Rubella Virus CSF ELISA IgG testkit

Order-No.: EC109L00 (IgG CSF- Testkit)
EC109L60 (IgG CSF-Standard set)
EN109L65 (IgG Antibody index controls)

Color Coding: red metallic /transparent

FOR IN-VITRO DIAGNOSIS ONLY

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1. Intended Use

The Rubella Virus CSF ELISA is used for the quantitative detection of endogenous IgG antibody synthesis in the CNS and may only be used for CSF diagnosis.

2. Diagnostic relevance

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (brain and bone marrow), which frequently occurs in young adults. The inflammation is caused by the cells of the body's immune system (antibodies, macrophages and T-cells) and leads to demyelination (removal of the nerve's protective coat) and to axonal damage (1, 2, 3, 4). There are multiple foci of inflammation in the CNS and these cause scarred tissue (sclerosis). As the inflammation can occur anywhere in the CNS, there is an extremely wide variety of symptoms. Frequent early symptoms include paralysis of the limbs (40%), inflammation of the optic nerve (22%), disturbed sensations (21%), double vision (12%), giddiness (5%) and bladder emptying (5%) (1). In more than 80% of patients, the disease starts with alternating phases and remissions (1, 2, 3). This means that the inflammation regresses within the first 6-8 weeks and that the symptoms then partially (25%) or totally disappear (50%) (3, 4). The symptoms may then reappear later. After 10 years, the clinical symptoms in about 40% of patients gradually become worse, even without additional phases (secondary progressive MS) (3). In rare cases, the clinical course is progressive from the start, without phases. This is known as primary progressive MS (1, 2, 3, 4). Although the disease cannot be cured, the clinical course can be alleviated by suitable therapy.

The objective is to use immunomodulatory or immunosuppressive drugs to prevent new phases of the disease, to suppress the symptoms associated with the phases as much as possible and to avoid the development of permanent deficits. This has been most successful with secondary progressive MS. The drugs are less active with the primary progressive form (1).

Early diagnosis is essential for effective treatment (3, 6). There may be an interval of months or even years between the first symptoms and the definitive diagnosis (3, 4). The reasons for this include:

- The complete disappearance of the symptoms, so that the patient does not take the symptoms seriously and does not contact a doctor.
- The variety of possible symptoms
- The highly individual and variable clinical course of MS
- The lack of specificity of the early symptoms, which could just as well be caused by other diseases (no specific MS markers).

The ideal sensitive and specific MS diagnosis would have to include the clinical presentation, paraclinical investigations and differential diagnosis. The combination of the different investigation methods and the necessary minimal criteria for the diagnosis of MS are specified in the so-called "McDonald Criteria" (5). These were laid down by an international team of experts in 2001 and then revised in 2005 on the basis of new scientific results (1, 4, 6). Magnetic resonance tomography (MRT) has become increasingly important as a paraclinical imaging procedure. Another important approach is to use evoked potentials (VEPs) to test the conductivity and thus the function of neuronal pathways.

CSF diagnosis is of the greatest importance in laboratory testing (3). Intrathecal IgG synthesis takes place in 98% of MS patients. The amount of synthesised antibody varies greatly between different patients and cannot be correlated with the severity of the disease. Nevertheless, the amount synthesised in the individual patient remains constant for decades from the time of the first clinical symptoms and is even detectable between the phases. This permits early diagnosis which is independent of the clinical course of the disease. Moreover, recent data have shown that CSF data is also relevant for MS diagnosis in children (10), which is of interest, as more and more children and adolescents are developing MS (3).

CSF diagnosis includes cytological studies, the detection of oligoclonal bands and the so-called MRZ reaction (3, 4). Detection of oligoclonal bands is regarded as being very sensitive (98%), but is non-specific, as it is found in many local and chronic conditions (8, 9, 10).

The MRZ [measles-rubella-zoster] reaction describes the extremely frequent occurrence of specific intrathecally produced antibodies to measles (78-79%), rubella (60-70%) and VZV [varicella zoster virus] (55-62%) (9, 10). Antibodies detected in CSF may either have diffused from plasma into the cerebrospinal fluid space or have been locally synthesised in the CNS (intrathecal antibody production). The specific antibody index (AI) is the ratio between the specific immunoglobulin quotient and the total immunoglobulin quotient and serves to clarify CNS infections. There is local antibody synthesis when the pathogen-specific antibody quotient for a specific antibody class is greater than the corresponding total immunoglobulin quotient. You will find more details in our brochure on CSF diagnosis. Increased AI values against two pathogens (M-R,
R+Z, M+Z), three pathogens (M+R+Z) or a single pathogen are found in 90% of all MS patients. As a two- or three-fold disease is implausible and the MRZ reaction has not been observed in any other disease, this test result can be regarded as typical for MS (7, 10). If only one AI value is increased, it must be established whether this is due to a pathogen-specific CNS infection. The MRZ findings must generally be seen in the context of the clinical presentation and all other laboratory tests (7).

Because of the non-specific symptoms of MS, differential diagnosis is both essential and manifold. Possibilities to be excluded include:

- Infectious diseases (neurosyphilis, neuroborreliosis, HIV)
- Chronic inflammatory diseases (collagenoses, vasculitis)
- Demyelinating diseases (neuromyelitis optica, tropical spastic paraparesis, acute disseminating encephalomyelitis (ADEM))
- Metabolic diseases (leukodystrophy)
- Psychiatric diseases (3)

3. Test Principle

The antibody searched for in the human serum and CSF forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added. The extinction (OD) of the color solution is directly proportional to the concentration of the analysed pathogen-specific IgG antibody in Serum and CSF. For the detection of CNS-own antibody-synthesis it is necessary to proceed a quantification of the antibody concentrations that are available in extinctions initially. For this, the arrays of standard sera with graded pathogen-specific antibody-concentration are provided. Those standard sera serve for the creation of a reference curve, that can be made manually or by using a suitable programme and allows the conversion of the detected OD-values into arbitrary defined non-dimensional measurement units (w ME). By allocating of the obtained measurement units (w ME) with the nephelometrical measured Sera- and CSF-Total-IgG concentrations the so called antibody index (AI) is detected (refer to the calculation of the AI in point 11.3). This antibody index mentions the searched pathogen-specific antibody quotient as a multiple respectively as a fraction of the respective Total-Immunoglobuline-Quotient. The value is therefore independent from the condition of the individual cerebral barrier-function. The antibody-index allows the conclusion to the presence and dimension of a CNS-own synthesis of pathogen-specific antibodies. This method is not valid in case of a poly-specific intrathecal immunoglobuline-synthesis, as then the total-IgX-quotient is no longer suitable as barrier parameter and has to be replaced by the so-called Limes value (refer to calculation of the Limes Quotient in point 11.3.4 B).

4. Package Contents

Package Contents (IgG CSF Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use)** 2x50ml, pH 7.2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated)** 50ml, pH 7.2, with preservative and Tween 20
4. **IgG-Conjugate (anti-human)**, 11ml, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
5. **Rubella ELISA IgG-Standards for the quantification of pathogen-specific antibody-concentrations**, 4 vials à 1000µl, human serum with protein-stabilizer and preservative, ready to use, 100w ME; 25w ME; 6.2w ME; 1.5w ME (w ME = arbitrary measurement units)
6. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB)**, 11ml, ready to use
7. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.
1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

<table>
<thead>
<tr>
<th>Material</th>
<th>Status</th>
<th>Storage</th>
<th>Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Samples</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>max. 6h</td>
</tr>
<tr>
<td></td>
<td>Undiluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Controls</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Microtitreplate</td>
<td>After Opening</td>
<td>+2 to +8°C (storage in the provided bag with desiccant bag)</td>
<td>3 months</td>
</tr>
<tr>
<td>Rheumatoid factor - Absorbent</td>
<td>Undiluted, After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td></td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Conjugate</td>
<td>After Opening</td>
<td>+2 to +8°C (protect from light)</td>
<td>3 months</td>
</tr>
<tr>
<td>Tetramethylbenzidine</td>
<td>After Opening</td>
<td>+2 to +8°C (protect from light)</td>
<td>3 months</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td></td>
<td>Final Dilution (ready-to-use)</td>
<td>+2 to +25°C</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples controls, conjugate and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. For internal quality assurance, we offer an Al control set.
   (Rubella Virus CSF Al Control Set; VT: Art. No.: EN109L65)
2. Aqua dest./demin.
3. Eight-channel pipette 50µl, 100µl
4. Micropipettes: 10µl, 100µl, 1000µl
5. Test tubes
6. Paper towels or absorbent paper
7. Cover for ELISA-plates
8. Disposal box for infectious material
9. ELISA handwasher or automated EIA plate washing device
10. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
11. Incubator

8. Test Procedure CSF-DIAGNOSTIC

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Consider the following for the CSF samples

1. Vein- and lumbar puncture should always be performed at approx. the same time.
2. Only optically clear and uncelled and not inactivated CSF may be used.
3. Do not use haemolytic or microbiologically contaminated or turbid CSF.
4. The use of deepfrozen CSF is possible if after thawing the conditions of items 2 and 3 are fulfilled.

8.2 Preparation of Reagents

The Sekisui Virotech System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer and washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters. The ready to use controls (positive control, negative control, cut-off control) are parameter specific and only to use with the plate lots they are related to. Please refer to Quality Control Certificate of the serum kit for possible combinations of plate lots and standard lots.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.

8.3 Virotech ELISA Test Procedure CSF-DIAGNOSTIC

- CSF/serum pairs are principally to be analysed next to each other in the same determination row on one test plate
- We recommend a double insertion for blank, standard sera, patient sera and CSF samples.
- To minimize matrix effects as much as possible, a working-dilution of 1+1 for CSF and 1+400 for serum is used.

1. For each test run pipette 100µl of each of the ready to use dilution buffer (blank), of the IgG ab-standard sera, the diluted CSF samples and the sera. Working dilution of sera: 1 + 400; e.g. 5µl serum + 2ml dilution buffer, working dilution of CSF: 1+1; e.g. 150µl CSF + 150µl dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350-400µl washing solvent per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to 3. above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to page 10 for Test Procedure Scheme

8.4 Usage of ELISA processors

All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Sekisui Virotech recommends the following procedure:

1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validation kit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each test run.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.
9. Test Evaluation CSF-DIAGNOSTIC

9.1 Test Function Control
To guarantee the optimal function of the test kit, the OD values of the 100w ME IgG Ab standard serums and the 6.2w ME IgG Ab standard serums should be above the minimal values given in the quality control certificate.

9.2 Interpretation
For the quantification of the pathogen-specific IgG antibody concentration of serum-CSF-pairs, a reference curve is created by hand on the enclosed halflogarithmic paper or by instrument using the IgG ab-standard sera. For this the OD-average values of the IgG ab-standard sera that have been carried along are transferred to the ordinate (y-axis) and the antibody concentration of the ready to use IgG ab-standard sera in w ME are transferred to the abscissa (x-axis). The reference curve, created by hand or by instrument (1,5w ME, 6,2w ME, 25w ME, 100w ME) shall have a sufficiently steep curve, a curve origin near the zero coordination point and acceptable deviation of all curve points from the extrapolished curve-course.

The OD-values of the Serum-CSF-Pairs may now be expressed in w ME by simply looking to the curve and correspond after multiplication with the dilution factors (400 for serum, 2 for CSF) to the concentrations of the pathogen-specific IgG antibody in serum and CSF. To obtain plausible antibody indices, OD-values below 0,05 and w ME-values below 1,5 or above 100 shall not be considered for the calculation. For OD-values, that lead to values above 100 w ME, a higher serum dilution than 1+400 and a higher CSF-dilution than 1+1 may be used considering the changed dilution ratio. During realisation of the CSF-diagnostic, a judgement of the 1+400 diluted patient-serum is impossible in the sense of an exceeding or fall short cut-off.

9.3 Calculation of the antibody index AI (with example)
Abbreviations:
\( \text{IgG\text{total}} = \text{Total IgG (mg/l)} \)
\( \text{IgG\text{spec.}} = \text{pathogen-specific IgG} \)
\( Q = \text{Quotient} \)
\( Q_{\text{alb}} = \text{Quotient resulting from the albumin content of CSF and albumin content of the serum (mg/l) /only necessary for calculation of Limes value!} \)

9.3.1 \( Q_{\text{IgG\text{spec.}}} \) (pathogen-specific antibody quotient)

**Serum**
- OD-values read: 0,700
- thus established concentration from the reference curve: 3,5 w ME
- dilution: 1:400

**CSF**
- OD-values read: 0,500
- thus established concentration from the reference curve: 2,5 w ME
- dilution: 1:2

\[
Q_{\text{IgG\text{spec.}}} = \frac{\text{IgG\text{spec.}}_{\text{CSF (wME)}} \times \text{dilution}}{\text{IgG\text{spec.}}_{\text{Serum (wME)}} \times \text{dilution}} = \frac{2,5 \text{ wME} \times 2}{3,5 \text{ wME} \times 400} = 3,6 \times 10^{-3}
\]

9.3.2 \( Q_{\text{IgG}} \) (Total Immunoglobuline Value; Value of the clinical chemistry)
- \( \text{IgG}_{\text{CSF}} = 33 \text{mg/l} \)
- \( \text{IgG}_{\text{Serum}} = 10000 \text{mg/l} \)
9.3.3 Calculation of the Limes Quotient ($Q_{\text{LIM}}$)

In case of an additional poly-specific intrathecal immunoglobulin synthesis, the Total-IgG-Quotient for the AI-determination is no longer suitable. The so called $Q_{\text{LIM}}$ has to be used instead of the Total-IgG-Quotient. Therefore it is necessary to determine the albumin quotient additionally (value of the clinical chemistry).

Calculation of the LIMES-Value (according to Reiber):

$$Q_{\text{LIM-IG}} = 0,93 \cdot \sqrt{Q_{\text{ab}}^2 + 6 \times 10^{-6}} - 1.7 \times 10^{-3}$$

9.3.4 Calculation of the Antibody Index (AI)

A. $Q_{\text{IGG}} < Q_{\text{LIM}}$

The antibody index (AI) states the relation between the pathogen-specific antibody quotient ($Q_{\text{spec}}$) and the total-immunoglobulin-quotient ($Q_{\text{total}}$). Thus, a pathogen-specific antibody-synthesis can be detected and quantified. In this case, the total-immunoglobulin-quotient is used as barrier-parameter.

$$AI = \frac{Q_{\text{IGG spec}}}{Q_{\text{IGG total}}} = \frac{\text{IgG spec.CSF} \times \text{dilution}}{\text{IgG spec.Serum} \times \text{dilution}} = \frac{3,6 \times 10^{-3}}{3,3 \times 10^{-3}} = 1,1$$

B. $Q_{\text{IGG}} > Q_{\text{LIM}}$

In case an additional poly-specific intrathecal immunoglobulin-synthesis is present the measured $Q_{\text{total}}$ must no longer be used for the AI-calculation as an antibody synthesis searched for or eventually present at the same time may be falsified in its extent or even get totally unrecognizable. In this cases the so called Limes value of the immunoglobulin quotient is calculated (see formula) or graphically determined by using the albumin quotient which has to be calculated additionally. This Limes value is used instead of the immunoglobulin quotient for calculation of AI-value.

$$AI = \frac{Q_{\text{IGG spec.}}}{Q_{\text{LIM}}}$$

9.4 Interpretation

<table>
<thead>
<tr>
<th>AI</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI &lt; 0,6</td>
<td>implausible: theoretically not to be expected, occurs in the routine occasionally, no pathological meaning, troubleshooting recommended</td>
</tr>
<tr>
<td>AI: 0,6 – 1,3</td>
<td>normal: an intrathecal ab-production is unlikely</td>
</tr>
<tr>
<td>AI: 1,4 – 1,5</td>
<td>borderline: it is recommended to re-test the sample or to test a second serum-CSF-pair in a follow-up test run</td>
</tr>
<tr>
<td>AI: &gt;1,5</td>
<td>pathologic: indication of an intrathecal ab-production</td>
</tr>
</tbody>
</table>

1. Since a minimum of four different results (pathogen specific CSF- and serum-antibody measurement-units, total serum- and CSF-IgG value, CSF- and serum-albumine in mg/l) are considered for the calculation of the diagnostical relevant AI-value, all methodic and coincidental errors add up here. In the most unfavourable case, a continuing mistake in the same
sense is possible; a double determination or better the measuring of two different test material dilutions are the best way to recognize this. For this reason, a clinical relevant limited Al-value of 1,5 has established as note for a local synthesis of pathogen-specific antibodies in the CSF.

2. Normally for virus-specific IgG antibodies there is the same ratio between CSF and serum as it is found for the summarised IgG-fraction. The theoretically expected Al-value is therefore 1,0. Corresponding tests have shown, that for all virus-specific antibodies a reference-range of 0,6 – 1,5 is valid. Al-values above 1,5 may be considered as pathologic in case of sufficient analytical quality of all incoming single values and may be characterized by a CNS-own synthesis of the corresponding virus-specific antibodies and their release into the cerebrospinal area.

3. Al-values below 0,6 are theoretically impossible and do normally point out an analytical mistake.

9.5 Limits of the Test

1. In case of very high pathogen-specific antibody concentrations in the cerebrospinal fluid or in the serum, a risk that the antigen-concentration in the wells is insufficient to fulfill the optimum conditions for a quantitative antibody detection is present. If an antibody-excess is suspected (please consider also Heidelberg curve and total CSF-result) a second determination with higher dilution of serum respectively CSF has to follow.

10. Performance Data CSF-Diagnostic

10.1 Sensitivity and Specificity

To determine the analytical sensitivity, 24 positive CSF/serum pairs were tested in the Rubella CSF IgG ELISA and in another ELISA as reference test.

<table>
<thead>
<tr>
<th>Rubella Virus CSF ELISA</th>
<th>Reference ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>positive</td>
<td>23</td>
</tr>
<tr>
<td>negative</td>
<td>1</td>
</tr>
<tr>
<td>borderline</td>
<td>0</td>
</tr>
</tbody>
</table>

This gives the analytical sensitivity of 96%.

To determine the analytical specificity, CSF/serum pairs (n = 35) were tested in the Rubella CSF IgG ELISA and in another ELISA as reference test.

<table>
<thead>
<tr>
<th>Rubella Virus CSF ELISA</th>
<th>Reference ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>positive</td>
<td>0</td>
</tr>
<tr>
<td>negative</td>
<td>0</td>
</tr>
<tr>
<td>borderline</td>
<td>0</td>
</tr>
</tbody>
</table>

This gives the analytical specificity of >99.9%

10.2 Intra-Assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation is < 9% (at an average OD-value of 0,25)

10.3 Inter-Assay Variation Coefficient (Reproducibility)

One CSF/serum pair with a normal Al value and one CSF/serum pair with a pathological value were tested in 10 independent tests in different laboratories and from different operators. The resulting variation coefficient was under 20%.

11. Literature

3. Deutsche Gesellschaft für Neurologie (DGN), Leitlinien zu Diagnostik und Therapie der Multiplen Sklerose
4. Deutsche Multiple Sklerose Gesellschaft Bundesverband E.V.; Homepage Stand 2008
6. Kahmann A. und Sindern E. (2003), psychoneuro, 29 (7+8), 332-335
12. Testprocedure Scheme – CSF-Diagnostic

Preparation of the Patient Samples and Washing Solution

▼ Washing Solution: Fill up concentrate to 1 liter with aqua dest./demin.

▼ IgG-Samples – Dilution
1:401

▼ CSF-Dilution
1:2
e.g.:
5 µl serum/plasma + 2000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

150 µl CSF-sample + 150 µl Dilution Buffer

Testprocedure

Samples Incubation 30 Minutes at 37°C 100 µl Patient Samples
blank (Dilution Buffer) and standards

Wash 4 times

Conjugate Incubation 30 Minutes at 37°C 100 µl Conjugate
IgG

Wash 4 times

Substrate Incubation 30 Minutes at 37°C 100 µl Substrate

Stopping

Measure Extinctions

400 µl Washing Solution
Remove Residues on a Cellulose Pad

400 µl Washing Solution
Remove Residues on a Cellulose Pad

50 µl Stopping Solution
shake carefully

Photometer at 450/620nm
(Reference Wavelength 620-690nm)