite **Manifestazioni tardive:** sintomi neurologici, dermatologici e/o cardiologici cronici; Acrodermatite cronica atrofica; Reazione di Herxheimer; Lyme-Artrite	Puntura di zecche Europa : Ixodes ricinus USA: Ixodes dammini

L'infezione o la presenza di un agente patogeno può essere identificata da:
- Microscopia
- PCR
- Sierologia: p. es. ELISA

2. USO PREVISTO

Il Borrelia burgdorferi IgG ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgG per Borrelia burgdorferi nel siero, plasma (citrato, eparina) umano e LCR (liquido cerebrospinale).
Per la determinazione di anticorpi IgG prodotti per via intratecale, l'istruzione per l'uso de LCR -liquido cerebrospinale- (BORL0040), possono essere ottenuti presso NovaTec.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).
Piastre di Microtitolazione sono rivestite con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu.
L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm è letto utilizzando un fotometro di Piastre di Microtitolazione ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Piastre di Microtitolazione:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni ricombinanti della Borrelia burgdorferi; dentro una busta d'alluminio richiudibile.
- **Tampone Diluizione del Campione IgG:** 1 flacone contenente 100 mL di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; colore giallo; pronto all'uso; tappo bianco; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Soluzione Bloccante:** 1 flacone contenente 15 mL di acido solforico, 0,2 mol/L, pronto all'uso; tappo rosso.
- **Tampone di Lavaggio (20x conc.):** 1 flacone contenente 50 mL di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato:** 1 flacone contenente 20 mL di anticorpi IgG anti-umani, coniugati a perossidasi in tampone fosfato (10 mM); colore azzurro; pronto all'uso; tappo nero.
- **Soluzione Substrato TMB:** 1 flacone contenente 15 mL di 3,3`,5,5`-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo.
- **Controllo Positivo:** 1 flacone da 2 mL controllo; colore giallo; tappo rosso; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Cut-off:** 1 flacone da 3 mL controllo; colore giallo; tappo verde; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Negativo:** 1 flacone da 2 mL controllo; colore giallo; tappo blu; pronto all'uso; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Le indicazioni di pericolo e consigli di prudenza vedi capitolo 12.1.

Per le sostanze potenziali pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 pellicola adesiva
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per Piastre di Microtitolazione con filtri da 450/620 nm
- Incubatrice 37 °C
- Lavatore, manuale o automatico, di Piastre di Microtitolazione
- Micropipette per l'uso tra 10-1000 µL
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20…25 °C) e mescoloare prima di iniziare il test.

6.1. Piastre di Microtitolazione

Le strisce divisibili sono rivestite con l'antigeni ricombinanti della Borrelia burgdorferi. Immediatamente dopo la rimozione degli strisces necessari, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di Lavaggio (20x conc.)

Diluire il Tampone di Lavaggio 1+19; per esempio: 10 mL del Tampone di Lavaggio + 190 mL di acqua distillata. Il Tampone di Lavaggio diluito è stabile per 5 giorni a temperatura ambiente (20…25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero, plasma (citrato, eparina) umano e LCR (liquido cerebrospinale). Per il CSF, si prega di utilizzare le istruzioni per l'uso BORL0040. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70…-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento.
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con Tampone Diluizione del Campione IgG. Per esempio, pipettare nelle provette 10 µL di campione + 1 mL di Tampone Diluizione del Campione IgG e mescolare bene (Vortex).

8. PROCEDIMENTO

Leggere bene le istruzioni per l'uso **prima** di iniziare il teste. L'affidabilità dei risultati dipende dalla stretta aderenza le istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a 5 volte e il volume della Tampone di Lavaggio da 300 a 350 µL per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a 37 ± 1 °C.

1. Pipettare 100 µL di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora ± 5 min a 37 ± 1 °C.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µL di Tampone di Lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.

   Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µL di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente (20...25 °C).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µL di Soluzione Substrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20...25 °C) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µL di Soluzione Bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Substrato TMB, in tal modo un cambiamento di colore dal blu al giallo si verifica.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione Bloccante.

8.1. Misurazione

Regolare il fotometro per le Piastre di Microtitolazione ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro per le Piastre di Microtitolazione a zero usando il Bianco-substrato, il valore de assorbanza de questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda de 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Affinché un test possa essere considerato valido, le presenti Istruzioni per l'uso devono essere rigorosamente seguite e devono essere soddisfatti i seguenti criteri:

- **Substrato Bianco (Blank)**: Valore di assorbanza **< 0,100**
- **Controllo Negativo**: Valore di assorbanza **< 0,200 e < Cut-off**
- **Controllo Cut-off**: Valore di assorbanza **0,150 – 1,300**
- **Controllo Positivo**: Valore di assorbanza **> Cut-off**

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli Cut-off.

Esempio: Valore di assorbanza del Controllo Cut-off 0,44 + valore di assorbanza del Controllo Cut-off 0,42 = 0,86/2 = 0,43
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = \text{[unità NovaTec = NTU]}$$

Esempio: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
Zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia il campione viene giudicato come **negativo**.
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

9.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: →suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	**n**	**Media (E)**	**CV (%)**
#1	24	0,517	4,00
#2	24	1,790	4,23
#3	24	2,339	9,04
Interdosaggio	**n**	**Media (NTU)**	**CV (%)**
#1	12	37,2	4,17
#2	12	45,3	9,28
#3	12	1,9	9,94

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analita specifici.
La specificità diagnostica è 95,48% (95% intervallo di confidenza: 90,92% - 98,17%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analita specifici.
La sensibilità diagnostica è 98,39% (95% intervallo di confidenza: 91,34% - 99,96%).

10.4. Possibili interferenze

Campioni emolitici, lipidici et itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,5 mg/mL di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

Utilizzando antigeni ricombinanti una reazione crociata con anticorpi contro le seguenti patogeni può escludere al del miglior modo possibile:
- Treponema pallidum
- Leptospira
- Borrelia recurrentis

Deve essere esclusa l'infezione da sifilide, perché potrebbero apparire gli anticorpi contro p41i.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- La procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti gli elementi di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e Piastre di Microtitolazione di lotti diversi.
- Non utilizzare reagenti d'altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione crociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- L'ELISA è progettato solo per il personale qualificato che segue le norme di buona pratica di laboratorio (Good Laboratory Practice, GLP).
- Per un ulteriore controllo di qualità interno ogni laboratorio dovrebbe inoltre utilizzare campioni noti.

12.1. Nota di sicurezza per i reagenti contenenti sostanze pericolose

I reagenti possono contenere CMIT/MIT (3:1) o MIT (vedi capitolo 4.1)
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza.

Attenzione

H317	Può provocare una reazione allergica cutanea.
P261	Evitare di respirare gli aerosol.
P280	Indossare guanti/ indumenti protettivi.
P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
P333+P313	In caso di irritazione o eruzione della pelle: consultare un medico.
P362+P364	Togliere tutti gli indumenti contaminati e lavarli prima di indossarli nuovamente.

Ulteriori informazioni sono disponibili nella scheda di dati di sicurezza.

12.2. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto:	BORG0040	Borrelia burgdorferi IgG ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

Las espiroquetas son bacterias gramnegativas mobiles de forma espiral que son desproporcionadamente largas (hasta 250µm) en comparación con su diámetro (0,1-3µm). Todas las especies patógenas pertenecen a la familia de las Treponemataceae que incluyen tres géneros: Treponema, Borrelia y Leptospira.

Las Treponemas son bacterias delgadas, flexibles, normalmente con una forma espiral caracteristica. Las Borrelias son las mas largas espiroquetas de las Treponematacae con espirales delicadas y irregulares. Borrelia burgdorferi es el causante de la enfermedad inflamatoria persistente tardía de Lyme que se reportó por primera vez en 1975 en el pueblo de Old Lyme, en el estado de Connecticut (Estados Unidos). La enfermedad se transmite sobre todo por la picadura de la garrapata, possiblemente también por otros insectos sanguijuelos. La propagación es a nivel mundial. Una prevención verdadera no existe. En Europa aprox. entre el 10 y el 20 % de las garrapatas están infectadas con Borrelia burgdorferi. El espacio vital son las regiones forrestales, humedas-calientes de norte America, Europa, norte de Africa, Australia y Japon. La población de mayor riesgo son obreros forestales, cazadores y agricultores.

Es una enfermedad difícil de diagnosticar ya que los síntomas se parecen a los de otras enfermedades. Generalmente aparece una erupción roja característica en el sitio de la picadura que puede pasar inadvertida. La enfermedad terciaria se desarrolla meses o años después de la infección inicial. Soló el diagnóstico temprano posibilita un control eficaz de la enfermedad con anitbioticos debido a que en la phase crónica las borrelias son casi inaccesibles.

Especies	Enfermedad	Síntomas (p.ej.)	Vía de transmisión
Borrelia burgdorferi	Enfermedad de Lyme	**Enfermedad multisistémica** **Manifestaciones tempranas**: Eritema migratorio; Síntomas similares a la gripe, como fiebre, mialgias y cefaleas; carditis; meningitis **Manifestaciones tardías:** Síntomas neurológicos, dermatológicos y/o cardiológicos crónicos; Acrodermatitis chronica atrophicans; Reacción de Herxheimer; Lyme-Artritis	Picadura de garrapatas Europa : Ixodes ricinus USA: Ixodes dammini

La infección o la presencia de un patógeno puede identificarse mediante:

- Microscópia
- PCR
- Serología: p.ej. ELISA

2. USO PREVISTO

El enzimoinmunoensayo Borrelia burgdorferi IgG ELISA se utiliza para la determinación cualitativa de anticuerpos IgG específicos contra Borrelia burgdorferi en suero, plasma (citrato, heparina) humano e y LCR (líquido cefalorraquídeo).
Para la determinación de anticuerpes IgG producidos de manera intratecal, una instrucción separada (BORL0040) para el uso de líquido cefaloraquídeo (LCR) se puede obtener en NovaTec.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).
Las Placas de Microtitulación están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unida, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualiza añadiendo substrato tetrametilbencidina (TMB), que da un producto de reacción azul.
La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un fotómetro de Placa de Microtitulación ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Placa de Microtitulacións:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos recombinante de Borrelia burgdorferi, en bolsa de aluminio.
- **Tampón Diluyente para la Muestra IgG:** 1 botella de 100 mL de solución de tampón de fosfato (10 mM) para diluir la muestra; pH 7,2 ± 0,2; color amarillo; listo para ser utilizado; tapa blanca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solución de Parada:** 1 botella de 15 mL de ácido sulfúrico, 0,2 mol/L, listo para ser utilizado; tapa roja.
- **Tampón de Lavado (20x conc.):** 1 botella de 50 mL de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH 7,2 ± 0,2; tapa blanca.
- **Conjugado:** 1 botella de 20 mL de conjugado de anticuerpos IgG anti-humano con peroxidasa en tampón de fosfato (10 mM); color azul; tapa negra; listo para ser utilizado.
- **Solución de Sustrato de TMB:** 1 botella de 15 mL 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 12.1.
Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de Placa de Microtitulación con filtros de 450/620 nm
- Incubadora 37 °C
- Dispositivo de lavado manual o automático para Placa de Microtitulación
- Micropipetas para uso de (10-1000 µL)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2... 8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2... 8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar todos los reactivos y las muestras a temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Placa de Microtitulación

Las tiras rompibles están recubiertas con antígeno recombinante de Borrelia burgdorferi. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita di dióxio de silicio y almacenar a 2... 8 °C.

6.2. Tampón de Lavado (20x conc.)

Diluir el Tampón de Lavado 1+19; por ejemplo 10 mL del Tampón de Lavado + 190 mL de agua destilada. El Tampón de Lavado diluido es estable durante 5 días a temperatura ambiente (20…25 °C). En caso de aparecer cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de Sustrato de TMB

La solución está lista para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizado en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero, plasma (citrato, heparina) humano o LCR. Las instrucciones de uso BORL0040 deben ser usadas para LCR. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas a 2...8 °C, en caso contrario deben ser alicotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.
No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el Tampón Diluyente para la Muestra IgG, por ejemplo 10 µL de la muestra con 1 mL de Tampón Diluyente para la Muestra IgG, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavados de tres hasta cinco veces y el volumen de Tampón de Lavado de 300 µL a 350 µL para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (se recomienda determinar en duplicado) en el esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pocillos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1 °C.

1. Pipetear 100 µL de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h ± 5 min a 37 ± 1 °C.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300 µL del Tampón de Lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetar 100 µL de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20…25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µL da Solución de Sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µL de la Solución de Parada en el mismo orden y mismo intervalo de tiempo como con el Solución de Sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la Solución de Parada.

8.1. Medición

Ajustar el fotómetro de Placa de Microtitulación ELISA al cero utilizando el Blanco.

Si por razones técnicas el fotómetro de Placa de Microtitulación de ELISA no se puede ajustar a cero utilizando el Blanco, el valor de la absorbancia de este debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en el esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CÁLCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

Para que un ensayo se considere válido, deben seguirse estrictamente las presentes instrucciones de uso y deben cumplirse los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control Negativo**: valor de la extinción **< 0,200 y < Cut-off**
- **Control Cut-off**: valor de la extinción **0,150 – 1,300**
- **Control Positivo:** valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es váida y deberá repetirse.

9.2. Cálculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de los dos Controles Cut-off.

Ejemplo: 0,42 OD Control Cut-off + 0,44 OD Control Cut-off = 0,86:2 = 0,43
Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

$$\frac{\text{Promedio valor de la extinción de la muestra x 10}}{\text{Cut-off}} = \text{[NovaTec-unidades = NTU]}$$

Ejemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como **negativa**.
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnésis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

9.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundaria del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en el grupo de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	**n**	**Promedio (E)**	**CV (%)**
#1	24	0,517	4,00
#2	24	1,790	4,23
#3	24	2,339	9,04
Inter ensayo	**n**	**Promedio (NTU)**	**CV (%)**
#1	12	37,2	4,17
#2	12	45,3	9,28
#3	12	1,9	9,94

10.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 95,48% (95% Intervalo de confianza: 90,92% - 98,17%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 98,39% (95% Intervalo de confianza: 91,34% - 99,96%).

10.4. Interferencias

Las muestras lipémicas, ictéricas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/mL para triglicéridos, de 0,5 mg/mL para bilirrubina y de 10 mg/mL hemoglobina.

10.5. Reactividad cruzada

Usando antígenos recombinantes una reacción cruzada con anticuerpos contra los siguientes patógenos puede ser excluido la mejor manera posible:
- Treponema pallidum
- Leptospira
- Borrelia recurrentis

La infección por sífilis debe excluirse, porque podrían aparecer los anticuerpos contra p41i.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- El procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y Placa de Microtitulación de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, Pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA sólo está diseñado para personal cualificado siguiendo las normas de buenas prácticas de laboratorio (Good Laboratory Practice,GLP).
- Para un mayor control de calidad interno, cada laboratorio deberá utilizar además muestras conocidas.

12.1. Nota de seguridad para los reactivos que contienen sustancias peligrosas

Los reactivos pueden contener CMIT/MIT (3:1) o MIT (consulte el cap. 4.1)
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención

H317	Puede provocar una reacción alérgica en la piel.
P261	Evitar respirar el aerosol.
P280	Llevar guantes/ prendas de protección.
P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
P333+P313	En caso de irritación o erupción cutánea: Consultar a un médico.
P362+P364	Quitar las prendas contaminadas y lavarlas antes de volver a usarlas.

Se puede encontrar más información en la ficha de datos de seguridad.

12.2. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto:	BORG0040	Borrelia burgdorferi IgG ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

As espiroquetas são bactérias móveis com um filamento axial periplasmático. Todas as espécies patogênicas pertencem à família Treponemataceae, que inclui os três gêneros: Treponema, Borrelia, e Leptospira. As Treponemae são células filamentosas extremamente longas e flexíveis que geralmente são mantidas em uma espiral característica, ou em forma de mola espiralada. As Borrelias são as maiores Treponemataceae com espirais muito grosseiras e irregulares. Borrelia burgdorferi, o agente causador da doença de Lyme, é transmitida principalmente por carrapatos, mas provavelmente também por outros insetos sugadores de sangue. Os habitats são as regiões arborizadas, úmidas e temperadas da América do Norte, Europa, Norte da África, Austrália e Japão; os silvicultores, agricultores e qualquer pessoa que entre nas florestas infestadas podem ser afetados.

O grau de contaminação dos carrapatos é de 3-60 %, dependendo das diferenças sazonais e regionais; até 30 % da população pode estar infectada (cerca de 1500 casos anualmente nos EUA, várias centenas na Europa).

Espécies	Doença	Sintomas (p.ex.)	Via de transmissão
Borrelia burgdorferi	doença de Lyme	**Doença multissistêmica** **Manifestações precoces:** Eritema migrante; sintomas semelhantes aos da gripe como febre, mialgias e dores de cabeça; cardite; meningite. **Manifestações tardias:** Sintomas neurológicos crônicos, dermatológicos e/ou cardiológicos; Acrodermatite crônica atrofiante; Herxheimer; Lyme-Arthritis.	Transmissão por picada de carrapato Europa: Ixodes ricinus EUA: Ixodes dammini

Infecção ou presença de patógeno pode ser identificada por:

- Microscopia
- PCR
- Serologia: p.ex. ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Borrelia burgdorferi IgG ELISA destina-se à determinação qualitativa dos anticorpos da classe IgG contra Borrelia burgdorferi em soro humano, plasma (citrato, heparina) e LCR (líquido cefalorraquidiano).
Para a determinação dos anticorpos IgG produzidos intratecalmente, um manual de instruções separado (BORL0040) pode ser solicitado à NovaTec.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).
As Placas de Microtitulação são revestidas com antigénios específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligada, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.
A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo. Absorvância a 450/620 nm é lida utilizando um fotômetro de Placa de Microtitulação ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Placa de Microtitulação:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antigénos recombinantes de Borrelia burgdorferi, em bolsas de folha de alumínio com fecho.
- **Tampão de Diluição de Amostra IgG:** 1 frasco contendo 100 mL de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; de cor amarela; pronto a usar; tampa branca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solução de Bloqueio:** 1 frasco contendo 15 mL ácido sulfúrico; 0,2 mol/L; pronto a usar; tampa vermelha.
- **Tampão de Lavagem (conc. 20x):** 1 frasco contendo 50 mL de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado:** 1 frasco contendo 20 mL de anticorpo para IgG humana marcados com peroxidase no tampão fosfato (10 mM); de cor azul, pronto a usar; tampa preta.
- **Solução Substrato TMB:** 1 frasco contendo 15 mL de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela.
- **Controle Positivo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa vermelha; ≤ 0,02% (v/v) MIT.
- **Controle Cut-off:** 1 frasco contendo 3 mL controle; de cor amarela; pronto a usar; tampa verde; ≤ 0,02% (v/v) MIT.
- **Controle Negativo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa azul; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para advertências de perigo e recomendaçaões de prudência ver capitulo 12.1.
Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Fotômetro de Placa de Microtitulação ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem de Placa de Microtitulação
- Pipetas para dispensar volumes entre 10 e 1000 µL
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Placa de Microtitulação

As tiras quebraveis são revestidas com antígenos recombinantes de Borrelia burgdorferi. Imediatamente após a remoção das tiras necessarias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silicio fornecido e armazenadar a 2...8 °C.

6.2. Tampão de Lavagem (conc. 20x)

Diluir o Tampão de Lavagem 1+19; por exemplo: 10 mL do Tampão de Lavagem + 190 mL de água destilada. O Tampão de Lavagem diluído é estavel durante 5 dias à temperatura ambiente (20…25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução Substrato TMB

A solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) e LCR (líquido cefalorraquidiano) humanos. Para LCR use a instrução de uso BORL0040. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2…8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente.
Não é recomendada a inactivação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Tampão de Diluição de Amostra IgG. Dispensar 10 µL de amostra e 1 mL de Tampão de Diluição de Amostra IgG em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três até cinco e o volume do Tampão de Lavagem de 300 µL para 350 µL para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µL dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora ± 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µL de Tampão de Lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!

   Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µL de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µL de Solução Substrato TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µL de Solução de Bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução Substrato TMB,desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da Solução de Bloqueio.

8.1. Medição

Ajustar o fotômetro para Placa de Microtitulação ELISA **a zero** usando o **Branco substrato .**

Se - devido à razões técnicas – o fotômetro para Placa de Microtitulação ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraido de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, estas Instruções de Uso devem ser rigorosamente seguidas, e os seguintes critérios devem ser cumpridos:

- **Branco substrato**: Valor de Absorvância **< 0,100**
- **Controle Negativo**: Valor de Absorvância **< 0,200 e < Cut-off**
- **Controle Cut-off**: Valor de Absorvância **0,150 – 1,300**
- **Controle Positivo**: Valor de Absorvância **> Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do Controle Cut-off.

Exemplo: Valor da absorvância do Controle Cut-off 0,42 + valor da absorvância do Controle Cut-off 0,44 = 0,86: 2 = 0,43

Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra} \times 10}{\text{Cut-off}} = \text{[Unidades NovaTec = NTU]}$$

Exemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antigénio (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como **negativa**.
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em pacientes imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

9.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra-ensaio	**n**	**Média (E)**	**CV (%)**
#1	24	0,517	4,00
#2	24	1,790	4,23
#3	24	2,339	9,04
Inter-ensaio	**n**	**Média (NTU)**	**CV (%)**
#1	12	37,2	4,17
#2	12	45,3	9,28
#3	12	1,9	9,94

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 95,48% (95% Intervalo de confiança: 90,92% - 98,17 %).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 98,39% (95% Intervalo de confiança: 91,34% - 99,96 %).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipémicas ou ictéricas até uma concentração de hemoglobina de 10 mg/mL, de triglicerídeos de 5 mg/mL e de bilirrubina de 0,5 mg/mL.

10.5. Reacção cruzada

Ao utilizar antígenos recombinantes, as reações cruzadas com anticorpos contra os seguintes patógenos são em sua grande maioria excluídas
- Treponema pallidum
- Leptospira
- Borrelia recurrentis

Uma infecção por Sífilis deve ser excluída, pois podem aparecer anticorpos contra p41i.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- O procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e/ou juntar reagentes ou Placa de Microtitulação de lotes de produção diferentes.
- Nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar o reagentes precisamente nos poços sem salpicar.
- O ELISA é projetado apenas para pessoal qualificado seguindo os padrões de boas práticas de laboratório (Good Laboratory Practice, GLP).
- Para um controle de qualidade interno adicional cada laboratório deve utilizar amostras conhecidas.

12.1. Nota de segurança para reagentes que contenham substâncias perigosas

Os reagentes podem conter CMIT/MIT (3:1) ou MIT (ver capitulo 4.1)
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção

H317	Pode provocar uma reacção alérgica cutânea.
P261	Evitar respirar os aerossóis.
P280	Usar luvas de protecção/ vestuário de protecção.
P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água.
P333+P313	Em caso de irritação ou erupção cutânea: consulte um médico.
P362+P364	Retirar a roupa contaminada e lavá-la antes de a voltar a usar.

Mais informações podem ser encontradas na ficha de dados de segurança.

12.2. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

Prod. No.:	BORG0040	Borrelia burgdorferi IgG ELISA	(96 Determinações)

BIBLIOGRAPHY / LITERATUR / BIBLIOGRAPHIE / BIBLIOGRAFIA / BIBLIOGRAFÍA / BIBLIOGRAFIA

Asbrink, Eva; Hovmark, Anders (1988): Early and late cutaneous manifestations in Ixodes-borne borreliosis (erythema migrans borreliosis, Lyme borreliosis). In *Annals of the New York Academy of Sciences* 539, pp. 4–15.

Barbour, Alan G. (2006): Relapsing Fever and Other Borrelia Diseases. In Richard L. Guerrant, David H. Walker, Peter F. Weller (Eds.): Tropical infectious diseases. Principles, pathogens & practice. 2nd ed. Philadelphia: Churchill Livingstone, pp. 499–510.

Deutsche Borreliose-Gesellschaft e.V. (2011): Diagnostik und Therapie der Lyme-Borreliose. Leitlinien der Deutschen Borreliose-Gesellschaft.

Eicken, Christoph; Sharma, Vivek; Klabunde, Thomas; Lawrenz, Matthew B.; Hardham, John M.; Norris, Steven J.; Sacchettini, James C. (2002): Crystal structure of Lyme disease variable surface antigen VlsE of Borrelia burgdorferi. In *The Journal of biological chemistry* 277 (24), pp. 21691–21696. DOI: 10.1074/jbc.M201547200.

Jauris-Heipke, Sigrid; Rößle, Birgit; Wanner, Gerhard; Habermann, Christiane; Rössler, Dieter; Fingerle, Volker et al. (1999): Osp17, a novel immunodominant outer surface protein of Borrelia afzelii: recombinant expression in Escherichia coli and its use as a diagnostic antigen for serodiagnosis of Lyme borreliosis. In *Medical microbiology and immunology* 187 (4), pp. 213–219.

Johnson, Russell C.; Schmid, George P.; Hyde, Fred W.; Steigerwalt, A. G.; Brenner, Don J. (1984): Borrelia burgdorferi sp. nov. Etiologic Agent of Lyme Disease. In *International journal of systematic bacteriology* 34 (4), pp. 496–497. DOI: 10.1099/00207713-34-4-496.

Karlsson, Mats; Hovind-Hougen, Kari; Svenungsson, Bo; Stiernstedt, Göran (1990): Cultivation and characterization of spirochetes from cerebrospinal fluid of patients with Lyme borreliosis. In *Journal of Clinical Microbiology* 28 (3), pp. 473–479.

Luther, Birgit; Moskophidis, Matthäus (1990): Antigenic cross-reactivity between Borrelia burgdorferi, Borrelia recurrentis, Treponema pallidum, and Treponema phagedenis. In *Zentralblatt fur Bakteriologie : international journal of medical microbiology* 274 (2), pp. 214–226.

Magnarelli, Louis A.; Fikrig, Erol; Padula, Steven J.; Anderson, John F.; Flavell, Richard A. (1996): Use of recombinant antigens of Borrelia burgdorferi in serologic tests for diagnosis of lyme borreliosis. In *Journal of Clinical Microbiology* 34 (2), pp. 237–240.

Ohnishi, Jun; Schneider, Brad; Messer, William B.; Piesman, Joseph; Silva, Aravinda M. de (2003): Genetic variation at the vlsE locus of Borrelia burgdorferi within ticks and mice over the course of a single transmission cycle. In *Journal of Bacteriology* 185 (15), pp. 4432–4441. DOI: 10.1128/JB.185.15.4432-4441.2003.

Preac-Mursic, V.; Wilske, Bettina; Schierz, G. (1986): European Borrelia burgdorferi isolated from humans and ticks culture conditions and antibiotic susceptibility. In *Zentralblatt fur Bakteriologie, Mikrobiologie, und Hygiene. Series A, Medical microbiology, infectious diseases, virology, parasitology* 263 (1-2), pp. 112–118.

Schulte-Spechtel, Ulrike; Lehnert, Gisela; Liegl, Gaby; Fingerle, Volker; Heimerl, Christiane; Johnson, Barbara J. B.; Wilske, Bettina (2003): Significant improvement of the recombinant Borrelia-specific immunoglobulin G immunoblot test by addition of VlsE and a DbpA homologue derived from Borrelia garinii for diagnosis of early neuroborreliosis. In *Journal of Clinical Microbiology* 41 (3), pp. 1299–1303. DOI: 10.1128/JCM.41.3.1299-1303.2003.

Wang, Dachun; Botkin, Douglas J.; Norris, Steven J. (2003): Characterization of the vls antigenic variation loci of the Lyme disease spirochaetes Borrelia garinii lp90 and Borrelia afzelii ACAI. In *Molecular Microbiology* 47 (5), pp. 1407–1417. DOI: 10.1046/j.1365-2958.2003.03386.x.

Wilske, Bettina (2003): Diagnosis of lyme borreliosis in europe. In *Vector borne and zoonotic diseases (Larchmont, N.Y.)* 3 (4), pp. 215–227. DOI: 10.1089/153036603322662200.

Wilske, Bettina; Preac-Mursic, V.; Fuchs, R.; Schierz, G. (1990): Diagnostik der Lyme Borreliose. Diagnose und Labor. In *Laboratoriumsblätter* 40, pp. 24–36.

ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulacións / Placa de Microtitulação
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL | -	Negative Control / Negativkontrolle / Contrôle Négatif / Controllo Negativo / Control Negativo / Controle Negativo
CONTROL | +	Positive Control / Positivkontrolle / Contrôle Positif / Controllo Positivo / Control Positivo / Controle Positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle Cut-off / Controllo Cut-off / Control Cut-off / Controle Cut-off
DIL | G	IgG Sample Dilution Buffer / IgG-Probenverdünnungspuffer / Tampon de Dilution d'Échantillon IgG / Tampone Diluizione del Campione IgG / Tampón Diluyente para la Muestra IgG / Tampão de Diluição de AmostraIgG
SOLN | STOP	Stop Solution / Stopplösung / Solution d'Arrêt / Soluzione Bloccante / Solución de Parada /Solução de Bloqueio
SUB | TMB	TMB Substrate Solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Substrato TMB / Solución de Substrato TMB / Solução Substrato TMB
WASH | BUF | 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampón de Lavado concentrado x20 / Tampão de Lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

Borrelia burgdorferi IgG ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 ± 1 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

NovaTec Immundiagnostica GmbH

Waldstraße 23 A6
63128 Dietzenbach, Germany

Tel.: +49 (0) 6074-48760 Fax: +49 (0) 6074-487629
Email: info@NovaTec-ID.com
Internet: www.NovaTec-ID.com

From: s22
To: s22 @paragoncare.com.au
Subject: DIR 90606 - ARTG 205351 - s41JA Requirement for Information for a DIR letter [SEC=OFFICIAL]
Date: Friday, 2 February 2024 10:46:00 AM
Attachments: DIR 90606 - ARTG 205351 - s41JA Requirement for Information for a DIR letter.pdf
image001.png
image002.png
image003.gif
image004.png

Dear s22

Good morning!

Please find attached the S41JA letter.

Your response is due 16 February 2024.

Kind regards,
s22

s22
Medical Devices Investigator – Devices Post Market Monitoring Section
Medical Devices Surveillance Branch

Medical Devices and Product Quality Division | Health Products Regulation Group
Australian Government, Department of Health and Aged Care
T: s22 | E: s22 @health.gov.au
Location: Melbourne, Victoria 3000
PO Box 100, Woden ACT 2606, Australia

The Department of Health and Aged Care acknowledges First Nations peoples as the Traditional Owners of Country throughout Australia, and their continuing connection to land, sea and community. We pay our respects to them and their cultures, and to all Elders both past and present.

Important: *This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error, please notify the author immediately and delete all copies of this transmission.*

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Our Reference: E23-275158

Sent by email

Dear s22

Subject: DEVICE INCIDENT REPORT DIR 90606 FOR ARTG 205351

NOTICE REQUIRING INFORMATION/DOCUMENTS TO BE PROVIDED UNDER SECTION 41JA OF THE THERAPEUTIC GOODS ACT

I have made a decision under subsection 41JA(1) of the *Therapeutic Goods Act 1989* (the Act) to require you to provide information/documents about Bacterial infectious disease IVDs (NovaTec NovaLisa Borrelia burgdorferi IgG-ELISA), included under ARTG number 205351. I am the delegate of the Secretary of the Department of Health and Aged Care for the purposes of section 41JA of the Act. The information is required for the purposes of the investigation of the above incident report, and to determine whether the certifications made about the devices under section 41FD are correct, including compliance with the Essential Principles and Conformity Assessment Procedures.

You are requested to provide the information/documents listed below including details of any action you have taken in relation to this matter:

1. The IFU you provided to the TGA on 6 December 2023 is for the NovaLisa Borrelia burgdorferi IgM – ELISA (recombinant). The attachment in the questionnaire letter sent to you on 16 October 2023 indicated the device involved in this DIR is NovaTec NovaLisa Borrelia burgdorferi IgG ELISA (Novatec Novalisa).

   Provide the Novatec Novalisa Borrelia burgdorferi IgG ELISA IFU to ensure it includes a statement indicating that the device cannot be used in isolation to diagnose Lyme disease.

2. ***Provide the manufacturer's investigation for the reported issue in this DIR.***

3. ***Provide comment on the clinical event stated in this DIR.*** For your ease, "I am writing to formally request the recall and investigation of the medical device NovaTec NovaLisa Borrelia burgdorferi IgG ELISA (Novatec Novalisa), bearing ARTG 205351, due to the occurrence of discordant serological false negative results that have raised serious concerns regarding its performance and safety." This statement can be found in the attachment of the questionnaire letter sent to you on 16 October 2023.

The requested information must be provided within **10** working days from the date of this letter and no later than **16 February 2024**.

Address for sending the requested information

Please submit all documents in electronic format only. The requested information should be sent to s22 via:

- Email to: s22 @health.gov.au

For large size documents, please post a universal serial bus (USB), compact disc (CD), or digital versatile disc (DVD) via:

- post to:

Devices Post Market Monitoring Section
Medical Devices Surveillance Branch
Therapeutic Goods Administration
PO Box 100
Woden ACT 2606

- courier to:

Devices Post Market Monitoring Section
Medical Devices Surveillance Branch
Therapeutic Goods Administration
1 Tindal Lane
Canberra Airport ACT 2609

Please quote file reference E23-275158 in all correspondence relating to this request. This information is essential for the TGA to track and identify all pieces of data in relation to this device.

Please note that failure to comply with this request for information may attract a range of sanctions. These are outlined in Attachment A.

Please note that in the event that medical devices are cancelled from the ARTG following a notice under subsection 41JA(1) of the Act requiring information/documents to be provided and a proposal to cancel, the Secretary is required under 41GP of the Act to publish in the Commonwealth Gazette or the Department's website particulars of the cancellation of the entry from the ARTG as soon as practicable after the cancellation. The TGA's practice is to publish information on its website about that cancellation including the name of the sponsor and product and the grounds on which the goods were cancelled. This information is updated in the event that the cancellation is subject to internal review or review by the Administrative Appeals Tribunal, or other form of legal review. [For more information see https://www.tga.gov.au/how-we-regulate/compliance-and-enforcement-hub]

Please contact the undersigned s22 on s22 or email: s22 @health.gov.au if any aspect of this request for information requires clarification.

Yours sincerely

Signed electronically by

s22

Delegate of the Secretary

Devices Post Market Monitoring Section
Medical Devices Surveillance Branch
Therapeutic Goods Administration

2 February 2024

ATTACHMENT A

Legislative extracts

Therapeutic Goods Act 1989

41JA Secretary may require information or documents

(1) The Secretary may, by written notice given to a person:

(a) who is an applicant for a conformity assessment certificate that would relate to a kind of medical device; or

(b) who holds a conformity assessment certificate, or an Australian conformity assessment body certificate, that relates to a kind of medical device; or

(ba) who held, at any time during the notice period under subsection (2), a conformity assessment certificate, or an Australian conformity assessment body certificate, that related to a kind of medical device; or

(c) who is an applicant for the inclusion of a kind of medical device in the Register; or

(d) in relation to whom a kind of medical device is included in the Register; or

(da) in relation to whom a kind of medical device was, at any time during the notice period under subsection (2), included in the Register;

require the person to give to the Secretary information or documents, relating to devices of that kind, that are relevant to one or more of the following:

(e) whether the devices comply with the essential principles;

(f) whether the conformity assessment procedures have been applied to the devices or whether requirements, comparable to those procedures, have been applied to the devices;

(g) whether the devices comply with conditions (if any) imposed on a conformity assessment certificate issued in respect of the device or the inclusion of the device in the Register;

(h) whether either of the following has not been complied with in relation to the devices:

(i) an applicable provision of the Therapeutic Goods Advertising Code;

(ii) any other requirement relating to advertising applicable under Part 5-1 or under the regulations;

(i) if the kind of medical device is included in the Register in relation to the person—whether medical devices of that kind are being:

(i) supplied in Australia; or

(ii) imported into Australia; or

(iii) exported from Australia;

(iaa) if the kind of medical device is included in the Register in relation to the person and there are one or more absolute prohibitions in force for the purposes of subsection 9K(1) or (3)—whether any supplies in Australia, any imports into Australia, any exports from Australia or any manufacture in Australia of medical devices of that kind contravene those prohibitions;

(iab) if the kind of medical device is included in the Register in relation to the person and there are one or more prohibitions in force for the

purposes of subsection 9K(1) or (3) that are subject to conditions—whether any supplies in Australia, any imports into Australia, any exports from Australia or any manufacture in Australia of medical devices of that kind contravene those conditions;

(ia) the safety and efficacy of the devices for the purposes for which they are to be used;

(ib) the regulatory history of the devices in another country;

(j) any other matter prescribed by the regulations for the purposes of this paragraph.

(1AA) If a notice is given under subsection (1) to a person covered by paragraph (1)(ba), then paragraphs (1)(e) to (j) (to the extent to which they are relevant) apply in relation to the period the person held the certificate.

(1AB) If a notice is given under subsection (1) to a person covered by paragraph (1)(da), then paragraphs (1)(e) to (j) (to the extent to which they are relevant) apply in relation to the period the kind of medical device was included in the Register.

(1A) The Secretary may, by written notice given to a person who is an applicant for a conformity assessment certificate, require the person to give to the Secretary such further information concerning the application as is specified in the notice.

(1B) Requirements under subsections (1) and (1A) may be included in the same notice.

(1C) The Secretary may, by written notice given to a person who holds a conformity assessment certificate, require the person to give to the Secretary specified information to be used by the Secretary in deciding whether to suspend the certificate under section 41EM, or to revoke the certificate under section 41ET, in relation to the circumstances referred to in paragraph 41ET(1)(e).

(1D) Requirements under subsections (1) and (1C) may be included in the same notice.

(1E) The Secretary may, by written notice given to an Australian corporation that has been an Australian conformity assessment body require the corporation to give to the Secretary specified information, or specified documents, relating to:

(a) the certification-related activities carried on by the corporation while the corporation was an Australian conformity assessment body; or

(b) the conditions referred to in subsection 41EWA(5) that applied while the corporation was an Australian conformity assessment body.

(2) For the purposes of paragraphs (1)(ba) and (da), the notice period is the period:

(a) of the length specified in the regulations; and

(b) ending on the day before the Secretary gives the notice under subsection (1).

(3) Nothing in this section affects the operation of Part VIIC of the *Crimes Act 1914* (which includes provisions that, in certain circumstances, relieve persons from the requirement to disclose spent convictions and require persons aware of such convictions to disregard them).

41JB Complying with the Secretary's requirements

(1) The person must give the information or documents to the Secretary:
  (a) within such reasonable time, being not less than 10 working days from the day on which the notice is given, as is specified in the notice; and
  (b) in such form as is specified in the notice.

(2) The form may require or permit information to be given in accordance with specified software requirements:
  (a) on a specified kind of data processing device; or
  (b) by way of a specified kind of electronic transmission.

Offence for failing to comply with a notice

(3) A person commits an offence if:
  (a) the person is given a notice under section 41JA; and
  (aa) the person is covered by paragraph 41JA(1)(b), (ba), (d) or (da) or subsection 41JA(1E); and
  (b) the person fails to comply with the notice.

Penalty: 500 penalty units.

Note: Failure to comply with the notice might also lead to suspension or revocation of a conformity assessment certificate (see Divisions 3 and 4 of Part 4-4) or suspension or cancellation of the entry of a kind of medical device in the Register (see Part 4-6).

(3A) Subsection (3) does not apply if the person has a reasonable excuse.

Note: A defendant bears an evidential burden in relation to the matter in subsection (3A): see subsection 13.3(3) of the *Criminal Code*.

(3B) A person commits an offence if:
  (a) the person is given a notice under section 41JA; and
  (b) the person is covered by paragraph 41JA(1)(b), (ba), (d) or (da) or subsection 41JA(1E); and
  (d) the person fails to comply with the notice.

Penalty: 100 penalty units.

(3C) An offence against subsection (3B) is an offence of strict liability.

(3D) Subsection (3B) does not apply if the person has a reasonable excuse.

Note: A defendant bears an evidential burden in relation to the matter in subsection (3D): see subsection 13.3(3) of the *Criminal Code*.

Offences for giving false or misleading information in purported compliance with a notice

(4) A person commits an offence if:
  (a) the person is given a notice under section 41JA in relation to a kind of medical device; and
  (b) the person gives information in purported compliance with the notice; and
  (c) the information is false or misleading in a material particular; and
  (d) either:
    (i) the use of the kind of medical device has resulted in, will result in, or is likely to result in, harm or injury to any person; or

(ii) the use of the kind of medical device, if the kind of medical device were used, would result in, or would be likely to result in, harm or injury to any person.

Penalty: Imprisonment for 5 years or 4,000 penalty units, or both.

Note 1: A jury may acquit a person of an offence against this subsection and may convict the person of an offence against subsection (7) instead: see section 53A.

Note 2: For the liability of an executive officer of a body corporate, see sections 54B and 54BA.

(7) A person commits an offence if:

(a) the person is given a notice under section 41JA; and

(b) the person gives information in purported compliance with the notice; and

(c) the information is false or misleading in a material particular.

Penalty: Imprisonment for 12 months or 1,000 penalty units, or both.

(8) A person commits an offence if:

(a) the person is given a notice under section 41JA; and

(b) the person gives information in purported compliance with the notice; and

(c) the information is false or misleading in a material particular.

Penalty: 100 penalty units.

(9) An offence against subsection (8) is an offence of strict liability.

From: s22
To: IRIS
Cc: PGC Regulatory; PGC Quality
Subject: RE: DIR 90606 - Questionnaire Letter - Response due by 13.11.23 [SEC=OFFICIAL, ACCESS=Personal-Privacy]
Date: Wednesday, 6 December 2023 2:59:35 PM
Attachments: image002.png
image003.png
DIR 90606 - Questionnaire Letter completed.rtf
NOV-DATA-NOVBORM0040.pdf

Dear s22

Please see attached manufacturers response and IFU.

Let me know if you have any questions.

Kind Regards
s22

s22
s22
Paragon Care Group Australia Pty Ltd | 1/17 Rodborough Rd, Frenchs Forest, NSW 2086
M_ s22 s22 E_ s22 @paragoncare.com.au W_ paragoncare.com.au

ParagonCare
Health. Covered.

BECOVIDSAFE

At Paragon we work flexibly, so whilst it suits me to email you now, I do not expect a response outside of your normal working hours.

Please consider the environment before printing this email.

This email and any attachments are intended only for the use of the individual or entity to which it is addressed and may contain proprietary information that is privileged, confidential or copyright. You are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited without the authority of the sender. If you have received this e-mail message in error or are not the intended recipient, please delete and destroy all copies and notify us immediately by return mail. Any loss or damage incurred as a result of prohibited use is not the sender's responsibility. E-mail communications are not guaranteed as secure or error free, the sender accepts no liability for any damage caused by this email or its attachments due to viruses, interference, interception, corruption or unauthorised access. The sender's entire liability is limited to resending this email.

From: IRIS <IRIS@health.gov.au>
Sent: Monday, December 4, 2023 1:36 PM
To: s22 @paragoncare.com.au>
Subject: RE: DIR 90606 - Questionnaire Letter - Response due by 13.11.23 [SEC=OFFICIAL, ACCESS=Personal-Privacy]

Dear s22

Thank you for your email, please accept my apology for the delayed response.

Your request for an extension has been granted on this occasion, please provide the entire Questionnaire response by **no later than COB 06 December 2023.**

Please note no further extensions will be considered. Additional information regarding sponsor responsibilities is available here Post market responsibilities for manufacturers and sponsors of medical devices | Therapeutic Goods Administration (TGA)

Kind regards,

s22

Device Support Team
Medical Device Incident Report Investigation Scheme (IRIS)
Devices Post Market Monitoring | Medical Devices Surveillance Branch
Medical Devices and Product Quality Division | Health Products Regulation Group
Therapeutic Goods Administration (TGA)
Australian Government, Department of Health and Aged Care
Email: IRIS@health.gov.au
Phone: DST s22
Phone: IRIS 1800 809 361
27 Scherger Drive, Canberra Airport, ACT 2609
PO Box 100, Woden ACT 2606
www.tga.gov.au

The Department of Health and Aged Care acknowledges First Nations peoples as the Traditional Owners of Country throughout Australia, and their continuing connection to land, sea and community. We pay our respects to them and their cultures, and to all Elders both past and present.

Important: *This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error, please notify the author immediately and delete all copies of this transmission.*

Please Note: Medical device sponsors and manufacturers are encouraged to submit adverse events via the online Medical Device Incident Reporting (MDIR) system. The MDIR allows sponsors and manufacturers to submit initial, follow-up and final reports and review reports already submitted to the TGA. The MDIR can be accessed via https://apps.tga.gov.au/prod/mdir/MDIRSummary.aspx, using a TGA eBusiness Service (eBS) user name and password. MDIR guidance documents and FAQ are available from the 'Training' section of the TGA eBS portal, or from the TGA website. MDIR Technical assistance is available via 02 6289 4642 or email (IRIS@health.gov.au). If you are a sponsor or manufacturer requiring a new account to gain access to the MDIR system or need to update your details with TGA eBS, please contact TBS Helpdesk via 1800 010 624 or email (eBS@health.gov.au).

Online reporting forms for Medical Device Healthcare Professionals/Users can be accessed via:
https://apps.tga.gov.au/prod/mdir/udir03.aspx
For ongoing information and updates please subscribe to the TGA's Medical Devices Information and IVDs Information email subscription services.

From: s22 @paragoncare.com.au>
Sent: Friday, 24 November 2023 4:53 PM
To: IRIS <IRIS@health.gov.au>
Subject: RE: DIR 90606 - Questionnaire Letter - Response due by 13.11.23 [SEC=OFFICIAL, ACCESS=Personal-Privacy]

REMINDER: Think before you click! This email originated from outside our organisation. Only click links or open attachments if you recognise the sender and know the content is safe.

Dear s22

The manufacturer requires additional time to complete this questionnaire. Can we please request an extension until 1 DEC 2023?

Thank you for your patience.

Regards
s22

s22
s22
Paragon Care Group Australia Pty Ltd | 1/17 Rodborough Rd, Frenchs Forest, NSW 2086
M_ s22 **D_** s22 **E** s22 **@paragoncare.com.au W_ paragoncare.com.au**

ParagonCare
Health. Covered.

BECOVIDSAFE

At Paragon we work flexibly, so whilst it suits me to email you now, I do not expect a response outside of your normal working hours.

Please consider the environment before printing this email.

This email and any attachments are intended only for the use of the individual or entity to which it is addressed and may contain proprietary information that is privileged, confidential or copyright. You are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited without the authority of the sender. If you have received this e-mail message in error or are not the intended recipient, please delete and destroy all copies and notify us immediately by return mail. Any loss or damage incurred as a result of prohibited use is not the sender's responsibility. E-mail communications are not guaranteed as secure or error free, the sender accepts no liability for any damage caused by this email or its attachments due to viruses, interference, interception, corruption or unauthorised access. The sender's entire liability is limited to resending this email.

From: IRIS <IRIS@health.gov.au>
Sent: Monday, October 16, 2023 2:11 PM
To: s22 @paragoncare.com.au>
Subject: DIR 90606 - Questionnaire Letter - Response due by 13.11.23 [SEC=OFFICIAL, ACCESS=Personal-Privacy]

Dear s22

Please see the attached Questionnaire Letter for action. **Please note the due date for a response is 13 November 2023.**

Kind regards,
s22

Device Support Team
Medical Device Incident Report Investigation Scheme (IRIS)
Devices Post Market Monitoring | Medical Devices Surveillance Branch

Medical Devices and Product Quality Division | Health Products Regulation Group
Therapeutic Goods Administration (TGA)
Australian Government, Department of Health and Aged Care

Email: IRIS@health.gov.au
Phone: DST s22
Phone: IRIS 1800 809 361
Post: 27 Scherger Drive, Canberra Airport, ACT 2609
Courier: 1 Tindal Lane, Canberra Airport, ACT 2609
www.tga.gov.au

The Department of Health and Aged Care acknowledges First Nations peoples as the Traditional Owners of Country throughout Australia, and their continuing connection to land, sea and community. We pay our respects to them and their cultures, and to all Elders both past and present.

Important: *This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error, please notify the author immediately and delete all copies of this transmission.*

Please Note: Medical device sponsors and manufacturers are encouraged to submit adverse events via the online Medical Device Incident Reporting (MDIR) system. The MDIR allows sponsors and manufacturers to submit initial, follow-up and final reports and review reports already submitted to the TGA. The MDIR can be accessed via https://apps.tga.gov.au/prod/mdir/MDIRSummary.aspx, using a TGA eBusiness Service (eBS) user name and password. MDIR guidance documents and FAQ are available from the 'Training' section of the TGA eBS portal, or from the TGA website. MDIR Technical assistance is available via 02 6289 4642 or email (IRIS@health.gov.au). If you are a sponsor or manufacturer requiring a new account to gain access to the MDIR system or need to update your details with TGA eBS, please contact TBS Helpdesk via 1800 010 624 or email (eBS@health.gov.au).

Online reporting forms for Medical Device Healthcare Professionals/Users can be accessed via:
https://apps.tga.gov.au/prod/mdir/udir03.aspx
For ongoing information and updates please subscribe to the TGA's Medical Devices Information and IVDs Information email subscription services.

"Important: This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error please notify the author immediately and delete all copies of this transmission."

"Important: This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error please notify the author immediately and delete all copies of this transmission."

FOI 26-3320 - Document 3

Australian Government

Department of Health and Aged Care
Therapeutic Goods Administration

Australian Medical Device
Incident Report Investigation Scheme

s22

Immuno Pty Ltd
63 Poplar Rd
Parkville VIC 3052
Email: s22 @paragoncare.com.au

File Reference: E23-275158
Sent by email

Dear s22

DEVICE INCIDENT REPORT DIR 90606 - ARTG # 205351 - Bacterial infectious disease IVDs

The Therapeutic Goods Administration has been advised of an incident involving the above product. A copy of the Device Incident Report (DIR) is attached.

To assist in the evaluation and resolution of this report, please provide the information requested in the attached questionnaire and return it to this office **within 20 working days of the date of this letter,** and no later than COB 13/11/2023.

Please send responses via email to IRIS@health.gov.au, referencing the DIR number.

If you are unable to respond with all the information requested by the due date please advise, **within the 20 days**, when a full response will be provided. Extensions of a reasonable time frame will be accepted depending on the seriousness of the complaint and the time requested.

Thank you for your cooperation. If you require further information please contact me on

s22 or email: IRIS@health.gov.au.

Yours sincerely
Signed electronically by

Administration Officer

Incident Report Investigation Scheme
Devices Post Market Monitoring Section
Therapeutic Goods Administration

16/10/2023

FOI 26-3320 - Document 3

INITIAL REQUEST FOR INFORMATION FROM LISTED SPONSOR **Date:** 16/10/2023

DIR: 90606 Manufacturer Name: Nova Tec Immundiagnostica GmbH [44194]

Question/Requirement			
1) Please confirm the device's Australian Register of Therapeutic Goods (ARTG) number		**ARTG: 205351**	
2) Do you currently supply or have you previously supplied this product, with the indicated Model/Serial/Batch/Lot numbers:		**YES**	**NO**
a) To the Australian Market		x	
b) For Export			x
3) How many of this model have been supplied	In Australia:	16	
	Worldwide:	420	
4) How many of this batch (if applicable) have been supplied:	In Australia:		
	Worldwide:		
5) Are you aware of this problem, as reported?			x
6) If deemed necessary, is a sample of the mentioned device available for review and/or testing?		x	
7) Have you had any other reports of similar* problems with this product in Australia?			x

If **YES**, how many:

If **YES**, please give details:

* Similar events are based on the clinical event description and not the cause of an event. Both the confirmed and unconfirmed rates for similar events are often required and beneficial to show the full outlook of the device and its use.

FOI 26-3320 - Document 3

8) Is the manufacturer aware of reports of similar problems with this product? ☐ ☒ X

If **YES**, how many:

If **YES**, please give details:

9) Please provide details of any action you have taken, or intend to take, regarding this problem

We currently do not have any complaints world-wide in regards to the performance of the Borrelia burgdorferi IgG ELISA Kit.

10) Please provide details of the manufacturer's investigation to date, including expected **Manufacturer's investigation completion date**

Date: 30/11/2023

No investigation as no lot or user details provided.

11) When returning this response to the office of the Therapeutic Goods Administration, you are requested to attach the following (if checked):

- ☐ Sample of the product/device
- ☐ Product Specifications
- ☐ Descriptive product promotional documentation
- ☐ Instructions for use, as supplied with the device
- ☐ Device Packaging with printed instructions
- ☐ Operator's manual
- ☐ Technical Service Manual
- ☐ Clinical training manual in printed or video form
- ☐ In-house training documentation
- ☐ Evidence of compliance with the Essential Principles
- ☐ A summary of risk assessment activities performed by the manufacturer for the device, eg Risk Management Report required by Clause 8 of ISO 14971:2007.
- ☐ Service History, and Safety and Performance Test Results
- ☐ Configuration Information and Documentation
- ☐ HHE (Health Hazard Evaluation)

12) Additional Information required:

1. Provide rates for similar DIRs in Australia and overseas.

2. Provide the IFU that accompanies the ARTG ID.

13) If your device is an implantable pacemaker/defibrillator and/or associated lead you are asked to provide the following additional information:

1. Both published and unpublished clinical trial data where events of this type are analysed.
2. The number of reported events of ALL types (including unconfirmed events), the number of devices sold and the cumulative implant months for each device in this product family.
3. What material has been used to insulate, both internally and externally, the lead? (applicable to leads only)

Information Supplied By:

14)

Name s22

Signature s22

Position s22

Phone s22

Fax

Email s22 @paragoncare.com.au

This questionnaire and any appended documents should be returned to the TGA within **20 working days or as specified on page 1 of this letter.**

Electronic submission of all information is preferred.
Please send the requested information to email address: IRIS@health.gov.au

For large size documents, please post a universal serial bus (USB), compact disc (CD), or digital versatile disc (DVD) via postal address:

Incident Report Investigation Scheme
Devices Post Market Monitoring Section
Therapeutic Goods Administration
PO Box 100
Woden ACT 2606

If you are sending a device/s to the TGA please follow the instructions via link:

https://www.tga.gov.au/form/report-medical-device-adverse-event-medical-device-use

---

Sponsors of products listed or registered on the Australian Register of Therapeutic Goods (ARTG) are reminded of their responsibilities under Section 31 and/or 41JA (as appropriate) of the Therapeutic Goods Act of 1989, to provide information relating to their product's formulation, composition, design specification, quality, method and place of manufacture, presentation, safety and efficacy, conformity to advertising regulations under the Act,

Reporter Reference #:

Date of Adverse Event:

Date of Final Report:
11/09/2023

ARTG #:
205351

Brand Name:
NovaTec NovaLisa Borrelia burgdorferi IgG-ELISA (Novatec Novalisa) - Bacterial infectious disease IVDs

Device Class:
Class 2

Software Version:

Batch #:

Lot #:

Manufacturer:
Nova Tec Immundiagnostica GmbH [44194]

Sponsor:
Immuno Pty Ltd [12706]
63 Poplar Rd
Parkville VIC 3052

Contact Name: s22

Phone: s22

Email: s22 @paragoncare.com.au

Fax:

Reporter:

Confidential: Yes

Clinical Event Information:
I am writing to formally request the recall and investigation of the medical device NovaTec NovaLisa Borrelia burgdorferi IgG-ELISA (Novatec Novalisa), bearing ARTG 205351, due to the occurrence of discordant serological false-negative results that have raised serious concerns regarding its performance and safety.

Patient Outcome/Consequences:

Additional Event Description:

Device Analysis Results:

Corrective/Preventative Actions:

Details of Similar Events:

Number of Similar Events:

Rate of Similar Events:

Countries Similar Events Also Occurred:

FOI 26-3320 - Document 3

SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

Lieferung: frei Haus
Bestellung auf Rechnung

Lieferung ab € 30,-
Erstbestellung Vorauskassa

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC
HandelsgmbH
Quellenstraße 110, A-1100 Wien
T. +43(0)1 489 3961-0
F. +43(0)1 489 3961-7
mail@szabo-scandic.com
www.szabo-scandic.com

linkedin.com/company/szaboscandic

NovaTec Immundiagnostica GmbH

NovaLisa ™

Borrelia burgdorferi

IgM – ELISA (recombinant) CE

Enzyme immunoassay for the qualitative determination of IgM-class antibodies against Borrelia burgdorferi in human serum or plasma and CSF (cerebrospinal fluid)

Enzymimmunoassay zur qualitativen immunenzymatischen Bestimmung von IgM-Antikörpern gegen Borrelia burgdorferi in Humanserum oder Plasma und Liquor

Test immunoenzimatico per la determinazione qualitativa degli anticorpi della classe IgM per Borrelia burgdorferi nel siero o plasma umano e CSF (liquido cerebrospinale)

Enzimoinmunoensayo para la determinación cualitativa de anticuerpos IgM contra –Borrelia burgdorferi en suero o plasma humano y LCR (líquido cefalorraquídeo)

Only for in-vitro diagnostic use

English:	Page	2 to 6
Deutsch:	Seite	7 bis 12
Italiano:	da Pagina	13 a 17
Espanol:	Página	18 a 23

For further languages please contact our authorized distributors.

Bibliography / Literatur / Bibliografia / Bibliografía	Page / Seite / Pagina / Página	26
Symbols Key / Symbolschlüssel / Legenda / Símbolos	Page / Seite / Pagina / Página	27
Summary of Test Procedure/ Kurzanleitung Testdurchführung/ Schema della procedura/ Resumen de la técnica	Page / Seite / Pagina / Página	28

Product Number: BORM0040 (96 Determinations)

ENGLISH

CE

1. INTRODUCTION

Spirochetes are motile bacteria with a periplasmatic axial filament. All pathogenic species belong to the family Treponemataceae, which includes the three genera: Treponema, Borrelia, and Leptospira. The Treponemae are extremely long, flexible, filamentous cells that are usually held in a characteristic spiral, or coiled-spring shape. Borreliae are the largest Treponemataceae with very coarse and irregular spirals. Borrelia burgdorferi, the causative agent of Lyme disease, is transmitted mainly by ticks but probably also by other blood-sucking insects. Habitats are the wooded, humid and temperate regions of North America, Europe, North Africa, Australia and Japan; foresters, farmers, and anybody entering infested forests may be affected.
The degree of contamination of ticks amounts to 3-60% dependent on seasonal and regional differences; up to 30% of the population may be infected (about 1500 cases annually in USA, several hundred in Europe).

Species	Diseases	Symptoms	Mechanism of infection
Borrelia burgdorferi	Lyme diseases	Erythema chronicum migrans (ECM), Bannwarth Syndrome;dermatologic,rheumatic,cardiologic and neurologic: multisystem discover	Transmission by tick bites infected with Borrelia Borrelia burgdorderi (Ixodes dammini,USA:Ixodes ricinus,Eurpoe)

Because of its multisystemic character, unequivocal diagnosis of Lyme disease is difficult. Only early detection permits efficient control by antibiotics, since in the chronic phase borreliae are nearly inaccessible. The presence of bacteria resp. infection may be identified by:

- Microscopy: Giemsa or Wright-stained blood smears
- PCR
- Serology: Detection of antibodies by IF, ELISA, immunoblotting

2. INTENDED USE

The **recombinant** NovaTec Borrelia burgdorferi IgM-ELISA is intended for the qualitative determination of IgM class antibodies against Borrelia burgdorferi in human serum or plasma (citrate) and CSF (cerebrospinal fluid) offering increased diagnostic specificity and sensitivity by employing immunodominant antigens. In combination with the recombinant IgG ELISA each clinical phase of Lyme disease - from Erythma chronicum migrans over Bannwarth-Syndrom up to Lyme arthritis - may be detected even in borderland cases providing unerring results and diagnostic aid for the clinical staff.
For the determination of intrathecally produced IgG antibodies a separate instruction for use for CSF can be obtained at NovaTec.

3. PRINCIPLE OF THE ASSAY

The major constituent of B. burgdorferi flagella is flagellin (41 kDa, p41). Whereas the lipoprotein OspC (22 kDa, p22) within the outer membrane of the spirochete induces an early antibody formation in the ECM-phase of Lyme disease. Recombinant p41i is included in the presented antigens to avoid crossreactivity with antibodies of syphilitic sera.
The NovaTec Borrelia burgdorferi IgM-ELISA contains the recombinant epitope OspC of the phylum PKo (B. afzelii) and 20047 (B. garinii) and p41i of the phylum PBi (B. garinii).
Microtiter strip wells are pre-coated with **recombinant** Borrelia burgdorferi antigens. Diluted patient specimens and ready to use controls are added to these wells and antibodies recognizing the immobilized B. burgdorferi antigen bind during the first incubation. After washing the wells to remove all unbound sample and control material horseradish peroxidase labelled anti-human IgM conjugate is added. During a second incubation this conjugate binds to the captured antibodies, and the excess unbound conjugate is removed by a further wash step. The immune complex formed by the bound conjugate is visualized with TMB Substrate Solution which gives a blue reaction product, the intensity of which is proportional to the amount of B. burgdorferi-specific IgM antibody in the patient specimen. Sulphuric acid is added to each well to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Borrelia burgdorferi Coated Wells (IgM):** 12 breakapart 8-well snap-off strips coated with Borrelia burgdorferi antigen; in resealable aluminium foil.
- **IgM Sample Diluent** ***: 1 bottle containing 100 ml of ready to use buffer for sample dilution; pH 7.2 ± 0.2; coloured green; white cap.
- **Stop Solution**: 1 bottle containing 15 ml. Ready to use sulphuric acid, 0.2 mol/l; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle each containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.

- **Borrelia burgdorferi anti-IgM Conjugate**:** 1 bottle containing 20 ml of peroxidase labelled rabbit antibody to human IgM.; coloured red, Ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Borrelia burgdorferi IgM Positive Control***:** 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.
- **Borrelia burgdorferi IgM Cut-off Control***:** 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.
- **Borrelia burgdorferi IgM Negative Control***:** 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.

* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foil
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37℃
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 ℃.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (20…25℃) before starting the test run!

6.1. Coated snap-off strips

The ready to use breakapart snap-off strips are coated with Borrelia burgdorferi antigen. Store at 2...8℃. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 ℃; stability until expiry date.*

6.2. Borrelia burgdorferi anti-IgM Conjugate

The bottle contains 20 ml of a solution with anti-human-IgM horseradish peroxidase, buffer, stabilizers, preservatives and an inert red dye. The solution is ready to use. Store at 2...8℃. *After first opening stability expiry date when stored at 2…8℃.*

6.3. Controls

The bottles labelled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2...8℃. *After first opening stability until expiry date when stored at 2…8℃.*

6.4. IgM Sample Diluent

The bottle contains 100 ml phosphate buffer, anti human-IgG, stabilizers, preservatives and an inert green dye. It is used for the dilution of the patient specimen. The solution contains anti human IgG class antibodies to eliminate competitive inhibition from specific IgG class antibody to remove rheumatoid factor. This ready to use solution has to be stored at 2…8℃. *After first opening stability expiry date when stored at 2…8℃.*

6.5. Washing solution (20xconc.)

The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute Washing Solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh and germ free redistilled water. The diluted buffer is stable for 5 days at room temperature. *After first opening stability until expiry date when stored at 2…8℃.*

6.6. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8℃, away from the light. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening the concentrate is stable until the expiry date when stored at 2…8℃.*

6.7. Stop Solution

The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8℃.
After first opening stability until expiry date.

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2...8℃; otherwise they should be aliquoted and stored deep-frozen (-20 to -70℃). If samples are stored frozen, mix thawed sam ples well before testing. *Avoid repeated freezing and thawing.*
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10µl sample and 1 ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
1 well	(e.g. B1)	for the negative control,
2 wells	(e.g. C1+D1)	for the cut-off control and
1 well	(e.g. E1)	for the positive control.

It is left to the user to determine controls and patient samples in duplicate, if necessary.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37°± 1℃.

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1℃.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl Borrelia burgdorferi anti-IgM Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.** *Do not expose to direct sunlight.*
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
    Any blue colour developed during the incubation turns into yellow.

    Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.

11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Assay Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance value **< 0.100.**
- **Negative control** in B1: Absorbance value **< 0.200 and < cut-off**
- **Cut-off control** in C1 and D1: Absorbance value **0.150 – 1.30.**
- **Positive control** in E1: Absorbance value **> cut-off**.

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 =0.76 / 2 = 0.38

Cut-off = 0.38

9.3. Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative

→ **grey zone**

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

9.3.1. Results in NovaTec Units

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{cut-off}} = \text{[NovaTec-Units = NTU]}$$

Example: $\frac{1.216 \times 10}{0.38} = 32$ *NTU (NovaTec Units)*

Cut-off :	10	NTU	
Grey zone:		9-11	NTU
Negative:	<9	NTU	
Positive:	>11	NTU	

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

Intraassay	**n**	**Mean value**	**CV (%)**
Pos. Serum	8	1.06	8.6
Interassay	**n**	**Mean value**	**CV (%)**
Pos. Serum	14	0.76	4.1

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 98.8%

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 93%.

10.4. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunsuppremized patients and newborns serological data only have restricted value. A neg. result (IgM or IgG) cannot exclude an infection with B. burgdorferi. Especially in the early phase of infection there is the possibility that none or no detection quantities of the antibodies do exist. In the case of infection or a grey zone result we recommend a further sample after 2-3 weeks. A positive result of IgM does not always mean that an acute infection exists because the antibodies of a previous infection can persist. The use of recombinant antigens avoid extensively cross reactions with the following antibodies: Treponema pallidum, Leptospira, Borrelia recurrentis. Antibodies of a lues-infection to p41i were occasionally determinated. Therefore a lues-infection should be excluded.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The NovaLisa™ ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.:	BORM0040	Borrelia burgdorferi IgM-ELISA (96 Determinations)

DEUTSCH

CE

1. EINLEITUNG

Spirochäten sind spiralig gekrümmte, im Vergleich zu ihrem Durchmesser (0,1-3µm) unproportional lange (bis 250 µm) gram-negative Bakterien.
Alle pathogenen Spezies gehören zur Familie der Treponemataceae, zu denen folgende Gattungen zählen: Treponema, Borrelia und Leptospira. Die Treponemen sind schlanke, biegsame Bakterien gewöhnlich in charakteristischer Spiralform. Borrelien sind zarte (0,2-0,5µm), relativ lange (bis 250 µm) Spirochäten, mit 3-10 ungleichmäßigen Windungen.
Borrelia burgdorferi, der Verursacher der Borreliose-Erkrankung (Lyme-Erkrankung), wird hauptsächlich durch Zecken übertragen, möglicherweise auch durch andere blutsaugende Insekten. Hauptvektor in Europa ist die Schildzecke Ixodes ricinus, in den USA Ixodes damini.
Außer B. burgdorferi verursachen auch B. garinii und B. afzelii Lyme-Borreliosen. Die Erkrankung verläuft klassischerweise in drei Stadien. Sie wurde 1975 in der Kleinstadt Lyme im US-Bundesstaat Connecticut beschrieben. Burgdorfer et al. konnten 1983 den Erreger erstmals isolieren.
Die Lyme-Borreliose ist eine weit verbreitete Krankheit. Eine echte Prophylaxe existiert nicht. Ca. 10-20 % der Zecken in Europa sind mit Borrelia burgdorferi durchseucht. Die Erkrankungen an Borreliose sind endemisch und treten z.B. in Süddeutschland, entlang von Flussläufen und im Einzugsgebiet großer Städte gehäuft auf. Den natürlichen Lebensraum stellen vor allem bewaldete, feucht-warme Regionen Nordamerikas, Europas, Nordafrikas, Australiens und Japans dar.
Saisonale Gipfel akuter Erkrankungen sind die Monate Juni bis September, weitgehend erkrankungsfreie Monate Januar bis März.
Bevorzugter Personenkreis sind Waldarbeiter, Förster, Jäger und in der Landwirtschaft tätige Personen.
Bedingt durch die Vielzahl möglicher uncharakteristischer Symptome (neurologische, dermatologische, kardiale, rheumatische Manifestationen möglich) ist die Diagnose der Lyme-Borreliose häufig schwer und erfolgt erst relativ spät.
Nur die frühzeitige Diagnosestellung jedoch ermöglicht eine effiziente Kontrolle der Erkrankung mittels Antibiotika, in der chronischen Phase sind die Borrelien nahezu unangreifbar.

Spezies	Erkrankung	Inkubationszeit	Symptome	Infektionsmodus
Borrelia burgdorferi	Lyme-Borreliose	5 -29 Tage (ECM)	Erkrankung verläuft in drei Stadien: 1. Erythema chronicum migrans 2. Morbus Bannwarth (dermat., kardiale, rheumatische Manifestationen möglich) 3. Lyme-Arthritis, Enzephalomyelitis	Übertragung durch Zeckenbiss. In Europa durch Ixodes ricinus, In den USA durch Ixodes damini.

Nachweis:

- Mikroskopie : nach Giemsa oder Wright gefärbte Blutausstriche
- PCR
- Serologie: Antikörpernachweis mittels IF, ELISA Immunoblotting

Zuverlässig wird die Diagnose durch den Nachweis von IgG-Antikörpern im Serum in Verbindung mit dem klinischen Befund gestellt. Während der verschiedenen Stadien dominieren jeweils Antikörper gegen unterschiedliche bakterielle Antigene, z.B. auf den Geißeln oder der äußeren Membran.

2. VERWENDUNGSZWECK

Der NovaTec Borrelia burgdorferi IgM ELISA ist für den qualitativen Nachweis spezifischer IgM-Antikörper gegen Borrelia burgdorferi in humanem Serum oder Plasma (Citrat) und Liquor bestimmt. Für die Bestimmung intrathekal produzierter IgG Antikörper kann bei NovaTec eine separate Arbeitsanleitung angefordert werden.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von gegen B. burgdorferi gerichtetem spezifischem IgM beruht auf der ELISA (Enzyme-linked Immunosorbent Assay)-Technik. Hauptbestandteil der B. burgdorferi Flagelle ist **Flagellin** (41 kDA, p41). Dieses Protein zusammen mit dem spezies-spezifischen Lipoprotein **OspC** der Außenmembran der Spirochäte induziert die Bildung einer frühen Antikörperformation in der ECM-Phase der Erkrankung (Frühphase, Stadium I).
Kreuzreaktionen mit syphilitischen Seren werden durch die Verwendung von **p41i** als Antigen vermieden.
Der vorliegende Kit enthält die rekombinant hergestellten Epitope OspC der Stämme PKo (B. afzelii) und 20047 (B. garinii) sowie p41i des Stammes PBi (B. garinii).
Mikrotiterstreifen als solide Phase sind beschichtet mit den oben aufgeführten Antigenen. Vorhandene spezifische Antikörper in der Probe binden an die immobilisierten Antigene der Mikrotiterplatte. Peroxidase-konjugierte anti-human IgM-Antikörper binden an Antigen-Antikörperkomplexe in positiven Proben. Die entstanden Immunkomplexe werden durch Blaufärbung nach Inkubation mit TMB-Substratlösung nachgewiesen. Stoppen der enzymatischen Reaktion mit Schwefelsäure führt zu einem Farbumschlag von blau zu gelb, der einfach nachgewiesen und mit einem ELISA-Reader bei 450 nm gemessen werden kann.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **B. burgdorferi beschichtete Mikrotiterstreifen (IgM):** 12 teilbare 8er-Streifen, beschichtet mit rekombinanten B. burgdorferi-Antigenen; in wiederverschließbarem Aluminiumbeutel.
- **IgM-Probenverdünnungspuffer***:** 1 Flasche mit 100 ml Puffer zur Probenverdünnung; enthält anti-human-IgG-Antikörper; pH 7.2 ± 0.2; grün gefärbt; gebrauchsfertig; weiße Verschlusskappe.
- **Stopplösung:** 1 Fläschchen mit 15 ml Schwefelsäure, 0.2 mol/l; gebrauchsfertig; rote Verschlusskappe.
- **Waschlösung (20x konz.)*:** 1 Flasche mit 50 ml eines 20-fach konzentrierten Puffers zum Waschen der Kavitäten; pH 7.2 ± 0.2; weiße Verschlusskappe.
- **B. burgdorferi anti-IgM-Konjugat**:** 1 Flasche mit 20 ml Peroxidase-konjugierten Antikörper gegen humanes IgM; rot gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Fläschchen mit 15 ml 3,3`,5,5`-Tetramethylbenzidin (TMB); gebrauchsfertig; gelbe Verschlusskappe.
- **B. burgdorferi IgM Positivkontrolle***:** 1 Fläschchen mit 2 ml; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig.
- **B. burgdorferi IgM Cut-off Kontrolle***:** 1 Fläschchen mit 3 ml; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig.
- **B. burgdorferi IgM Negativkontrolle***:** 1 Fläschchen mit 2 ml; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig.

* enthält 0.1 % Bronidox L nach Verdünnung
** enthält 0.2 % Bronidox L
*** enthält 0.1 % Kathon

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Rahmenhalter
- 1 Arbeitsanleitung
- 1 Ergebnisblatt

4.3. Erforderliche Materialien und Geräte

- Photometer mit Filtern 450/620 nm
- Feuchtkammer/Brutschrank mit Thermostat
- Manuelle oder automatische Waschvorrichtung
- Mikropipetten mit Einmalspitzen (10, 100, 200, 1000 µl)
- Vortex-Mischer
- Plastikröhrchen für den einmaligen Gebrauch
- Röhrchen-Ständer
- Aqua dest.
- Timer

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8℃ lagern. Die Reagenzien nicht nach den angegebenen Verfallsdaten verwenden. Die Verfallsdaten sind jeweils auf den Flaschenetiketten und auf dem Außenetikett angegeben.

6. VORBEREITUNG DER REAGENZIEN

Alle Reagenzien, Proben und Kontrollen sind vor ihrer Verwendung auf Raumtemperatur(20...25℃) zu bringen!

6.1. Beschichtete Streifen

Die abbrechbaren Streifen sind mit inaktivierten, rekombinanten B. burgdorferi Antigenen beschichtet. Die gebrauchsfertigen Vertiefungen sind bei 2...8℃ aufzubewahren. *Nichtverbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8° C lagern. Haltbarkeit bis zum angegebenen Verfallsdatum.*

6.2. B. burgdorferi anti-IgM-Konjugat

Die Flasche enthält 20 ml einer Lösung von anti-human IgM-Meerrettichperoxidase, Puffer, Stabilisatoren, Konservierungsmittel und einen inerten roten Farbstoff. Die gebrauchsfertige Lösung ist bei 2...8℃ aufzubewahren. *Nach dem ersten Öffnen haltbar bis zum angegebenen Verfallsdatum (bei 2...8℃).*

6.3. Kontrollen

Die Fläschchen mit Kontrollen enthalten 2 bzw. 3 ml gebrauchsfertige Kontrolllösung. Die gebrauchsfertigen Lösungen sind bei 2...8℃ aufzubewahren und enthalten 0.1 % Kathon. *Nach dem ersten Öffnen haltbar bis zum angegebenen Verfallsdatum (bei 2...8℃).*

6.4. IgM-Probenverdünnungspuffer

Die Flasche enthält 100 ml Phosphatpuffer, Stabilisatoren, Konservierungsmittel und einen inerten grünen Farbstoff. Die gebrauchsfertige Lösung enthält anti-human-IgG-Antikörper, um den Proben spezifische IgG-Anteile sowie IgG-gebundene Rheumafaktoren zu entziehen. Sie wird für die Verdünnung der Proben eingesetzt und ist bei 2...8℃ aufzubewahren. *Nach dem ersten Öffnen haltbar bis zum angegebenen Verfallsdatum (bei 2...8℃).*

6.5. Waschlösung (20x konz.)

Die Flasche enthält 50 ml konzentrierten Puffer, Detergenzien und Konservierungsmittel. Der Inhalt wird auf einen Liter mit Aqua dest. verdünnt (1+19). Der verdünnte Puffer ist bei Raumtemperatur 5 Tage haltbar. Die Waschlösung wird zum Waschen der Streifen eingesetzt. *Sollte eine Kristallisation im Konzentrat auftreten, die Waschlösung auf 37℃ erwärmen und vor dem Verdünnen gut mischen. Nach dem ersten Öffnen, Konzentrat haltbar bis zum angegebenen Verfallsdatum (bei 2...8℃).*

6.6. TMB-Substratlösung

Das Fläschchen enthält 15 ml eines Tetramethylbenzidin- Wasserstoffperoxidgemisches. Die gebrauchsfertige Lösung ist bei 2...8℃ vor Licht geschützt aufzubewahren. *Die Lösung ist leicht bläulich. Sollte die TMB-Substratlösung dunkelblau sein, ist sie kontaminiert und kann nicht im Test verwendet werden. Nach dem ersten Öffnen haltbar bis zum Verfallsdatum bei sachgerechter Lagerung von 2…8℃.*

6.7. Stopplösung

Das Fläschchen enthält 15 ml 0,2 M Schwefelsäure (R36/38, S26). Die gebrauchsfertige Lösung ist bei 2...8℃ aufzubewahren. *Nach dem ersten Öffnen haltbar bis zum angegebenen Verfallsdatum (bei 2...8℃).*

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat) verwendet werden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8℃ aufbewahrt werden, sonst tiefgefri eren (-70...-20℃). Wiederaufgetaute Proben vor dem Verdünnen gu t schütteln. *Wiederholtes Tiefgefrieren und Auftauen vermeiden*! Hitzeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1+100 mit IgM-Probenverdünnungspuffer verdünnen, z.B. 10µl Probe und 1 ml IgM-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1+100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

8.1. Testvorbereitung

Gebrauchsinformation **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei auf fünf und das Volumen der Waschlösung von 300 µl auf 350 µl zu erhöhen. Vor Testbeginn auf dem mitgelieferten Ergebnisblatt die Verteilung bzw. Position der Patientenproben und Standards auf den Mikrotiterstreifen genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Hierbei mindestens

1 Vertiefung	(z.B. A1)	für den Substratleerwert (Blank*),*
1 Vertiefungen	(z.B. B1)	für die Negativ Kontrolle und
2 Vertiefungen	(z.B. C1+D1)	für die Cut-off Kontrolle und
1 Vertiefung	(z.B. E1)	für die Positiv Kontrolle vorsehen.

Prinzipien der Qualitätssicherung in der Laboratoriumsmedizin erfordern zur höheren Sicherheit für Kontrollen und Patientenproben mindestens Doppelbestimmungen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Brutschrank auf 37 ± 1℃ einstellen.

1. Je 100 µl Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ℃ inkubieren.**

4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300µl Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Intervall zwischen Waschen und Absaugen sollte mindestens 5 sec betragen. Nach dem Waschen die Teststreifen mit den Öffnungen nach unten kurz auf Fliesspapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: *Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falsch erhöhten Messergebnissen führt!*
5. 100µl B. burgdorferi anti-IgM Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes vorgesehenen, pipettieren. Mit Folie abdecken.
6. **30 min bei Raumtemperatur (20...25℃) inkubieren.** *Nicht dem direkten Sonnenlicht aussetzen*.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100µl TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25℃) inkubieren.**
10. In alle Vertiefungen 100µl Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei der TMB-Substratlösung Zugabe pipettieren. *Während der Inkubation gebildete blaue Farbe schlägt in gelb um.*

    Hinweis: *Hochpositive Patientenproben können schwärzliche Präzipitate des Chromogens verursachen! Diese Präzipitate beeinflussen die Messwerte. Es wird empfohlen, die Patientenprobe mit physiologischer Kochsalzlösung 1 + 1 zu verdünnen und anschließend die verdünnte Probe mit IgM-Probenverdünnungspuffer 1 + 100 für den Test vorzubereiten. Das Ergebnis in NTU wird in diesem Fall mit zwei multipliziert.*
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen

8.2. Messung

Mit Hilfe des Substratleerwertes (Blank) **in A1** den **Nullabgleich** des Mikrotiterplatten-Photometers (ELISA-Readers) vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert der Position A1 von allen anderen Extinktionswerten abgezogen werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Kontrollen und Proben in das Ergebnisblatt eintragen.

*Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.*

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Der Test wurde richtig durchgeführt, wenn er folgende Kriterien erfüllt:

- **Substrat-Leerwert** in A1: Extinktion **niedriger als 0,100**
- **Negativ Kontrolle** in B1: Extinktion **< 0,200 und < cut-off**
- **Cut-off Kontrolle** in C1 und D1: Extinktion **0,150 – 1,300**
- **Positiv Kontrolle** in E1: Extinktion **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der beiden Cut-off Kontrollen.

Beispiel: 0.37 OD Cut-off Kontrolle + 0.39 OD Cut-off Kontrolle = 0.76 : 2 = 0.38

Cut-off = 0.38

9.3. Interpretation der Ergebnisse

Patientenproben gelten als **positiv**, wenn der Extinktionswert mindestens 10 % höher liegt als der Cut-Off.

Patientenproben mit Extinktionswerten 10 % über bzw. unter dem Cut-Off können nicht eindeutig als positiv bzw. negativ angesehen werden → **Grauzone**
Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut innerhalb der Grauzone, gilt die Probe als **negativ**.

Patientenproben gelten als **negativ**, wenn der Extinktionswert mindestens 10 % unterhalb des Cut-Offs liegt.

9.3.1. Ergebnisse in NovaTec-Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Patientenprobe} \times 10}{\text{Cut-Off}} = \text{[NovaTec-Einheiten = NTU]}$$

Beispiel: $\frac{1.216 \times 10}{0.38} = 32$ *NTU (NovaTec Units)*

Cut-Off:	10	NTU	
Grauzone:		9-11	NTU
Negativ:	<9	NTU	
Positiv:	>11	NTU	

10. TESTMERKMALE

10.1. Präzision

Interassay	**n**	**Mittelwert**	**Vk (%)**
Pos. Serum	14	0.76	4.1

Intraassay	**n**	**Mittelwert**	**Vk (%)**
Pos. Serum	8	1.06	8.6

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 98.8 %.

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie beträgt 93 %.

10.4. Interferenzen

Hämolytische,. lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/ml für Hämoglobin, von 5 mg/ml Triglyceride und von 0,2 mg/ml für Bilirubin keine Interferenzen im vorliegenden ELISA.

Hinweis: Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden.
Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse der serologischen Tests nur einen begrenzten Wert.
Ein negatives Ergebnis kann eine Infektion nicht ausschließen. Besonders in der frühen Infektionsphase können Antikörper in Konzentrationen unterhalb der Nachweisgrenze vorliegen. Es wird daher empfohlen, bei begründetem Verdacht auf Borreliose bzw. einem Befund im Graubereich nach 2-3 Wochen eine weitere Probe zu testen.
Bei einem positiven Ergebnis wird empfohlen dieses mit einem weiteren Testsystem (Blot) zu bestätigen.
Durch den Einsatz von rekombinanten Antigenen sind Kreuzreaktionen mitfolgenden Erregern weitestgehend ausgeschlossen:

- Treponema pallidum
- Borrelia recurrentis
- Leptospira

Bei Lues-Infektionen wurden vereinzelt Antikörper gegen p41i nachgewiesen. Daher sollte eine Lues-Infektion ausgeschlossen werden.
Bei einer infektiösen Mononukleose (EBV-Infektion) kann es zu einer Kollektion einer Stimulierung von B-Lymphozyten kommen. Dies kann zu unspezifischen Reaktionen beim IgM-Nachweis führen. Es wird daher empfohlen beim Vorliegen einer schwachen IgM Antwort und unklarem klinischen Bild eine EBV IgM Infektion auszuschließen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Gemäß Art. 1 Abs. 2b der EU-Richtlinie 98/79/EG legt der Hersteller die Zweckbestimmung von In-vitro-Diagnostika fest, um deren Eignung, Leistung und Sicherheit sicherzustellen. Daher sind die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Gebrauchsanweisung strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAG nicht-reaktiv getestet. Dennoch sind alle Materialien als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat- und Standardfläschchen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten Patientenproben und Konjugat sorgfältig in die Kavitäten pipettieren.
- Der NovaLisa™ ELISA ist nur für die Anwendung durch Fachpersonal vorgesehen, welches die Arbeitstechniken einwandfrei beherrscht.

WARNUNG:	Bronidox L zeigt in der verwendeten Konzentration nahezu keine toxikologischen Risiken an Haut bzw. Schleimhaut.
WARNUNG:	Schwefelsäure reizt Augen und Haut! Nach Berührung mit den Augen gründlich mit Wasser spülen und einen Arzt aufsuchen.

12.1. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: BORM0040 Borrelia burgdorferi IgM-ELISA (96 Bestimmungen)

ITALIANO

CE

1. INTRODUZIONE

Le Spirochete appartengono alla famiglia dei Treponomataceae che comprende le specie Treponema, Borrelia e Leptospira. Hanno una lunghezza di 250µm, sono gram negativi con un diametro di 0,1-3µm. La Borrelia burgdorferi é l'agente eziologico del morbo di Lyme. Viene trasmessa soprattutto dalle zecche Ixodes dammini e Ixodes pacificus. Nel 1975 è stata scoperta per la prima volta a Lyme USA. Burgdorfer et al. hanno isolato per la prima volta il batterio nel 1983. Il morbo di Lyme è una malattia molto diffusa nel mondo. Non esiste una vera profilassi. Il 10-20 % circa delle zecche europee sono contaminate. I focolai naturali sono boschi umidi e caldi in Europa, Nord America, Africa, Australia e Giappone. La distribuzione stagionale della malattia individua un picco costantemente presente in tarda primavera-inizio estate (Giugno-Luglio), mentre i sintomi più tardivi si concentrano in autunno-inizio inverno. A causa dei sintomi poco specifici la diagnosi è difficile e viene fatta spesso troppo tardi. La terapia con antibiotici è efficace soltanto dopo una diagnosi precoce, nella fase cronica la cura diventa molto difficile.

Specie	Malattia	Sintomi	Modo d'infezione
Borrelia burgdorferi	Morbo di Lyme	Tre stadi: 1. Erythema cronicum migrans 2. Morbo di Bannwarth 3. Artrite di Lyme, Encefalomielite	Morso di zecche.

Diagnosi
- Microscopia: colorazione di Giemsa; colorazione di Wright per cellule ematiche
- PCR
- Sierologia: ELISA; immunofluorescenza indiretta IFA

2. USO PREVISTO

Il NovaTec Borrelia burgdorferi IgM ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgM per Borrelia burgdorferi nel siero o plasma (citrato) umano e CSF (liquido cerebrospinale).
Presso il NovaTec sono disponibili le istruzioni corrispondenti.

3. PRINCIPIO DEL TEST

La determinazione qualitativa degli anticorpi IgM per Borrelia burgdorferi si basa sul principio ELISA. La parte preponderante del flagello di B. burgdorferi è la **flagellina** (41kDa, p41). Questa proteina insieme con lipoproteine specifiche (OspC) della spirocheta induce la formazione di anticorpi nella prima fase della malattia. Reazioni incrociate con il siero positivo da sifilide vengono evitate mediante l'uso dell'antigene p41i.
Il presente kit contiene epitopi **OspC** delle specie PKo (B. afzelii) e 20047 (B. garinii), e **p41i** della specia PBi (B. garinii).
I pozzetti delle micropiastre contengono una fase solida con antigeni specifici della Borrelia burgdorferi. Anticorpi specifici nel campione si legano agli antigeni immobilizzati nei pozzetti. Gli anticorpi del coniugato (perossidasi di rafano-anticorpi anti-IgM umani) si legano ai complessi antigene (fase solida)-anticorpo (paziente) nei campioni positivi. Questi complessi vengono evidenziati da una colorazione blu dopo l'incubazione con la soluzione TMB. L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici per la Borrelia burgdorferi di classe IgM presenti nel campione. Fermando la reazione enzimatica con acido solforico si causa un cambiamento di colore dal blu al giallo che può essere misurato facilmente con un fotometro per l'ELISA a 450 nm.

4. MATERIALI

4.1. Reagenti forniti

- **Micropiastre con antigeni dell Borrelia burgdorferi (IgM):** 12 strisce divisibili in 8 pozzetti, con adesi antigeni ricombinanti della Borrelia burgdorferi; dentro una busta d'alluminio richiudibile.
- **Tampone diluente IgM***:** 1 flacone contenente 100 ml di tampone per diluire i campioni; pH 7.2 ± 0.2; color verde; pronto all'uso; tappo bianco; contiene anticorpi anti IgG umani.
- **Soluzione stop:** 1 flacone contenente 15 ml di acido solforico, 0.2 mol/l, pronto all'uso; tappo rosso.
- **Tampone di lavaggio (20x conc.)*:** 1 flacone contenente 50 ml di un tampone concentrato 20 volte per il lavaggio dei pozzetti; pH 7.2 ± 0.2; tappo bianco.
- **Coniugato Borrelia burgdorferi anti IgM**:** 1 flacone contenente 20 ml di anticorpi di coniglio anti-IgM umani, coniugati a perossidasi; color rosso; pronto all'uso; tappo nero.
- **Soluzione TMB:** 1 flacone contenente 15 ml di 3,3`,5,5`-Tetrametilbenzidina (TMB); pronto all'uso; tappo giallo.
- **Borrelia burgdorferi IgM Controllo positivo***:** 1 flacone da 2 ml; color giallo; tappo rosso; pronto all'uso.
- **Borrelia burgdorferi IgM Controllo Cut-off***:** 1 flacone da 3 ml; color giallo; tappo verde; pronto all'uso.
- **Borrelia burgdorferi IgM Controllo negativo***:** 1 flacone da 2 ml; color giallo; tappo blu; pronto all'uso.

* contiene 0.1 % Bronidox L dopo diluizione
** contiene 0.2 % Bronidox L
*** contiene 0.1 % Kathon

4.2. Accessori forniti

- 1 pellicola adesiva
- 1 supporto per micropiastre
- 1 istruzione per l'uso
- 1 foglio di controllo

4.3. Materiali e attrezzature necessari

- Fotometro per micropiastre con filtri da 450/620 nm
- Incubatore a 37℃
- Lavatore di micropiastre
- Micropipette con punte monouso (10, 100, 200, 1000 µl)
- Vortex-Mixer
- Provette monouso
- Supporto per provette
- Acqua deionizzata o distillata.
- Timer

5. MODALITÀ DI CONSERVAZIONE

I reagenti devono essere conservati tra 2-8℃. Non usare i reagenti dopo la scadenza. La data di scadenza è stampata sull'etichetta di ogni componente e sull'etichetta esterna della confezione.

6. PREPARAZIONE DEI REAGENTI

Portare tutti i reagenti a temperatura ambiente (20-25℃) prima dell'uso!

6.1. Micropiastre

I pozzetti sono separabili e sotto vuoto. Contengono adesi antigeni ricombinanti inattivati della Borrelia burgdorferi. I pozzetti, pronti all'uso, devono essere conservati tra 2-8℃. *Riporre i pozzetti non utilizzati nel sacchetto con il gel essiccante di silice. Il prodotto è stabile fino alla data di scadenza se conservato tra 2-8℃.*

6.2. Coniugato Borrelia burgdorferi IgM

Il flacone contiene 20 ml di anticorpi anti-IgM umani coniugati a perossidasi di rafano, stabilizzanti, conservanti e un colorante inerte rosso. *Una volta aperto, il prodotto é stabile fino alla data di scadenza se conservato tra 2-8℃.*

6.3. Controlli

I flaconi dei controlli contengono di soluzione pronta all'uso. Contengono 0,1% Kathon. *Una volta aperto, il prodotto é stabile fino alla data di scadenza se conservato tra 2-8℃.*

6.4. Tampone diluente IgM

Il flacone contiene 100 ml di tampone fosfato, stabilizzanti, conservanti e un colorante verde inerte. La soluzione contiene anticorpi anti IgG umani per togliere dagli campioni gli anticorpi specifici della classe IgG ed i fattori reumatici legati ad anticorpi IgG. Viene usata per diluire i campioni . Una volta aperto, il prodotto é stabile fino alla data di scadenza se conservato tra 2-8℃.

6.5. Tampone di lavaggio (20x conc.)

Il flacone contiene 50 ml di un tampone concentrato, detergenti e conservanti. Il contenuto viene diluito con acqua deionizzata o distillata (1 + 19). Il tampone diluito é stabile fino 5 giorni se conservato a temperatura ambiente. *Se sono presenti cristalli, scioglierli a 37℃ prima di dil uire. Una volta aperto, il prodotto é stabile fino alla data di scadenza se conservato tra 2-8℃.*

6.6. Soluzione TMB

Il flacone contiene 15 ml di 3,3`,5,5`-Tetrametilbenzidina (TMB) e perossido di idrogeno pronto all'uso. Conservare al buio. *La soluzione é incolore o celeste chiaro. Nel caso in cui diventasse blu significa che é contaminata e non può essere più usata. Una volta aperto, il prodotto é stabile fino alla data di scadenza se conservato tra 2-8℃.*

6.7. Soluzione Stop

Il flacone contiene 15 ml di acido solforico, 0.2 mol/l (R36/38, S26), pronto all'uso. *Una volta aperto, il prodotto é stabile fino alla data di scadenza se conservato tra 2-8℃.*

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Usare campioni di siero o plasma (citrato) umano. Se il test viene fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2-8℃. Altrimenti devono esse re aliquotati e congelati tra -70…-20℃. Agitare bene i campioni scongelati prima di diluirli. *Evitare cicli ripetuti di congelamento/scongelamento.*
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1 + 100 con tampone diluente IgM. Per esempio, pipettare nelle provette 10 µl di campione + 1 ml di tampone e mescolare bene (Vortex).

8. PROCEDIMENTO

8.1. Preparazione del test

Leggere bene le istruzioni prima di iniziare il dosaggio. Per ottenere risultati validi é indispensabile seguire esattamente le istruzioni. La seguente procedura è stata validata per l'esecuzione manuale. Per una esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a5 volte e il volume della soluzione di lavaggio da 300 a 350µl per evitare interferenze. Stabilire innanzitutto il piano di distribuzione ed identificazione dei campioni e controlli sul foglio di lavoro fornito con il kit. Inserire i pozzetti necessari nel supporto micropiastre

Utilizzare almeno:

1 pozzetto	(es. A1)	per il bianco-substrato (blank)
1 pozzetti	(es. B1)	per il controllo negativo
2 pozzetti	(es. C1+D1)	per il controllo Cut-off
1 pozzetto	(es. E1)	per il controllo positivo.

È consigliato effettuare ogni analisi in duplicato.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza pause.

Utilizzare puntali nuovi e puliti per ogni campione e controllo.

Regolare l'incubatore a 37° ± 1℃

1. Pipettare 100 µl di controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva.
3. **Incubare 1 ora ± 5 min a 37° ± 1℃.**
4. Al termine dell'incubazione, togliere la pellicola ed aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µl di tampone di lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere almeno di 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti con l'apertura verso il basso su una carta assorbente per togliere completamente il liquido.

   Attenzione: *Il lavaggio é una fase critica. Un lavaggio non accurato determina una cattiva precisione del test ed un innalzamento falsato delle densità ottiche.*
5. Pipettare 100µl di Coniugato Borrelia burgdorferi anti-IgM in tutti i pozzetti, escludendo quello con il bianco-substrato (blank). Coprire i pozzetti con la pellicola adesiva.
6. **Incubare 30 min a temperatura ambiente (20°...25 ℃).** *Non esporre a fonti di luce diretta.*
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100µl di Soluzione TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20°...25℃) al buio.**
10. Pipettare 100µl di Soluzione Stop in tutti i pozzetti, nello stesso ordine della soluzione TMB. *Durante l'incubazione il colore cambia dal blu al giallo.*

    Attenzione: *Campioni con un risultato positivo molto alto possono causare precipitati scuri del cromogeno! Questi precipitati influenzano la lettura delle densità ottiche. È consigliato diluire i campioni con soluzione fisiologica NaCl, esempio 1+1. Poi diluire normalmente 1 + 100 con tampone diluente IgM. Il risultato NTU viene moltiplicato per due.*
11. Misurare l'assorbanza di tutti i pozzetti a 450/620 nm entro 30 min dopo l'aggiunta della soluzione stop.

8.2. Misurazione

Regolare il fotometro per le micropiastre (ELISA-Reader) **a zero** usando il substrato-bianco (blank) **in A1**. *Se, per motivi tecnici, non é possibile regolare il fotometro sottrarre l'assorbanza del bianco-substrato da tutti i valori delle altre assorbanze.*

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nel foglio di lavoro.

É raccomandato fare una misurazione delle densità ottiche a doppia lunghezza d'onda utilizzando i 620 nm come lunghezza di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze.**

9. RISULTATI

9.1. Validazione del test

Il test é valido se risponde ai prossimi criteri:

- **Substrato bianco** in A1: Valore di assorbanza **< 0.100**
- **Controllo negativo** in B1: Valore di assorbanza **< 0.200 e< cut-off**
- **Controllo Cut-off** in C1 e D1: Valore di assorbanza **0.150 – 1.30**
- **Controllo positivo** in E1: Valore di assorbanza **>Cut-Off**

Se non vengono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-Off e' la media dei valori di assorbanza dei controlli Cut-off.

Esempio: Valore di assorbanza del controllo Cut-off 0.39 + valore di assorbanza del controllo Cut-off 0.37 = 0.76/2= 0.38

Cut-Off = 0.38

9.3. Interpretazione dei risultati

I campioni sono **positivi**, se l'assorbanza supera il Cut-Off almeno del 10 %.

Campioni con assorbanze del 10 % al di sopra o al di sotto del Cut-Off non sono identificabili come positivi o negativi → **Dubbio**
In questo caso é raccomandato di ripetere il test dopo 2 o 4 settimane con un campione fresco. Se il risultato é ancora incerto viene considerato **negativo**.

I campioni sono **negativi**, se l'assorbanza risulta inferiore del Cut-Off almeno del 10 %.

9.3.1. Risultati in unità NovaTec [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-Off}} = [\text{unità NovaTec = NTU}]$$

Esempio: $\frac{1.216 \times 10}{0.38} = 32$ *NTU (NovaTec Units)*

Cut-Off :	10	NTU
Dubbio:	9-11	NTU
Negativo:	<9	NTU
Positivo:	>11	NTU

10. CARATTERISTICHE DEL TEST

10.1. Precisione

Interdosaggio	**n**	**Media**	**Cv (%)**
Siero pos.	14	0.76	4.1
Intradosaggio	**n**	**Media**	**CV (%)**
Siero pos.	8	1.06	8.6

10.2. Specificità diagnostica

La specificità diagnostica é la probabilità del test di fornire un risultato negativo in assenza di anticorpi specifici. La specificità diagnostica é pari a 98.8%.

10.3. Sensibilità diagnostica

La sensibilità diagnostica é la probabilità del test di fornire un risultato positivo in presenza di anticorpi specifici. La sensibilità diagnostica é pari a 93%.

10.4. Possibili interferenze

Campioni emolitici, lipidici ed itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,2 mg/mL di bilirubina non hanno presentato fenomeni di interferenza nel presente test.

Nota: I risultati si riferiscono al gruppo di campioni realizzati, questi non sono specifiche garantite.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze. La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato. Il risultato negativo non esclude la possibilità di essere infetto dal virus. Specialmente all'inizio dell'infezione è possibile che la presenza di anticorpi nel siero sia insufficiente per una determinazione esatta. Se vi è il sospetto clinico si raccomanda di ripetere il test dopo 2-3 settimane con un campione nuovo. Si raccomanda di verificare i risultati positivi con un altro metodo di determinazione. Con l'uso di antigeni ricombinanti si escludono quasi completamente le reazioni incrociate con i seguenti microorganismi:

Treponema pallidum

Borrelia recurrentis

Leptospira

Si raccomanda di escludere un'infezione da sifilide, poiché talvolta si sono ritrovati anticorpi contro p41i. A causa di un'infezione da Mononucleosi (Epstein-Barr) è possibile che si verifichi una stimolazione dei linfociti B che puó generare una reazione poco specifica per la determinazione degli anticorpi IgM. Si raccomanda di escludere un'infezione da Epstein-Barr in caso di un risultato lievemente positivo (IgM) e un quadro clinico poco specifico.

12. PRECAUZIONI E AVVERTENZE

- In ottemperanza all'articolo 1, paragrafo 2 della direttiva Europea 98/79/EC, l'uso dei diagnostici medici in vitro è inteso da parte del produttore ad assicurare la congruenza, le prestazioni e la sicurezza del prodotto. Di conseguenza la procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i componenti di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg. Nonostante ciò e tutti i materiali devono comunque essere considerati potenzialmente contagiosi e infettivi.
- Non scambiare reagenti e micropiastre di lotti diversi.
- Non utilizzare reagenti di altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto attrezzatura pulita.
- Non scambiare i tappi dei flaconi.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti.
- Il NovaLisa™ ELISA è previsto soltanto per essere impiegato da parte di personale specializzato che conosce perfettamente le tecniche di lavoro.

ATTENZIONE:	Bronidox L, nella concentrazione usata, mostra quasi assenza di tossicità sulla pelle e sulle mucose.
ATTENZIONE:	L'acido solforico irrita occhi e pelle! Dopo il contatto sciacquare immediatamente e abbondantemente. Contattare un medico.

12.1. Smaltimento

In genere tutte le sostanze chimiche vengono considerate rifiuti tossici. Lo smaltimento viene regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto: BORM0040 Borrelia burgdorferi IgM-ELISA (96 determinazioni)

ESPANOL

CE

1. INTRODUCCIÓN

Las espiroquetas son bacterias gramnegativas mobiles de forma espiral que son desproporcionadamente largas (hasta 250µm) en comparación con su diámetro (0,1-3µm). Todas las especies patógenas pertenecen a la familia de las Treponemataceae que incluyen tres géneros: Treponema, Borrelia y Leptospira.
Las Treponemas son bacterias delgadas, flexibles, normalmente con una forma espiral caracteristica. Las Borrelias son las mas largas espiroquetas de las Treponematacae con espirales delicadas y irregulares. Borrelia burgdorferi es el causante de la enfermedad inflamatoria persistente tardía de Lyme que se reportó por primera vez en 1975 en el pueblo de Old Lyme, en el estado de Connecticut (Estados Unidos). La enfermedad se transmite sobre todo por la picadura de la garrapata, possiblemente también por otros insectos sanguijuelos. La propagación es a nivel mundial. Una prevención verdadera no existe. En Europa aprox. entre el 10 y el 20 % de las garrapatas están infectadas con Borrelia burgdorferi. El espacio vital son las regiones forrestales, humedas-calientes de norte America, Europa, norte de Africa, Australia y Japon. La población de mayor riesgo son obreros forestales, cazadores y agricultores.
Es una enfermedad difícil de diagnosticar ya que los síntomas se parecen a los de otras enfermedades. Generalmente aparece una erupción roja característica en el sitio de la picadura que puede pasar inadvertida. La enfermedad terciaria se desarrolla meses o años después de la infección inicial. Soló el diagnóstico temprano posibilita un control eficaz de la enfermedad con anitbioticos debido a que en la phase crónica las borrelias son casi inaccesibles.

Especies	Enfermedad	Síntomas	Vía de transmisión
Borrelia burgdorferi	Enfermedad de Lyme Infecciones genito-urinarias Otras infecciones	Erytema crónicum migrans, lesiones cutáneas, cardiológicos, neurológicas y musculoesqueléticas	Picadura de garrapatas infectadas por Borrelia burgdorferi (Ixodes dammini, USA; Ixodes ricinus, Europa)

La infección puede ser detectada por:

- Microscópia: Frotis de sangre colorados de Giemsa o Wright
- PCR
- Serología: Detección de anticuerpos a través de IF, ELISA

2. USO PREVISTO

El enzimoinmunoensayo de Nova Tec se utiliza para la determinación cualitativa de anticuerpos IgM específicos contra Borrelia burgdorferi en suero o plasma (citrato) humano y LCR (líquido cefalorraquídeo).
Para la determinación de anticuerpes IgG producidos de manera intratecal, una instrucción separada para el uso de líquido cefaloraquídeo (LCR) se puede obtener en NovaTec.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimatica cualitativa de anticuerpos especificos contra Borrelia burgdorferi se basa en la tecnica del ELISA (Enzym linked Immunosorbent Assay). El componente principal de los flagelos de B. burgdorferi es la flagelina (41 kDA, p41). Esta proteína junto con la lipoproteína especifica **OspC** de la membrana exterior induce la forma temprana de anticuerpos en la phase I de la enfermedad (Erytema crónicum migrans). Los marcadores especificos de la especie, p100 y p18 son muy seguros en detectar la respuesta IgM, p18 es responable para la alta sensibilidad. Se usa de forma adicionalmente el antígeno p41i para evitar recciónes cruzadadas con sueros sifilíticos. Este testkit contiene los epitopos **OspC** del filo B31 (B. sensu stricto), 20047 y T25 (B. garinii), **p100** y **p18** de PKO (B.arzelii) y **p41i** del filo Pbi (B. garinii).
La determinación inmunoenzimatica cualitativa de anticuerpos específicos contra Borrelia burgdorferi se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay). Las tiras de micropocillos que se usan como fase sólida están recubiertas con antígenos específicos de Borrelia burgdorferi. Los anticuerpos existentes en la muestra se usan a los antígenos inmovilizados de la placa de microtítulación. El conjugado de anticuerpos IgM anti humano con peroxidasa de rábano, se une con los complejos antígeno-anticuerpo en muestras positivas. Estos complejos inmunológicos desarrollan una coloración azul después de incubarlos con sustrato de tetrametilbenzidina (TMB). Finalmente se añade ácido sulfúrico para detener la reacción, causando un cambio de coloración de azul a amarillo. La densidad óptica se mide con un lector de ELISA a 450nm.

4. MATERIALES

4.1. Reactivos suministrados

- **Microtiras (IgM) recubiertas de antígeno de Borrelia burgdorferi:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Borrelia burgdorferi, en bolsa de aluminio.
- **Diluyente para IgM de la muestra***:** 1 botella de 100ml de solución de tampón para diluir la muestra; contiene IgG anti-humana; pH 7.2 ± 0.2; color verde; listo para el uso; tapón blanco.
- **Solución de parada:** 1 botella de 15ml de ácido sulfúrico, 0.2mol/l, listo para ser utilizado; tapa roja.
- **Solución de lavado (20x conc.)*:** 1 botella de 50ml de una solución de tampón 20x concentrado para lavar los pocillos; pH 7.2 ± 0.2; tapa blanca.
- **Conjugado IgM anti-humano (Borrelia burgdorferi)**:** 1 botella de 20ml de conjugado de anticuerpos IgM anti-humano con peroxidasa; color rojo; tapa negra; listo para ser utilizado.
- **Solución de sustrato de TMB:** 1 botella de 15ml 3,3',5,5'-tetrametilbenzindina (TMB); listo para ser utilizado; tapa amarilla.
- **Control positivo de IgM (Borrelia burgdorferi)***:** 1 botella de 2ml; color amarillo; tapa roja; listo para ser utilizado.
- **Control cut-off de IgM (Borrelia burgdorferi)***:** 1 botella de 3ml; color amarillo; tapa verde; listo para ser utilizado
- **Control negativo de IgM (Borrelia burgdorferi)***:** 1 botella de 2ml; color amarillo; tapa azul; listo para ser utilizado.

* contiene 0.1% de Bronidox L después de diluir
** contiene 0.2% Bronidox L
*** contiene 0.1% Catón

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 soporte
- 1 hoja de instrucciones
- 1 hoja de resultados

4.3. Materiales e instrumentos necesarios

- Fotómetro con filtros de 450/620 nm
- Incubadora/cámara húmeda con termostato
- Dispositivo de lavado manual o automático
- Micropipetas con jeringuillas desechables (10, 100, 200, 1000 µl)
- Mezcladora Vortex
- Tubos de plástico desechables
- Gradilla para los tubos
- Agua destilada
- Cronómetro

5. ESTABILIDAD Y ALMACENAJE

El test tiene que estar almacenado de 2...8℃. No usar los reactivos después de la fecha de caducidad indicada en la etiqueta de las botellas y en el exterior.

6. PREPARACIÓN DE LOS REACTIVOS

Todos los reactivos, las muestras y los controles tienen que estar a la temperatura ambiente (20...25°C) antes de ser utilizados!

6.1. Tiras reactivas

Las tiras separables recubiertas con antígeno de Borrelia burgdorferi. Los pocillos listos para ser utilizados tienen que estar almacenados de 2...8℃. *Mantener los pocillos no utilizados en la bolsa de aluminio junto con el desecante y conservar de 2...8℃. El producto se conserva hasta la fecha de caducidad indicada.*

6.2. Conjugado de IgM anti-humano (Borrelia burgdorferi)

La botella contiene 20ml de una solución de IgM anti-humano conjugada con peroxidasa de rábano, tampón, estabilizadores, conservante y un colorante rojo inerte. La solución está lista para ser utilizada y tiene que estar almacenada de 2...8℃. *Después de la primera abertura, el producto se conserva hasta la fecha de caducidad si esta almacenado de 2...8℃.*

6.3. Controles

Las botellas de los controles contienen de solución de control listas para ser utilizadas. Las soluciones tienen que estar almacenadas de 2...8℃ y contienen 0.1% de Catón. *Después de la primera abertura, el producto se conserva hasta la fecha de caducidad si esta almacenado de 2...8℃.*

6.4. Diluyente de muestra IgM

La botella contiene 100ml de tampón de fosfato, estabilizadores, conservantes y un colorante verde inerte se utiliza para la dilución de la muestra del paciente. La solución contiene anticuerpos del tipo IgG anti-humanos para eliminar la inhibición competitiva de los anticuerpos de la clase IgG específicos y para retirar el factor reumatoide. Esta solución lista para ser utilizada ha de almacenarse entre 2...8℃. La solución se usa para diluir las muestras. *Después de la primera abertura, el producto se conserva hasta la fecha de caducidad si está almacenado entre 2...8℃.*

6.5. Solución para lavar (20x conc.)

La botella contiene 50ml de tampón concentrado, detergentes y conservantes. El contenido se diluye con un litro de agua destilada (1+19). La solucíon diluida es estable 5 días a temperatura ambiente. *La cristalización en el concentrado desaparece al calentarla a 37℃ y mezclarla bien antes de usarla. Después de la primera abertura, el producto se conserva hasta la fecha de caducidad si esta almacenado de 2...8℃.*

6.6. Solución de TMB

La botella contiene 15ml de una mezcla de tetrametilbenzidina con peróxido de hidrógeno. La solución lista para ser utilizada se tiene que almacenar entre 2...8℃ protegida de la luz. *La solución es levemente azulada. En caso de contaminación cambia a una coloración azul más intensa no pudiendo ser utilizada en el ensayo.*

6.7. Solución de parada

La botella contiene 15ml de 0.2 M de ácido sulfúrico (R36/38, S26). La solución lista para ser utilizada se tiene que almacenar entre 2...8℃. *Después de la primera abertura, el producto se conserva hasta la fecha de caducidad si esta almacenado de 2...8℃.*

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato) humano. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas de 2...8℃, en caso contrario hay que congelarlas (-20℃). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.
No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1+100 con el tampón de dilución para la muestra de IgM, p.e. 10µl de la muestra con 1ml de tampón, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

8.1. Preparación del ensayo

Por favor, leer cuidadosamente las instrucciones del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido para el método manual. Para excluir efectos de lavado en caso de utilizar los automáticos ELISA elevas el número de lavado de 3 a 5veces y el volumen de solución de lavado de 300 µl a 350 µl. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los controles en la hoja de resultados suministrada. Usar la cantidad necesaria de tiras o pocillos en el soporte.

En este caso por lo menos

1 pocillo	(z.B. A1)	para el blanco,
1 pocillo	(z.B. B1)	para el control negativo,
2 pocillos	(z.B. C1+D1)	para el control cut-off y
1 pocillo	(z.B. E1)	para el control positivo

Para mayor seguridad es necesario hacer doble ensayo de controles y muestras del paciente.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1℃

1. Pipetear 100 µl de controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. Incubar **1 h ± 5 min a 37℃.**

4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300µl de la solución de lavado. Evitar el rebosamiento de los pocillos. El tiempo entre cada lavado y cada aspiración tiene que ser por lo menos de 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: El lavado es muy importante! Un mal lavado provoca una mala precisión y resultados erróneamente aumentados!

5. Pipetar 100µl de conjugado anti-IgM (Borrelia burgdorferi) en cada pocillo con excepción del blanco. Cubrir con una lámina adhesiva.
6. **Incubar 30 min a la temperatura ambiente (20…25℃).** *Evitar la luz solar directa.*
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100µl de sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 C).**
10. Pipetear en todos los pocillos 100µl de la solución de parada en el mismo orden y mismo intervalo de tiempo como con el sustrato de TMB. *Toda coloración azul formada durante la incubación se convierte en amarilla.*

    Nota: Muestras que son altamente positivas pueden causar precipitados negros del cromógeno! Estos precipitados influyen en los valores de las mediciones. Se recomienda diluir las muestras del paciente con solución salina 1+1. Después, preparar la muestra diluida con el tampón de dilución para la prueba de IgM 1+100. En este caso, el resultado se multiplica por 2.
11. Medir la extinción de la solución en cada pocillo con 450/620nm en un periodo de 30 min después de añadir la solución de parada.

8.2. Medición

Efectuar con ayuda del blanco en el pocillo **A1** la **calibración al cero** del fotómetro (lector de ELISA).

Para obtener resultados correctos, si la calibración no es posible por causas técnicas, hay que sustraer el valor de la extinción de la posición A1 del resto de los valores de extinción!
Medir la **extinción** de todos los pocillos con **450nm** y anotar los resultados de los controles y de las muestras en la hoja de resultados.

*Es aconsejable la medición **bicromática** a una longitud de onda de referencia de 620nm.*

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CALCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

El ensayo es válido si se cumplen los siguientes criterios:

- **Blanco** en A1 extinción **< 0.100**
- **Control negativo** en B1 extinción **< 0.200 y < cut-off**
- **Control cut-off** en C1 y D1 extinción **0,150 – 1,30**
- **Control positivo** en E1 extinción **>cut-off**

Si estos criterios no se cumplen, la prueba no es válida y deberá repetirse.

9.2. Calculo del valor de la medición

El *cut-off* se obtiene de los volores de la extinción de los dos controles Cut-off.

Ejemplo: 0,42 OD Cut-off Control + 0,44 OD Cut-off Control = 0,86:2 = 0.43

Cut-off= 0.43

9.3. Interpretación de los resultados

Las muestras se consideran positivas cuando el valor de la extinción es como mínimo mayor al 10% del valor del *cut-off*.

Las muestras con valores de extinción **±**10% del *cut-off* no pueden ser consideradas claramente positivas o negativas → **Zona intermedia**

Se recomienda entonces repetir el ensayo con nuevas muestras del paciente de 2 a 4 semanas más tarde. Si de nuevo se encuentran resultados en la zona intermedia, la muestra tiene que estar valorada como **negativa**.

Las muestras se consideran **negativas** si el valor de la extinción esta por lo menos un 10% por debajo del *cut-off*.

9.3.1. Resultados en unidades Nova Tec [NTU]

$$\frac{\text{Promedio de la extinción de la muestra x 10}}{\textit{Cut-Off}} = \text{[NovaTec-Einheiten = NTU]}$$

Ejemplo: $\frac{1.204 \times 10}{0.43} = 28$ *NTU (NovaTec Units)*

Cut-Off :	10	NTU
Zona intermedia:	9-11	NTU
Negativo:	<9	NTU
Positivo:	>11	NTU

10. CARACTERÍSTICAS DEL ENSAYO

10.1. Precisión

Inter ensayo	n	Promedio	CV (%)
Suero pos.	14	0.76	4.1

Intra ensayo	n	Promedio	CV (%)
Suero pos.	8	1.06	8.6

10.2. Especificad del ensayo

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia de la sustancia a analizar específicamente (98,8 %).

10.3. Sensibilidad del ensayo

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico (93 %).

10.4. Interferencias

Las muestras lipémicas e ictéricas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/ml para triglicéridos y de 0,2 mg/ml para bilirrubina.

Los resultados están basados en pruebas de ensayos querales: No se trata de especificaciones garantizadas.

11. LIMITACIONES DEL ENSAYO

La contaminación bacteriana o repetidos ciclos de congelación-descongelación de la muestra pueden afectar a los valores de absorvancia. El diagnóstico de una enfermedad infecciosa no debe establecerse con base al resultado de una sola prueba. Un diagnóstico preciso debe tener en consideración historia clínica, sintomatología y datos serológicos. En pacientes immunosuprimidos y neonatos los datos serológicos tienen valor limitado.
Un resultado negativo (IgG o IgM) no puede excluir una infección de B. burgdorferi. Especialmente en la fase inicial de la infección existe la posibilidad de que no se detecten anticuerpos. En el caso de que exista infección o resultado equívoco, se recomienda un nuevo muestreo después de 2-3 semanas. Un resultado positivo de IgG no siempre implica que exista una infección aguda ya que los anticuerpos de una infección anterior pueden persistir. El uso de antígenos recombinantes evita en gran medida reacciones cruzadas con los siguientes anticuerpos: Treponema pallidum, Leptospira, Borrelia recurrentis. Los anticuerpos de la infección de lues a p41i se determinaron ocasionalmente. Por lo tanto, una infección de lues debe ser excluida.

12. PRECAUCIONES Y ADVERTENCIAS

- En cumplimiento con el artículo 1 párrafo 2b de la directiva europea 98/79/EC, la utilización de sistemas médicos para diagnóstico in vitro tiene la intención por parte del fabricante de asegurar la adecuación, realizaciones y seguridad del producto. Por lo tanto, el procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG. No obstante, todos los materiales se deben considerar y tratar como potencialmente infecciosos.
- No intercambiar reactivos y placas de microtítulo de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.

- No intercambiar las tapas de los diferentes reactivos.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Pipetear cuidadosamente las muestras y el conjugado en los pocillos para evitar contaminaciones cruzadas y resultados erróneamente aumentados.
- El NovaLisa™ ELISA está pensado exclusivamente para su uso por personal expecializado que domine perfectamente las técnicas de trabajo.

ADVERTENCIA:	Bronidox L, en la concentración utilizada, casi no muestra riesgos tóxicos en la piel y en las mucosas.
ADVERTENCIA:	El ácido sulfúrico irrita los ojos y la piel! En caso de contacto con los ojos lavar abundantemente con agua y consultar a un médico.

12.1. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto: BORM0040 Borrelia burgdorferi IgM-ELISA (96 determinaciones)

BIBLIOGRAPHY / LITERATUR / BIBLIOGRAFIA / BIBLIOGRAFÍA

Johnson R.C.,Schmid G.P.,Hyde F.W.,Steigerwaldt A.G.,Brenner D.J.(1984) Borrelia burgdorferi sp.nov.:etiologic agent of Lyme disease.Int J Syst Bacteriol 34,496-497

AsbrinkE.,Hovmark A.(1988) Early and late cutaneous manifestations of Ixodes-born borreliosis (erythema migrans borreliosis, Lyme Borreliose).Ann NY Acad Sci 539,4-15

Karlson M.,Hovind-Hougan K.,Svenungsen B.,Stiernsted G.(1990) Cultivation and characterization of Spirochetes from cerebrospinal fluid of patients with Lyme borreliosis. J Clin Microbiol 28, 473-479

Preac-Mursicd V.,Wilske B.,Schierz G. (1986) Eurpean Borrelia Burgdorferi isolated from human and ticks:Culture conditions and antibiotic susceptibility.Zbl Bakt Hyg A 263,112-118

WilskeB.,Preac-Mursic V.,FuchsR.,Schierz G.(1990) Diagnostik der Lyme Borreliose.Diagnose und Labor,Laboratoriumsblätter 40,24-36

FOI 26-3320 - Document 3

Symbols Key/ Symbolschlüssel/ Legenda / Símbolos	
	Manufactured by / Hergestellt von/ / Prodotto da/ Fabricado por
IVD	In Vitro Diagnostic Medical Device/ In Vitro Diagnosticum/ Diganostico *in vitro*/ Producto para diagnóstico In vitro
LOT	Lot Number/ Chargenbezeichnung/ Lotto/ Número de lote
	Expiration Date/ Verfallsdatum/ Scadenza/ Fecha de caducidad
	Storage Temperature/ Lagertemperatur/ Temperatura di conservazione / Temperatura de almacenamiento
CE	CE Mark/ CE-Zeichen/ Marchio CE/ MarcaCE
[REF]	Catalogue Number/ Katalog Nummer/ Numero di codice/ Número de Catálogo
	Consult Instructions for Use/ Gebrauchsanweisung beachten/ Consultare le istruzioni/ Consulte las Instrucciones de Uso
MTP	Microplate/ Mikrotiterplatte/ Micropiastra/ Microplaca
CONJ	Conjugate/ Konjugat / Coniugato/ Conjugado
CONTROL –	Control serum, negative/ Kontrollserum, negative/ siero di controllo, negativo /Suero control negativo/ Soro de controle negativo
CONTROL +	Control serum, positive/ Kontrollserum, positiv/ siero di controllo, positivo/ Suero de control positivo
CUT OFF	Cut off control serum/ Cut off Kontrollserum/ siero di controllo, cut-off/ Suero control Cut-off
DIL G	Sample diluent buffer IgG/ IgG-Probenverdünnungspuffer/ soluzione tampone per i campioni IgG/ solución tampón para muestras IgG
SOLN STOP	Stop solution/ Stopplösung/ Soluzione bloccante
SUB TMB	TMB Substrate solution/ TMB-Substratlösung/ soluzione substrato TMB/ solción substrato TMB
WASHBUF 20x	Washing solution 20x concentrated/ Waschlösung 20x konzentriert/ soluzione di lavaggio concentrazione x20/ solución de lavado concentrado x20
	Contains sufficient for “n” tests/ Ausreichend für “n” Tests/ Contenuto sufficiente per “n” saggi/ Contenido suficiente para ”n” tests

SCHEME OF THE ASSAY

Borrelia burdorferi IgM-ELISA

Assay preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Cut-off control	Sample (diluted 1+100)
Negative control	-	100µl	-	-	-
Positive control	-	-	100µl	-	-
Cut-off control	-	-	-	100µl	-
Sample (diluted 1+100)	-	-	-	-	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37℃** Wash each well three times with 300µl of washing solution					
Conjugate	-	100µl	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution					
TMB Substrate	100µl	100µl	100µl	100µl	100µl
Incubate for exactly 15 min at room temperature in the dark					
Stop Solution	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

NovaTec Immundiagnostica GmbH

Technologie & Waldpark

Waldstr. 23 A6
D-63128 Dietzenbach, Germany

Tel.: +49 (0) 6074-48760 Fax: +49 (0) 6074-487629
Email : info@NovaTec-ID.com
Internet: www.NovaTec-ID.com

BORM0040engl,dt,it,es-13012011-CR

From: IRIS
To: s22 @paragoncare.com.au
Subject: DIR 90606 - Questionnaire Letter - Response due by 13.11.23 [SEC=OFFICIAL, ACCESS=Personal-Privacy]
Date: Monday, 16 October 2023 2:10:57 PM
Attachments: image001.png
DIR 90606 - Questionnaire Letter - Response due by 13.11.23.rtf

---

Dear s22

Please see the attached Questionnaire Letter for action. **Please note the due date for a response is 13 November 2023.**

Kind regards,

s22

Device Support Team
Medical Device Incident Report Investigation Scheme (IRIS)
Devices Post Market Monitoring | Medical Devices Surveillance Branch

Medical Devices and Product Quality Division | Health Products Regulation Group
Therapeutic Goods Administration (TGA)

Australian Government, Department of Health and Aged Care
Email: IRIS@health.gov.au
Phone: DST s22
Phone: IRIS 1800 809 361
Post: 27 Scherger Drive, Canberra Airport, ACT 2609
Courier: 1 Tindal Lane, Canberra Airport, ACT 2609
www.tga.gov.au

The Department of Health and Aged Care acknowledges First Nations peoples as the Traditional Owners of Country throughout Australia, and their continuing connection to land, sea and community. We pay our respects to them and their cultures, and to all Elders both past and present.

Important: *This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error, please notify the author immediately and delete all copies of this transmission.*

Please Note: Medical device sponsors and manufacturers are encouraged to submit adverse events via the online Medical Device Incident Reporting (MDIR) system. The MDIR allows sponsors and manufacturers to submit initial, follow-up and final reports and review reports already submitted to the TGA. The MDIR can be accessed via https://apps.tga.gov.au/prod/mdir/MDIRSummary.aspx, using a TGA eBusiness Service (eBS) user name and password. MDIR guidance documents and FAQ are available from the 'Training' section of the TGA eBS portal, or from the TGA website.
MDIR Technical assistance is available via 02 6289 4642 or email (IRIS@health.gov.au). If you are a sponsor or manufacturer requiring a new account to gain access to the MDIR system or need to update your details with TGA eBS, please contact TBS Helpdesk via 1800 010 624 or email (eBS@health.gov.au).

Online reporting forms for Medical Device Healthcare Professionals/Users can be accessed via:
https://apps.tga.gov.au/prod/mdir/udir03.aspx
For ongoing information and updates please subscribe to the TGA's Medical Devices Information and IVDs Information email subscription services.

FOI 26-3320 - Document 4

Australian Government

Department of Health and Aged Care
Therapeutic Goods Administration

Australian Medical Device
Incident Report Investigation Scheme

s22

Immuno Pty Ltd
63 Poplar Rd
Parkville VIC 3052
Email: s22 @paragoncare.com.au

File Reference: E23-275158
Sent by email

Dear s22

DEVICE INCIDENT REPORT DIR 90606 - ARTG # 205351 - Bacterial infectious disease IVDs

The Therapeutic Goods Administration has been advised of an incident involving the above product. A copy of the Device Incident Report (DIR) is attached.

To assist in the evaluation and resolution of this report, please provide the information requested in the attached questionnaire and return it to this office **within 20 working days of the date of this letter,** and no later than COB 13/11/2023.

Please send responses via email to IRIS@health.gov.au, referencing the DIR number.

If you are unable to respond with all the information requested by the due date please advise, **within the 20 days**, when a full response will be provided. Extensions of a reasonable time frame will be accepted depending on the seriousness of the complaint and the time requested.

Thank you for your cooperation. If you require further information please contact me on

s22 or email: IRIS@health.gov.au.

Yours sincerely
Signed electronically by

Administration Officer

Incident Report Investigation Scheme
Devices Post Market Monitoring Section
Therapeutic Goods Administration

16/10/2023

FOI 26-3320 - Document 4

INITIAL REQUEST FOR INFORMATION FROM LISTED SPONSOR **Date:** 16/10/2023

DIR: 90606 Manufacturer Name: Nova Tec Immundiagnostica GmbH [44194]

Question/Requirement		
1) Please confirm the device's Australian Register of Therapeutic Goods (ARTG) number	**ARTG:**	
2) Do you currently supply or have you previously supplied this product, with the indicated Model/Serial/Batch/Lot numbers: a) To the Australian Market b) For Export	**YES** ☐ ☐	**NO** ☐ ☐
3) How many of this model have been supplied	In Australia: Worldwide:	
4) How many of this batch (if applicable) have been supplied:	In Australia: Worldwide:	
5) Are you aware of this problem, as reported?	☐	☐
6) If deemed necessary, is a sample of the mentioned device available for review and/or testing?	☐	☐
7) Have you had any other reports of similar* problems with this product in Australia?	☐	☐

If **YES**, how many:

If **YES**, please give details:

* Similar events are based on the clinical event description and not the cause of an event. Both the confirmed and unconfirmed rates for similar events are often required and beneficial to show the full outlook of the device and its use.

FOI 26-3320 - Document 4

8) Is the manufacturer aware of reports of similar problems with this product? ☐ ☐

If **YES**, how many:

If **YES**, please give details:

9) Please provide details of any action you have taken, or intend to take, regarding this problem

10) Please provide details of the manufacturer's investigation to date, including expected **Manufacturer's investigation completion date**

Date:

11) When returning this response to the office of the Therapeutic Goods Administration, you are requested to attach the following (if checked):

- ☐ Sample of the product/device
- ☐ Product Specifications
- ☐ Descriptive product promotional documentation
- ☐ Instructions for use, as supplied with the device
- ☐ Device Packaging with printed instructions
- ☐ Operator's manual
- ☐ Technical Service Manual
- ☐ Clinical training manual in printed or video form
- ☐ In-house training documentation
- ☐ Evidence of compliance with the Essential Principles
- ☐ A summary of risk assessment activities performed by the manufacturer for the device, eg Risk Management Report required by Clause 8 of ISO 14971:2007.
- ☐ Service History, and Safety and Performance Test Results
- ☐ Configuration Information and Documentation
- ☐ HHE (Health Hazard Evaluation)

12) Additional Information required:

1. Provide rates for similar DIRs in Australia and overseas.

2. Provide the IFU that accompanies the ARTG ID.

13) If your device is an implantable pacemaker/defibrillator and/or associated lead you are asked to provide the following additional information:

1. Both published and unpublished clinical trial data where events of this type are analysed.
2. The number of reported events of ALL types (including unconfirmed events), the number of devices sold and the cumulative implant months for each device in this product family.
3. What material has been used to insulate, both internally and externally, the lead? (applicable to leads only)

Information Supplied By:

14)

Name		Phone	
Signature		Fax	
		Email	
Position			

This questionnaire and any appended documents should be returned to the TGA within **20 working days or as specified on page 1 of this letter.**

Electronic submission of all information is preferred.
Please send the requested information to email address: IRIS@health.gov.au

For large size documents, please post a universal serial bus (USB), compact disc (CD), or digital versatile disc (DVD) via postal address:

Incident Report Investigation Scheme
Devices Post Market Monitoring Section
Therapeutic Goods Administration
PO Box 100
Woden ACT 2606

If you are sending a device/s to the TGA please follow the instructions via link:

https://www.tga.gov.au/form/report-medical-device-adverse-event-medical-device-use

---

Sponsors of products listed or registered on the Australian Register of Therapeutic Goods (ARTG) are reminded of their responsibilities under Section 31 and/or 41JA (as appropriate) of the Therapeutic Goods Act of 1989, to provide information relating to their product's formulation, composition, design specification, quality, method and place of manufacture, presentation, safety and efficacy, conformity to advertising regulations under the Act,

Reporter Reference #:

Date of Adverse Event:

Date of Final Report:
11/09/2023

ARTG #:
205351

Brand Name:
NovaTec NovaLisa Borrelia burgdorferi IgG-ELISA (Novatec Novalisa) - Bacterial infectious disease IVDs

Device Class:
Class 2

Software Version:

Batch #:

Lot #:

Manufacturer:
Nova Tec Immundiagnostica GmbH [44194]

Sponsor:
Immuno Pty Ltd [12706]
63 Poplar Rd
Parkville VIC 3052

Contact Name: s22

Phone: s22

Email: s22 @paragoncare.com.au

Fax:

Reporter:

Confidential: Yes

Clinical Event Information:
I am writing to formally request the recall and investigation of the medical device NovaTec NovaLisa Borrelia burgdorferi IgG-ELISA (Novatec Novalisa), bearing ARTG 205351, due to the occurrence of discordant serological false-negative results that have raised serious concerns regarding its performance and safety.

Patient Outcome/Consequences:

Additional Event Description:

Device Analysis Results:

Corrective/Preventative Actions:

Details of Similar Events:

Number of Similar Events:

Rate of Similar Events:

Countries Similar Events Also Occurred: