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Comparison of the Immunoperoxidase-Slide-Test with the Immunofluorescence-Test and ELISA for the Detection of Antibodies against *Borrelia burgdorferi*

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Abbreviations

ELISA = enzyme-linked immunosorbent assay
IFT = immunofluorescence-test
IPT = immunoperoxidase-test
PBS = phosphate-buffered saline

Summary

The demonstration of IgG- and IgM-antibodies against *Borrelia burgdorferi* by IFT or ELISA requires special technical equipment and experience in the evaluation of the results. The immunoperoxidase slide test presented in this paper is easily performed and can be read with a simple light microscope. The binding of specific IgG-, IgM-, or IgA-antibodies to smears of *Borrelia burgdorferi (strain* B31) fixed on slides by acetone is visualized by subsequent incubation with peroxidase conjugated antihuman IgG, IgM, or IgA, respectively, followed by a chromogenic substrate. This technique was tested in seras of 90 patients in comparison to IFT and ELISA. IgG antibody titers of IPT and IFT showed an excellent correlation ($r = 0.981$, $p\ 0.001$). With ELISA, the correspondence of IPT was high ($r = 0.770$, $p\ 0.001$). IgM-antibody titers in IPT and IFT also showed excellent correspondence ($r = 0.967$, $p\ 0.001$), whereas the correlation of IPT with ELISA was poor ($r = 0.346$, p. 0.001), pobably due to other components of the sera (e. g. rheumatoid factors, antinuclear antibodies, immune complexes). Circulating IgA-antibodies were neither detected with IPT nor ELISA.

In view of almost identical results with the reference techniques, the IPT looks promising as simple screening assay and may be suitable for every serological laboratory.

Introduction

In the course of the establishment of *Borrelia burgdorferi* as causative agent of Lyme disease (2), it became possible to determine circulating antibodies against this agent in

an immunofluorescence assay (1). Further development of borrelia research led to the introduction of an enzyme-linked immunosorbent assay (3). Today, both methods are used with comparable sensitivity and specifity (3, 6). The IFT is not automatizableand needs a fluorescence microscope for the reading of the results. With the ELISA a large number of tests can be run in a short time, but special equipment with a photometer requires a certain number of sera being tested for this technique to be time and money saving. Moreover an elaborate preparation of a standardized antigen is of great importance.

In 1974 Felsenfeld and Wolf demonstrated that the immunoperoxidase technique is valuable for the detection of antibodies against different borreliae (4). In our laboratory we use an immunoperoxidase-slide-test (Ipazyme®) for the determination of circulating antibodies against *Chlamydia trachomatis* (5). Thus, we tried the same method to determine antibodies against *Borrelia burgdorferi* and compared the results with the two well established methods, the IFT and ELISA.

Materials and methods

Sera

Ninty sera were tested for antibodies against *Borrelia burgdorferi* in the serological laboratory of the department of dermatology, Berne. IgG-, IgM, and in selected sera IgA-antibodies were determined. No selection was made according to clinical diagnosis and no correlation between serological results and clinical findings was established.

Antigen

Borrelia burgdorferi strain B 31 was grown in modified Kelly's medium (BSK II). The spirochetes were washed three times and suspended in bidistilled water, then diluted to a number of about 1 Million/ml. 10 µl of this suspension was brought to masked slides (Bio Mérieux), air dried and fixed with pure acetone. Until used, the slides were stored at −30 °C. For the ELISA technique the antigen was prepared according to the methods described elsewhere (6).

Test methods

a) Immunoperoxidase slide test. The prepared slides were covered with a log2-dilution of the sera (IgG from 1 : 64 to 1 : 2048, IgM from 1 : 16 to 1 : 512) in PBS, incubated for 45 minutes at 37 °C in a humid chamber, washed twice with PBS and once with bidistilled water, and subsequently incubated as mentioned above for 45 minutes with peroxidase-labelled goat antihuman IgG, IgM or IgA (Medac, Hamburg, FRG), respectively. The bound antibodies were visualized by adding a chromogenic substrate (Medac, Hamburg, FRG) for 15 minutes at room temperature. The determination of the result was performed with a light microscope (× 400). The final titer was defined as maximal dilution in which stained borrelia were distinctly visible (Fig. 1a). In doubtful cases, the borreliae were demonstrated with phase-contrast and the pictures compared (Fig. 1b).

b) IFT. The IFT was performed according to the methods described elsewhere (6), using the slides prepared as mentioned above. FITC-labelled goat antihuman IgG and IgM was obtained from Bio Mérieux.

c) ELISA. The ELISA was performed according to the methods described elsewhere (6). Briefly, *Borrelia burgdorferi* antibodies (IgM and IgG) were verified with an enzyme linked immunosorbent assay (ELISA) using the supernatant of the ultrasonicated *B. burgdorferi strain* B31. The optical densitiy was measured at a wave length of 492 nm by means of an SLT 210 ELISA microplate reader (SLT, Austria).

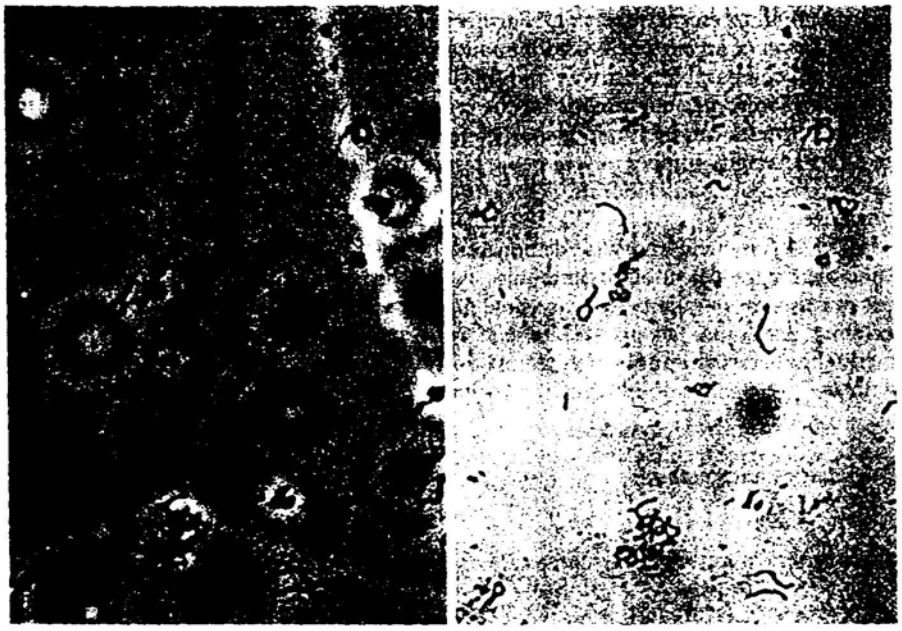

Fig. 1. Positive immunoperoxidase-slide-test: a) light microscope (× 400), b) phase contrast (× 400) as a control.

IgM-fractions. The chromatographic isolation of the IgM-antibodies of 10 sera was performed on an IgM-Combicolumn from Biochrom, Germany.

Other serologic parameters. For the determination of rheumatoid factors, antinuclear antibodies, and circulating immune complexes standard techniques were used.

Statistical evaluation. Correlation between IFT, IPT and ELISA titers were determined after – log transformations using Pearson correlation coefficients. Statistical significance of correlation coefficients was established assuming bivariante normal distributions of the -log titers by t-tests with n-2 degrees of freedom.

Results

IgG antibody titers with IPT and IFT show an excellent correlation (Fig. 2). The correlation of IPT and ELISA is lower but still very high (Fig. 3).

For the IgM-results, correlation between IPT and IFT is excellent (Fig. 4). On the other hand, IgM-results of IPT and ELISA correlate only poorly (Fig. 5). After chromatographic isolation of the IgM-fraction of 10 sera with high IgM-titers in ELISA ($-\log \geq 2.95$), the correlation of IPT and ELISA becomes equal to that obtained with IgG-titers (Fig. 6). In 3 of these sera, in which a high amount of antinuclear antibodies, rheumatoid factors or circulating immune complexes, respectively, could be detected prior to the separation of the IgM, no IgM-antibodies to *Borrelia burgdorferi* were detectable after this procedure.

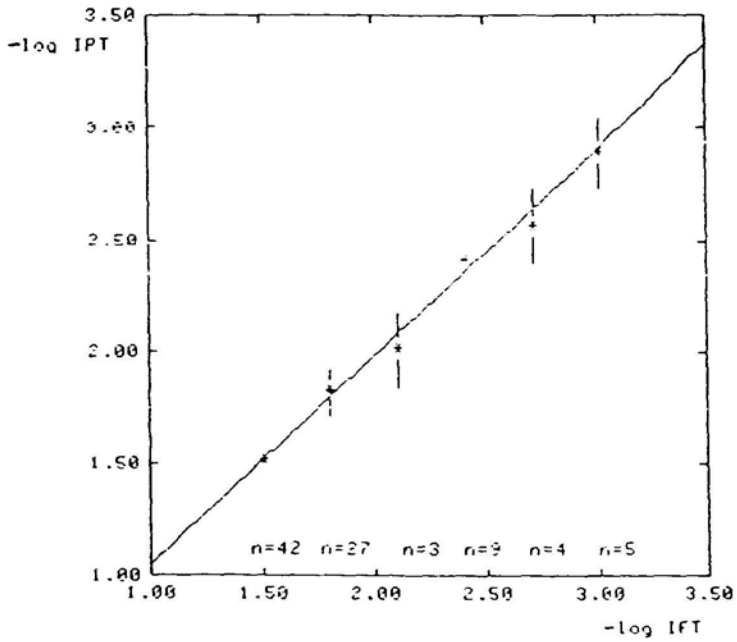

Fig. 2. Correlation of IgG-titers against *Borrelia burgdorferi* in IPT and IFT, mean ± 2 sd (n = 90, r = 0.981, $p < 0.001$; 1.51 = −log ≤ 1 : 32, 1.81 = −log 1 : 64, 2.11 = -log 1. : 128, 2.41 = -log 1 : 256, 2.71 = -log 1 : 512, 3.01 = -log 1 : 1024).

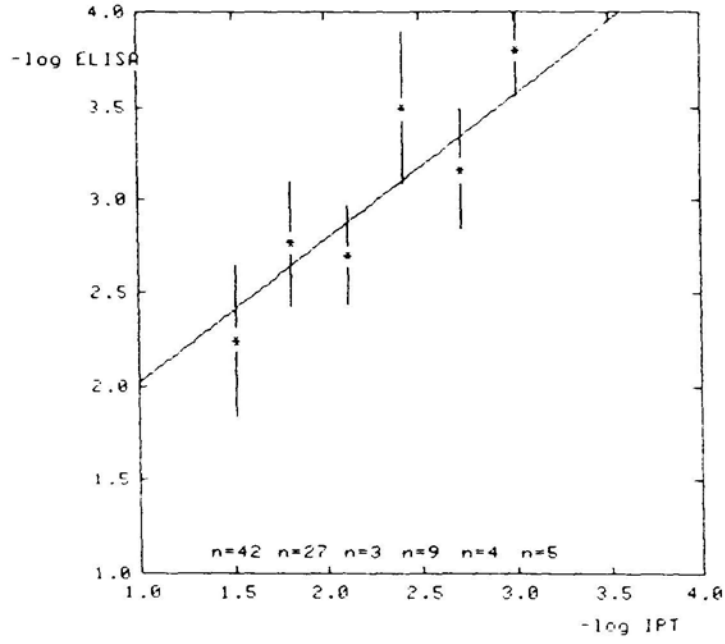

Fig. 3. Correlation of IgG-titers against *Borrelia burgdorferi* in IPT and ELISA, mean ± 2 sd (n = 90, r = 0.770, $p < 0.001$; 1.51 = -log ≤ 1 : 32, 1.81 = -log 1 : 64, 2.11 = -log 1 : 128, 2.41 = -log 1 : 256, 2.71 = -log 1 : 512, 3.01 = -log 1 : 1024).

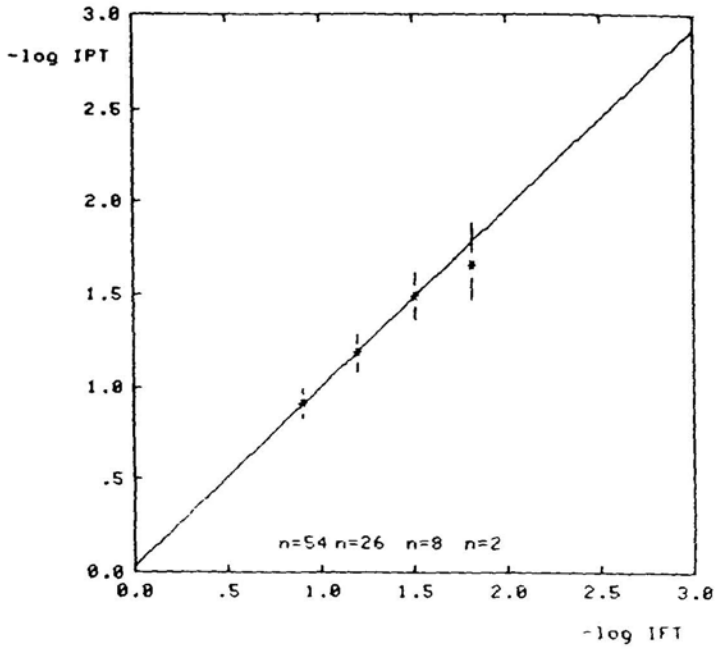

Fig. 4. Correlation of IgM-titers against *Borrelia burgdorferi* in IPT and IFT, mean ± 2 sd (n = 90, r = 0.967, p < 0.001; 0.90 = -log ≤ 1 : 8, 1.20 = -log 1 : 16, 1.51 = -log 1 : 32, 1.81 = -log 1 : 64).

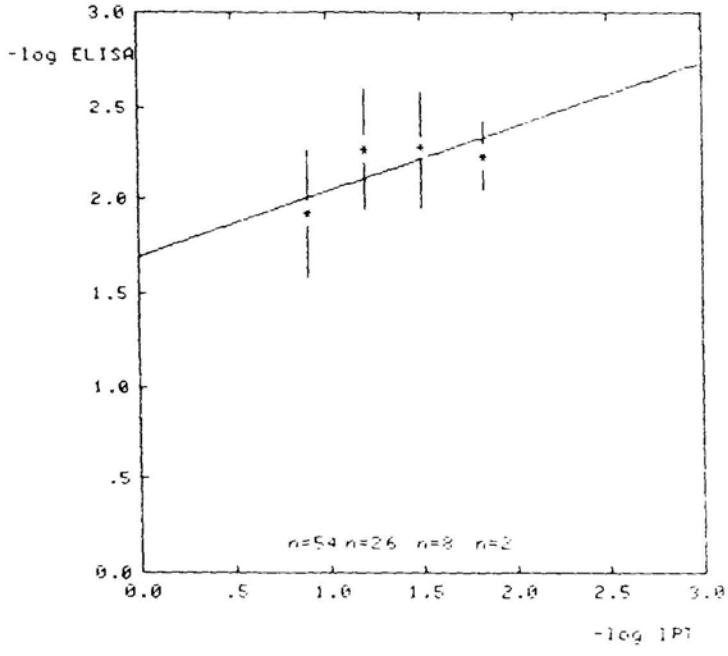

Fig. 5. Correlation of IgM-titers against *Borrelia burgdorferi* in IPT and ELISA, mean ± 2 sd (n = 90, r = 0.346, p < 0.001; 0.90 = -log ≤ 1 : 8, 1.20 = -log 1 : 16, 1.51 = -log 1 : 32, 1.81 = -log 1 : 64).

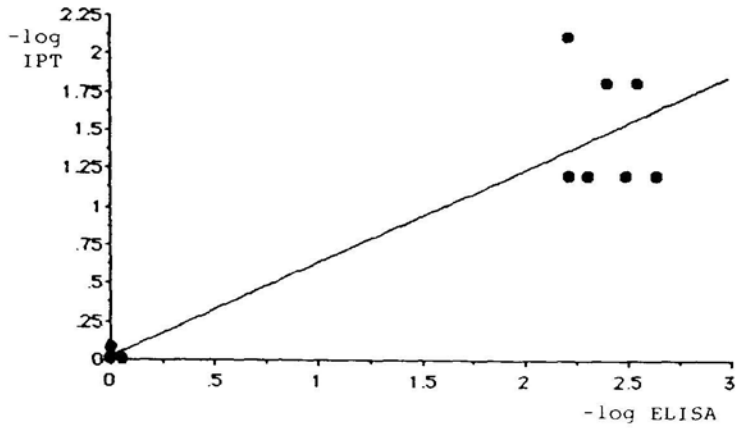

Fig. 6. Correlation of IgM-titers against *Borrelia burgdorferi* in IPT and ELISA after chromatographic isolation of the IgM-fraction ($n = 10$, $r = 0.808$, $p < 0.005$).

In every 10 sera with high IgG-or IgM-titers in IPT (-log $\geq$ 2.11 or $\geq$ 1.51 resp.) and ELISA (-log $\geq$ 2.95), no significant amounts of IgA-antibodies could be detected (-log $<$ 1.20 in IPT, -log $<$ 2.71 in ELISA).

Discussion

For the detection of IgG-antibodies against *Borrelia burgdorferi* the IPT and IFT give identical results. Differences to the ELISA technique are small, as are those between IFT and ELISA reported by others (3,6). The limit for positive IgG-titers in ELISA (-log $\geq$ 2.95) derived from a study in 1000 healthy controls (7), corresponds to IgG-titers of $\geq$ 1 : 128 (-log $\geq$ 2.11) in IPT.

So far, the determination of IgM-antibodies to *Borrelia burgdorferi* is difficult with all three of the methods, the results not always being reliable. Our studies with isolated IgM-fractions show that the results may be influenced by other components of the sera, as rheumatoid factors, antinuclear antibodies, circulating immune complexes and eventually a high amount of IgG-antibodies to *Borrelia burgdorferi* – as known in the serology of syphilis. Pretreatment of the sera by absorption of IgG may improve the reliability of IgM-determinations. Limits for IgM-titers have still to be established.

The fact that no circulating IgA antibodies against *Borrelia burgdorferi* could be detected may be due to the route of infection, which is transepidermal by a tick or insect bite and usually does not involve mucous membranes. Further studies are needed to support this hypothesis.

In view of our results, we recommend the IPT as a simple screening assay for the reliable detection of antibodies against *Borrelia burgdorferi*. Compared to IFT, the reading of the results is performed with a simple light microscope. Moreover, the slides are stable for a longer period without loss of the staining intensity.

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