

# Detection of Rubella-specific intrathecally produced IgG antibodies using Rubella-IgG-ELISA PKS medac

Klaus Zimmermann 1), E Linke 2), S Dettlaff 3), M Radtke 3), D Franke 3)

1) Laborpraxis im Ärztehaus Mickten, Dresden, Germany, 2) LFKH für Psychiatrie u. Neurologie, Stadtroda, Germany, 3) medac GmbH, Hamburg, Germany

## Background and Objective:

Investigation of cerebrospinal fluid (CSF) is an essential part of neurologic diagnosis. The detection of an intrathecal synthesis of pathogen-specific antibodies as part of this diagnosis provides relevant information regarding the cause of a neurological disorder, which can be an acute infection, an opportunistic infection or a chronic immune disease. In acute infection like Rubella encephalitis a monospecific immune response with intrathecally produced antibodies towards the causative agent will be detected. In chronic immune disease of the central nervous system like Multiple Sclerosis a polyspecific local antibody response is detected. In case of Multiple Sclerosis the detection of an intrathecal antibody synthesis against measles, rubella and/or zoster virus is considered as a diagnostic tool of high clinical specificity.

The aim of the study was to evaluate the Rubella-IgG-ELISA PKS medac regarding its suitability for CSF diagnosis using single point quantification (SPQ).

## Methods:

The Rubella-IgG-ELISA PKS medac is a CE-marked indirect ELISA, which is approved for the investigation of CSF. The assay uses purified virus coated to the microtitre plate. It is calibrated towards the WHO standard preparation. IU values are calculated by a SPQ method without the need to utilize a calibration curve with each test run. In parallel a calibration curve (CC) made by titration of a serum pool with 100 arbitrary units (AU) was used during this investigation to examine the SPQ. Sera are diluted 1:200. For the investigation of serum/CSF pairs the CSF is diluted 1:7 and a second serum dilution of 1:1500 is used to avoid frequent retesting of sera above the measuring range (100 IU). Sera  $\geq 5$  IU are considered positive while CSF with an activity compared to  $\geq 2$  IU can be used for antibody index (AI) calculation.

Total IgG determination: Total IgG in serum and CSF was determined using reagents and the nephelometers BN 100 and BN 2 resp. from Dade-Behring (Marburg, Germany).

Antibody index (AI) determination: AI calculation was performed according to Reiber (Lab. med. 19:444;1995) as described in the package insert using SPQ as well as (CC) for the calculations. Only AI values were used for the different comparisons.

Reference method: Samples were characterized regarding antibody index using an in-house method based on the ETI-Rubek-G (DiaSorin GmbH, Düsseldorf, Germany).

## Results:

The laboratory comparison resulted in 99 out of 110 (90%) reactive samples within the measuring range, which could be evaluated. From the remaining 11 samples 5 were below and 6 samples were above the measuring range of the assay regarding Rubella-specific IgG. Retesting of these samples was not possible. The overall agreement regarding the AI results calculated using SPQ and using CC respectively was high within each CSF laboratory ( $\geq 97\%$ ). Regression analysis showed a high correlation for the whole AI range (fig. 1). The agreement between both labs was lower (88%) but equal for SPQ and CC results. Only few deviations were real assessment differences (table II). These samples could not be repeated. The agreement with the AI results of the reference assay was between 77% and 81% comparable in both laboratories and for both quantification methods (table I). More than 90% of the differences were due to higher AI values with the reference assay.

From the 62 sample pairs that were investigated additionally 48 (77%) were initially assessable. With these samples we obtained 100% agreement between SPQ and CC (table III). From the remaining 14 samples 2 were above and 12 were below the measuring range. Retesting with modified dilutions yielded in 12/14 (86%) corresponding valid results. From the remaining 2 sample pairs the CSF activity was even at a 1:2 dilution below the measuring range.

**Table I: Evaluation of the laboratory comparison:**

Quantification	Intralaboratory Concordance <sup>a</sup>		Agreement	
	Lab 1	Lab 2	Interlaboratory concordance <sup>a</sup>	With reference assay <sup>b</sup>
			Lab 1	Lab 2
SPQ <sup>c</sup> vs. CC <sup>d</sup>	98%	97%		
SPQ			88%	77%
CC			88%	80%

a) N = 99/110 (90%; 5 below and 6 above the measuring range)

c) Single point quantification

b) N = 95/99 (96%)

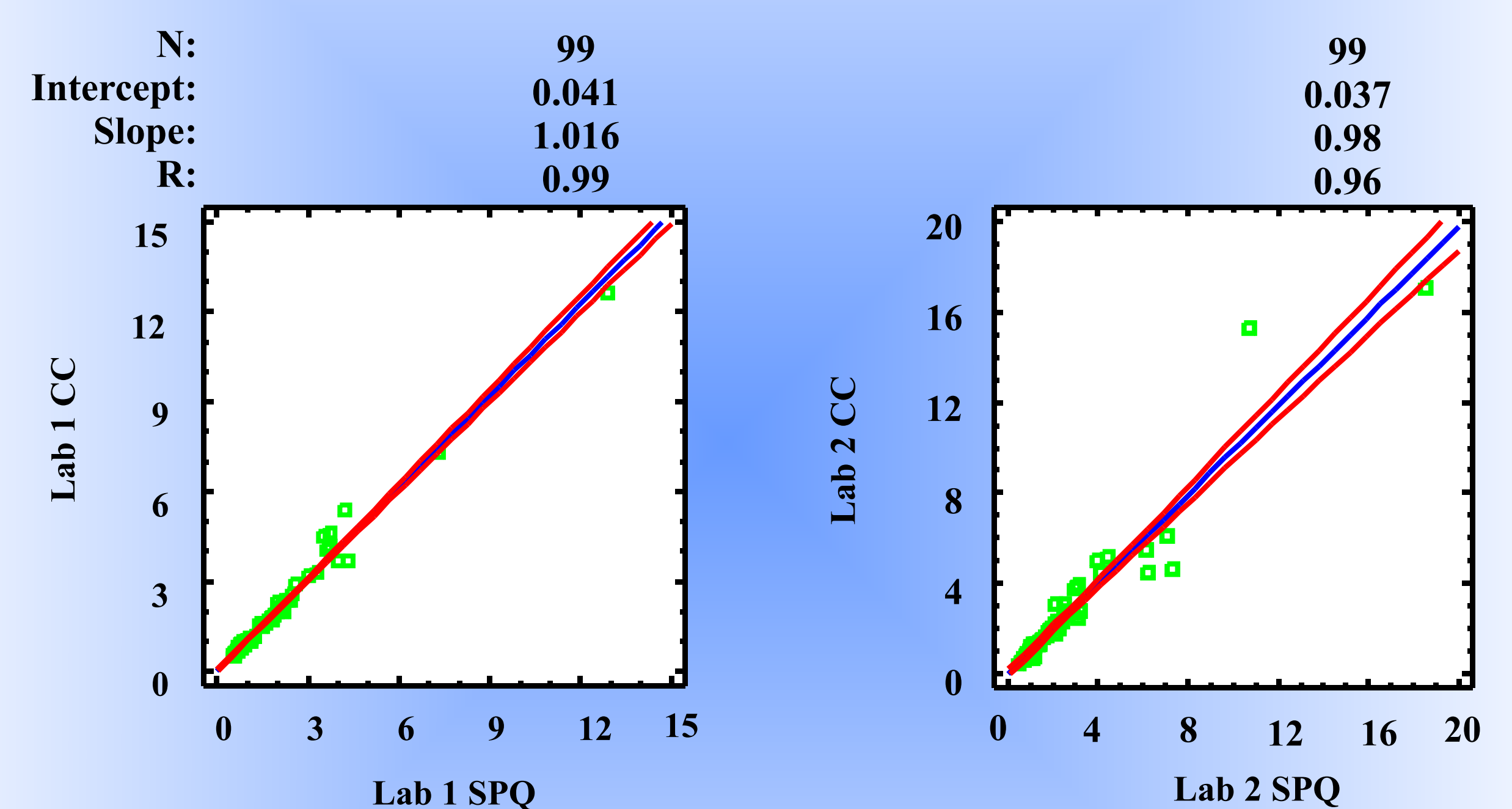
d) Calibration curve

**Table II: Laboratory comparison: Interlaboratory concordance using SPQ and CC**

		AI (CC) Lab 1			
		AI > 1.5 (+)	1.3 < AI ≤ 1.5 (±)	AI ≤ 1.3 (-)	Σ
Lab 2	AI > 1.5 (+)	31	1	1	33
	AI > 1.3 – 1.5 (±)	-	-	5	5
	AI ≤ 1.3 (-)	5	-	56	61
	Σ	36	1	62	99

		AI (SPQ) Lab 1			
		AI > 1.5 (+)	1.3 < AI ≤ 1.5 (±)	AI ≤ 1.3 (-)	Σ
Lab 2	AI > 1.5 (+)	29	3	1	33
	AI > 1.3 – 1.5 (±)	1	-	3	4
	AI ≤ 1.3 (-)	4	-	58	62
	Σ	34	3	62	99



**Figure 1: Regression Analysis for SPQ vs. CC**

**Table III: Rubella-IgG: Evaluation of 62<sup>a</sup> serum/CSF samples**

Quantification	Results			Agreement	
	Seronegative	Intrathecal antibody synthesis		Intralab. concordance <sup>b</sup>	With ref. assay <sup>c</sup>
		+	±		
SPQ	2	7	0	39	80%
CC		7	0	39	80%
SPQ vs. CC					100%

a) 12 CSF sample were initially below and 2 above the measuring range. After retesting with modified dilutions 12/14 (86%) revealed valid results

b) N = 46

c) N = 10 (after retesting the 2 samples outside the measuring range with modified dilutions the agreement was 10/12 (83%))

## Conclusion:

Our investigations demonstrate that the Rubella-IgG-ELISA PKS medac with its easy and material saving SPQ method is well suited for CSF diagnosis. Using a calibration curve with each test run showed no advantage towards the single point quantification. CSF samples from seropositive patients below the measuring range can be tested more concentrated. Samples above the measuring range can be diluted further. The assay provides reliable results in less than three hours.