15TH CONGRESS
JULY 11-16, 2004

PROGRAM

Mycoplasma pneumoniae-ELISA medac: New assays for the detection of specific IgG, IgA, and IgM antibodies

Thorsten König[1], Martha Böttcher[1], Gunna Christiansen[2,4], Mette Drasbek[4], Walter Däubener[3], Svend Birkelund[2,4], Dieter Franke[1]

[1]medac GmbH, Diagnostic Division, Hamburg, Germany, [2]Department of Medical Microbiology and Immunology, University of Aarhus, Denmark, [3]Institute for Medical Microbiology, University of Düsseldorf, Germany, [4]Loke Diagnostics ApS, Risskov, Denmark

Introduction

Serology is frequently used to support the diagnosis of *Mycoplasma pneumoniae (M.pn.)* infection. Complement fixation tests, agglutination assays and ELISA are alternative methods for this purpose. But only the ELISA has the potential to detect an isotype-specific antibody response and is best suited to fulfill the requirements for routine use like suitability for automation and reliable, simple interpretation.

We developed and evaluated 3 indirect ELISA using highly purified bacterial (IgM assay) and recombinant major *M.pn.* antigens (IgG/IgA assay). IgG and IgA assay provide quantitative results without the need for a calibration curve in the test run (single-point quantitation method, SPQ).

Material & Methods

Diagnostic evaluation: We determined the prevalence of *M.pn.*-specific antibodies in sera of 300 healthy blood donors, 166 children with acute respiratory infections, and 189 hospitalized patients with the medac assays. 193 patient sera pre-defined with a *M.pn.* particle agglutination assay (PAA, Fujirebio Inc., Japan) were used to compare ELISA vs. PAA.

Precision/Automation: Inter- and intra-assay variation were investigated both manually and using an automatic device (Dynatech Immunoassay System, DIAS). Furthermore, person-to-person variation and, regarding the quantitative assays, dilution linearity, limit of quantitation (LOQ) and the correlation of SPQ with data obtained using a calibration curve were studied. In order to verify suitability for automation parallel test runs performed manually vs. using the DIAS and the Behring ELISA Processor III (BEP III), respectively, were accomplished. Therefor samples covering a broad reactivity range were chosen.

Table 1:
Prevalences obtained in different sample cohorts using the *M.pn.*-IgG/A/M-ELISA medac.

		IgG	IgA	IgM
Healthy blood donors	+	39%	6%	2%
N = 300 sera	±	11%	3%	2%
	–	50%	91%	96%
Children with acute	+	67%	9%	28%
respiratory infections	±	4%	3%	5%
N = 166 sera	–	29%	88%	66%
Hospitalized patients with	+	44%	10%	21%
respiratory infections	±	5%	3%	5%
N = 189 sera	–	51%	87%	74%

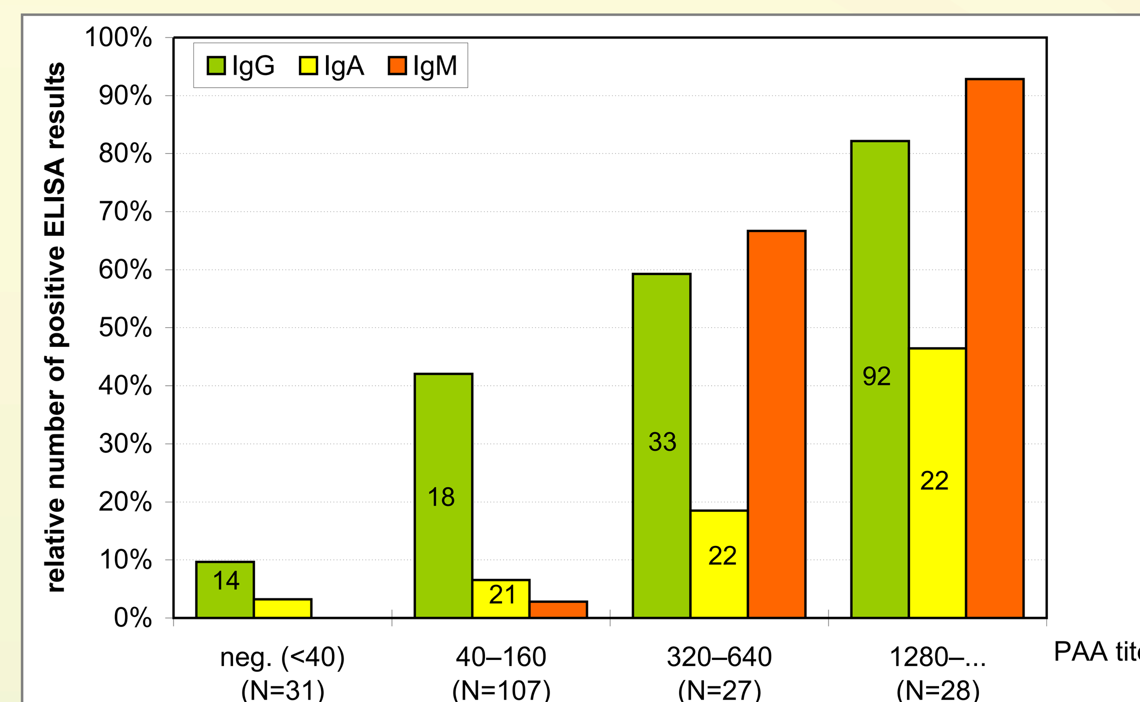

Fig. 1: Comparison of *M.pn.*-IgG/A/M-ELISA medac vs. PAA. Numbers in bars indicate the median concentration (AU/mL) of IgG and IgA positive results.

Table 2:
Precision data for *M.pn.*-IgG/A/M-ELISA medac.

	IgG		IgA		IgM	
	CV	N	CV	N	CV	N
Intra-assay variation (manually)	≤ 8%	21	≤ 7%	21	≤ 8%	21
Intra-assay variation (DIAS)	≤ 9%	10	≤ 9%	10	≤ 4%	10
Interassay variation (manually)	≤ 8%	5	≤ 7%	5	≤ 16%	6
Interassay variation (DIAS)	≤ 10%	5	≤ 9%	5	≤ 6%	5
Person-to-person variation	≤ 11%	3	≤ 6%	3	≤ 12%	3

CV calculation based on AU/mL for IgG/IgA and on OD values for IgM, respectively. Only the highest CV of several investigated positive/ borderline sera is stated.

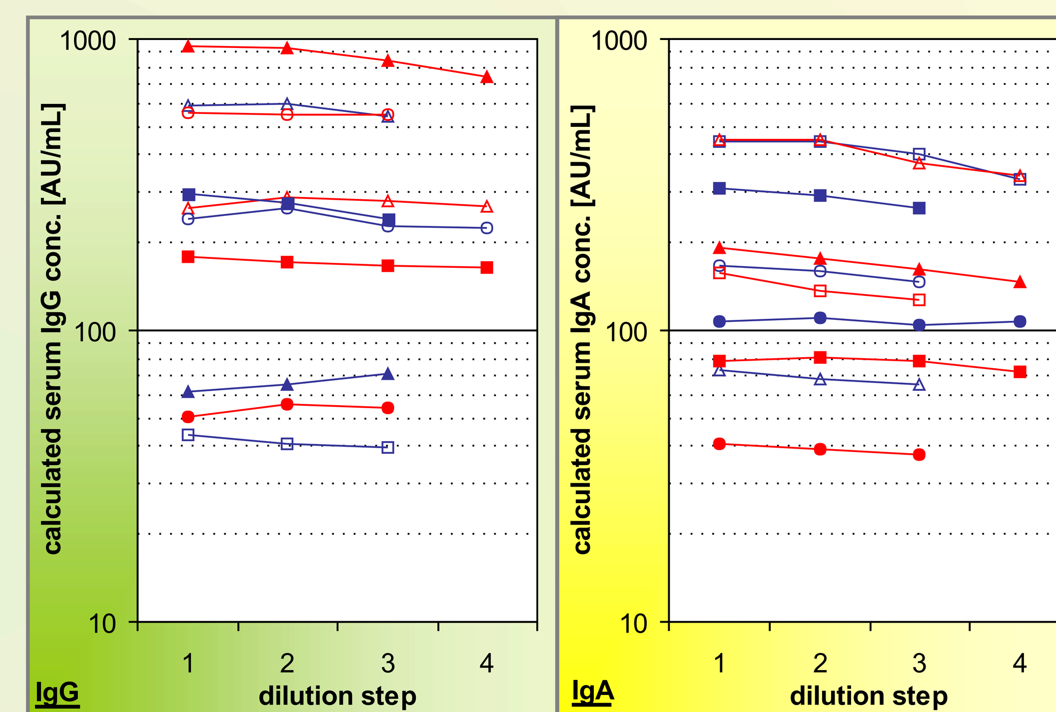

Fig. 2: Dilution linearity. 10 reactive sera were titrated in 1:2 dilution steps. Only results within the measuring range (9–130 AU/mL) were used for calculation.

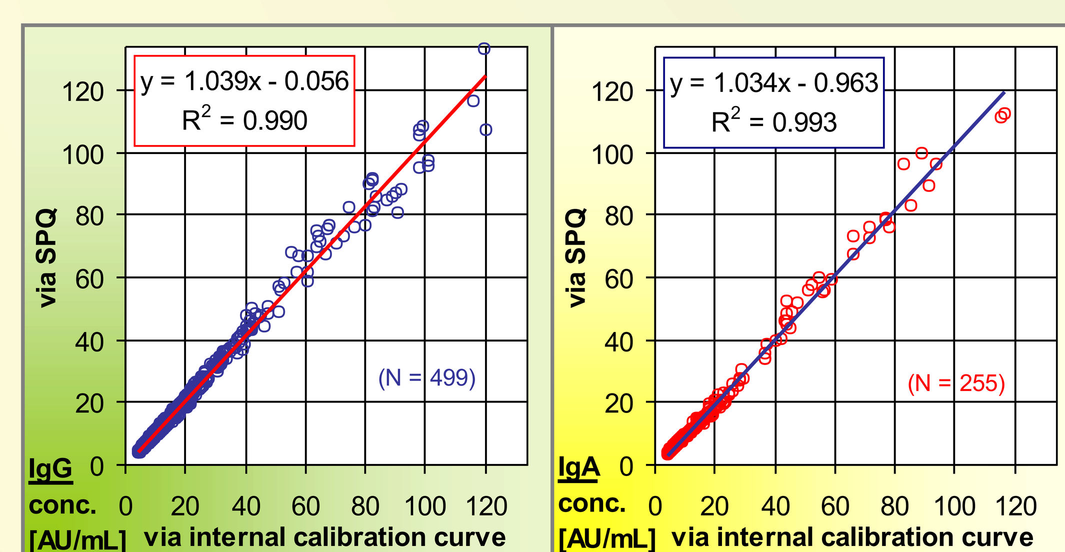

Fig. 3: Single-point quantitation. Antibody concentrations were calculated by SPQ and using an internal calibration curve (4–130 AU/mL), respectively.

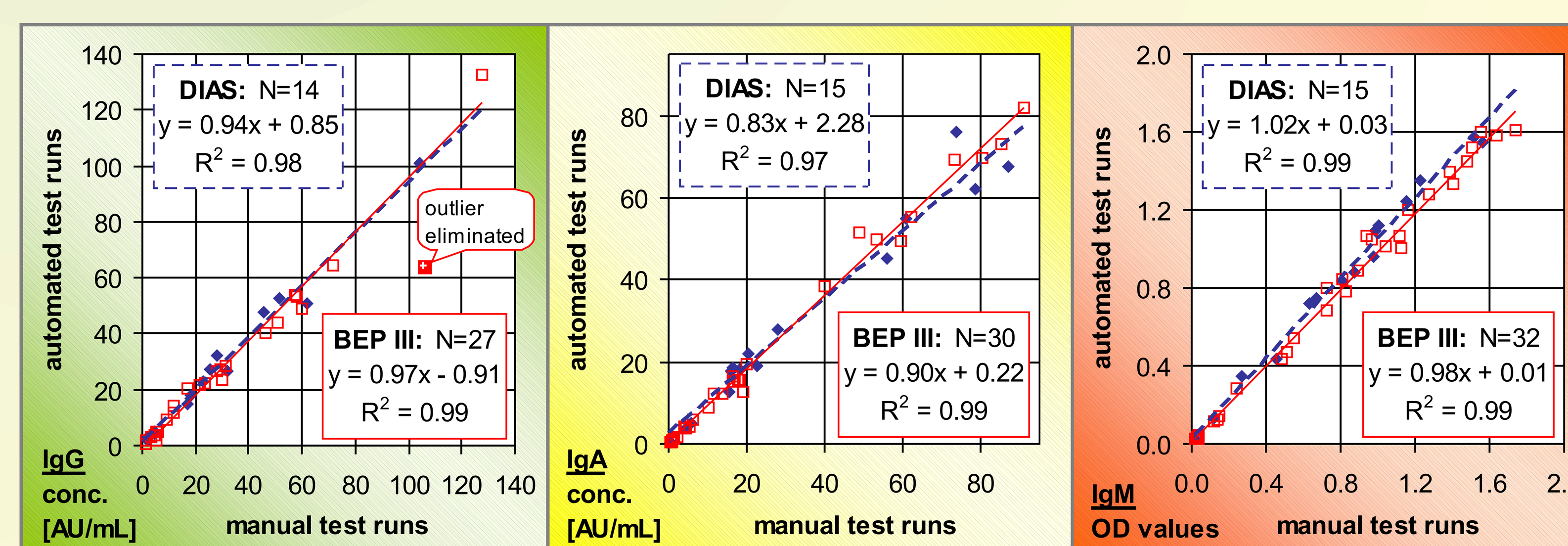

Fig. 4: Correlation of automated vs. manually performed test runs.

Results

Diagnostic evaluation: For blood donors our investigations revealed prevalences as expected. Highly elevated IgM prevalences were found in the symptomatic patient cohorts (s. Table 1). Positive correlations between relative number of positive ELISA results and PAA titer were observed for all isotypes. For IgG also reactivity strongly increases. Below titer 1:320 the IgM positivity rate was identical to the prevalence found with blood donors (2%; Fig. 1).

Precision/Automation: Coefficients of variation (CV) for samples in the relevant reactivity range (positive or borderline) were below 10% for intra-assay variation. For interassay and person-to-person variation only one CV slightly exceeded 15% (Table 2). The dilution linearity of both quantitative assays was very good. The variation of the respective dilutions of each serum was low (CV <14%; Fig. 2). LOQ was determined in 3 measurements each and was always found <4 AU/mL. This is far below the limit of reporting (9 AU/mL), documenting the validity of the lower limit of the measuring ranges. Correlations of quantitative results obtained using calibrators and SPQ, respectively, were excellent (Fig. 3). Comparison of manually and automatically performed test runs revealed a good concordance for all three assays (Fig. 4).

Conclusions

Our evaluation shows that the *M.pn.*-IgG/A/M-ELISA medac have the attributes to fulfill the needs for modern routine diagnostics in laboratories with small and large sample size. They are easy to perform, providing precise qualitative (IgM) and quantitative (IgG/IgA) results in less than three hours.

The observed elevated prevalences in patients with respiratory tract infection compared to blood donors as well as the evident correlation to PAA demonstrates the usefulness of the 3 new ELISA.

Isotype-specific antibody detection seems to be superior to other serological methods in defining the state of infection, like specific IgM indicates acute (primary) infection.