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Evaluation of a new serological test for the detection of anti-*Coxiella* and anti-*Rickettsia* antibodies

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Abstract

Coxiella burnetii and members of the genus *Rickettsia* are obligate intracellular bacteria. Since cultivation of these organisms requires dedicated techniques, their diagnosis usually relies on serological or molecular biology methods. Immunofluorescence is considered the gold standard to detect antibody-reactivity towards these organisms. Here, we assessed the performance of a new automated epifluorescence immunoassay (InoDiag) to detect IgM and IgG against *C. burnetii*, *Rickettsia typhi* and *Rickettsia conorii*.

Samples were tested with the InoDiag assay. A total of 213 sera were tested, of which 63 samples from Q fever, 20 from spotted fever rickettsiosis, 6 from murine typhus and 124 controls. InoDiag results were compared to micro-immunofluorescence.

For acute Q fever, the sensitivity of phase 2 IgG was only of 30% with a cutoff of 1 arbitrary unit (AU). In patients with acute Q fever with positive IF IgM, sensitivity reached 83% with the same cutoff. Sensitivity for chronic Q fever was 100% whereas sensitivity for past Q fever was 65%. Sensitivity for spotted Mediterranean fever and murine typhus were 91% and 100%, respectively. Both assays exhibited a good specificity in control groups, ranging from 79% in sera from patients with unrelated diseases or EBV positivity to 100% in sera from healthy patients.

In conclusion, the InoDiag assay exhibits an excellent performance for the diagnosis of chronic Q fever but a very low IgG sensitivity for acute Q fever likely due to low reactivity of phase 2 antigens present on the glass slide. This defect is partially compensated by the detection of IgM. Because it exhibits a good negative predictive value, the InoDiag assay is valuable to rule out a chronic Q fever. For the diagnosis of rickettsial diseases, the sensitivity of the InoDiag method is similar to conventional immunofluorescence.

Keywords: Multiplexed serology; Immunofluorescence; *Coxiella burnetii*; *Rickettsia typhi*; *Rickettsia conorii*

1. Introduction

Coxiella burnetii, *Rickettsia typhi* and *Rickettsia conorii* are the causative agents of Q fever, murine typhus and Mediterranean spotted fever, respectively [18,5,16]. These obligate intracellular bacteria are difficult to diagnose and culture-based methods are restricted to specialized BSL3 laboratories. PCR-based detection methods have been described but are essentially found in larger reference diagnostic laboratories [15,17]. Therefore, screening for Q fever and rickettsial diseases often relies on serologic techniques such as Enzyme-linked immunosorbent assays (ELISA), Western-blots (WB) and immunofluorescence assays (IF). Serology is especially useful for the diagnosis of chronic Q fever, in particular when a biopsy of the affected organ (e.g. cardiac valve, liver) is not

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available. *C. burnetii* serological diagnosis is based on the presence of antibodies against phase 2 and phase 1 antigens, for the diagnosis of acute and chronic Q fever, respectively. The serological diagnosis of rickettsial infections is more complex, because of the common occurrence of cross-reactions between the different spotted fever group rickettsia. Conventional IFs are labor-intensive and reading of the assay is operator-dependent. Recently, a new innovative automated epifluorescence assay has been made available by InoDiag (Signes, France) for the diagnosis of *C. burnetii*, *R. typhi* and *R. conorii*. This multiplexed antigen microarray uses standardized quantities of antigens, spotted on glass slides with appropriate controls [9]. Except for the initial deposition of the serum samples, all subsequent steps (i.e. secondary antibody depositions, incubations, washing, drying, reading and interpretation) are performed automatically. This assay has previously been shown to be a promising tool for the serodiagnosis of *Chlamydia trachomatis* infection, culture-negative endocarditis and atypical pneumonia [8,9,3].

In the present study, we compared the performance of the automated InoDiag serological test with a gold standard indirect micro-immunofluorescence technique on sera taken from patients with serologically and clinically proven Q fever or rickettsial diseases, as well as on sera from pregnant women and control patients.

2. Materials and methods

2.1. Patient population and controls

A total of 213 sera were studied. Tested sera from acute, chronic or past Q fever infections as well as rickettsiosis cases that were positive for IgM and/or IgG using a reference indirect micro-immunofluorescence technique (described below) were selected from the routine serological laboratories of Lausanne and Sion, Switzerland (seroconversion, clinically confirmed or compatible cases as well as sera from previous studies [2,6,13]). This included 20 sera from spotted fever rickettsiosis (11 spotted Mediterranean fever, 9 African tick-bite fever), 6 sera from murine typhus, 63 sera from Q fever (12 acute, 22 chronic, 29 past infections). Clinical cases corresponding to some of these sera have been previously published [1,4,10,11]. A total of 124 control samples were included. These consisted in 10 samples from EBV IgM+ sera, 101 sera from pregnant women and 13 sera from patients with unrelated infections.

2.2. InoDiag

The tested InoDiag assay is a fully automated multiplexed immunofluorescent assay consisting of glass slides spotted with nanolitre spots of antigens of *R. typhi*, *R. conorii*, *Rickettsia felis*, and *C. burnetii* antigens (Fig. 1). The sensitivity of the assay was not evaluated for *R. felis*, the causative agent of cat-flea typhus. Slides also contain four control spots: (i) *Staphylococcus aureus* ATCC 29213 to assess serum deposition, (ii) human IgG to confirm the adequate distribution of the secondary anti-human IgG antibody, (iii) human IgM to confirm the adequate distribution of the secondary anti-human IgM antibody and detect the eventual presence of rheumatoid factor, (iv) double-stranded DNA to detect antinuclear antibodies. Serum samples were diluted at a ratio of 1:16 and applied on the slide. All subsequent steps were performed automatically as previously described [6]. After the final drying step, slides were imaged with an automated InoDiag fluorescent camera analyzer and the data processed using the software Inosoft (InoDiag). The end-point is a fluorescence index. For *C. burnetii*, two-cutoffs for positivity were considered: a fluorescence index > 1 arbitrary units (AU) and >2 AU. For *Rickettsia*, only the cutoff of 2 AU was considered. Receiver operating characteristics (ROC) curves were used to precise the diagnosis performance of the assay.

2.3. Indirect micro-immunofluorescence

Sera were tested for the presence of antibodies directed against *C. burnetii* or *Rickettsia* spp. using indirect micro-immunofluorescence (IF). Briefly, sera were screened at 1:20 to 1:80 dilution in two-fold steps using *C. burnetii* phase I and II antigens (strain Nine Miles, kindly provided by Dr W. Burgdorfer, Rocky Mountain Laboratories, Hamilton, USA), *R. conorii* and *R. typhi* (kindly provided by Dr W. Burgdorfer, Rocky Mountain Laboratories, Hamilton, USA) and *Rickettsia africae* (kindly provided by Dr D. Raoult and Dr. P-E. Fournier, Marseille, France). We used fluorescein isothiocyanate goat anti-human specific IgG and IgM conjugates (Bio-Mérieux, Marcy-l'Etoile, France). Positive sera were then diluted in two-fold steps from 1:20 to final dilutions.

3. Results

3.1. Performance of the InoDiag assay in the Q fever group

The assay has been evaluated for the three categories of Q fever disease (acute, chronic and past infection). The sensitivity and specificity for *C. burnetii* of the InoDiag assay for the various tested groups are shown in Table 1. In the acute Q fever group (n = 12), 100% (12/12) and 83% (10/12) sera were positive by IF for phase 2 IgM and phase 2 IgG, respectively. The sensitivity of the InoDiag assay for acute Q fever with positive IgG detected by IF (irrespective of the presence of IgM) was of 20% (2/10) and 30% (3/10) with cutoffs of 2 and 1 AU, respectively. Considering sera positive in IgM by IF in patients with acute Q fever, the sensitivity reached 75% (9/12) and 83% (10/12) with cutoffs of 2 AU and 1 AU, respectively. Two early seroconversions that were detected by IF already on the first sera (one positive for both IgG and IgM; one positive for phase 2 IgM only) were missed by the InoDiag assay.

In the chronic Q fever group, 22 sera were positive for IgG against both phase 1 and phase 2 antigens by IF. Among these, the InoDiag test showed a sensitivity of 100% (22/22) for IgG detection. Of note, 4 sera exhibited traces of IgM with the IF.