

Evaluation of pELISA medac for the detection of *Chlamydia trachomatis*-specific IgA and IgG

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Introduction

In many situations where *C. trachomatis* is suspected to contribute to or to be the cause of disease the bacterium is not directly detectable. For these cases of chronic persistent infection with *C. trachomatis*, serology is an important tool of diagnosis. This means diagnostic assays for routine use are needed which fulfill the demands of high precision and specificity as well as good sensitivity together with the suitability for automatic processing. Regarding specificity a lack of cross-reactivity with antibodies against *Chlamydia pneumoniae* is of particular importance. The basis of serologic diagnosis is the detection of specific IgG which should be supplemented by detection of specific IgA. The aim of this study was to evaluate two new ELISA for the detection of *C. trachomatis* antibodies, *Chlamydia trachomatis*-IgG-pELISA medac and *Chlamydia trachomatis*-IgA-pELISA medac (medac, Hamburg, Germany).

Material & Methods

Both evaluated assays are ELISA using *C. trachomatis*-specific synthetic antigens coated to the solid phase.

Reference Tests: Microimmunofluorescence (MIF) assays: MRL Diagnostics, Labsystems OY, Dept. Clin. Microbiol., University Hospital Malmö. *C. trachomatis*-specific ELISA: Savyon, Labsystems OY. Anti-chlamydial LPS ELISA: Chlamydien rELISA medac. Nested PCR: Extracted DNA was used in a standard nested PCR involving the Seattle HL-1/HR-1 primers, which amplify a genomic 438 bp *C. pneumoniae* target sequence, and, for enhanced specificity, the nested oligonucleotide primer pair IN-1 and IN-2 which yields a 128 bp product. For confirmation, non-radioactive DNA hybridization was performed (Maass M, et al. J Am Coll Cardiol 1998; 31: 827-832).

Precision: Precision experiments were performed manually and using the Dynex Immunoassay system (DIAS, using a slightly modified protocol). Lot to lot variation, person dependent variation, intra-assay variation and inter-assay variation were investigated.

Specificity: Specimens from children (1 month to 6 years), sera from *Chlamydia* negative persons, from patients with PCR-proven *C. pneumoniae* infection and/or elevated MIF titers and potentially cross-reactive specimens ("Tricky panel") including sera containing rheumatoid factor (N=20), heterophilic antibodies (N=10), anti-nuclear antibodies (N=13) and anti-*Treponema pallidum* (N=27) positive sera were used to determine the specificity of the assays.

Prevalence: In the following panels prevalences of anti-*C. trachomatis* IgA and IgG antibodies were determined: Sera from volunteer whole-blood donors (151 female, 148 male, each group 15-65 years), 85 sera from patients with the diagnosis of secondary infertility, 83 sera from patients with proven or suspected rheumatoid diseases, selected for anti-chlamydial LPS (IgG) reactivity, sera from patients attending a STD clinic, which were *C. trachomatis* culture positive (57 female, 57 male, 16-30 years) and culture negative (51 female, 61 male, 16-30 years).

Results

Table 1: Specificity results obtained with different panels

Panel	N	<i>Chlamydia</i> negative	<i>C. pneumoniae</i> PCR and/or MIF positive	pELISA positive or equivocal	Confirmed <i>C. trachomatis</i> positive	Specificity
IgG						
Children's sera	100	100	ND	1	0	99%
<i>C. pneumoniae</i> infected	55	0	55	7	7	100%
<i>Chlamydia</i> negative	5	5	0	0	-	100%
"Tricky Panel"	70	ND	ND	17	15	97%
IgA						
Children's sera	100	100	ND	2	0	98%
<i>C. pneumoniae</i> infected	55	0	55	4	4	100%
<i>Chlamydia</i> negative	5	5	0	0	-	100%
"Tricky Panel"	70	ND	ND	14	13	99%

Table 2: Prevalences of *C. trachomatis* antibodies in different panels

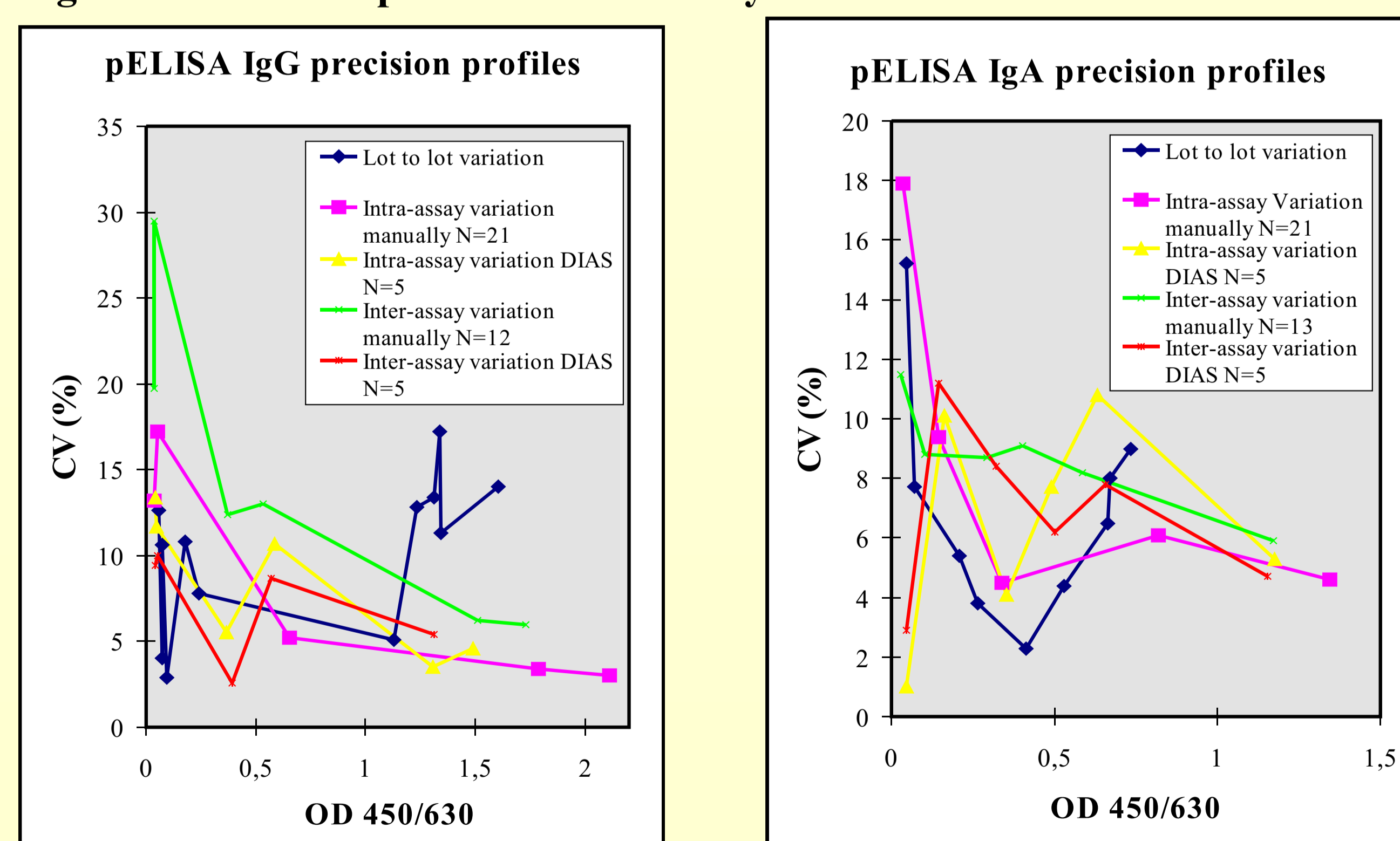
Panel	N	Results (%)				MIF
		+	pELISA ±	-	+	
IgG						
Blood donors	299	14.4	1.3	84.3	ND	ND
STD patients ¹	226	41.2	4.4	54.4	43.6	56.4
- Culture neg. ²	112	21.4	3.6	75.0	24.0	76.0
- Culture pos. ³	114	60.5	5.3	34.2	62.1	37.9
Sec. infertility patients	85	60.0	2.3	37.7	ND	ND
Rheum. disease patients	83	48.2	2.4	49.4	ND	ND
IgA						
Blood donors	299	6.4	4.0	89.6	ND	ND
STD patients ¹	226	15.5	4.9	79.6	12.5	87.5
- Culture neg. ²	112	6.2	4.5	89.3	3.2	96.8
- Culture pos. ³	114	24.6	5.3	70.1	21.2	78.8
Sec. infertility patients	85	15.3	5.9	78.8	ND	ND
Rheum. disease patients	83	41.0	4.8	54.2	ND	ND

¹ Specificity and sensitivity compared to culture: IgG: Sensitivity: pELISA 61%, MIF 62%; Specificity: pELISA 75%, MIF 76%.

IgA: Sensitivity: pELISA 25%, MIF 21%. Specificity: pELISA 89%, MIF 97%

² MIF: N=132; ³ MIF: N=125

Figure 1: Precision profiles of both assays



Precision:

Person dependent variation as well as the other precision measurements revealed no differences regarding positive equivocal and negative results with low CV values in the relevant OD range (fig. 1).

Specificity:

Both assays show high specificity. Unconfirmed reactivity within the "Tricky panel" was found with 1 *T. pallidum* positive serum and 1 serum with rheumatoid factor (IgG) and furthermore with 1 serum containing heterophilic antibodies (IgA, table 1).

Prevalences:

Apart from the culture negative STD patients who showed similar prevalences all patient groups revealed clearly increased anti-chlamydial reactivity compared to the blood donors. Culture negative and culture positive STD patients showed strong differences in chlamydial seroprevalence in IgA and IgG which was similar to the MIF results (table 2).

Conclusions

Chlamydia trachomatis-IgG-pELISA medac and *Chlamydia trachomatis*-IgA-pELISA medac are easy to perform assays that provide reliable results within less than three hours. The assay format which is identical for both assays is convenient to handle for small as well as for large sample sizes because of the suitability to perform the assays on automatic devices. Both assays exhibit good reproducibility and overall precision. The assays show excellent specificity with regard to unspecific binding and cross-reactivity with *C. pneumoniae* antibodies.

The prevalence of positive results in sera of patients with positive *C. trachomatis* culture as well as in sera of patients with potentially persistent chlamydial infection is significantly higher than in sera of healthy blood donors.

The results of this evaluation comprising the investigation of more than 900 sera illustrate the usefulness of both assays as a supportive tool for *C. trachomatis* diagnosis especially in patients with persistent infection.