

**MIKROGEN**

molekularbiologische Entwicklungs-GmbH

## **Liquor diagnosis *Borrelia burgdorferi***

**Instructions for use**

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Liquor diagnosis Borrelia	
	Instructions for use AVBBL0011.doc Valid from: April 2004
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## Liquor diagnosis

*recomWell* Borrelia IgG and IgM for quantitative determination and *recomBlot* Borrelia IgG and IgM for reliable identification of intrathecally produced human IgG and IgM antibodies to *Borrelia burgdorferi sensu lato*.

### General aspects / Intended use

In cases of clinically suspected neuroborreliosis, the detection of intrathecally produced Borrelia-specific IgG and IgM antibodies is indicated. For this purpose serum-CSF pairs are analysed in parallel in a quantifiable test (e.g. *recomWell* Borrelia). Following the recommended 2-tier serodiagnosis, the results can then be confirmed using *recomBlot* Borrelia. An excel program developed to facilitate the calculations for liquor diagnosis (acc. to Reiber) is available from **MIKROGEN**. See also Chapter 6.

First, serum and CSF are included in the standard *Borrelia* screening test with *recomWell* Borrelia. If the CSF is positive, the values for total IgG and/or IgM and albumin must be requested for serum and CSF as the basis for the determination of the *Borrelia*-specific antibody index. In addition to the detailed set of procedural specifications, the enclosed protocol sheet is a convenient aid in performing the necessary calculations.

By this method based on the *Borrelia*-specific antibody index, an intrathecally produced antibody fraction can be differentiated from a fraction that has passed the blood-CSF barrier passively to enter the subarachnoid space. Applying the limiting value QLim from the quotient diagram according to Reiber (1,2) ensures that no false-negative antibody index values will be calculated in case of a polyspecific intrathecal immune reaction.

In cases of serum-CSF pairs showing a pathologically elevated antibody index in the screening test, confirmation with *recomBlot* Borrelia IgG/IgM is recommended. In this test, serum and CSF are diluted to the same total IgG / total IgM concentrations to allow qualitative comparison of the corresponding *Borrelia*-specific bands.

In addition to these procedural specifications, document matrices for producing patient protocol copies are provided for documentation of all test results and calculations involved in analysis of the serum-CSF pairs in both *recomWell* Borrelia and *recomBlot* Borrelia.

Alternatively, an Excel program developed by **MIKROGEN** can be used for this purpose. This program is described in Chapter 4.

## 2. Screening of serum and liquor in *recomWell* Borrelia

Serum and liquor must be withdrawn from the patient at the same day. To avoid test-to-test variations, parallel testing of serum and liquor in the same run is recommended. To increase diagnostic safety, patient sera and liquors should be tested in duplicate.

### 2.1 Sample dilution

<b>IgG</b>	Serum 1:101	In accordance to the instructions for use, the <b>control sera</b> from the kit (positive control, cutoff control, negative control) and the <b>patient sera</b> are diluted 1:101 with dilution buffer. 100 µl of each of these dilutions are pipetted per well.
<b>IgG</b>	Liquor 1:4	25 µl liquor + 75 µl dilution buffer  75 µl of dilution buffer are first pipetted into the well, followed by 25 µl of liquor and the solution is then mixed well.

<b>IgM</b>	Serum 1:101	In accordance with the instructions for use, the <b>control sera</b> from the kit (positive control, cutoff control, negative control) and the <b>patient sera</b> are diluted 1:101 with dilution buffer. 100 µl of each of these dilutions are pipetted per well.
<b>IgM</b>	Liquor undiluted 1:1	100 µl of the undiluted liquor are directly pipetted into a well.

The further test procedure is performed according to the instructions for use, *recomWell* Borrelia IgG/IgM.

Please note, that the CSF should not be diluted in the IgM test.

### 2.2 Evaluation

The test is valid if the criteria for the control sera are fulfilled according to the instructions for use, *recomWell* Borrelia IgG/IgM.

With respect to the qualitative evaluation the marginal values for sera and CSF in IgG and IgM are calculated as following:

<b>IgG / IgM</b>	marginal value for serum: OD cutoff control
<b>IgG / IgM</b>	marginal value for liquor: 0,5 x OD cutoff control

### 2.3 Test interpretation

#### IgG determination:

Result of <i>recomWell Borrelia IgG</i>	Evaluation and further action
<p>OD<sub>serum</sub> 1:101 &lt; marginal value for serum</p> <p>and</p> <p>OD<sub>liquor</sub> 1:4 &lt; marginal value for liquor</p>	<p>No detection of Bb-spec. IgG antibodies in serum</p> <p>No detection of Bb-spec. IgG antibodies in liquor</p> <p>No indication of neuroborreliosis</p> <p><b>No determination of antibody index AI (IgG)</b></p>
<p>OD<sub>serum</sub> 1:101 ≥ marginal value for serum</p> <p>and</p> <p>OD<sub>liquor</sub> 1:4 &lt; marginal value for liquor</p>	<p>Detection of Bb-spec. IgG antibodies in serum</p> <p>No detection of Bb-spec. IgG antibodies in liquor</p> <p>Indication of an advanced or past Borrelia infection</p> <p>No indication of neuroborreliosis</p> <p><b>No determination of antibody index AI (IgG)</b></p>
<p>OD<sub>serum</sub> 1:101 &lt; marginal value for serum</p> <p>and</p> <p>OD<sub>liquor</sub> 1:4 ≥ marginal value for liquor</p>	<p>No detection of Bb-spec. IgG antibodies in serum</p> <p>Detection of Bb-spec. IgG antibodies in liquor</p> <p>Neuroborreliosis is possible</p> <p><b>Determination of antibody index AI (IgG)</b></p>
<p>OD<sub>serum</sub> 1:101 ≥ marginal value for serum</p> <p>and</p> <p>OD<sub>liquor</sub> 1:4 ≥ marginal value for liquor</p>	<p>Detection of Bb-spec. IgG antibodies in serum</p> <p>Detection of Bb-spec. IgG antibodies in liquor</p> <p>Neuroborreliosis is possible</p> <p><b>Determination of antibody index AI (IgG)</b></p>

**IgM determination:**

<b>Result of <i>recomWell</i> Borrelia IgM</b>	<b>Evaluation an further action</b>
OD <sub>serum</sub> 1:101 < marginal value for serum and OD <sub>liquor</sub> 1:4 < marginal value for liquor	No detection of Bb-spec. IgM antibodies in serum No detection of Bb-spec. IgM antibodies in liquor No indication of neuroborreliosis <b>No determination of antibody index AI (IgM)</b>
OD <sub>serum</sub> 1:101 ≥ marginal value for serum and OD <sub>liquor</sub> 1:4 < marginal value for liquor	Detection of Bb-spec. IgM antibodies in serum No detection of Bb-spec. IgM antibodies in liquor Indication of a fresh Borrelia infection No indication of neuroborreliosis <b>No determination of antibody index AI (IgM)</b>
OD <sub>serum</sub> 1:101 < marginal value for serum and OD <sub>liquor</sub> 1:4 ≥ marginal value for liquor	No detection of Bb-spec. IgM antibodies in serum Detection of Bb-spec. IgM antibodies in liquor Neuroborreliosis is possible <b>Determination of antibody index AI (IgM)</b>
OD <sub>serum</sub> 1:101 ≥ marginal value for serum and OD <sub>liquor</sub> 1:4 ≥ marginal value for liquor	Detection of Bb-spec. IgM antibodies in serum Detection of Bb-spec. IgM antibodies in liquor Neuroborreliosis is possible <b>Determination of antibody index AI (IgM)</b>

**The test can be terminated if serum and liquor give negative IgG and IgM results in screening. The Borrelia-specific antibody index cannot be determined.**

**In case the liquor OD exceeds the marginal value, the procedure continues. In the following, IgG and IgM tests will be described in parallel. However, the antibody index is to be determined only for the immunoglobulin class in which a positive screening result for the liquor has been obtained.**

## 2.4 Determination of Borrelia-specific antibody concentrations in serum and liquor

For the conversion of the ODs of liquor and serum into units, extinction values between 0.100 and 2.000 are required (linear part of a dilution curve).

If the liquor is positive and the value for the serum < 0.100, an OD of 0.100 is assumed in the unit calculation for the serum.

If the OD value for the serum or liquor is larger than 2.000 or even outside the measurable range, the serum-liquor pair must be retested in **one** run after appropriately higher dilution. The following dilutions are recommended:

### *recomWell* Borrelia IgG

OD	Serum dilution	Liquor dilution
OD ≤ 2,000	1:101	1:4
2,000 < OD ≤ 3,000	1:500	1:20
OD > 3,000	1:1000, 1:2000, 1:4000 geometric dilution	1:40, 1:80, 1:160 geometric dilution

### *recomWell* Borrelia IgM

OD	Serum dilution	Liquor dilution
OD ≤ 2,000	1:101	1:1 undiluted
2,000 < OD ≤ 3,000	1:500	1:5
OD > 3,000	1:1000, 1:2000, 1:4000 geometric dilution	1:10, 1:20, 1:40 geometric dilution

The geometric dilution of the liquor in both IgG and IgM can be carried out directly in the well on the microtiter plate.

In practice, the one-point quantification method via the cutoff control has proved useful in *recomWell* Borrelia. In one-point quantification, the ODs of serum and liquor obtained in the evaluable range between 0.100 and 2.000 are converted into units using Formula [1]. The formula is valid for IgG and IgM. After the geometric dilution of serum or liquor, if several values are in this range, the value with the OD closest to 1.000 is selected (highest precision with respect to dilution errors and linearity of the standard curve).

**As the cutoff in this formula the value of the cutoff control (cutoff for serum) of the corresponding IgG or IgM determination is to be used for serum and for liquor. The marginal value for liquor from the qualitative evaluation is not valid here.**

All calculations following can also be performance by the use of the Excel program; (see chapter 4)

$$[1] \quad \text{U/ml serum or liquor} = \frac{\text{OD}_{\text{Serum resp. Liquor}} \times 20 \times \text{dilution factor}^{1)}}{\text{Cutoff}^{2)} \times 101^{3)}}$$

<sup>1)</sup> Dilution factor = reciprocal value of dilution, e.g., dilution factor 500 corresponds to a dilution of 1:500

<sup>2)</sup> For the cutoff the value of the respective cutoff control has to be used for **serum** and for **liquor**.

<sup>3)</sup> Standard dilution in *recomWell* Borrelia IgG/IgM

This gives the Borrelia-specific IgG and IgM concentration in the serum and liquor in units/ml.

Measurements compared with a standard curve show only slight differences which are not meaningful. If quantification via a standard curve is still required, a geometric dilution series of the positive control is recommended. This requires at least 4 values: 1:100, 1:200, 1:400 and 1:800. The units 100, 50, 25 and 12.5 are arbitrarily assigned to these dilutions and the units of the patient samples are read off on the corresponding curve. The respective dilutions of serum and liquor have to be considered.

Thus, the Borrelia-specific IgG quotient  $Q_{\text{spec}}(\text{IgG})$  and the Borrelia-specific IgM quotient  $Q_{\text{spec}}(\text{IgM})$  are calculated according to Formulas [2] and [3]:

$$[2] \quad \mathbf{Q_{\text{spec}}(\text{IgG})} = \text{Bb-spec. IgG liquor (U/ml)} / \text{Bb-spec. IgG serum (U/ml)}$$

$$[3] \quad \mathbf{Q_{\text{spec}}(\text{IgM})} = \text{Bb-spec. IgM liquor (U/ml)} / \text{Bb-spec. IgM serum (U/ml)}$$

## 2.5 Calculation of $Q_{\text{IgG}}$ , $Q_{\text{IgM}}$ , $Q_{\text{Alb}}$ , $Q_{\text{Lim}}(\text{IgG})$ and $Q_{\text{Lim}}(\text{IgM})$

Total IgG, total IgM and albumin in both serum and liquor are determined by using a suitable method.

From the values obtained, the IgG quotient  $Q_{\text{IgG}}$ , the IgM quotient  $Q_{\text{IgM}}$  and the albumin quotient  $Q_{\text{Alb}}$  are calculated according to Formulas [4], [5], and [6].

$$[4] \quad \mathbf{Q_{\text{IgG}}} = \text{IgG-conc. liquor} / \text{IgG-conc. serum}$$

$$[5] \quad \mathbf{Q_{\text{IgM}}} = \text{IgM-conc. liquor} / \text{IgM-conc. serum}$$

$$[6] \quad \mathbf{Q_{\text{Alb}}} = \text{albumin-conc. liquor} / \text{albumin-conc. serum}$$

From  $Q_{\text{Alb}}$ , the limiting values  $Q_{\text{Lim}}(\text{IgG})$  and  $Q_{\text{Lim}}(\text{IgM})$  are calculated according to Formulas [7] and [8].

$$[7] \quad \mathbf{Q_{\text{Lim}}(\text{IgG})} = 0,93 \times \sqrt{Q_{\text{Alb}}^2 + (6 \times 10^{-6})} - 1,7 \times 10^{-3}$$

$$[8] \quad \mathbf{Q_{\text{Lim}}(\text{IgM})} = 0,67 \times \sqrt{Q_{\text{Alb}}^2 + (120 \times 10^{-6})} - 7,1 \times 10^{-3}$$

## 2.6 Calculation of the *Borrelia*-specific IgG antibody index AI (IgG) and the *Borrelia*-specific IgM antibody index AI (IgM)

The values obtained for QIgG and QLim (IgG) are compared and AI (IgG) is calculated using formula [9] or [10].

Correspondingly, QIgM and QLim (IgM) are compared and AI (IgM) is calculated using formula [11] or [12].

if QIgG < QLim (IgG) [9] <b>AI (IgG) = Qspec (IgG) / QIgG</b>	if QIgM < QLim (IgM) [11] <b>AI (IgM) = Qspec (IgM) / QIgM</b>
if QIgG > QLim (IgG) [10] <b>AI (IgG) = Qspec (IgG) / QLim (IgG)</b>	if QIgM > QLim (IgM) [12] <b>AI (IgM) = Qspec (IgM) / QLim (IgM)</b>

## 2.7 Interpretation of the antibody index values

<b>AI (IgG) ≤ 1,3</b>	normal	<b>AI (IgM) ≤ 1,3</b>	normal
<b>1,3 &lt; AI (IgG) &lt; 1,5</b>	borderline	<b>1,3 &lt; AI (IgM) &lt; 1,5</b>	borderline
<b>AI (IgG) ≥ 1,5</b>	pathological	<b>AI (IgM) ≥ 1,5</b>	pathological

Antibody index values ≤ 1.3 are in the normal range. An intrathecal synthesis of antibodies against *Borrelia* is unlikely. The detected *Borrelia*-antibodies in the liquor originate from the serum and have passively passed into the liquor space through the blood-liquor barrier.

Antibody index values between 1.3 and 1.5 are borderline cases. The serum-liquor pair should be retested. Or if a clinical doubt exists, it is recommended to test another serum-liquor pair in the course of the infection.

Antibody index values ≥ 1.5 are to be classified as pathological. They indicate intrathecal synthesis of *Borrelia*-antibodies as found in patients suffering from neuroborreliosis.

### 3. Confirmation of positive serum-liquor pairs with *recomBlot* Borrelia IgG/IgM

For serum-liquor pairs with a pathological elevated antibody index in the screening test, the confirmation with *recomBlot* Borrelia IgG/IgM is recommended.

Serum and liquor are diluted to same total IgG and total IgM concentrations, respectively. Thus, possibly present Borrelia-specific bands can be compared qualitatively.

For an intrathecal antibody synthesis in the liquor of the patient with an elevated IgG quotient (QIgG) and IgM quotient (QIgM), respectively, compared with the albumin quotient QAlb, the dilution is calculated according to the limiting value QLim (IgG) and QLim (IgM), respectively. Besides, the serum is tested in the standard dilution compared to the weak positive control in accordance with the instructions for use, if it has not been tested before during the normal routine.

#### 3.1 Calculation of QIgG, QIgM, QAlb, QLim (IgG) and QLim (IgM)

If not determined yet for the calculation of the Borrelia-specific antibody index in the screening test, see Chapter 2.5.

#### 3.2 Test procedure

If not performed before, the serum is tested in both IgG and IgM in standard dilution compared to the weak positive control in accordance with the instructions for use, *recomBlot* Borrelia IgG/IgM.

If the antibody index is pathological in the screening test in IgG and/or IgM, serum and liquor will be tested in *recomBlot* in IgG and/or IgM in the same immunoglobulin concentration.

Procedure:

- The liquor is tested as concentrated as possible, dependant on the material available:
  - minimum 1:2 and maximum 1:20 in IgG
  - minimum 1:2 and maximum 1:10 in IgM
- If the liquor is tested in both IgG and IgM, the amount of liquor will be divided up. It is not allowed to incubate the strips for IgG and IgM in one well of the incubation tray
- Due to the limited amount of the CSF, the incubation volume for CSF is only **1 ml** (instead of 2 ml) during the first incubation, for serum it is 2 ml.
- To calculate the serum dilution in IgG and IgM, respectively, the liquor dilution is divided by the IgG quotient QIgG or the limiting value QLim (IgG) and the IgM quotient QIgM or the limiting value QLim (IgM), respectively. The respective values calculated above will be compared and the lower one will be used in the formula below.

- Sometimes the serum amounts to pipet are smaller than 10 µl. In these cases it is recommended to make a 1:10 or even 1:100 predilution of the serum in wash and dilution buffer.
- The first incubation step is always over night.
- The further test procedure after the first incubation step is performed according to the instructions for use, *recomBlot Borrelia IgG/IgM*. For the second and third incubation (conjugate and substrate incubation) the incubation volume is 2 ml again for both serum and liquor.
- The dilutions and volumes to pipet for serum and liquor are calculated in the following scheme: (alternatively the dilutions can be calculated by the Excel program, see chapter 4)

**IgG**

<b>Test of the serum in standard dilution compared to the weak positive control in IgG</b>			
1 + 200	→	10 µl serum + 2000 µl wash and dilution buffer	→ Incubation over night
or			
1 + 66	→	30 µl serum + 2000 µl wash and dilution buffer	→ Incubation 2 hours

<b>Test of liquor and serum in the same IgG concentration</b>			
Dilution liquor in IgG			
minimum 1 : 2 (DF 2) <sup>1)</sup>		1000 µl / <b>DF liquor IgG</b> = x µl liquor	
maximum 1 : 20 (DF 20)		x µl liquor ad 1000 µl wash and dilution buffer	→ Incubation over night
Dilution serum in IgG			
if Q <sub>IgG</sub> < Q <sub>Lim</sub> (IgG)	→	<b>DF serum IgG</b> = DF liquor IgG / Q <sub>IgG</sub>	
or			
if Q <sub>IgG</sub> > Q <sub>Lim</sub> (IgG)	→	<b>DF serum IgG</b> = DF liquor IgG / Q <sub>Lim</sub> (IgG)	
1 : DF serum IgG		2000 µl / <b>DF serum IgG</b> = x µl serum	
		x µl serum ad 2000 µl wash and dilution buffer	→ Incubation over night

<sup>1)</sup> DF = dilution factor = reciprocal value of dilution, e.g., dilution factor 10 corresponds to a dilution of 1:10

**IgM**

Test of the serum in standard dilution compared to the weak positive control in IgM			
1 + 100	→	20 µl serum + 2000 µl wash and dilution buffer	→ Incubation over night
or			
1 + 33	→	60 µl serum + 2000 µl wash and dilution buffer	→ Incubation 2 hours

Test of liquor and serum in the same IgM concentration			
Dilution liquor in IgM			
minimum 1 : 2 (DF 2)		1000 µl / <b>DF liquor IgM</b> = x µl Liquor	
maximum 1 : 10 (DF 10)		x µl liquor ad 1000 µl wash and dilution buffer	→ Incubation over night
Dilution serum in IgM			
if $Q_{IgM} < Q_{Lim} (IgM)$	→	<b>DF serum IgM</b> = DF liquor IgM / $Q_{IgM}$	
or			
if $Q_{IgM} > Q_{Lim} (IgM)$	→	<b>DF serum IgM</b> = DF liquor IgM / $Q_{Lim} (IgM)$	
1 : DF serum IgM		2000 µl / <b>DF serum IgM</b> = x µl serum	
		x µl serum ad 2000 µl wash and dilution buffer	→ Incubation over night

**3.3 Evaluation of the test**

The evaluation of the serum in standard dilution is performed according to the point evaluation in the instructions for use, *recomBlot* Borrelia IgG/IgM.

The respective bands and their intensity are identified on the strips of serum and liquor, tested in the same immunoglobulin concentration in IgG and/or IgM. It is not allowed to use the weak positive control and the point evaluation because this method is only valid for standard dilutions.

### 3.4 Test interpretation

Evaluation of the bands	Interpretation
No bands in liquor present.	No <i>Borrelia</i> -specific antibodies detectable in the liquor space.
Bands in liquor present, intensity of the bands equal or weaker than the bands in serum.	The detected <i>Borrelia</i> -specific antibodies have passively passed from the serum into the liquor space (blood brain barrier failure)
Bands in liquor present, intensity of the bands stronger than bands in serum. The pattern of the bands may be different in liquor.	The intrathecal synthesis of <i>Borrelia</i> -specific antibodies is likely. A differing pattern of bands in liquor is highly significant.  Isolated p41 may be unspecific. However, intrathecal synthesis can not be excluded.

#### 4. Description of the Excel program

Into the first window the patient data, the values of the Clinical Chemistry and the results of the *recomWell* are entered.

Regarding the values from Clinical Chemistry the respective units have to be selected (g/l; mg/l; g/dl; mg/dl)

The results of the *recomWell* may be entered with the corresponding dilution factors. The default value for the serum dilution is 1:101, for the liquor dilution in IgG 1:4, respectively in IgM 1:1. The extinction of the cutoff control is taken over by the program for the liquor calculation as well as serum and liquor are to be examined in the same.

From the values of clinical chemistry and the data of the *recomWell* the program automatically calculates the quotients and the antibody indices (AI).

MIKROGEN									
Liquor diagnostics with <i>recomWell</i> Borrelia IgG & IgM									
Version 7.0									
Calculation of blot dilution			Setting back all values			Preview		Print	
<b>Probe</b>									
Patient	Friedrich Wilhelm Mustermann				Date of birth	12.12.12			
Sender	Dr. N. Borrel				Sample collection	11.11.03			
<b>Clinical chemistry</b>									
	Albumine	IgG	IgM						
Serum	4300,00	1180,00	116,00	mg/dl					
Liquor	401,00	85,50	6,30	mg/l					Concentration ratio 1: 10
<b>Specific antibodies</b>									
	Extinction		Dilution factor		Cut-off		U/ml		
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	
Serum	0,168	0,726	101	500	0,353	0,295	9,52	243,67	
Liquor	0,067	0,675	4	10	0,353	0,295	0,15	4,53	
<b>Results</b>									
Quotients	QAib	9,33 /1000							
	QIgG	7,25 /1000							
	QIgM	5,43 /1000							
	QLim (IgG)	7,27 /1000							
	QLim (IgM)	2,54 /1000							
	QSpec. (IgG)	15,79 /1000							
	QSpec. (IgM)	18,60 /1000							
Antibody index	AI (IgG)	no determination of AI (IgG)							
	AI (IgM)	7,32			>= 1,5 pathological findings!				

By pressing the button „Calculation of blot dilution“ a second calculation sheet will be opened where the available amount of liquor can be entered. In addition the dilution of the liquor may be changed as desired.

On the basis of the quotients from the first calculation sheet the program calculates the serum dilution for the *recomBlot* Borrelia, that corresponds to the respective total immunoglobulin concentration of IgG or IgM in liquor.

MIKROGEN	
<b>Serum and liquor dilution for recomBlot Borrelia IgG/IgM</b> <span style="float: right;">Version 7.0</span>	
<a href="#">Back to calculation of antibody</a>	<a href="#">Preview</a> <a href="#">Print</a>
<b>Sample</b> (data are taken from the form "Antibody index calculation")	
<b>Patient</b> Friedrich Wilhelm Mustermann	<b>Date of birth</b> 12.12.12
<b>Sender</b> Dr. N. Borrel	<b>Sample collection</b> 11.11.03
<b>Liquor dilution</b>	
How much liquor is available?	<input type="text" value="1000"/> µl
How should the liquor be diluted?	
<b>IgG</b> <input type="text" value="1:4"/>	= 250 µl Liquor + 750 µl dilution buffer
<b>IgM</b> <input type="text" value="1:4"/>	= 250 µl Liquor + 750 µl dilution buffer
<b>Serum dilution</b>	
<b>For a dilution according to the liquor Ig-concentration serum has to be diluted as the following:</b>	
Basis of the calculation are QlgG and QLim (IgG) or QlgM und QLim (IgM) from the antibody index calculation	
<b>IgG</b>	
<b>without predilution</b>	1:552 = 3,6 µl Serum + 1996,4 µl dilution buffer
<b>alternatively, serum 1:10 prediluted</b> <small>(100 µl serum + 900 µl dilution buffer)</small>	1:55 = 36,2 µl serum dilution + 1963,8 µl dilution buffer
<b>alternatively, serum 1:100 prediluted</b> <small>(10 µl Serum + 990 µl dilution buffer)</small>	1:6 = 362,3 µl serum dilution + 1637,7 µl dilution buffer
<b>IgM</b>	
<b>without predilution</b>	1:1576 = 1,3 µl Serum + 1998,7 µl dilution buffer
<b>alternatively, serum 1:10 prediluted</b> <small>(100 µl serum + 900 µl dilution buffer)</small>	1:158 = 12,7 µl serum dilution + 1987,3 µl dilution buffer
<b>alternatively, serum 1:100 prediluted</b> <small>(10 µl Serum + 990 µl dilution buffer)</small>	1:16 = 126,9 µl serum dilution + 1873,1 µl dilution buffer

## 5. Literature

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We will be pleased to send you further literature on liquor serum diagnosis

