研究用試薬

Antibodies of the IgG class against Measles virus in cerebrospinal fluid Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2610-9601-L G	Measles virus	IgG	Ag-coated microplate wells	96 x 01 (96)

Principles of the test: The ELISA test kit provides a quantitative in vitro assay for human antibodies of the IgG class against measles virus in cerebrospinal fluid (CSF). It is the same assay as for the determination of antibodies against measles virus in human serum, but additionally contains calibrators for CSF diagnostics. The test kit contains microtiter strips each with 8 break-off reagent wells coated with measles virus antigens.

In the first reaction step, diluted patient sample and cerebrospinal fluids are incubated in parallel with the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

The concentration of antibodies against Measles virus in cerebrospinal fluid and serum is measured by means of a calibration curve established by incubating the CSF calibrators C (100 U) to F (5 U). A wider measurement range is obtained by additional incubation of calibrators A (230 U) and B (175 U). This also increases the number of evaluable serum pairs per run. If human antibodies against Measles virus are to be detected exclusively in human serum, a calibration curve established with calibrators 1 to 3 must be used (see EUROIMMUN Anti-Measles virus ELISA, order number EI 2610-9601 G).
For optional checking and documentation of correctness and precision of the determination of the relative CSF/serum quotient (CSQ_{rel}), CSF/serum control pairs (CSQ control pair) in the “normal range” are separately available.
These can be acquired under the EUROIMMUN order number CK 2610-0220-L G.

Contents of the test system: EI 2610-9601-L G

Component	Colour	Format	Symbol
1. Test kit Anti-Measles virus ELISA (IgG), Order number EI 2610-9601 G	---	---	---
2. CSF calibrator A 230 U (human IgG), ready for use	Purple coloured in decreasing intensity.	1 x 2.0 ml	CAL A
3. CSF calibrator B 175 U (human IgG), ready for use		1 x 2.0 ml	CAL B
4. CSF calibrator C 100 U (human IgG), ready for use		1 x 2.0 ml	CAL C
5. CSF calibrator D 50 U (human IgG), ready for use		1 x 2.0 ml	CAL D
6. CSF calibrator E 25 U (human IgG), ready for use		1 x 2.0 ml	CAL E
7. CSF calibrator F 5 U (human IgG), ready for use		1 x 2.0 ml	CAL F
8. Test instruction	---	1 booklet	---
9. Quality control certificate	---	1 protocol	---

LOT	Lot description		Storage temperature
IVD	In vitro diagnostics	CE	Unopened usable until

Updates with respect to the previous version are marked in grey.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Cerebrospinal fluid calibrators:** Ready for use. The calibrators must be mixed thoroughly before use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: Calibrators and controls used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays or indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the samples

Samples: Human serum and cerebrospinal fluid (CSF), preferably taken at the same time.

Stability: The recommended storage time at +2°C to +8°C is up to 14 days for the patient sera to be investigated and up to 6 days for the CSF samples. [1]

Sample dilution, method A:
When using this method patient samples are diluted according to one of the dilution schemes recommended by EUROIMMUN, independent of the IgG concentration that is included later into the calculation of the relative CSF/serum quotient (see section 3 of the chapter „Instruction for the calculation of the relative CSF/serum quotient“, page 6).

Serum samples for analysis are diluted **1:404** in sample buffer.
For example, first mix the serum sample thoroughly, then add 10 µl serum to 1.0 ml sample buffer and mix thoroughly (1:101 dilution). Transfer 250 µl of this prediluted sample into 750 µl sample buffer and mix thoroughly again (1:404 dilution).

CSF samples for analysis are diluted **1:2** in sample buffer.
For example, first mix the CSF sample thoroughly, then add 100 µl CSF to 100 µl sample buffer and mix thoroughly (dilution 1:2).

Alternative sample dilution, method B:
CSF and serum samples can be alternatively diluted in such a way that the same IgG concentrations are achieved. It is nevertheless necessary to check if the CSF/serum quotient of the total immunoglobulin can be taken into account for the calculation of the relative CSF/serum quotient or if the CSF/serum quotient of total albumin should also be included (see section 4 of the chapter “Instruction for the calculation of the relative CSF/serum quotient”, page 6). The calculations of the upper discrimination value $CSQ_{lim.}$ (IgG) on the basis of the CSF/serum quotient of total albumin concentrations must also be made when performing the alternative sample dilutions. The calculation of the relative CSF/serum quotient when using the alternate sample dilution is described in section 6 of the chapter „Instruction for the calculation of the relative CSF/serum quotient” (page 9).

Artificial blood contamination of CSF samples:
Slight artificial blood contamination (<1000 erythrocytes/µl) are negligible in protein analysis. [2]

研究用試薬

Incubation

Sample incubation: (1st step)
Transfer 100 µl of the CSF calibrators, diluted sample serum or cerebrospinal fluid into the individual microplate wells and incubate for **60 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Empty the wells and wash reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **60 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C C 100 E**	**S 3 1:404**	**S 7 1:404**	**S 11 1:404**					**C A 230 E**	**S 2 1:404**	**S 6 1:404**	**S 10 1:404**
B	**C D 50 E**	**C 3 1:2**	**C 7 1:2**	**C 11 1:2**					**C B 175 E**	**C 2 1:2**	**C 6 1:2**	**C 10 1:2**
C	**C E 25 E**	**S 4 1:404**	**S 8 1:404**	**S 12 1:404**					**C C 100 E**	**S 3 1:404**	**S 7 1:404**	**S 11 1:404**
D	**C F 5 E**	**C 4 1:2**	**C 8 1:2**	**C 12 1:2**					**C D 50 E**	**C 3 1:2**	**C 7 1:2**	**C 11 1:2**
E	**S 1 1:404**	**S 5 1:404**	**S 9 1:404**						**C E 25 E**	**S 4 1:404**	**S 8 1:404**	**S 12 1:404**
F	**C 1 1:2**	**C 5 1:2**	**C 9 1:2**						**C F 5 E**	**C 4 1:2**	**C 8 1:2**	**C 12 1:2**
G	**S 2 1:404**	**S 6 1:404**	**S 10 1:404**						**S 1 1:404**	**S 5 1:404**	**S 9 1:404**	
H	**C 2 1:2**	**C 6 1:2**	**C 10 1:2**						**C 1 1:2**	**C 5 1:2**	**C 9 1:2**	

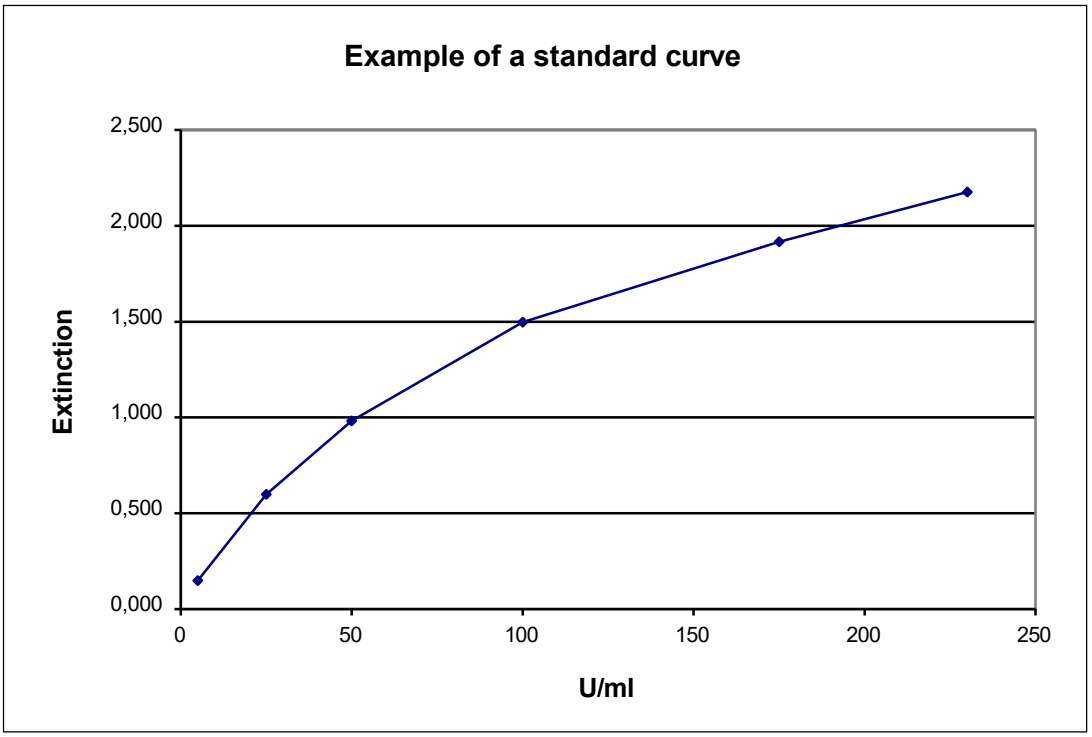
The pipetting scheme given is an example for the analysis of 12 patient sera (S 1 to S 12) and the 12 corresponding CSF samples (C 1 to C 12). The scheme on the left shows the CSF calibration for the measurement range from 5 U to 100 U and the scheme on the right shows the CSF calibration for the extended measurement range from 5 U to 230 U.

CSF calibrators and patient samples are incubated each in a single determination per dilution. The accuracy of the calibration curve can be increased by performing duplicate determinations of the calibrators.
The CSF calibrators should be incubated with every new test run and a new standard curve determined. Sera and corresponding CSF samples must be investigated in the same test run.
Wells can be broken off individually from the microtiter strips. This allows the number of test substrates used to be matched to the number of samples, minimizing reagent wastage.

Calculation of results

The **CSF/serum quotient of pathogen-specific antibodies $CSQ_{path.-spec.}$ (IgG)** can be determined with this test system. For particular requirements in the determination of pathogen-specific antibodies in CSF see chapter “Basics of the CSF diagnostics” (page 5).

The standard curve from which the concentration of antibodies in the CSF and patient sample pair can be taken is obtained by point-to-point plotting of the extinction values measured for the 4 or 6 CSF calibrators against the corresponding units. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in your patient samples.



The relative CSF/serum quotient can only be calculated correctly if the extinctions of both samples are within the measurement range. The measurement range is the extinction range between the calibrator with the highest OD and the calibrator with the lowest OD.

If the values determined for the samples lie outside the measurement range, proceed as follows:

1. If the value obtained for the serum sample and/or the CSF sample is under the measurement range (extinction of sample < extinction of calibrator L F): no specific antibodies are detectable in the sample and it is not possible to calculate the CSF/serum quotient.

2. If the value obtained for the serum sample and/or the CSF sample is above the measurement range (extinction of sample > extinction of calibrator C A): the sample must be tested at a higher dilution, according to the same scheme. For example, use a serum dilution of 1:808 or a CSF dilution of 1:4. Serum and cerebro-spinal fluid must be retested together in the same test run. The new dilution factor must be taken into account for the calculation of the CSF/serum quotient.

If values outside the measurement range are used, the CSQrel. might not be determined correctly, resulting in an incorrect interpretation of values.

Instruction for the calculation of the relative CSF/serum quotient ($CSQ_{rel.}$)

1. Basics of the CSF diagnostics

In order to identify a specific humoral immunoreaction in an infection of the central nervous system (CNS), concentrations of pathogen-specific antibodies, the corresponding class of immunoglobulins, and albumin in the patient's cerebrospinal fluid (CSF) and in the serum are determined.

During an infection of the central nervous system with measles virus an enrichment of pathogen-specific antibodies in the cerebrospinal fluid occurs. If the infection is not restricted to the brain and there is no dysfunction in the blood-CSF barrier, pathogen-specific antibodies have the same distribution in cerebrospinal fluid and serum as total IgG. In this case, all immunoglobulins in the cerebrospinal fluid come from the blood. For the detection of an infection of the central nervous system with measles virus it is necessary to distinguish between intrathecally produced antibodies and antibodies passed from blood into CSF.

A value for the intrathecal pathogen-specific antibody production is the **relative CSF/serum quotient CSQrel.** (synonym: antibody specificity index). This value is the quotient of the portion of pathogen-specific antibodies in total IgG of cerebrospinal fluid and the portion of pathogen-specific antibodies in total IgG of serum.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

$$CSQ_{rel.} = \frac{\frac{\text{pathogen-specific IgG in CSF}}{\text{total IgG in CSF}}}{\frac{\text{pathogen-specific IgG in serum}}{\text{total IgG in serum}}} = \frac{\frac{\text{pathogen-specific IgG in CSF}}{\text{total IgG in CSF}}}{\frac{\text{pathogen-specific IgG in serum}}{\text{total IgG in serum}}} = \frac{CSQ_{path.-spec.}(IgG)}{CSQ_{total}(IgG)}$$

The CSF/serum quotient of pathogen-specific antibodies $CSQ_{path.-spec.}$ (IgG) is put into relation to the CSF/serum quotient of the total IgG concentration CSQ_{total} (IgG). A relative CSQ value up to 1.3 indicates that there are no specific antibodies produced in the central nervous system. A relative CSQ value above 1.5 indicates that pathogen-specific antibodies are produced in the central nervous system due to a local infection.

The antibody production in the central nervous system can occur under different pathologic conditions, for example multiple myeloma or encephalomyelitis disseminata (multiple sclerosis). If there is an indication of intrathecal IgG production or a defect in the blood-brain barrier, the CSF/serum quotient diagram of Reiber (1991; see section 4 of this chapter) would be helpful.

2. Calculation of CSF/serum quotient of pathogen-specific antibodies $CSQ_{path.-spec.}$ (IgG)

Pathogen-specific antibodies of immunoglobulin classes IgG in the CSF can be determined with the corrresponding EUROIMMUN ELISA and the CSF/serum quotient $CSQ_{path.-spec.}$ (IgG) is calculated using the following formula:

$$CSQ_{path.-spec.}(IgG) = \frac{\text{units (CSF)} \times \text{dilution factor (CSF)}}{\text{units (serum)} \times \text{dilution factor (serum)}}$$

Calculation example:

CSF 1:2 Extinction 0.675: 55 U
Serum 1:404 Extinction 0.530: 42 U

$$CSQ_{path.-spec.}(IgG) = \frac{55 \text{ units(CSF)} \times 2}{42 \text{ units (serum)} \times 404} = 6.48 * 10^{-3}$$

3. Calculation of CSF/serum quotient of total immunoglobulin CSQ_{total} (IgG)

The IgG concentrations (in mg/l) in CSF and serum can be determined using e.g. nephelometric methods. The CSF/serum quotient CSQ_{total} (IgG) is calculated using the following formula:

$$CSQ_{total}(IgG) = \frac{\text{IgG-concentration in CSF}}{\text{IgG-concentration in serum}} = \frac{\text{TotalIgG in CSF}}{\text{TotalIgG in serum}}$$

Calculation example:
Total IgG concentration in CSF: 13.3 mg/l
Total IgG concentration in serum: 8.53 g/l

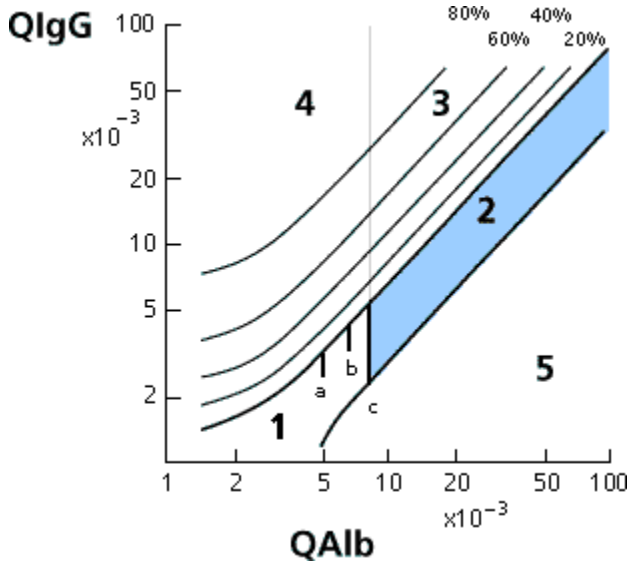
$$CSQ_{total}\,(IgG) = \frac{13.3\ mg/l}{8530\ mg/l} = 1.56 \times 10^{-3}$$

4. Calculation of the upper discrimination value $CSQ_{lim.}$ (IgG) on the basis of the CSF/serum quotient of total albumin concentrations $CSQ_{alb.}$

During an antibody production in the central nervous system (e.g. multiple myeloma or multiple sclerosis) the CSF/serum quotient of total immunoglobulin CSQ_{total} (IgG) is extraordinarily high and must not be used for determination of the pathogen-specific antibody production in the CNS. In such a case the CSF/serum quotient of total albumin $CSQ_{alb.}$ is used because albumin is produced exclusively in the liver and not intrathecally. In a large number of CSF/serum pairs of probands without symptoms the albumin quotient and the corresponding highest normal immunoglobulin quotient designated as upper discrimination value $CSQ_{lim.}$ (limes quotient for IgG) were calculated. If the total immunoglobulin quotient CSQ_{total} (IgG) is above $CSQ_{lim.}$ (IgG), an additional IgA, IgG or IgM production is present in the central nervous system. In this case, $CSQ_{lim.}$ (IgG) is used instead of the CSQ_{total} (IgG) for the calculation of the pathogen-specific antibody production in the CNS (see section 5 of this chapter).

The empirical data of CSF/serum pairs (Q_{IgG} and $Q_{alb.}$) are shown according to the CSF/serum quotient diagrams of Reiber and Lange (1991). In these diagrams the CSF/serum quotient of the immunoglobulin concentrations is plotted versus the CSF/serum quotient of the albumin concentrations. This allows the calculation of the highest normal IgA, IgG or IgM quotient (upper discrimination value, Qlim.) for every albumin quotient.

The CSF/serum quotient diagram shown below presents an example for the immunoglobulin class IgG. The curve above the areas 1 and 2 is the upper discrimation line of the reference range without additional immunoglobulin production in the CNS.



The measured data can be subdivided into 5 categories:

1. Reference range of normal values, intact blood-CSF barrier
2. Dysfunction of the blood-CSF barrier, no immunoglobulin production in the CNS
3. Dysfunction of the blood-CSF barrier, additional immunoglobulin production in the CNS
4. Intact blood-CSF barrier, immunoglobulin production in the CNS
5. Mistakes during preparation of the sera or the analytical steps

CSQ_{lim} (IgA, IgG or IgM) can also be calculated. Owing to the distinct diffusion constants of the immunoglobulins for the calculation of the quotients for IgA, IgG and IgM, three different formulas are used (Beuche and Felgenhauer, 1999).

$$CSQ_{lim.}(IgA) = 0.77 \times \sqrt{(CSQalb)^2 + 23 \times 10^{-6}} - 3.1 \times 10^{-3}$$

$$CSQ_{lim.}(IgG) = 0.93 \times \sqrt{(CSQalb)^2 + 6 \times 10^{-6}} - 1.7 \times 10^{-3}$$

$$CSQ_{lim.}(IgM) = 0.67 \times \sqrt{(CSQalb)^2 + 120 \times 10^{-6}} - 7.1 \times 10^{-3}$$

5. Calculation of the relative CSF/serum quotient $CSQ_{rel.}$

Depending on the relation of CSQ_{total} and $CSQ_{lim.}$ the relative CSF/serum quotient $CSQ_{rel.}$ can be calculated using one of the following formulas:

5.1 CSQ_{total} (IgG) < $CSQ_{lim.}$ (IgG)

If the CSF/serum quotient of the total immunoglobulin CSQ_{total} (IgG) is below the upper discrimination value $CSQ_{lim.}$ (IgA, IgG or IgM), the relative CSF/serum quotient is calculated according to the following formula:

$$CSQ_{rel.} = \frac{CSQ_{path.-spec.}(IgG)}{CSQ_{total}(IgG)}$$

Calculation example:

$CSQ_{path.-spec.}$ (IgG) = 6.48 x 10^{-3}
CSQ_{total} (IgG) = 1.56 x 10^{-3}
$CSQ_{alb.}$ = 6.0 x 10^{-3}
$CSQ_{lim.}$ = 4.33 x 10^{-3}

This results in: CSQ_{total} (IgG) = 1.56 x 10^{-3} < $CSQ_{lim.}$ (IgG) = 4.33 x 10^{-3}.

$$CSQ_{rel.} = \frac{CSQ_{path.-spec.}(IgG)}{CSQ_{total}(IgG)} = \frac{6.48 \times 10^{-3}}{1.56 \times 10^{-3}} = 4.16$$

5.2 CSQ_{total} (IgG) > $CSQ_{lim.}$ (IgG)

If the CSF/serum quotient of the total immunoglobulin CSQ_{total} (IgG) is above the upper discrimination value $CSQ_{lim.}$ (IgG), the relative CSF/serum quotient is calculated according to the following formula:

$$CSQ_{rel.} = \frac{CSQ_{path.-spec.}(IgG)}{CSQ_{lim.}(IgG)}$$

Calculation example:
$CSQ_{path.-spec.}$ (IgG) = 6.48 x 10^{-3}
CSQ_{total} (IgG) = 11.6 x 10^{-3}
$CSQ_{alb.}$ = 6.0 x 10^{-3}

$$CSQ_{lim}(IgG) = 0.93 \times \sqrt{(6.0 \times 10^{-3})^2 + 6 \times 10^{-6}} - 1.7 \times 10^{-3} = 4.33 \times 10^{-3}$$

This results in: CSQ_{total} (IgG) = 11.6 x 10^{-3} > $CSQ_{lim.}$ (IgG) = 4,33 x 10^{-3}

$$CSQ_{rel.} = \frac{CSQ_{path.-spec.}(IgG)}{CSQ_{lim.}(IgG)} = \frac{6.48 \times 10^{-3}}{4.33 \times 10^{-3}} = 1.50$$

6. Alternative method to determine the CSF/serum quotient $CSQ_{rel.}$ if alternative sample dilution, method B, is used

In order to achieve a comparable relationship between the CSF and serum for the determination of specific IgG, the concentration of total IgG in CSF and serum can first be determined. The IgG concentration in the serum can be matched to that in the 1:2 diluted CSF by appropriate dilution of the serum. To calculate the individual serum dilution for each patient (with a CSF dilution of 1:2) the following formula is used.

$$\text{Dilutionfactor of the serum} = \frac{\text{total-IgG in serum}}{\text{total-IgG in CSF}} \times 2$$

Calculation example:
IgG concentration in CSF: 57 mg/l
IgG concentration in serum: 11.100 mg/l

this means that:

$$\text{Dilution factor of the serum} = \frac{11.100\ \text{mg/l}}{57\ \text{mg/l}} \times 2 = 389$$

In this example the serum sample should be diluted 1:389 in order to achieve the same IgG concentration as in the 1:2 diluted CSF sample. Since the IgG concentration in the diluted serum-sample is identical to the IgG concentration in the 1:2 diluted CSF sample, the relative CSF/serum quotient becomes the quotient of the measured specific antibody concentrations.

$$CSQ_{rel.} = \frac{\text{units(CSF)}}{\text{units(serum)}}$$

Calculation example:

CSF	1:2	extinction 0.836:	77 U
Serum	1:389	extinction 0.442:	36 U

$$CSQ_{rel.} = \frac{77\ \text{Units (CSF)}}{36\ \text{Units (serum)}} = 2.1$$

Interpretation

According to the recommendation of Reiber and Lange (1991) interpretation is made using the following scale. The upper limit of the reference range for individuals without intrathecal pathogen-specific antibody production is 1.5:

$CSQ_{rel.}$ < 0,6	**implausible result; cause analysis recommended**
$CSQ_{rel.}$ 0,6 to < 1,3	**normal range**
$CSQ_{rel.}$ 1,3 to 1,5	**equivocal range**
$CSQ_{rel.}$ > 1,5	**indication of pathogen-specific antibody production in the CNS**

⇒ **For evaluation of the data EUROIMMUN offers a program (requires MS Excel) which calculates the relative CSF/serum quotient $CSQ_{rel.}$ automatically.**

Test characteristics

Cross-reactivity: Cross-reactivities with other pathogens cannot be ruled out.

Interference: Influences of haemolytic, lipaemic and icteric samples on the result cannot be excluded.

Reproducibility: Intra-assay and inter-assay reproducibility are ensured.

Sensitivity and specificity: Study I: The performance of MRZ-reaction, an intrathecal humoral immune response against-Measles (M), Rubella (R) and Varicella Zoster (Z) viruses, were investigated in patients diagnosed either with multiple sclerosis (MS), clinically isolated syndrome (CIS) or other neurological diseases.

Patient panel	n	$CSQ_{rel.}$ > 1.5		
		Measles Virus	Rubella Virus	VZV
Patients diagnosed with multiple sclerosis (MS)	28	14 (50.0%)	13 (46.4%)	15 (53.6%)
Patients diagnosed with clinically isolated syndrome (CIS: high risk of developing MS)	10	3 (30.0%)	5 (50.0%)	3 (30.0%)
Patients with other neurological diseases	23	1 (4.3%)	2 (8.7%)	2 (9.1%)

		$CSQ_{rel.}$ > 1.5 for			
Patient panel	n	1 pathogen	2 pathogens	3 pathogens	1, 2 oder 3 pathogens
Patients diagnosed with multiple sclerosis (MS)	28	11 (39.3%)	11 (39.3%)	3 (10.7%)	25 (89.3%)
Patients diagnosed with clinically isolated syndrome (CIS: high risk of developing MS)	10	2 (20.0%)	3 (30.0%)	1 (10.0%)	6 (60.0%)
Patients with other neurological diseases	23	0 (0.0%)	1* (4.3%)	1** (4.3%)	2 (8.7%)

* no further information available

** patient diagnosed with brain metastases

A positive MRZ reaction, defined as a positive intrathecal response to at least two of the three pathogens, was found in 14 of 28 MS patients, but only in 2 of 23 controls corresponding to a sensitivity of 50% and specificity of 91%, which is in similar range as reported in literature (Feki et al. 2018; Reiber et al. 2016).

研究用試薬

Study II: The $CSQ_{rel.}$ for anti-measles virus IgG were determined for 14 patients with suspected subacute sclerosing panencephalitis (SSPE) caused by measles virus infection of the CNS.

Patient panel	n	$CSQ_{rel.}$ > 1.5
Patients with suspicion of SSPE	14	14

All patients with suspected SSPE achieved a pathological CSQrel. for anti-measles virus IgG corresponding to a sensitivity of 100%.

Study III: 36 CSF/serum pairs provided by INSTAND e.V. quality assessment scheme (QAS) were investigated. A correct interpretation of the $CSQ_{rel.}$ for intrathecal anti-Measles virus IgG were achieved in 100%.

INSTAND e.V. QAS (2000-2017)	n	Correlation with target value*	Correct interpretation **
Measles	36	92%	100%

* accepted deviation from the target value: ± 30% (since May 2007; before: ± 45%)
** pathological / non pathological

Literature references

1. Zimmermann K., Kühn H.-J., Linke E. **Praktische Liquordiagnostik in Frage und Antwort.** 1. Auflage, Dresden (2010).
2. Wildemann B., Oschman P, Reiber H. **Neurologische Labordiagnostik.** Thieme Verlag, Stuttgart (2006).
3. Felgenhauer K, Beuche W. **Labordiagnostik neurologischer Erkrankungen.** Georg Thieme Verlag, Stuttgart (1999).
4. Reiber H., Lange P. **Virus-spezifische Antikörper in Liquor und Serum. ELISA-Analytik und Auswertung mittels Antikörper-Index und Quotientendiagramm.** Lab Med 15: 204-207 (1991).
5. Reiber H. **The hyperbolic function: a mathematical solution of the protein flux/CSF flow model for blood-CSF barrier function.** J Neurol Sci 126: 243-245 (1994).