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Interference of Immunoglobulin G (IgG) Antibodies in IgA Antibody Determinations for *Chlamydia pneumoniae* by Microimmunofluorescence Test

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In the microimmunofluorescence test for measuring immunoglobulin A (IgA) antibodies against *Chlamydia pneumoniae*, removal of interfering IgG antibodies made IgA antibody reactivity patterns in 952 serum samples easier to interpret, prozone effects disappeared, and titers increased, especially in the sera with high IgG titers. IgA rheumatoid factors did not interfere in the assay.

Elevated or persisting titers of specific immunoglobulin A (IgA) antibodies have been suggested to be markers of chronic bacterial infections in yersinial reactive arthritis (4), in cystic fibrosis pulmonary infections caused by *Pseudomonas aeruginosa* (1, 5), and in chronic pelvic inflammatory disease caused by *Chlamydia trachomatis* (3, 9). Recently, chronic *Chlamydia pneumoniae* infection has been associated with coronary heart disease (7), and specific elevated IgA antibody titers were suggested to be better markers of chronic infection than respective IgG titers (8).

IgA titers in the microimmunofluorescence (micro-IF) test (12) are often difficult to read. There is a marked tendency to prozone effect, and the fluorescence pattern is often much weaker than in IgG titrations. This phenomenon, also seen in IgM titrations by this test, has been related to interfering IgG antibodies of higher affinity (6). A similar effect on IgA titrations in enzyme immunoassay (EIA) has also been found (2). Moreover, IgM rheumatoid factors (IgM-RFs) are known to lead to false-positive IgM findings (11), and positive IgM titers should always be verified after the removal or inactivation of IgG antibodies. In this study we studied the effect of IgG removal on IgA antibody titers to *C. pneumoniae* in the micro-IF test.

Altogether, 952 serum samples from Jewish residents of Jerusalem aged 25 to 64 were studied. One-third of the sera were obtained from cases of first myocardial infarction during their hospitalization, and the remainder were obtained from an age-weighted control group representative of Jerusalem residents. The male to female ratio was about 2:1. Chlamydial serum IgG and IgA antibodies were determined by the micro-IF test (13). For IgA antibody measurements, all sera were at first titrated untreated and then after treatment with GullSORB (Gull Laboratories, Salt Lake City, Utah) according to the instructions of the producer to remove interfering IgG antibodies by immunoprecipitation with anti-human IgG. Twofold serum dilutions starting from 1:10 were incubated for 30 min with *C. pneumoniae* TWAR (Washington Research Foundation, Seattle, Wash.) antigen on Teflon-coated slides with 30 wells (Danlab, Helsinki, Finland). After washing and drying, fluorescein isothiocyanate-conjugated anti-human IgA and IgG (Sigma, St. Louis, Mo.) with Evans blue as counterstain were incubated for 30 min and washing and drying steps were repeated. Cover plates were mounted with glycerol veronal buffer (1:1), pH 8.5. A Zeiss Axiophot Photo microscope with 10× ocular and 40× objective and 50 W HBO mercury lamp illumination with a 450- to 490-nm-pore-size filter was used for determining specific fluorescence of elementary bodies.

The presence of IgA-RF was tested by EIA (10). Human IgG, Fc fraction (Jackson), 2.5 μg/ml in carbonate buffer, pH 9.6, was used as coating antigen. EIA plates were postcoated with 1% human serum albumin by incubation for 30 min at room temperature. Sera were diluted 1:20 with phosphate buffer, pH 7.2, containing 0.05% Tween, 0.2% human serum albumin, and 4% polyethylene glycol. Alkaline phosphatase conjugated $F(ab')_2$ fragments of rabbit anti-human IgA (Jackson) were used in a dilution of 1:3,000. Sera and conjugate were incubated for 1 h at room temperature, and *p*-nitrophenyl phosphate (Sigma) was used as a substrate.

The prevalences of positive IgA (≥10) and IgG (≥16) antibody titers to *C. pneumoniae* were high in serum samples, 52.7 and 79.3% respectively, and geometric mean titers were 37 and 156. Removal of IgG antibodies made IgA titrations easier to read; fluorescence was brighter and more clearcut, and prozone effect disappeared. In most cases there were no changes in IgA titer after treatment with GullSORB, and two IgA titers correlated well ($r = 0.83$, $P < 0.01$). However, 85 (8.9%) of 952 samples showed marked (fourfold or more) change in titer, and this correlated to high IgG titers in the sera (Tables 1 and 2).

All of the 28 sera with decreasing titer and, as a control, 10 with increasing titer after IgG inactivation were tested by

TABLE 1. Changes in IgA titers after GullSORB treatment and the respective IgG titers

Change in IgA titer	No. (%) of subjects with change	Mean IgG titer (95% confidence interval)
Fourfold decrease	5 (0.5)	146 (63–230)
Twofold decrease	23 (2.4)	
No change	673 (70.7)	102 (88–116)
Twofold rise	171 (17.9)	245 (195–295)
Fourfold rise	54 (5.7)	420 (316–523)
More than fourfold rise	26 (2.7)	
Total	952 100	156 (139–173)

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TABLE 2. Change in IgA titer in relation to IgG titer

IgG titer	No. of subjects	No. (%) of subjects with changed titer	IgA_{GS}/IgA (95% confidence interval)[a]
<32	204	9 (4.4)	1.02 (0.99–1.06)
32–64	245	41 (16.7)	1.24 (1.10–1.39)
128–256	378	155 (41.0)	1.70 (1.53–1.87)
512–1,024	117	68 (58.1)	3.03 (1.90–4.16)
>1,024	8	6 (75.0)	7.50 (−1.77–16.8)
Total	952	279 (29.3)	

[a] IgA_{GS}, after GullSORB treatment.

IgA-RF EIA (Table 3). Differences between groups were insignificant, suggesting that IgA-RF is not responsible for decreasing titer. Five (0.5%) weakly false-positive sera were found: for four of them titers fell from 20 to <10, and for one of them the titer fell from 10 to <10.

The results of this study showed that IgG antibodies interfere in IgA determinations by the micro-IF method. When measuring specific IgM antibodies in the presence of IgG, IgM-RF often causes false-positive reactions which can be eliminated by removing IgG from sera (11). Only 0.5% of false-positive sera were found in this study. Most of the changes seen were minimal (twofold), but in 8.9% of the samples the change was marked (fourfold or more). The change was positively correlated with increased IgG titers. This suggests that the higher binding affinity of IgG or a much higher concentration of IgG than IgA in sera alters IgA reactivity in the micro-IF test. When using this test is useful to remove IgG antibodies before measuring IgA. The only disadvantage is the high cost of GullSORB reagent. Results from the IgA-RF EIA test suggest that IgA-RF does not interfere in IgA measurement by micro-IF.

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TABLE 3. Optical density at 405 nm (OD_{405}) in IgA rheumatoid factor enzyme immunoassay in sera representing different groups of IgA titer changes after GullSORB treatment

Change in IgA titer	No. of subjects	OD_{405} (95% CI)[a]
Rise	10	0.258 (0.197–0.319)
No change	10	0.265 (0.165–0.365)
Decrease	28	0.285 (0.244–0.326)
False positive	5	0.258 (0.084–0.433)

[a] Reference cutoff OD for positivity (95th percentile of blood donors, $n = 100$) ≥0.524.

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