# IDEIA™ Lyme Neuroborreliosis

REF K602811-2

Σ 96 IVD CE

An enzyme immunoassay for determination of human intrathecal IgG and IgM antibodies to *Borrelia burgdorferi* sensu lato.

Dosage immuno-enzymatique pour la détermination des anticorps IgG et IgM intrathécaux humains dirigés contre *Borrelia burgdorferi* sensu lato.

Ein Enzymimmunoassay für den Nachweis von humanen, intrathekalen IgG- und IgM-Antikörpern gegen *Borrelia burgdorferi* sensu lato



OXOID

IDEIA™

TECHNICAL ADVICE AND CUSTOMER SERVICE

CONSEIL TECHNIQUE ET SERVICE CLIENTELE

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IDEIA™ Lyme Neuroborreliosis
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Manufactured by / Fabrique par / Hersteller:

OXOID

DEDICATED TO MICROBIOLOGY

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## 1 INTENDED USE

The IDEIA™ Lyme Neuroborreliosis test is an enzyme immunoassay for the detection of intrathecally produced human IgG and IgM antibodies to *Borrelia burgdorferi* sensu lato. The kit is intended as an aid in the diagnosis of Lyme Neuroborreliosis.

## 2 SUMMARY

Lyme borreliosis is a multisystem infection caused by the tickborne spirochete *B. burgdorferi sensu lato*[1,2]. Lyme borreliosis is the most common vector-transmitted human disease in Europe and North America. The disease has been recognized also in the former USSR, China and Japan.

Involvement of the nervous system, neuroborreliosis, is a common and serious manifestation of Lyme borreliosis[3]. Neuroborreliosis develops in approximately 10% of patients who have not received treatment for the primary stage lesion, erythema migrans. *B. burgdorferi* causes a variety of neurological disorders such as painful lymphocytic meningoradiculitis with or without cranial nerve palsies and paresis of the extremities (Bannwarth's syndrome), chronic lymphocytic meningitis, myelitis and chronic progressive encephalomyelitis[4,5].

A sensitive and reliable diagnostic test for neuroborreliosis is needed as a number of other diseases with similar symptoms exist and because neuroborreliosis, in contrast to many of these diseases, responds to antibiotic treatment.

Currently, the best indicator of active neuroborreliosis is an inflammatory cerebrospinal fluid (CSF) change, particularly an increase in the number of mononuclear cells (mononuclear pleocytosis) combined with the occurrence of intrathecally produced *Borrelia*-specific antibodies in the CSF. The detection of a specific intrathecal immune response is diagnostically more significant than the measurement of specific antibodies in serum. Moreover, in neuroborreliosis, specific antibody synthesis is frequently detected earlier in CSF than in serum[6,7].

Intrathecal, specific antibody synthesis is very rarely a non-significant finding in patients with neurological symptoms.

IDEIA™ Lyme Neuroborreliosis is designed for the sensitive and direct detection of intrathecally produced IgG and IgM antibodies to *B. burgdorferi*[8]. Measurement of reference substances (like albumin etc) and complicated corrections for transudation of serum antibodies to CSF caused by damage to the blood CSF barrier are unnecessary. Even blood contamination of CSF during a spinal tap will not lead to false positive results.

The assay uses purified, native *B. afzelii* strain DK1 flagellum as test antigen. The flagellum is highly immunogenic, elicits an early, strong and persistent immune response [9,10] and is the major antigen for the intrathecal antibody response[11,12]. Compared with the conventional antigen, which is based on whole cell extract of the spirochete, the purified, native flagellar antigen improves the diagnostic sensitivity and specificity of serological assays for Lyme borreliosis[7,13]. Furthermore, the use of flagellum as test antigen is appropriate in all geographical areas as the flagellum shows no significant variation between *B. burgdorferi* strains.

## 3 PRINCIPLE OF THE TEST

The test is based on the capture ELISA principle and consists of a human IgG capture assay and a human IgM capture assay for determination of intrathecally produced IgG and IgM antibodies to *B. burgdorferi*, respectively. The human IgG capture assay is described in detail:

Paired CSF and serum specimens are added to microwells coated with antibody specific to human IgG. The dilution of CSF and serum ensures that the anti-human IgG capture antibody is saturated with either CSF or serum IgG. Therefore, the same amount of total IgG from either CSF or serum is captured in the microwells.

After washing of the microwells to remove excess protein, biotinylated, native *Borrelia* flagella complexed with peroxidase-conjugated streptavidin (Flagellum Conjugate) is added to the microwells. The Flagellum Conjugate will be bound by *B. burgdorferi*-specific IgG only, whereas non-*B. burgdorferi*-specific IgG will not bind the Flagellum Conjugate.

Excess Flagellum Conjugate is removed by washing. The amount of Flagellum Conjugate bound per microwell is visualized by the addition of a chromogenic substrate, which develops a blue colour. The reaction is stopped by the addition of acid, changing the blue colour to yellow. The intensity of the colour corresponds to the concentration of *B. burgdorferi*-specific IgG captured on the solid phase.

The human IgM capture assay is similar to the human IgG capture assay except that the microwells are coated with an anti-human IgM capture antibody.

Normally, the total IgG and IgM concentrations in CSF are very low compared with levels in serum. Therefore, when intrathecal *B. burgdorferi*-specific antibody production takes place, the amount of specific IgG or IgM antibody in CSF will constitute a much larger proportion of the total amount of IgG and IgM, respectively, than the proportions found in serum. If there is no intrathecal, specific antibody production, or when specific antibodies in the CSF are due to either blood contamination or transudation from serum, then the OD value for CSF minus the OD value for serum will be ≤0.

Consequently, in the case of neuroborreliosis, a significantly higher OD value will be obtained for the CSF compared with the OD value obtained for the serum specimen. In such cases the OD value for CSF minus the OD value for serum will, therefore, be >0.

Comparison of OD values from paired serum and CSF specimens, tested in parallel, make it possible to determine whether or not *B. burgdorferi*-specific antibodies have been produced intrathecally. The formula for calculation of test results has been designed to take into account any intra-assay variation when comparing OD value differences between paired CSF and serum specimens[8].

Due to the selective immunocapture of IgG and IgM antibodies, there will be no competitive interference from other immunoglobulin classes. The use of conjugated test antigen furthermore prevents interference from rheumatoid factor (RF).

## 4 DEFINITIONS

The following symbols have been used throughout the product information.

| | |
|---|---|
| REF | Product code and catalogue number |
| | Consult the instructions for use |
| Σ N | Contains sufficient for <N> tests |

| | |
|---|---|
| | Manufactured by |
| IVD | *In vitro* diagnostic medical device |
| | Use by |
| LOT | Batch Code |
| 2°C 8°C | Storage temperature limitations |
| | Add water |

## 5 REAGENTS PROVIDED

Σ 96 - Each kit contains sufficient materials for 96 determinations. - The shelf life of the kit is as indicated on the outer box label.

### 5.1 IDEIA™ LYME NEUROBORRELIOSIS TEST CONTENTS

| | |
|---|---|
| | One Instructions For Use Booklet. |
| MICROTITRATION PLATE | 48 microwells (6 strips of 8 microwells) coated with anti-human IgG (green colour code) and 48 microwells (6 strips of 8 microwells) coated with anti-human IgM (yellow colour code). A resealable plastic pouch is provided for storage of unused microwells. |

One bottle of each of the following:

| | |
|---|---|
| SAMPLE DILUENT | 100mL Sample Diluent: Buffered solution with detergent, antimicrobial agent and coloured red dye. |
| WASH BUFFER (X25) | 50mL Wash Buffer concentrate x25: Buffered solution with detergent and antimicrobial agent. |
| IgG POSITIVE CONTROL | 1.5mL IgG Positive Control: Human serum in buffer with antimicrobial agent and coloured green dye. |
| IgM POSITIVE CONTROL | 1.5mL IgM Positive Control: Human serum in buffer with antimicrobial agent and coloured yellow. |
| FLAGELLUM CONJUGATE | Lyophilized Flagellum Conjugate: Biotinylated *B. afzelii* flagellum to be reconstituted with Reconstitution Buffer and complexed with peroxidase-conjugated streptavidin prior to use. |
| RECONSTITUTION BUFFER | 12.5mL Reconstitution Buffer: Buffered solution with antimicrobial agent and coloured blue. |
| PEROXIDASE COMPLEX | 1mL Peroxidase Complex: Peroxidase-conjugated streptavidin in buffer containing carrier proteins and antimicrobial agent. |
| SUBSTRATE TMB | 12mL Substrate: stabilized peroxide and 3,3'-5,5'-tetramethylbenzidine in a dilute buffer solution. TMB has been reported to be non-carcinogenic. However, personal protective equipment is recommended to avoid direct exposure. |
| STOP SOLUTION | 25mL Stop Solution: 0.46mol/L sulphuric acid. |

## 5.2 PREPARATION, STORAGE AND RE-USE OF KIT COMPONENTS

The IDEIA™ Lyme Neuroborreliosis kit format allows for up to 6 individual runs during any 3 month period within the expiry date given on the kit label. In order to ensure optimal kit performance, it is important that unused kit components are prepared and stored according to the following instructions:

### 5.2.1 Microwells - MICROTITRATION PLATE

Open the plate pouch by cutting along the seal. Break-off the required number of anti-human IgG strips (green colour code) and anti-human IgM strips (yellow colour code) and relocate them into the frame. One strip allows double determination of CSF and serum from one patient; the remaining four microwells of the strip are used for positive and buffer controls. Each additional IgG and IgM strip allows paired samples from two patients to be analysed. When the whole frame of 6 IgG and 6 IgM strips is used at one time, CSF and serum samples from 11 patients can be tested for IgG and IgM antibodies.

Place unused microwells in the resealable plastic pouch with the dessicant, carefully reseal the pouch and store at 2-8°C. Microwells may be used for up to 12 weeks after initial opening, provided they are stored in this manner.

### 5.2.2 Sample Diluent - SAMPLE DILUENT

Ready to use. Store unused Sample Diluent at 2-8°C.

### 5.2.3 Wash Buffer Concentrate - WASH BUFFER (X25)

Provided x25 concentrate. Prepare working strength Wash Buffer by adding 1 part of Wash Buffer Concentrate to 24 parts of fresh deionised or distilled water (or add the contents of Wash Buffer concentrate to 1200mL fresh deionised or distilled water). **Prepare working strength Wash Buffer as required on the day of use**. Store unused concentrate at 2-8°C.

**Do not store unused working strength Wash Buffer for subsequent use** (see Section 8.2.11).

### 5.2.4 IgG Positive Control - IgG POSITIVE CONTROL

Ready to use. Store unused IgG Positive Control at 2-8°C.

### 5.2.5 IgM Positive Control - IgM POSITIVE CONTROL

Ready to use. Store unused IgM Positive Control at 2-8°C.

### 5.2.6 Flagellum Conjugate, Peroxidase and Reconstitution Buffer – FLAGELLUM CONJUGATE / PEROXIDASE COMPLEX / RECONSTITUTION BUFFER

Reconstitute the lyophilized, biotinylated *Borrelia* Flagellum Conjugate with the contents of Reconstitution Buffer and add 650µL Peroxidase Complex. Mix the contents by inversion. **The reconstituted Flagellum Conjugate must be prepared at least 1 hour prior to use.** The reconstituted Flagellum Conjugate should be stored at 2-8°C and used within 3 months.

### 5.2.7 Substrate - SUBSTRATE TMB

Ready to use. Store unused substrate at 2-8°C.

### 5.2.8 Stop Solution - STOP SOLUTION

Ready to use. Store unused Stop Solution at 2-8°C.

## 6 ADDITIONAL REAGENTS

### 6.1 REAGENTS

Fresh deionised or distilled water

## 7 EQUIPMENT

The following equipment is required:

Measuring cylinder (2L)

Test tubes of approximately 5mL capacity

Precision micropipettes and disposable tips to deliver 10-1000µL volumes

8-channel pipette to deliver 100µL (optional)

Reagent reservoirs for 8-channel pipette (optional)

Clean absorbent paper (onto which microwells can be tapped dry)

Plastic lid for microwell plate

Microtitration plate shaker capable of a minimum speed of 500rpm with an orbital diameter of 3-4mm. For information on suitability of plate shakers contact your local Oxoid subsidiary or distributor.

Timer

Automated plate washer (optional) or suitable equipment for washing 8 microwell strips (Section 10.2.3).

**Note: If washing less than 8 test microwells in a strip using an automated washer with an 8 microwell head, it is important to completely fill the strip with blank microwells.**

Spectrophotometer or EIA plate reader able to read a 96 microwell plate of 8 microwell strips at an absorbance of 450nm with a reference at 620-650nm. (Optional, Section 10.3, Reading the Test Results).

Application notes for use on open automated systems are available for this assay. Contact your local Oxoid subsidiary or distributor.

## 8 PRECAUTIONS

IVD - For *in vitro* diagnostic use. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.

## 8.1 SAFETY PRECAUTIONS

**8.1.1** Each donor serum used for the preparation of the Positive Controls has been tested individually and found negative for hepatitis B virus surface antigen and antibodies to HIV and hepatitis C.

**8.1.2** Stop Solution contains sulphuric acid (0.46mol/L). Avoid eye and skin contact by wearing protective clothing and eye protection.

**8.1.3** Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.

**8.1.4** Do not pipette materials by mouth.

**8.1.5** Wear disposable gloves whilst handling clinical specimens and always wash hands after working with infectious materials.

**8.1.6** Dispose of all clinical specimens in accordance with local legislation.

**8.1.7** Safety data sheet available for professional user on request.

## 8.2 TECHNICAL PRECAUTIONS

**8.2.1** Components must not be used after the expiry date printed on the labels. Do not mix or interchange different batches/lots of reagents, with the exception of standard reagents (Sample Diluent, Wash Buffer Concentrate, Substrate and Stop Solution) which are also used in IDEIA™ *Borrelia burgdorferi* IgG (Code No. K602911-2) and IDEIA™ *Borrelia burgdorferi* IgM (Code No. K603011-2).

**8.2.2** The reagents are provided at fixed working concentrations. Test performance will be affected if the reagents are modified or stored under conditions other than those detailed in Section 5.2.

**8.2.3** Avoid contamination of reagents.

**8.2.4** Use separate disposable pipettes or pipette tips for each specimen, control or reagent in order to avoid cross-contamination of either specimen or controls which could cause erroneous results.

**8.2.5** Avoid contamination with metal ions and oxidising agents.

**8.2.6** Store deionised or distilled water for dilution of concentrated reagents in clean containers to prevent microbial contamination.

**8.2.7** Do not use Substrate showing a blue colour prior to its addition to the microwells.

**8.2.8** Protect Substrate from light.

**8.2.9** Microwells cannot be re-used.

**8.2.10** Manual or automated washing equipment, must be free of microbial contamination, and correctly calibrated and maintained according to manufacturer's instructions.

**8.2.11** Do not store unused working strength Wash Buffer for subsequent use. When not in use Wash Buffer reservoirs should be rinsed in deionised or distilled water and left to dry.

# 9 COLLECTION AND PREPARATION OF SPECIMENS

## 9.1 SPECIMEN COLLECTION

The IDEIA™ Lyme Neuroborreliosis kit is for the testing of paired human CSF and serum specimens simultaneously.

The paired CSF and serum specimens should be obtained from the patient at the same time. A minimum of 0.5mL CSF is required; preferably 1-2mL CSF should be requested, because this will allow for repetition of the test and/or performance of the test with a lower CSF dilution. For serum 0.5mL is sufficient.

Testing of turbid and viscous specimens may lead to unreliable results due to pipetting errors.

Paired CSF and serum specimens may be stored for 14 days at 2-8°C prior to testing, and for at least 6 months at –20°C or below.

## 9.2 PREPARATION OF SPECIMENS

Dilute CSF specimens 1+4 by adding 100µL of CSF to 400µL of Sample Diluent.

Dilute serum specimens 1+200 by adding10µL of serum to 2mL of Sample Diluent.

# 10 TEST PROCEDURE

**PLEASE REFER TO SECTION 8.2, TECHNICAL PRECAUTIONS, BEFORE PERFORMING THE TEST PROCEDURE.**

## 10.1 PROCEDURAL NOTES

**10.1.1** Standard reagents (Sample Diluent, Wash Buffer Concentrate, Substrate and Stop Solution) and a similar assay procedure are also used in IDEIA™ *Borrelia burgdorferi* IgM (Code No. K603011-2) and IDEIA™ *Borrelia burgdorferi* IgG (Code No. K602911-2). This offers the opportunity to run each of the assays in parallel using a single dilution of patient serum.

**10.1.2** The validation of the assay performance is based on concurrent duplicate testing of all specimens. If the patient specimens are to be run singly, it is recommended that users do so only after becoming familiar with the performance characteristics of the assay. The intra-assay variation (CV%) is approximately 50% higher when based on single determination.

**10.1.3** If a shaker capable of 500rpm is not available, the speed of shaking of microwell strips during the 2 incubation steps may be reduced to 300rpm without adverse effect on the assay performance, or a static incubation protocol is available, please contact your local Oxoid subsidiary for further information on suitable protocols.

## 10.2 ASSAY PROCEDURE

**NOTE:** The assay procedure requires the use of a Microtitration Plate shaker. For information on suitability of shakers contact your local Oxoid subsidiary or distributor.

If multiple strips are used it is recommended that an 8-channel pipette is used for addition of Conjugate, Substrate and Stop Solution.

### 10.2.1 Specimen and Control Addition

Locate the required number of microwells into the microwell holder. Add 100µL of diluted patient CSF and serum to duplicate microwells of both the green anti IgG strip and the yellow anti IgM strip microwells. Add 100µL of IgG Positive Control to two anti IgG microwells and 100µL of the IgM Positive Control to 2 anti IgM microwells. Add Sample Diluent to separate IgG and IgM microwells. (At least 1 Sample Diluent microwell should be included with each batch of specimens tested).

### 10.2.2 Specimen Incubation

Cover and incubate the microwells on a shaker at room temperature (**20-25°C**) with **shaking** for **60 minutes**.

### 10.2.3 Washing the Microwells

The microwells should be washed using working strength Wash Buffer (see Section 5.2.3).

The washing technique is critical to the test performance (see Section 8.2.10) and should be carried out so as to ensure complete filling (with a minimum of **350µL** of working strength Wash Buffer) and emptying of the microwells.

Four wash cycles are essential, by either automated or manual washing techniques, which should include a 2 minute soak period during the second wash or a total of a 2 minute soak period during the complete cycle.

**Manual Washing**
If washing microwells manually, aspirate or shake out the contents of the microwells and using freshly prepared Wash Buffer, ensure complete filling and emptying of microwells. Between each wash step remove all remaining Wash Buffer by tapping the inverted microwells on to clean absorbent paper. Manual washing efficiency is further ensured if the Wash Buffer is delivered at an angle so as to produce a vortex in the microwells. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of wash buffer.

**Automated Washing**
Automated washers should be programmed to complete 4 wash cycles and to incorporate the equivalent of 2 minutes soaking time during the complete washing cycle. Washers must be correctly calibrated to ensure complete filling and emptying of microwells between each wash. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of Wash Buffer.

### 10.2.4 Conjugate Addition

Mix the Flagellum Conjugate by inversion. Add 100µL of Flagellum Conjugate to each microwell.

### 10.2.5 Conjugate Incubation

Cover and incubate the microwells on a shaker at room temperature (**20-25°C**) with **shaking** for **60 minutes**.

### 10.2.6 Washing the Microwells

Wash the microwells as described in 10.2.3.

### 10.2.7 Substrate Addition and Incubation

Add 100µL of Substrate to each microwell.

Cover and incubate the microwells at room temperature **(20–25°C) without shaking** for **10 minutes**.

### 10.2.8 Stopping the Reaction

Add 100µL of Stop Solution to each microwell. Ensure thorough mixing in the microwells. The coloured product is stable for 30 minutes. **Do not expose to direct sunlight** as photobleaching of the coloured product may occur.

## 10.3 READING THE TEST RESULTS

### 10.3.1 Photometric Reading

The microwells should be read photometrically within 30 minutes after addition of the Stop Solution. Mix the contents of the microwells and read the absorbance of each microwell using a suitable spectrophotometer or EIA plate reader set at 450nm. Ensure that the bottoms of the microwells are clean before reading and check that no foreign matter is present in the microwells. The reader should be blanked on air (ie with no plate in the carriage) before the plate is scanned.

Alternatively, if the spectrophotometer or EIA plate reader allows for the use of a reference wavelength (at 620 to 650nm), dual wavelength reading should be performed as this eliminates any potential interference caused by aberrations, such as dirt or marks, on the optical surface of the microwells.

## 10.4 SUMMARY OF IDEIA™ LYME NEUROBORRELIOSIS ASSAY PROCEDURE

Ensure reagents reach room temperature (15–30°C) before use

Add 100µL of Control or test serum/CSF

↓ **Cover and incubate at 20–25°C with shaking for 60 minutes**

Wash (x4)

↓

Add 100µL of Flagellum Conjugate **FLAGELLUM CONJUGATE**

↓ **Cover and incubate at 20–25°C with shaking for 60 minutes**

Wash (x4)

↓

Add 100µL of Substrate **SUBSTRATE TMB**

↓ **Cover and incubate at 20-25°C without shaking for 10 minutes**

Add 100µL of Stop Solution **STOP SOLUTION**

↓

Read absorbance photometrically at 450nm (reference 620-650nm)

# 11 QUALITY CONTROL AND INTERPRETATION OF RESULTS

## 11.1 BUFFER CONTROL

Calculate the mean OD values of the 2 Buffer Control microwells.

The mean OD value of the Buffer Control must be less than 0.100, but greater than 0.000 (dual wavelength reading).

If the value is above 0.100, inadequate washing or contamination of the Substrate may be the cause. If the value is less than 0.000, the ELISA reader should be re-blanked on air and the microwells re-read.

If the quality control requirements are not satisfied, test results are invalid and the assay should be repeated.

## 11.2 IgG POSITIVE CONTROL AND IgM POSITIVE CONTROL

Calculate the mean OD values for IgG Positive Control and IgM Positive Control. Individual OD values of duplicate test should not differ more than 15% from the mean OD value.

The mean OD value of IgG Positive Control and IgM Positive Control, respectively must be at least 0.500. If these values are less than 0.500 it may be caused by inadequate washing, inadequate shaking during incubations, or low ambient temperature, particularly during incubation with the Substrate solution.

If the quality control requirements are not satisfied, the test results are invalid and the assay should be repeated.

## 11.3 PATIENT SPECIMENS

Calculate the mean OD value for each patient CSF ($OD_{CSF}$) and serum ($OD_{serum}$) specimen for both the IgG and IgM determination. The OD values should not differ more than 15% from the mean. Any such specimens should be retested. However, if both test microwells show a negative result a difference of more than 15% may be accepted without retesting because low OD values are measured with less precision.

The specific antibody index formula is given below. Calculate the specific antibody index for IgG ($I_{IgG}$) and IgM ($I_{IgM}$), respectively. The index calculation should not be performed if the mean OD value of the CSF determination is less than 0.150. Any such test result should be reported as negative.

$$\text{Index} = \frac{OD_{CSF}}{OD_{serum}} \times (OD_{CSF} - OD_{serum})$$

## 11.4 INTERPRETATION OF RESULTS

### 11.4.1 Qualitative Interpretation

Interpret as follows:

Negative for production of intrathecal IgG antibodies to *B. burgdorferi*:

$I_{IgG}$ <0.3

**Or** $OD_{CSF}$ <0.150

Positive for production of intrathecal IgG antibodies to *B. burgdorferi*:

$I_{IgG} \geq 0.3$

Negative for production of intrathecal IgM antibodies to *B. burgdorferi*:

$I_{IgM}$ <0.3

**Or** $OD_{CSF}$ <0.150

Positive for production of intrathecal IgM antibodies to *B. burgdorferi*:

$I_{IgM} \geq 0.3$

### 11.4.2 Semiquantitative Interpretation

The higher the obtained index value is, the more pronounced is the specific intrathecal antibody production. Index values may be 100 or even more.

The specific antibody index is a highly sensitive, semiquantitative, measure of intrathecal antibody production because it depends on the rate of intrathecal antibody production, the blood CSF barrier permeability and the level of specific serum antibodies. Thus, in consecutive post-therapy specimens a change should be considered significant only if a positive index becomes <0.3, or if it decreases or increases more than 5 times. These are guidelines only.

### 11.4.3 Comments on Interpretation of Results

**Expression of Results**

Intrathecal antibody production is theoretically present if $OD_{CSF}/OD_{Serum} > 1$. However, this assumption is not safe, because of the intra-assay variation, especially in the lower OD range. The net OD difference ($OD_{CSF}$ - $OD_{Serum}$) gives a more precise information, but, owing to the intra-assay variation, the minimal OD difference indicative of intrathecal antibody synthesis will increase with increasing OD values. These drawbacks are eliminated by multiplication of OD ratio by OD difference as expressed by the specific antibody index (I). The lower limit of index value at 0.3 for a positive result ensures that $OD_{CSF}$ is significantly higher than $OD_{Serum}$[8].

**Positive Results**

An IgG and/or IgM index ≥0.3 with concomitant mononuclear pleocytosis in CSF is highly suggestive of Lyme neuroborreliosis. (A high CSF-OD value by itself is not indicative of intrathecal antibody production).

An IgM index ≥0.3 is usually compatible with a disease duration of less than 6 months. Intrathecal synthesis of specific IgM is a strong indication of neuroborreliosis, but IgM is not an obligatory finding. Patients with active neuroborreliosis of more than 6 months duration will usually have intrathecal synthesis of specific IgG only, and an IgG index ≥0.3 will be detected.

**Negative Results**

A negative result, that is an IgG and IgM index <0.3, with concomitant mononuclear pleocytosis in CSF does not exclude the clinical diagnosis of Lyme neuroborreliosis. This applies particularly when the time between onset of neurological symptoms and sampling of serum and CSF is short.

In most untreated patients with definite clinical signs of neuroborreliosis, specific antibodies in CSF become detectable in the 2nd week after onset of neurological symptoms[8]. In

patients with early neuroborreliosis and a negative test result, a later specimen may often prove positive, even after institution of treatment.
An IgM index <0.3 does not always exclude specific intrathecal IgM synthesis. The finding of a high (≥1.0) IgM OD value in CSF that is either equal to or even lower than the corresponding serum IgM value might still be indicative of intrathecal antibody synthesis if a severe impairment of the blood CSF barrier can be excluded[8].

**Post-treatment Specimens**
Evaluation of consecutive post-treatment CSF/serum specimens: After appropriate antibiotic therapy, a specific IgM index will decrease, and after 6-9 months the index is usually <0.3. An elevated IgG index will often persist for years despite complete recovery.

An IgG index ≥0.3 without concomitant mononuclear pleocytosis in CSF is a rare finding, except in post-treatment specimens. Therefore, this finding may indicate a previous episode of neuroborreliosis.

## 12 PERFORMANCE LIMITATIONS

**12.1** Excessive blood contamination of the CSF sample may lead to falsely low, specific antibody indices.

**12.2** Due to the antigenic relationship between *B. burgdorferi* and *Treponema pallidum*, serological cross-reactions, although rare, may occur in patients with a recent or past history of neurosyphilis. Serological discrimination is possible using the *T. pallidum* haemagglutination assay (TPHA) or the non-treponemal cardiolipin tests: VDRL, rapid plasma reagin test (RPR), and the Wassermann reaction. These tests will be negative in patients with a *B. burgdorferi* infection only.

**12.3** Prior antibiotic therapy may abrogate the antibody response and, thus, make the serological findings less predictable.

**12.4** Collection of specimens early in the course of disease may lead to negative test results (see Section 11).

**12.5** Test performance will be adversely affected if reagents are modified or stored under conditions other than those detailed in Section 5.2.

**12.6** Test results should be interpreted in conjunction with information available from epidemiological studies, clinical assessment of the patient and other diagnostic procedures.

## 13 EXPECTED VALUES

A specific antibody index ≥ 0.3 always indicates intrathecal synthesis of specific antibodies.

In patients with neuroborreliosis, intrathecal antibody synthesis usually begins in the second week after onset of neurological symptoms. Specific IgG and/or IgM production is detectable in ≈ 80% of patients with definite neuroborreliosis by the beginning of the third week and in all patients 6-8 weeks after onset of neurological symptoms[8].

Absence of intrathecal antibody production or the presence of specific antibodies in CSF due to transudation from serum or blood contamination of CSF will give antibody indices ≤0.

## 14 SPECIFIC PERFORMANCE CHARACTERISTICS

Paired CSF and serum specimens from 25 patients with clinically defined early Lyme neuroborreliosis were tested with the IDEIA™ Lyme Neuroborreliosis.

| IDEIA™ Lyme Neuroborreliosis | Number of patients |
|---|---|
| Positive for intrathecally produced IgG antibodies | 18 |
| Negative for intrathecally produced IgG antibodies | 7 |
| Positive for intrathecally produced IgM antibodies | 12 |
| Negative for intrathecally produced IgM antibodies | 13 |
| Positive for intrathecally produced IgG and/or IgM antibodies | 19 |
| Negative for intrathecally produced IgG and IgM antibodies | 6 |

A specific antibody index ≥0.3 was found for IgG in 72% and for IgM in 48% of the 25 patients with early Lyme neuroborreliosis. Only one patient had specific IgM synthesis without concomitant IgG synthesis. However, some patients with low IgG indices showed IgM indices ≥0.3. Therefore, the diagnostic evidence is increased by examining for both specific IgG and IgM synthesis. 19 of 25 patients (76%) with early Lyme neuroborreliosis were identified by IDEIA™ Lyme Neuroborreliosis.

### 14.1 CROSS REACTIVITY

A positive result always indicates intrathecal antibody synthesis to *B. burgdorferi* except when specimens from patients with neurosyphilis are tested. Specimens from these patients may give false positive results. However, the *B. burgdorferi*-specific antibody index usually remains low. Furthermore, it is a consistent finding that cross-reacting CSF antibodies as well as serum antibodies belong to the IgG class[3].

The significance of intrathecal polyclonal B cell activation as a cause of "false positive" IgM results is unknown[3].

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