Sources of error in the determination and calculation of the antibody index (AI)

Q_{spez} ? Q_{IgG}

? Q_{IgG} 80 % 60 40 20 ?

Q_{ges}

Q_{IgM}

?

Q_{Alb}

AI

? Q_{Alb} Q_{Lim}

?
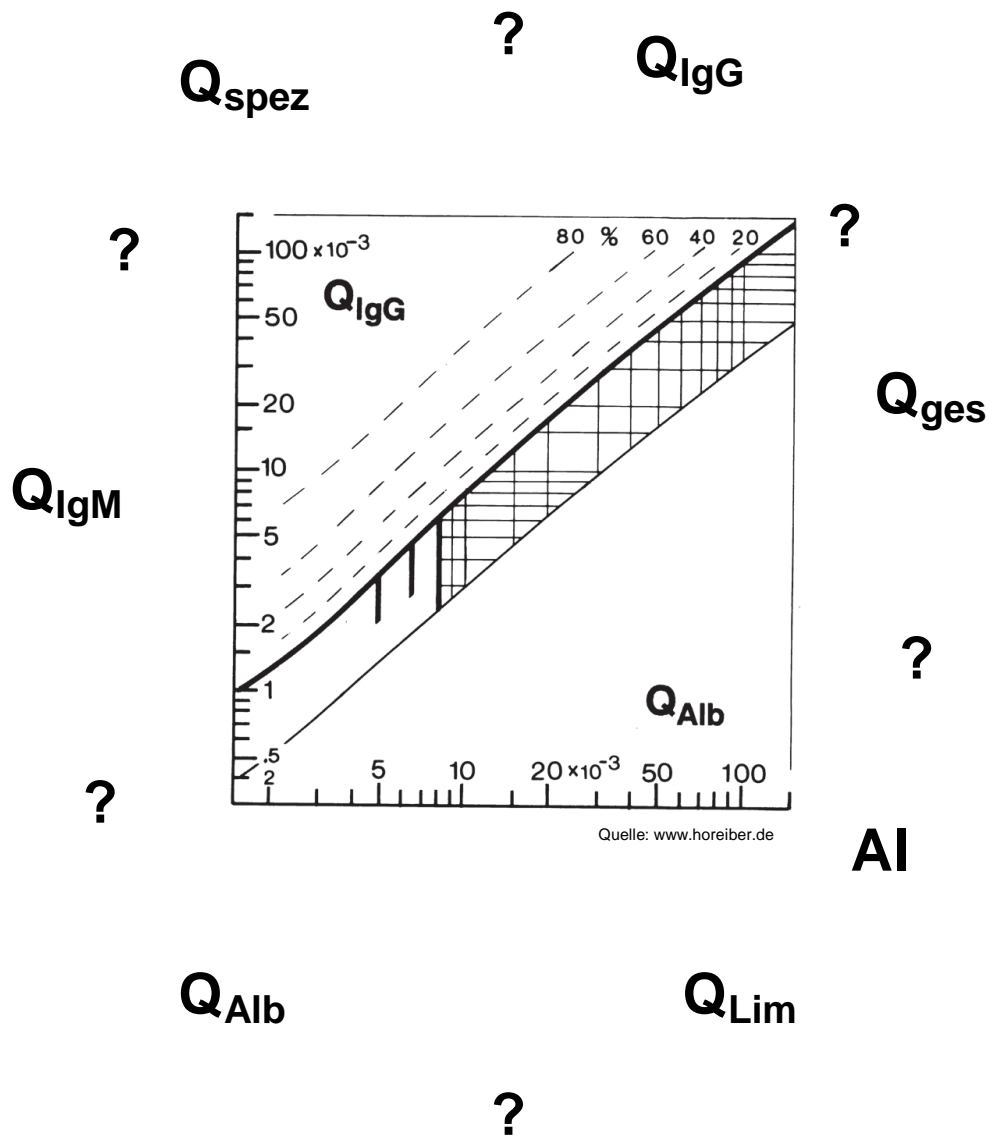
1. General sources of error:

- ▼ **Do not** use blood-containing cerebrospinal fluid (erythrocyte count >8000 Mpt/l), (simulating positive immunoglobulin syntheses)
- ▼ Use all reagents at **room temperature**
- ▼ Analyze cerebrospinal fluid/serum pairs (promptly taken) in **one** test, measure in the cerebrospinal fluid range
- ▼ Do not use a sample volume **below 5µl** → dilution error!
- ▼ For controls/standards and Li/Se pairs, a **double approach** is recommended
- ▼ **AI controls** should be carried as long as available and check results with QC **certificate**
- ▼ Use **controls** and **standards** only for **specific batches**
- ▼ Pre-adsorption with RF SorboTech in IgM recommended
- ▼ **Cut off values** for the calculation of AI are **useless** for liquor diagnostics (blood-liquor barrier function is not taken into account)

2. Sources of errors in immunoglobulin determination

- ▼ Methodological limits (knowing/considering detection limits / dilution levels of the analytical instruments)
- ▼ Possible IgG infusion
- ▼ Measurement of false low immunoglobulin levels

3. Sources of error in the dilution of liquor and serum samples

- ▼ **Never** use liquor **undiluted** to avoid matrix effects
- ▼ Use **standard dilution:**

  Liquor: 1:2

  Serum: 1:1000 (IgM); 1:1000, 1:1400 (IgG/IgM)
- ▼ Check OD values / wME for **dilution authenticity** (the AI value should remain approximately the same for different liquor/serum dilutions; **(example 1)**)
- ▼ **OD values** of liquor and serum must be almost identical **(example 2)**
- ▼ **Use OD values between the second and third standard** (do not use OD values in the asymptotic range of the standard curve; **(example 3)**

▼ Exclude **antigen saturation** (taking into account the Heidelberg curve) (If the OD value of the 1:4000 dilution is not significantly lower, or even higher than the 1:1000 dilution, continue to dilute) **(example 4)**

▼ **Geometric dilution series**, if necessary use intermediate stages

▼ No use of OD values below the **lowest or above the highest** standard → no curve linearity

4. Practical information on liquor and serum dilution:

▼ If intrathecal synthesis (quotient diagram) is known, apply several liquor dilutions

▼ If there is little cerebrospinal fluid (e. g. in children) apply several serum dilutions so that a suitable pair of Li/Se can be found.

5. Sources of errors when calculating the AI:

▼ To calculate the AI **do not use the OD values**, conversion to wME is required!

▼ If $Q_{IgX} > Q_{IgXLim}$ (intrathecal Ig synthesis) must be used to calculate the AI of Q_{IgXLim} **(example 5)**

Examples from routine liquor diagnostics

Example 1: Test for dilution authenticity

Table 1: CSF / serum pairs must produce approximately the same AI at different dilutions.

	Dilution	OD	wME	$Q_{spec} \times 10^{-3}$	AI
CSF	1:2	1.61	82.6	29.3	6.8
Serum	1:100	1.5	2812	29.3	
CSF	1:30	0.39	78.6	27	6.6
Serum	1:1000	0.429	2905	27	

Example 2: Liquor and serum OD values far apart

Table 3: The OD values marked in red are too far apart, which is why a false negative AI is calculated. The OD values marked in green are close to each other, which is why a correct AI is calculated (Q_{IgG} = 2.9×10^{-3}).

	Dilution	OD	wME	$Q_{spec} \times 10^{-3}$	AI
CSF	1:2	0.61	17.75	2.72	0.93
Serum	1:1000	1.7	6519		
CSF	1:2	0.61	17.75	4.78	1.65
Serum	1:400	0.92	3711		

Example 3: Dependency of the AI on the standard curve

Table 3: Liquor and serum OD values in the asymptotic range of the standard curve
If OD values for CSF and serum, which lie in the asymptotic range of the standard curve, are used for the AI calculation, this leads to a false positive or false negative AI.

Standards OD		Standard curve HSV-1			
			Dilution	OD	wME
1 hour	0.286	Liquor	1:2	1.24	66.7
2 hours	0.804				
3 hours	1.197	Serum	1:4000	1.09	5194
4 hours	1.27	$Q_{spec} = 12.8 \times 10^{-3}$		$Q_{IgG} = 3.65 \times 10^{-3}$	
		AI = 3.5			

Table 4: Liquor and serum OD values in the <u>linear range</u> of the standard curve. OD curve for liquor and serum are between second and third standard of the standard curve.

Standards		Standard curve HSV-1			
	OD		**Dilution**	**OD**	**wME**
1 hour	0.368	Liquor	1:2	**1.17**	57
2 hours	0.891				
3 hours	1.376	Serum	1:600	**1.26**	11714
4 hours	1.575	$Q_{spec} = 4.86 \times 10^{-3}$		$Q_{IgG} = 3.65 \times 10^{-3}$	
		AI = 1.3			

Example 4: Working with antigen saturation

Table 5: If further dilution of the samples does not lead to a decrease in OD values or even to an increase, antigen saturation is achieved. Continue to dilute until OD values decrease is achieved (marked in red)

	Dilution	OD	wME	$Q_{spec} \times 10^{-3}$	AI
Liquor	1:2	1.117	33.4	2.48	0.23
	1:10	1.392	255	18.98	1.76
	1:20	1.697	971	72.3	6.69
	1:40	1.416	1053	78.4	7.26
	1:1000	0.966	1106	82.3	**7.62**
Serum	**1:1000**	1.064	13430		

Example 5: Consideration of the QLim for calculating the AI

Table 6: If $Q_{IgX} > Q_{IgXLim}$, the AI of Q_{IgXLim} must be used for the calculation of the AI. Considering Q_{Lim} results in a positive AI (marked green)! If Q_{IgG} is used instead of Q_{Lim} to calculate the AI, the result is a false negative AI (marked red).

	Dilution	OD	wME	$Q_{spec} \times 10^{-3}$
Liquor	1:5	0.698	38	7.21
Serum	1:800	0.623	5335	

$Q_{IgG} = 6.57 \times 10^{-3}$ $Q_{Lim} = 2.25 \times 10^{-3}$

$$Q_{IgG} > Q_{Lim}$$

$AI = Q_{spec}/Q_{IgG}$ $AI = Q_{spec}/Q_{Lim}$
AI = 1.1   **AI = 3.2**

CSF diagnostics with VIROTECH Diagnostics ELISA and LINE products

Responsible diagnostics & practical processing

▼ AI determination and determination of serum AK per Ig class on a microtitre plate and in one run

▼ Economical processing by means of fixed standard curve or ready-for-use standards

▼ Uniform serum and cerebrospinal fluid dilution and incubation times

▼ AI controls

▼ All tests with CE marking

▼ Automated processing possible, evaluation software available

▼ All VIROTECH Borrelia LINE Immunoblots are validated for CSF diagnostics

VIROTECH CSF-palett:

▼ Borrelia + VlsE IgG
▼ CMV IgG
▼ FSME/TBE IgG/IgM
▼ HSV 1 (gG1) IgG
▼ Mumps IgG
▼ VZV IgG/IgM/IgA

▼ Borrelia IgM
▼ EBV IgG
▼ HSV Screen IgG
▼ HSV 2 (gG2) IgG
▼ Masern/Measles IgG/IgM
▼ Rubella IgG

VIROTECH Antibody Index Controls

The determination of the antibody index is used to detect a synthesis of pathogen-specific antibodies in the central nervous system. It is calculated from the ratio of the quotient of the concentrations of pathogen-specific antibodies in liquor and serum and the associated total immunoglobulin quotient of liquor and serum.

Advantages of Antibody Index Control

▼ Verification of accuracy and precision of AI determination

▼ Quality control in daily liquor routine diagnostics

▼ Ready-to-use cerebrospinal fluid / serum control pair for the normal range (AI < 1.5)

▼ Ready-to-use cerebrospinal fluid / serum control pair for the pathological range (AI < 1.5)

▼ Contains all the information required for calculation on the quality control certificate

QUALITÄTSKONTROLL-ZERTIFIKAT
QUALITY CONTROL CERTIFICATE
CERT

HSV Screen ELISA
IgG Liquor Antikörper Index Kontrollen

CSF AI   108   E

Kit		MTP Platte/ Plate	
REF EN108L65 LOT 00819-01 ⧗ 2012/07		REF EC108.01 LOT ≥ 123 ⧗ 2011/09	

	IgG			
	CSF AI (<1,5)		CSF AI (≥1,5)	
	CSF AI L1	CSF AI S1	CSF AI L2	CSF AI S2
LOT	00819-01	00819-01	00819-02	00819-02
REF	EN108L81	EN108S61	EN108L62	EN108S62
⧗	2012/07	2012/07	2012/07	2012/07
AI	**0,6 – 1,4**		**2,4 – 5,4**	
ZW	**1**		**3,6**	
IgG CONC T Gesamt Konzentration / total concentration	CSF: 25 mg/l SERUM: 8928 mg/l		CSF: 40 mg/l SERUM: 13790 mg/l	
IgG Q Quotient IgG-Gesamt / total IgG quotient	**Q = 2,8 x 10⁻³**		**Q = 2,9 x 10⁻³**	
ALB CONC T Gesamt Konzentration / total concentration	CSF: 210 mg/l SERUM: 41700 mg/l		CSF: 210 mg/l SERUM: 41700 mg/l	
ALB Q Quotient Albumin-Gesamt / total albumin quotient	**Q = 5,0 x 10⁻³**		**Q = 5,0 x 10⁻³**	

Available for:

▼ Borrelia + VlsE IgG ▼ EBV IgG ▼ FSME/TBE IgG ▼
▼ HSV Screen IgG ▼ Masern/Measles IgG ▼ Rubella IgG ▼ VZV IgG

LabCheck CSF Analysis

Produkt:
Testdatum:
Kit-Lotnummer:
Patienten-ID

Formular zurücksetzen

IgG

	Std 1 Lotnr.:		1,5wME	
	Std 2 Lotnr.:		6,25wME	Zertifikatangabe fehlt
	Std 3 Lotnr.:		25wME	
	Std 4 Lotnr.:		100wME	Zertifikatangabe fehlt
	AI normal Lotnr.:			
	AI pathologisch Lotnr.:			

OD Standards

Standard	Sollwert lt. Zertifik

	AI normal		AI pathologisch		Patient		Patient		Patient	
	Liquor	Serum	Liquor	Serum	Liquor	Serum	Liquor	Serum	Liquor	Serum
Test-OD										
Probenverd.	2	400	2	400	2	400	2	400	2	400
Konz. IgG [mg/L]							0,0	0,0	0,0	0,0
Konz. Alb. [mg/L]							0,0	0,0	0,0	0,0
OD-Werte	kein Wert	kein Wert	kein Wert	kein Wert	kein Wert	kein Wert	kein Wert	kein Wert	kein Wert	kein Wert
Li/Se Relation	kein Wert		kein Wert		kein Wert		kein Wert		kein Wert	
Gesamt-beurteilung	kein Wert		kein Wert		kein Wert		kein Wert		kein Wert	
wME										
wME x Verd.										
Q spez. IgG										
Q IgG										
Q Albumin										
Q Lim										
AK-Index	kein Index		kein Index		kein Index		kein Index		kein Index	
Diagnose	kein Befund		kein Befund		kein Befund		kein Befund		kein Befund	
Patienten VE										

▼ Easy input of OD values

▼ Calculation possible with measured or fixed standard curve

▼ Automatic certificate matching

▼ Automatic transfer of the IgX and Alb concentration of the patient for easy evaluation of several parameters or dilutions (1 patient per sheet) **or**

▼ Automatic transfer of standard values for the diagnosis of a parameter for several patients (1 parameter per sheet)

▼ Comparison of the measured OD values with the range of the standard curve

▼ Checking the relation of liquor OD to serum OD

▼ Automatic conversion to wME

▼ Automatic consideration of the Q_{Lim}

▼ Direct calculation of the AI with indication of the diagnosis

▼ Software with CE marking