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Quantitative Chlamydia Serology with Single-Point Quantitation

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Abstract

Background: Chlamydia serology is primarily performed using MIF and ELISA. Besides state-of-the-art diagnostic performance modern routine diagnostics demand a simple quantitative evaluation, economic use and suitability for automation. MIF does not fulfill all of these requirements. Quantitation improves the potential to compare the results of different assays. The semiquantitative C. pneumoniae- and C. trachomatis-IgG/IgA-ELISA medac were improved to fulfill all a.m. demands. For the new quantitative test generation C. pneumoniae- and C. trachomatis-IgG/IgA-ELISA *plus* medac a single-point quantitation (SPQ) was validated.

We investigated the agreement of the old and new assay versions. Data for C. pneumoniae-IgG/IgA-ELISA *plus* medac are presented, including a comparison to MIF titers.

Methods: Intra- and interassay variation were determined manually and using an automatic device (DSX, Dynex). Person-to-person variation, precision data and a panel of 303 (IgG)/263 (IgA) sera were calculated with both protocols. Moreover, SPQ was investigated concerning dilution linearity, limit of quantitation (LOQ) and its correlation to data obtained using a calibration curve with arbitrary units (AU). Finally, different MIF IgG titers of 100 sera were compared with AU values.

Results: Precision data for IgG and IgA generated with both evaluation methods were very similar. Coefficients of variation for positive or borderline samples were below 12 % for intra- and interassay variation (manually and on DSX). Person-to-person variation was below 11 %. The diagnostic results of the panel sera obtained with both evaluation methods showed a very good concordance. LOQ determination and linearity documented the validity of the measuring ranges. The quantitative results obtained using calibrators and SPQ resulted in correlation indices (r^2) of 98 % (IgG and IgA). However, a well-defined attribution of units to MIF titers was not possible. Similar results were obtained with both new C. trachomatis ELISA.

Conclusions: Our SPQ provides precise and reliable results without the need for a calibration curve in the test run. Therefore the preconditions for follow-up measurements are given. However, the comparison of results obtained in assays of different manufacturers is still difficult due to the lack of reference sera.

Introduction

Chlamydia pneumoniae (C. pneumoniae) and *Chlamydia trachomatis* (C. trachomatis) are each associated with acute as well as chronic diseases. For the diagnosis, especially of chronic disease, species-specific serology is frequently used. Microimmunofluorescence (MIF) is considered the method of choice for serology. But like with other serological methods variable results are obtained with different MIF assays from different laboratories. Furthermore MIF is increasingly replaced by enzyme immuno assays (EIA) because different from MIF EIA fulfills all requirements for routine diagnostics like automation of test run and evaluation of the results. But also variability of results obtained with different commercial EIA has been observed. A prerequisite for an improved standardization of Chlamydia serology is a reliable quantitation, which is suited for routine diagnostics. Furthermore, national or international immunoglobulin standard preparations would certainly improve the comparability of all results received with assays from different manufacturers.

To fulfill the above described demand quantitative assays (**Chlamydia trachomatis-IgG/IgA-ELISA *plus* medac** and **Chlamydia pneumoniae-IgG/IgA-ELISA *plus* medac**) were developed to supplement the existing species-specific chlamydia serology panel (Chlamydia pneumoniae-IgG/IgA-sELISA medac and Chlamydia trachomatis-IgG/IgA-pELISA medac). A single-point quantitation (SPQ), which does not require a calibration curve in the test run, was validated and the results were compared to the results of qualitative assays and MIF. Data obtained with the **Chlamydia pneumoniae-IgG/IgA-ELISA *plus* medac** are presented to show the performance of the SPQ.

Methods

SPQ: For each batch a specific calibration curve is determined using internal calibrators representing arbitrary units. By appropriate curve fitting the characteristic curve parameters are estimated. Furthermore lot-specific nominal values for positive control and the single kit calibrator, which is used to balance test variations, are determined. Using the curve parameters and the corrected sample OD arbitrary units (AU) can be easily calculated. In each testkit a data sheet is included containing the calibration curve and the lot-specific data.

A panel of 303 IgG and 264 IgA sera were evaluated using the qualitative and quantitative assays, respectively. Validation measurements included determination of intra- and interassay variation with manually and automatically performed test runs as well as person-to-person variation. Suitability for automation was performed using two different devices (DSX, Dynex Technologies; BEP III, Dade Behring). Moreover, SPQ was evaluated concerning dilution linearity, limit of quantitation (LOQ) and its correlation to data obtained using a calibration curve in each respective test run. Finally, MIF-predefined sera (175 IgG/60 IgA) were investigated with the quantitative assays. MIF results were obtained using an in-house method (Dep. Clin. Microbiol., Malmö Univ. Hosp., Malmö, Sweden).

Results

IgG-sELISA (OD/Cut-off)

Chlamydia pneumoniae-IgG ELISA *plus* [AU/ml]	-	±	+	:
-	**85**	**0**	**0**	85
±	**1**	**8**	**0**	9
+	**0**	**3**	**206**	209
:	86	11	206	303

Concordance = 98.7 %

IgA-sELISA (OD/Cut-off)

Chlamydia pneumoniae-IgA ELISA *plus* [AU/ml]	-	±	+	:
-	**119**	**0**	**0**	119
±	**7**	**20**	**0**	27
+	**0**	**5**	**113**	118
:	126	25	113	264

Concordance = 95.5 %

Fig. 1: Concordance of the results obtained with Chlamydia pneumoniae-IgG/A-ELISA *plus* medac and C. pneumoniae-IgG/IgA-sELISA medac.

Table 1: Precision data for Chlamydia pneumoniae-IgG/IgA-ELISA *plus* medac.

	IgG		IgA	
	CV[a]	N[b]	CV[a]	N[b]
Intra-assay variation (manually)[c]	10.2 %	22	7.1 %	22
Intra-assay variation (DSX)[d]	5.8 %	22	9.7 %	22
Interassay variation (manually)[e]	11.3 %	11	7.2 %	11
Interassay variation (DSX)[f]	9.4 %	11	9.9 %	11
Person-to-person variation[g]	10.8 %	3	8.3 %	3
	AU/ml	N[b]	AU/ml	N[b]
Limit of quantitation (LOQ)	5.9	3	3.3	3

[a] CV calculation based on AU/ml. Only the highest CV of reactive sera is shown;
[b] No. of determinations;
No. of samples: [c,d] IgG 3, IgA 4; [e] IgG 6, IgA 5; [f] IgG 5, IgA 5; [g] IgG 6, IgA 8

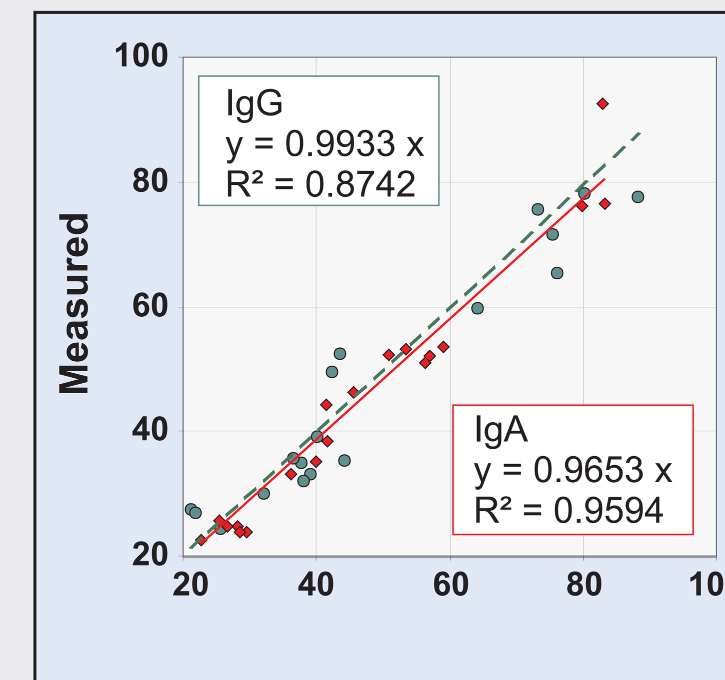

Fig. 3: Dilution linearity. 10 reactive sera for each test (IgG ● and IgA ◆) were titrated in 1:2 dilution steps. Only positive and borderline results within the measuring range were used for calculation.

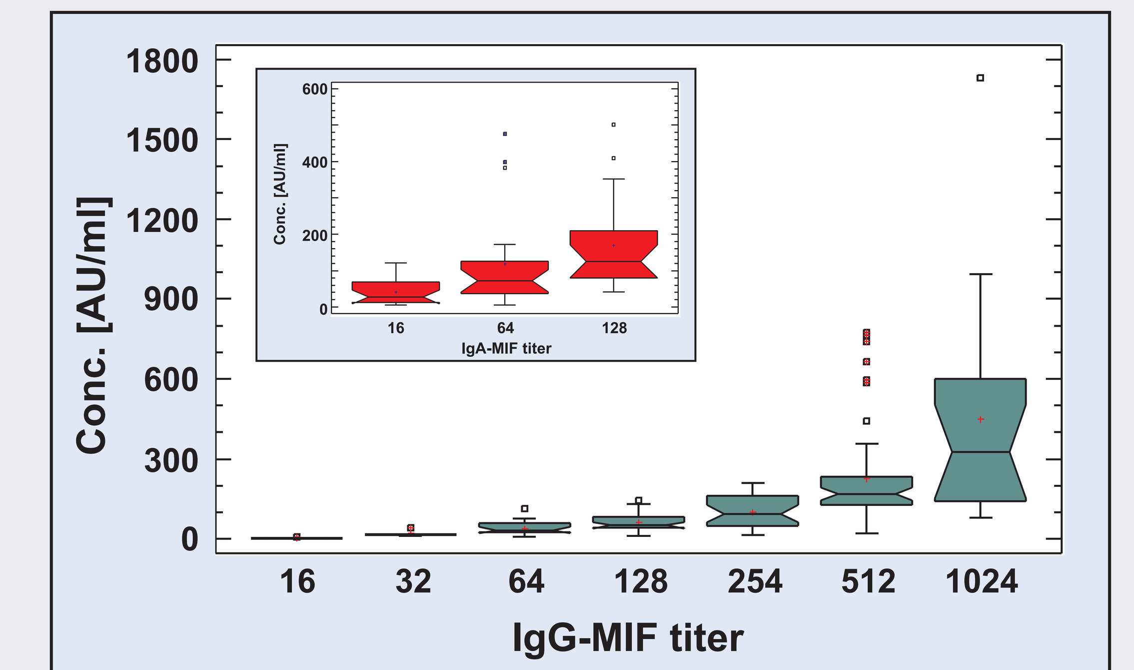

Fig. 5: Comparison of MIF titers with Chlamydia pneumoniae-IgG/A-ELISA *plus* medac results. 175 (IgG) and 60 (IgA) sera were investigated, respectively.

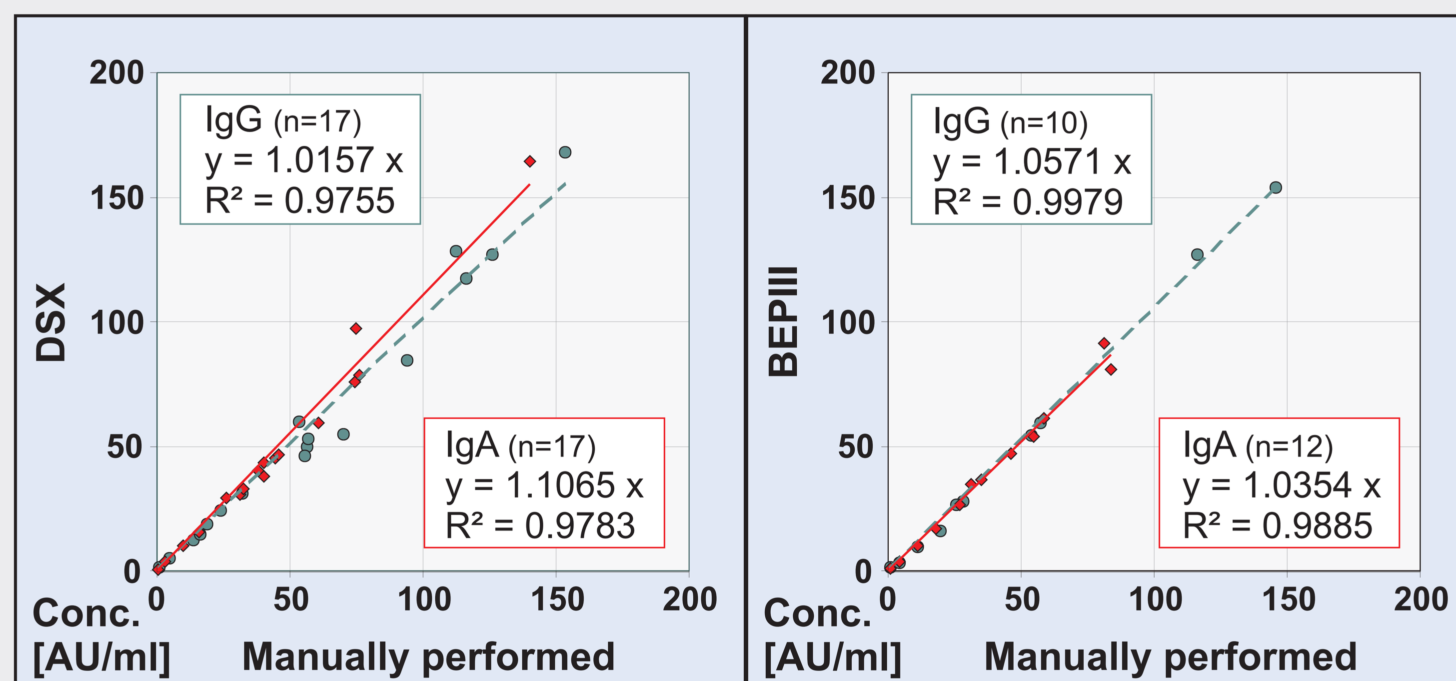

Fig. 2: Automation. Comparison of test results obtained through manually performed test runs with data received from parallel automatically performed test runs. IgG (●) and IgA (◆).

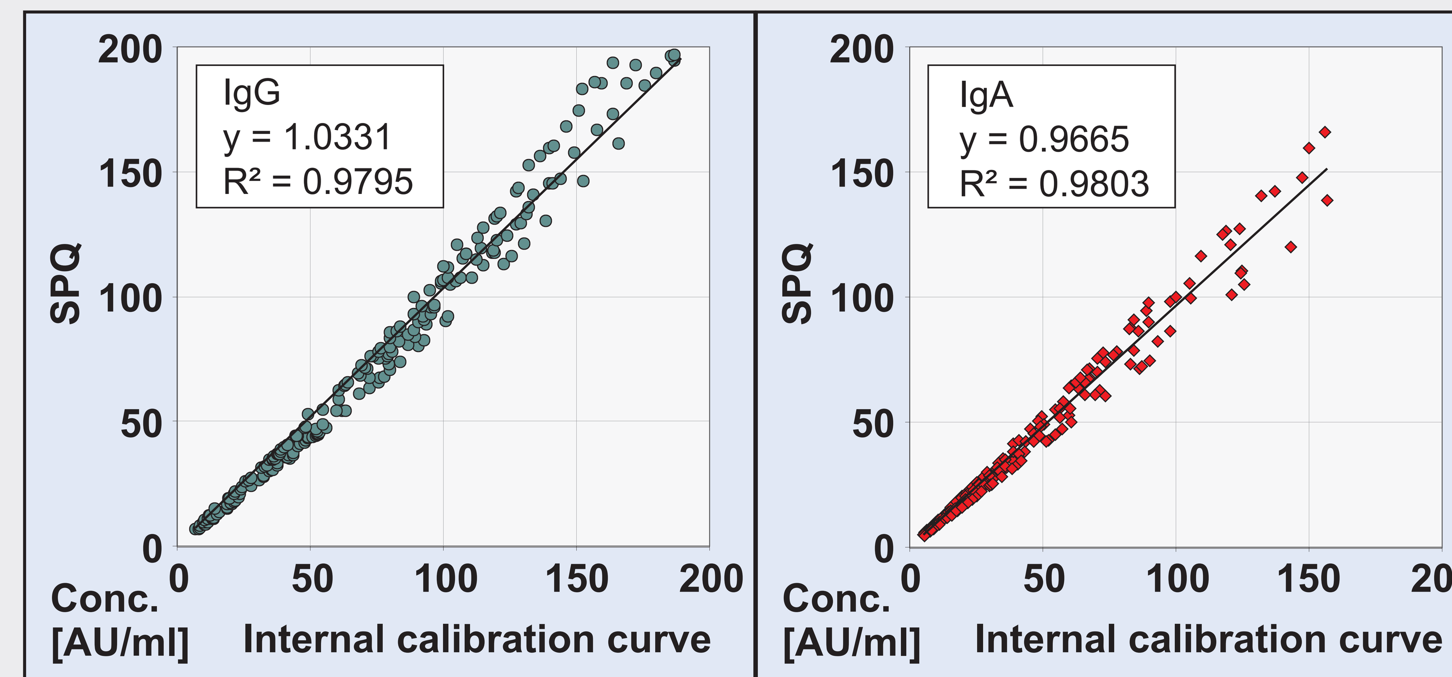

Fig. 4: Single-point quantitation (SPQ). Antibody concentration of 217 sera IgG (●) and 202 sera IgA (◆) were calculated by SPQ and using an internal calibration curve (IgG 6.25-200 AU/ml, IgA 5.25-167 AU/ml) in one test run, respectively.

Conclusions

The data show that the new quantitative assay generation provides results which are in very good concordance with the results obtained with the qualitative assays. The new assays equally fulfill the demands of modern routine diagnostics like suitability for automation and easy calculation of the quantitative results. SPQ provides reliable results over the whole measuring range, which is true for all assays of the new generation: **Chlamydia pneumoniae-IgG/IgA-ELISA *plus* medac** and **Chlamydia trachomatis-IgG/IgA-ELISA *plus* medac**. Therefore the assays fulfill this prerequisite towards a better standardization of Chlamydia serology. For further improvement of standardization reference preparations for anti-Chlamydia immunoglobulins are needed. The comparison to MIF showed a very good agreement with the ELISAs regarding the qualitative assessment of samples. But the variability of the ELISA units within the individual MIF titers does not allow to relate a quantitative ELISA result to a certain MIF titer.