

products were detected by agarose gel electrophoresis.

Results and Discussion. This prospective study confirmed the high incidence of septicaemia caused by MRSA in most parts of Europe. Methicillin resistance occurred in 0 to 50% of the *Staphylococcus aureus* strains isolated from blood cultures in each of the centres, with an overall proportion of 25% (170/676).

The agreement between the C-MRSA ID test and the reference test to detect the *mecA* gene, together with the sensitivity, specificity, and positive and negative predictive values of the C-MRSA ID, are presented in Table 1. A 97.8% correlation was observed between the results of both tests. The specificity, percentage agreement, positive predictive value, and negative predictive value were very satisfactory (> 95%). The sensitivity (93%) was also satisfactory but probably could be improved by an additional 1 h period of incubation, as suggested previously (5). Upon repeated testing, correct results were obtained for 13 of the 15 isolates from which results were initially discrepant.

However, two MRSA strains failed to produce a positive result, even after 5 h of incubation. One *mecA* gene-positive isolate was sensitive to the sodium chloride content of the Mueller-Hinton medium (agar or broth). The minimum inhibitory concentration of oxacillin for this isolate was > 4 mg/l or 0.06 mg/l, respectively, depending on the absence or presence of 4% sodium chloride. The other *mecA* gene-positive isolate demonstrated heterogeneous resistance to oxacillin detectable only with Mueller-Hinton agar containing 5% NaCl.

Since infection with MRSA can be extremely difficult to treat, the rapid identification of MRSA by the C-MRSA ID system may aid in the rational management of these infections in hospitals and in the prevention of MRSA spread. Technically, this test is easy to perform and to integrate into routine diagnostic workflow. It represents a useful alternative to other rapid antimicrobial susceptibility test systems (6–8) and molecular methods, especially since it does not require expensive equipment for interpretation.

References

1. Chambers HF: Methicillin-resistant staphylococci. *Clinical Microbiology Review* 1988, 1: 173–186.
2. Matsushashi M, Song MD, Ishino F, Wachi M, Doi M, In-

oue M, Ubukata K, Yamashita N, Konno M: Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to β -lactam antibiotics in *Staphylococcus aureus*. *Journal of Bacteriology* 1986, 167: 975–980.

3. Hartman BJ, Tomasz A: Low-affinity penicillin binding protein associated with β -lactam resistance in *Staphylococcus aureus*. *Journal of Bacteriology* 1984, 158: 513–516.
4. Zambardi G, Reverdy ME, Bland S, Bes M, Freney J, Fleurette J: Laboratory diagnosis of oxacillin resistance in *Staphylococcus aureus* by a multiplex polymerase chain reaction assay. *Diagnostic Microbiology of Infectious Diseases* 1994, 19: 25–31.
5. Knapp CC, Ludwig MD, Washington JA: Evaluation of BBL Crystal MRSA ID system. *Journal of Clinical Microbiology* 1994, 32: 2588–2589.
6. Woods GL, Latemple D, Cruz C: Evaluation of MicroScan rapid Gram-positive panels for detection of oxacillin-resistant staphylococci. *Journal of Clinical Microbiology* 1994, 32: 1058–1059.
7. Struelens MJ, Nonhoff C, Van der Auwera P, Mertens F, Serruys E: Evaluation of Rapid ATB Staph for 5-hour antimicrobial susceptibility testing of *Staphylococcus aureus*. *Journal of Clinical Microbiology* 1995, 33: 2395–2399.
8. Knapp CC, Ludwig MD, Washington JA: Evaluation of differential inoculum disk diffusion method and Vitek GPS-SA card for detection of oxacillin-resistant staphylococci. *Journal of Clinical Microbiology* 1994, 32: 433–436.

Comparison of Immunofluorescence with Enzyme Immunoassay for Detection of Q Fever

S. Cruchaga D'Harcourt, A. Buño Soto, V. Cascante Burgos, D. Lozano Calero, R. Martínez-Zapico*

To evaluate enzyme immunoassay (EIA) as an alternative to indirect immunofluorescence assay (IFA) to screen for Q fever in humans, 157 serum samples from patients suspected of having the disease were tested for immunoglobulin G antibodies

Microbiology Department, La Paz University Hospital, Paseo de la Castellana, 261, 28046 Madrid, Spain.

to *Coxiella burnetii*. The agreement between the tests and the sensitivity of EIA were excellent (96.8% and 98.4%, respectively) when an IFA titer of $> 1/160$ was considered positive. All serum samples with a titer of $> 1/320$ in the IFA were also positive by the EIA. The EIA seems to be an acceptable alternative to IFA for screening for Q fever.

Q fever, a zoonosis first described by Derrick (1) in 1937, is caused by the obligate intracellular bacterium *Coxiella burnetii*. High concentrations of this organism are found in the urine, feces, milk, and birth products of infected animals (cattle, sheep, goats). It has a spore-like cycle, which explains its high resistance to external agents and its extraordinary virulence. Inhalation of contaminated aerosols is the most frequent means of transmission to humans (2). There is no typical form of acute Q fever, and in about 50% of cases, exposure to *Coxiella burnetii* leads to asymptomatic infection. The chronic form, which is characterized by endocarditis, occurs in approximately 2% of cases.

Changes in the surface lipopolysaccharide of *Coxiella burnetii* result in an antigen shift called "phase variation". Infections in humans produce characteristic serological profiles. Anti-phase II antibodies predominate during acute Q fever, whereas high levels of anti-phase I antibodies exceed those of anti-phase II in chronic Q fever (3).

Because of the hazards associated with culture of *Coxiella burnetii*, diagnosis of Q fever is based mainly on clinical signs and is confirmed by demonstration of specific anti-*Coxiella burnetii* antibodies in serum. Therefore, an accurate serological test is essential to diagnose Q fever and to detect antibodies in both phase I and II. Three serological techniques are commonly used: complement fixation (CF), the indirect immunofluorescence assay (IFA), and the enzyme immunoassay (EIA). Complement fixation is very specific but lacks sensitivity and is laborious. The IFA remains the reference method for serological diagnosis and is capable of distinguishing between the various immunoglobulin isotypes produced following Q fever infection (4). Although this method is both highly specific and sensitive, it must be performed by an experienced technician. The IFA is not considered the optimal test for epidemiologic surveys of *Coxiella burnetii* infection because it is subjective and not easily adapted to test a large number of specimens. On the other hand, EIA is highly sensitive, is easy to perform, has the

potential for being adapted for automation, can be applied in epidemiologic surveys, and has been shown to be of value for the serological diagnosis of acute and chronic Q fever (3, 5–7).

The present study was undertaken to compare indirect EIA with IFA for detection of immunoglobulin G (IgG) antibodies to *Coxiella burnetii* phase II and to assess the usefulness of EIA in screening for Q fever in humans.

Materials and Methods. One hundred fifty-seven serum samples from patients suspected by our hospital clinicians to have Q fever were studied retrospectively. The most common clinical symptoms were pneumonia and prolonged fever. Sera were stored at -20°C (no longer than one month) until tested by EIA.

We considered the IFA the reference technique. It was based on standard fluorescent antibody methods. A serum specimen was placed on a *Coxiella burnetii*-spot IF substrate slide (bioMérieux, France), which was coated with phase II antigens obtained from culture on Vero cells. The antibody titer was the reciprocal of the highest dilution giving definitive specific fluorescence. Positive and negative control sera were included in each test run. A positive IFA result was indicated by a titer of $> 1/80$.

An indirect EIA was used to detect the presence of specific anti-phase II IgG antibodies to *Coxiella burnetii* in serum specimens. When present, these antibodies combine with a soluble *Coxiella burnetii* phase II antigen attached to the polystyrene surface of the microwell test strips (PanBio, Australia). The assays were performed according to the manufacturer's instructions. Positive and negative control sera as well as cut-off calibrators were included in each test run. Samples were prepared at a 1:100 dilution. Optical density was measured at 450 nm in a microplate reader (Bio-Whittaker, USA). When the ratio between specimen absorbance and cut-off absorbance was above 1.1, the EIA result was considered positive. Ratios between 0.9 and 1.1 were considered doubtful and were reassayed to exclude experimental error.

Statistical analyses included computations of frequencies, cross-tabulation, and testing for agreement between the IFA and EIA assays. Sensitivity (number of specimens positive by both methods/total number of positive specimens tested) and specificity (number of specimens negative by both methods/total number of negative specimens tested) were calculated. Total correct classi-

Table 1: Frequency distribution of the samples according to the indirect immunofluorescence (IFA) test titers and results obtained by enzyme immunoassay (EIA).

IFA test titer	No. of serum samples (%)	No. positive by EIA (%)	No. negative by EIA (%)
Negative	73 (46.5)	1 (1.4)	72 (98.6)
1/80	20 (12.7)	3 (15)	17 (85)
1/160	9 (5.7)	8 (89)	1 (11)
1/320	34 (21.7)	34 (100)	0
1/640	8 (5.1)	8 (100)	0
1/1280	6 (3.8)	6 (100)	0
1/2560	2 (1.3)	2 (100)	0
> 1/5120	5 (3.2)	5 (100)	0

Table 2: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and agreement of the enzyme immunoassay, considering three titers of the indirect immunofluorescence assay (IFA).

IFA titer	Sensitivity	Specificity	PPV	NPV	Agreement
1/80	78.6 %	98.6 %	98.5 %	80 %	87.9 %
1/160	98.4 %	95.7 %	94 %	98.9 %	96.8 %
1/320	100 %	88.2 %	82.1 %	100 %	92.4 %

fication (agreement between IFA and EIA) was calculated as the percentage of all patients who were correctly identified by both tests. The positive predictive value of the EIA determined the likelihood that a patient diagnosed with infection caused by *Coxiella burnetii* by means of IFA would be confirmed by EIA.

Results and Discussion. We studied the frequency distribution of 157 serum samples according to the IFA titers and EIA results (Table 1). When the IFA cut-off value was set at a titer of 1/80, the agreement between the tests was 87.9%. The sensitivity of EIA was 78.6%, the specificity 98.6%, and the positive and negative predictive values 98.5% and 80%, respectively. The highest agreement occurred when we set a titer of 1/160 as the cut-off value; with this cut-off, the sensitivity and specificity were 98.4% and 95.7%, respectively. The sensitivity obtained at a cut-off value of > 1/320 with IFA was 100%, indicating that no sample with a significant level of antibodies should be missed with EIA (Table 2).

An EIA for the detection of *Coxiella burnetii*-specific IgM was first described by Field et al. (7) in 1983. Since then, several studies comparing EIA with IFA have demonstrated good correlation between the two methods (8–11). Döller et al. (12) concluded that EIA is useful for the diagnosis of acute cases. Peter et al. (8) reported that EIA is the test of choice for epidemiological surveys and an excellent diagnostic test for chronic Q fever (13).

In our study we found excellent correlation between these techniques, as documented previously. However, 85% of the samples with titers of 1/80 by IFA were negative by EIA, which lowers the sensitivity of the latter technique to 78.6%. Considering the high prevalence of *Coxiella burnetii* infection in our area (14, 15), these low titers determined by IFA might be due to residual antibodies of past infection that were not detectable by EIA.

In conclusion, we suggest that EIA is an acceptable alternative to IFA for screening for human *Coxiella burnetii* infection. It is sensitive, adaptable for automation, easy to perform and correlates well with the IFA method. We intend to reassay the positive results with EIA, using IFA to confirm the antibody levels.

References

- Derrick EH: Q fever, a new fever entity: clinical features, diagnosis and laboratory investigation. *Medical Journal of Australia* 1937, 2: 281–299.
- Marrie TJ: *Coxiella burnetii* (Q fever). In: Mandell GL, Douglas RGJ, Bennett JE (ed): *Principles and practice of infectious diseases*. Churchill Livingstone, New York, 1995, p. 1727–1735.
- Raoult D, Marrie T: Q fever. *Clinical Infectious Diseases* 1995, 20: 489–496.
- Peacock MG, Philip RN, Williams MS, Faulkner RS: Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. *Infection and Immunity* 1983, 41: 1089–1098.

5. Roges G, Edlinger E: Immunoenzymatic test for Q fever. *Diagnostic Microbiology and Infectious Diseases* 1986, 4: 125–132.
6. Behymer DE, Ruppner R, Brooks D, Williams JC, Franti CE: Enzyme immunoassay for surveillance of Q fever. *American Journal of Veterinary Research* 1985, 46: 2413–2417.
7. Field PR, Hunt JG, Murphy AM: Detection and persistence of specific IgM antibody to *Coxiella burnetii* by enzyme-linked immunosorbent assay. A comparison with immunofluorescence and complement-fixation tests. *Journal of Infectious Diseases* 1983, 148: 477–487.
8. Peter O, DuPuis M, Peacock MG, Burgdorfer W: Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody test for detection of *Coxiella burnetii* antibody. *Journal of Clinical Microbiology* 1987, 25: 1063–1067.
9. Cowley R, Fernandez F, Freemantle W, Rutter D: Enzyme immunoassay for Q fever: comparison with complement fixation and immunofluorescence test and dot immunoblotting. *Journal of Clinical Microbiology* 1992, 30: 2451–2455.
10. Roges G, Edlinger E: Immunoenzymatic test for Q-fever. *Diagnostic Microbiology and Infectious Diseases* 1986, 4: 125–132.
11. Uhaa IJ, Fishbein DB, Olson JG, Rives CC, Waag DM, Williams JC: Evaluation of specificity of indirect enzyme-linked immunosorbent assay for diagnosis of human Q fever. *Journal of Clinical Microbiology* 1994, 32: 1560–1565.
12. Döller G, Döller PC, Gerth HJ: Early diagnosis of Q fever: detection of immunoglobulin M by radioimmunoassay and enzyme immunoassay. *European Journal of Clinical Microbiology* 1984, 3: 550–553.
13. Peter O, Dupuis G, Bee D, Luthy R, Nicolet J, Burgdorfer W: Enzyme-linked immunosorbent assay for diagnosis of chronic Q fever. *Journal of Clinical Microbiology* 1988, 26: 1978–1982.
14. Tellez A, Martin A, Anda P, de la Fuente L, Benitez P, Garcia C, León P: Study of *Coxiella burnetii* human and animal seroprevalence in a rural population in Madrid community. *European Journal of Epidemiology* 1989, 5: 444–446.
15. Ruiz-Beltrán R, Herrero-Herrero JI, Martín-Sánchez AM, Martín-González JA: Prevalence of antibodies to *Rickettsia conorii*, *Coxiella burnetii*, and *Rickettsia typhi* in Salamanca province (Spain). Serosurvey in the human population. *European Journal of Epidemiology* 1990, 6: 293–299.

Evaluation of an Antigen-Capture Enzyme Immunoassay for Detection of *Entamoeba histolytica* in Stool Samples

T. Jelinek, G. Peyerl, T. Löscher, H.-D. Nothdurft

In order to identify the prevalence of *Entamoeba histolytica* in tourists with diarrhoea returning from countries of the developing world, sensitivity and specificity of a commercially available enzyme immunoassay (EIA) kit for the detection of *Entamoeba histolytica* coproantigen in stool were evaluated. Five hundred seventy-seven specimens from 469 patients were examined by microscopy and EIA. Sixty-two specimens from 49 patients were considered positive for *Entamoeba histolytica*. Compared with microscopic examination of stool samples, the EIA was found to be slightly more sensitive (90.3% vs. 87.1%) and was 97.7% specific for *Entamoeba histolytica*.

Diagnosis of intestinal amoebiasis is based on the demonstration of *Entamoeba histolytica* in stool or in biopsy of mucosal tissue but is labour and time intensive and depends on the skill of an experienced microscopist (1, 2). It has recently been suggested that there are two distinct species of *Entamoeba histolytica* that are morphologically identical (1). *Entamoeba dispar*, the more prevalent of the two forms, appears to be associated solely with an asymptomatic carrier state. The pathogenic species, now referred to as *Entamoeba histolytica* sensu strictu, appears to have the capacity to invade tissue and cause symptomatic disease. Microscopical examination of a single stool specimen yields a sensitivity of 50 to 70%. At least three separate stool samples are required for a sensitivity of 90% (1). It is essential to have permanent stains of fresh or fixed faecal specimens in addition to a saline wet mount of stool (1). Specimens