

Evaluation of sELISA medac for the detection of *Chlamydia pneumoniae*-specific IgG, IgA and IgM in comparison to microimmunofluorescence (MIF)

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INTRODUCTION:

C. pneumoniae is a common cause of acute respiratory disease, including pneumonia, bronchitis, sinusitis, and pharyngitis throughout the world. Most infections are mildly symptomatic or asymptomatic and therefore often undetected with the risk to become chronic. An association of chronic *C. pneumoniae* infection and atherosclerosis and diseases of the central nervous system like multiple sclerosis and Alzheimer's disease, are discussed. Serology is the method of choice for the diagnosis of *C. pneumoniae* infections. The microimmunofluorescence (MIF) is accepted as the gold standard, but it is time consuming and evaluation requires a lot of experience. Furthermore it is not suitable for automation which is a necessity for today's routine diagnosis of *C. pneumoniae* infections.

AIM OF THE STUDY:

Three new *C. pneumoniae* EIAs, *Chlamydia pneumoniae* IgG, IgA, and IgM-sELISA medac (medac, Hamburg, Germany) were investigated regarding precision, sensitivity, specificity, and suitability for automation. Sensitivity and specificity were determined in comparison to MIF to establish ELISA as a reliable alternative in routine diagnosis suitable for automation.

MATERIAL AND METHODS:

C. pneumoniae-IgG-, IgA- and IgM-sELISA medac were evaluated. Each assay uses highly specific, LPS-reduced, native *C. pneumoniae* antigen.

Reference tests were microimmunofluorescence (MIF) assays (MRL Diagnostics, Cypress, CA, and in-house MIF, Dept. Clin. Microbiol., University Hospital Malmö, Malmö, Sweden, and Inst. Clin. Microbiol., F.S. University Jena, Jena, Germany).

Precision experiments were performed manually and using an automatic device (Dynex Immunoassay System/DIAS, using a slightly modified protocol). Intra-assay variation (21fold and 10fold, resp., determinations of 5 samples in one test run) and interassay variation (5 samples in 11 independent test runs) were determined.

In addition automation suitability was also investigated by using the DIAS and a second device (Behring Processor III/BEPIII) for measuring panel of sera with different reactivity in parallel to manually performed test runs.

Specificity and sensitivity were determined using 376 MIF-defined sera (IgG: 44 negative, 119 positive; IgA: 87 negative, 77 positive; IgM: 121 negative, 39 positive). 16 IgM sera with a titer of 1:20 in MIF were considered borderline.

Furthermore 11 (IgG), 14 (IgA), and 12 (IgM) MIF *Chlamydia trachomatis* (*C. tr.*) as well as 2 (IgM) MIF *Chlamydia psittaci* (*C. ps.*) positive sera were tested to investigate cross-reactivity. Borderline results were excluded from the calculations.

RESULTS:

Sensitivity and specificity: For the three assays high values were found in comparison to MIF (fig. 1). Cross-reactivity is low regarding *C. tr.* but both *C. ps.*-positive sera showed cross-reactivity with *C. pneumoniae* (table I).

Precision: Coefficients of variation (CV) within the relevant OD range are low with all three assays (table II/III).

Automation: All assays showed a good correlation between the results of manually and automatically performed test runs, which was true for both devices (fig. 2). No discrepant results were obtained between manually and automated test runs.

CONCLUSIONS:

- Chlamydia pneumoniae*-IgG-, IgA- and IgM-sELISAs medac are easy to perform assays, which provide reliable results in less than three hours. All three assays show good precision and are suitable for automation. Test results are in very good concordance with the MIF showing high sensitivity and specificity and only minimal cross-reactivity.

SPECIFICITY AND SENSITIVITY

IgG	MIF			Σ
	-	+	±	
sELISA medac	40	1	2	41
	2	2	2	4
	2	116	2	118
	44	119	1	163
Sensitivity	99%			
Specificity	95%			

IgA	MIF			Σ
	-	+	±	
sELISA medac	80	4	1	84
	1	3	1	4
	6	70	1	76
	87	77	1	164
Sensitivity	95%			
Specificity	93%			

IgM	MIF			Σ
	-	±	+	
sELISA medac	116	6	1	123
	2	0	1	3
	3	10	37	50
	121	16	39	176
Sensitivity	97%			
Specificity	97%			

Fig. 1: Determination of sensitivity and specificity in comparison to MIF. Calculations were performed without borderline results

CROSS-REACTIVITY

sELISA medac	IgG	IgA	IgM	
Species	<i>C. tr.</i>	<i>C. tr.</i>	<i>C. tr.</i>	<i>C. ps.</i>
No. of samples	11	14	12	2
Positive samples	1	1	-	2
%-cross-reactivity	9	7	-	100

PRECISION

TABLE II: INTRA-ASSAY VARIATION USING MANUALLY AND AUTOMATICALLY PERFORMED TEST PROCEDURES

Sample	IgG				IgA				IgM			
	man.*		DIAS**		man.*		DIAS**		man.*		DIAS**	
	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)
1	0.032	27	0.036	16	0.026	42	0.020	39	0.036	9	0.044	4
2	0.772	5	0.880	9	0.538	4	0.599	10	0.503	5	0.566	5
3	1.990	3	2.287	5	1.251	3	1.405	7	1.216	4	1.306	7
4	1.951	5	1.882	7	1.419	4	1.220	6	0.844	7	0.086	16
5	0.731	4	1.278	12	0.169	7	0.797	5	0.719	6	0.897	9

*) 21 determinations in one test run
**) 10 determinations in one test run

TABLE III: INTERASSAY VARIATION USING MANUALLY AND AUTOMATICALLY PERFORMED TEST PROCEDURES

Sample	IgG				IgA				IgM			
	man.*		DIAS*		man.*		DIAS*		man.*		DIAS*	
	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)	OD	CV (%)
1	0.035	11	0.031	21	0.027	28	0.022	11	0.035	10	0.038	9
2	0.824	9	0.824	8	0.533	8	0.570	8	0.521	5	0.554	5
3	1.027	8	1.054	8	0.663	7	0.711	10	1.225	5	1.228	7
4	1.655	8	1.642	7	0.975	9	1.059	10	0.051	9	0.061	31
5	2.133	7	2.136	6	1.243	9	1.298	8	0.848	7	0.816	8

*) 11 independent test runs

AUTOMATION

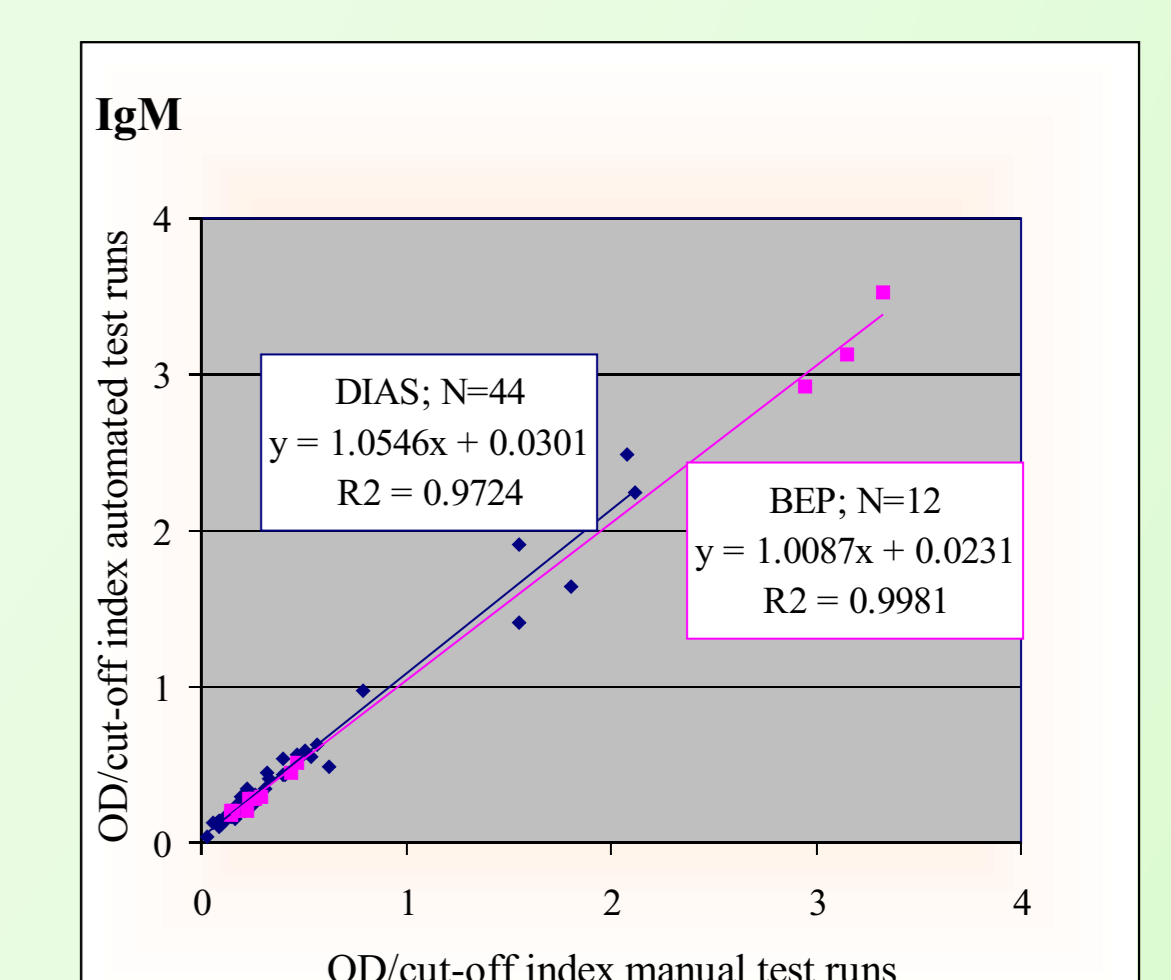
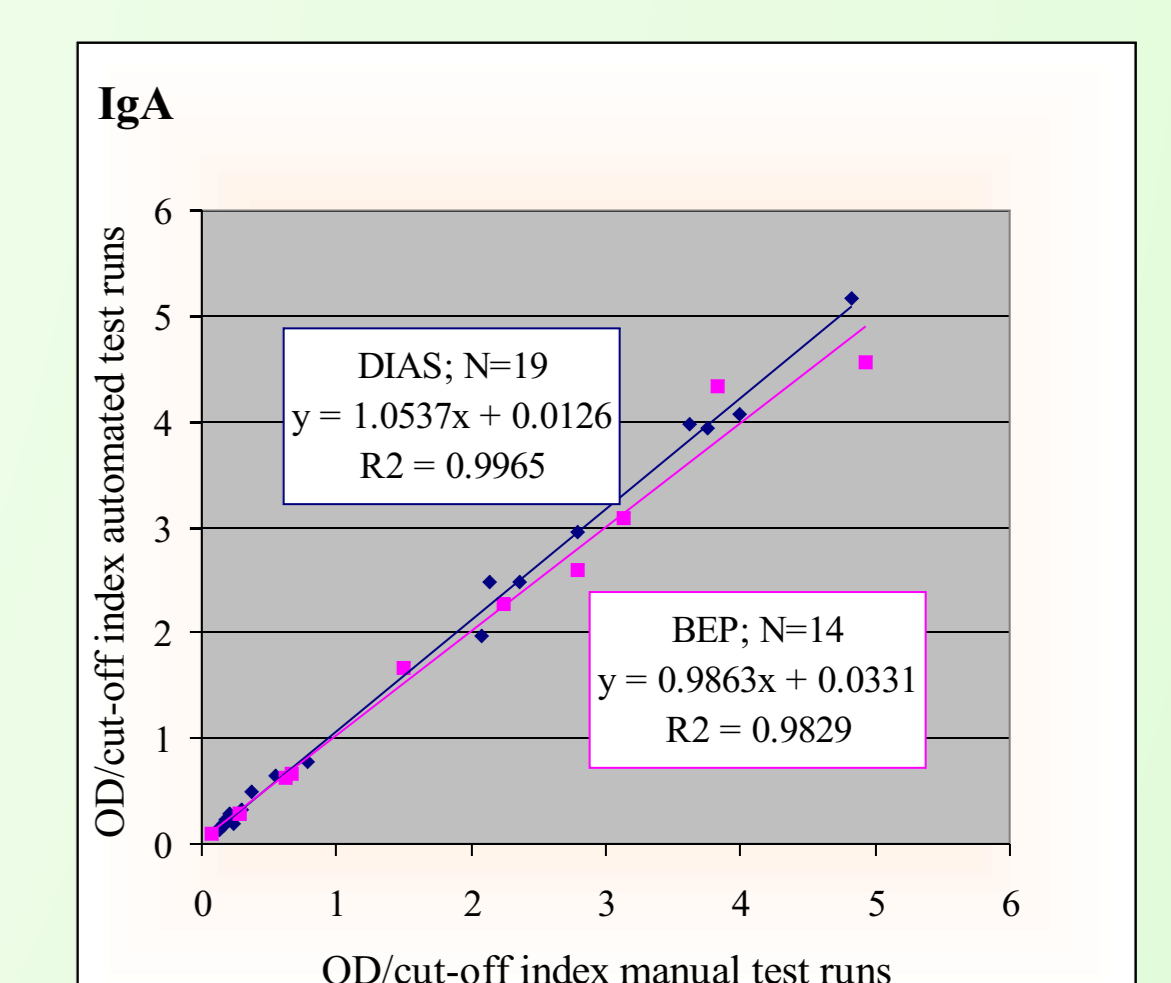
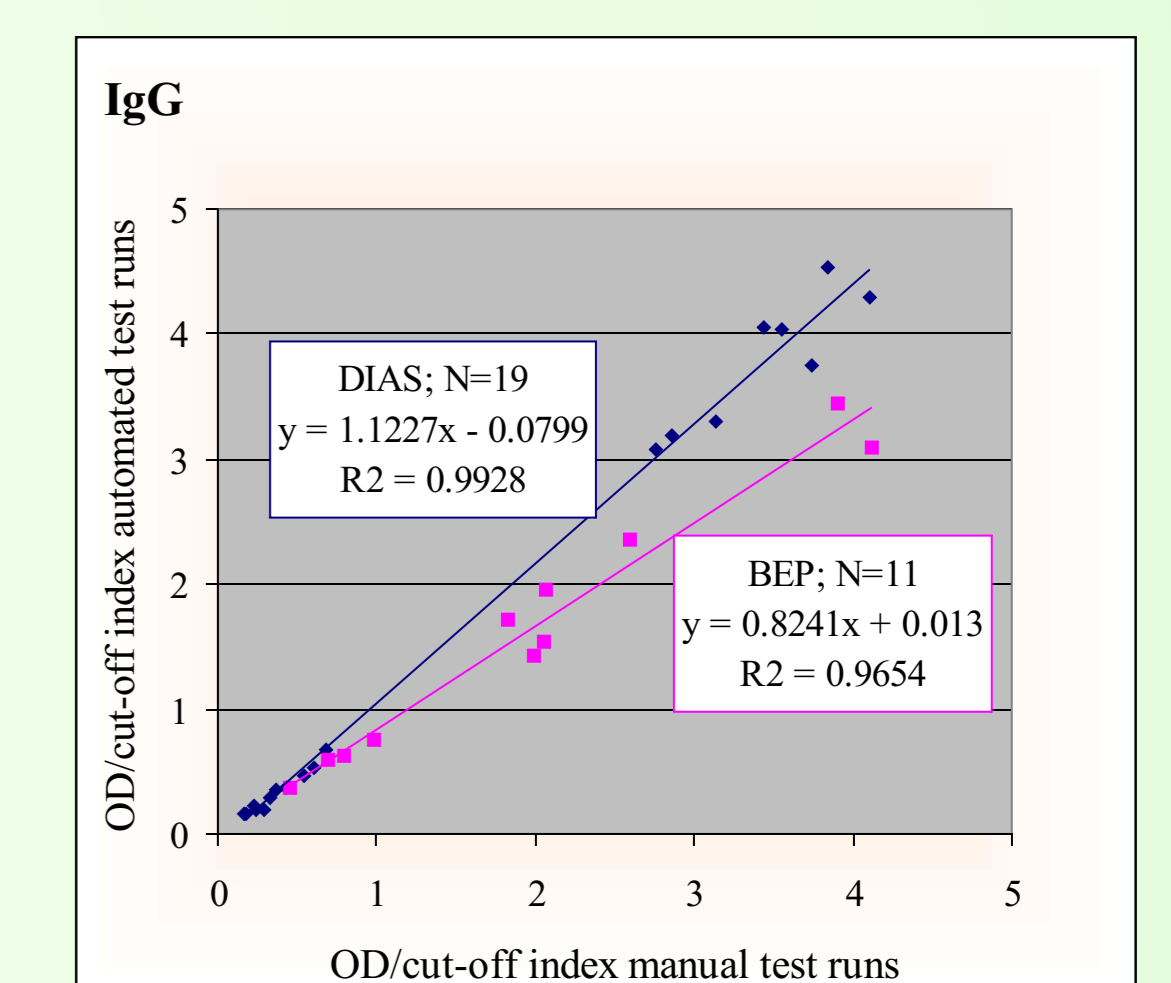


Fig. 2: Correlation of OD/cut-off indices obtained with manually performed and automated test runs.