Eur. J. Clin. Microbiol. Infect. Dis., 1997, 16: 648–652

Evaluation of Commercial Slides for Detection of Immunoglobulin G against *Bartonella henselae* by Indirect Immunofluorescence

R. Zbinden[1]*, N. Michael[1], M. Sekulovski[1], A. von Graevenitz[1], D. Nadal[2]

Four commercial slides were compared with in-house slides for the detection of immunoglobulin G (IgG) against *Bartonella henselae* in 58 healthy persons from a rural region by an indirect immunofluorescence assay. MRL-BA slides (MRL Diagnostics, USA) and Virion slides (Virion, Switzerland) with agar-derived *Bartonella henselae* showed IgG titers of ≥ 1:256 in 44.8% and 51.7%, respectively, whereas Bion slides (Bios, Germany), MRL-Vero slides (MRL Diagnostics), and in-house slides with cell-associated *Bartonella henselae* showed such titers in 3.4%, 5.1% and 3.4%, respectively. The MRL-Vero slides (*Bartonella* IgG substrate slides, MRL Diagnostics) were further evaluated with 26 patients with cat scratch disease, 20 patients with lymphadenopathy not due to cat scratch disease, 100 blood donors from an urban area, and 120 blood donors from a mixed urban/rural area. In our mixed urban/rural population the IgG titer of 1:256 had a sensitivity of 84.6% and a specificity of 93.4% for the serodiagnosis of cat scratch disease. Seroprevalence was higher in blood donors from the mixed area (50.8%) than from the urban area (37%). MRL-Vero slides were considered useful for the serodiagnosis of cat scratch disease by indirect immunofluorescence and have replaced our in-house system. However, patients with low IgG titers should be retested three to four weeks after initial sampling to demonstrate a possible rise of IgG titers in paired sera.

Cat scratch disease (CSD) has traditionally been diagnosed in patients with regional lymphadenopathy if three of the following criteria are met: contact with a cat and the presence of a scratch or a primary lesion; negative studies for other possible causes of lymphadenopathy; a positive skin test; and characteristic histopathological findings (1). By means of culture and the polymerase chain reaction (PCR), *Bartonella henselae* has been recently recognized as the major causative agent of CSD (2, 3). The CSD skin test has been shown to have a lower sensitivity than PCR for *Bartonella henselae* and may, for this and for safety reasons, have lost its usefulness (4). Histopathological analyses are only possible following surgical biopsy. Serology has made important contributions to recognition of the causative agent (5), epidemiological studies (6), and a better delineation of the clinical spectrum of CSD (7). Thus, serology may become the simplest and most feasible way to diagnose CSD, provided the tests are reliable (8).

We recently demonstrated the intracellular location of *Bartonella henselae* after cocultivation with Vero cells. Fixed slides with monolayers of Vero cells with intracellular *Bartonella henselae* were used in an indirect immunofluorescence assay (IFA) for detecting immunoglobulin G (IgG) to *Bartonella henselae* (9). The workload associated with cocultivation of *Bartonella henselae* with Vero cells on chamber slides for the in-house IFA was considered reasonable as long as no alternatives were available. In the meantime, several commercial slides for IFA have been developed for the detection of IgG to *Bartonella henselae*. In the present study we compared our in-house IFA with four commercial IFAs. Furthermore, we determined the diagnostic value of one commercial IFA with Vero cell-associated *Bartonella henselae*.

[1] Department of Medical Microbiology, University of Zurich, Gloriastrasse 32, CH-8028 Zurich, Switzerland.
[2] Infectious Diseases Unit, University Children's Hospital, CH-8032 Zurich, Switzerland.

Table 1: Immunoglobulin G titers to *Bartonella henselae* of 58 persons from the rural region determined by indirect immunofluorescence with four commercial slides and an in-house slide.

	Agar-derived		Cell-associated		
Titer	MRL-BA	Virion	Bion	MRL-Vero	In-house
< 1:64	6	7	20	25	24
1:64	12	7	27	20	29
1:128	14	14	9	10	3
1:256	7	9	1	2	2
1:512	14	16	1	1	
1:1024	3	4			
1:2048	2	1			

Material and Methods

Serum Specimens. Fifty-eight sera (supplied by J. Zihler in 1994, stored at –20°C) from individuals living in a rural region in central Switzerland were tested for IgG antibodies to *Bartonella henselae* with four commercial IFAs and the results compared with those obtained with our in-house system. The individuals had consulted their physicians because of symptoms not suggestive of CSD. Thirty-three of them were cat owners. Eight sera from four patients with lymphadenopathy who, after cat contact, presented a clear rise of IgG against *Bartonella henselae* with the in-house IFA, were included in this comparison.

Sera collected from 1994 to 1996 from 22 patients with CSD (age range, 3–35 years; mean, 13 years), 20 immunocompetent children with lymphadenopathy without CSD, 100 blood donors from Zurich (urban area; supplied by P. Schenker in 1994), and 120 blood donors from Aarau (mixed urban/rural area; supplied by K. Giger in 1994) were tested with a commercial cell-associated IFA. Sera were stored at –20°C. Sera from 15 of the 22 patients with CSD and from the blood donors had been tested previously with the inhouse system (10). The remaining seven patients with CSD had either subacute lymphadenitis with typical histopathological changes or a positive PCR for *Bartonella henselae.*

Indirect Immunofluorescence Assay. Immunoglobulin G to *Bartonella henselae* was detected by an IFA with in-house slides and the following four commercial slides: MRL-BA slides (prototype #4, lot #R017194; MRL Diagnostics, USA); Virion slides (experimental lot #B:381718.09; Institut Virion, Switzerland) containing blood agar-derived *Bartonella henselae;* MRL-Vero slides (lot #R049395, lot #R067095, and lot #R028196, *Bartonella* IgG substrate slides, MRL Diagnostics); and Bion slides (Bios, Germany) containing cell-associated *Bartonella henselae.* Each of the MRL-BA and MRL-Vero slides with eight wells contained one test field with *Bartonella henselae* and one test field with *Bartonella quintana.* Results of the IFA for *Bartonella quintana* were not evaluated. The in-house slides were based on intracellular *Bartonella henselae* cocultivated with Vero cells as described previously (9). In brief, *Bartonella henselae* (strain G-5436; kindly provided by R. Weyant, Centers for Disease Control and Prevention, Atlanta, USA) was cocultivated with Vero cells in tissue chamber slides. After two days the slides were fixed with acetone-ethanol (1:1) and used for the IFA.

Sera were overlaid onto the slides at dilutions of 1:64 (if necessary, up to 1:2048) and allowed to rest for 30 min at 37°C. After washing, IgG was detected by a fluorescein-isothiocyanate (FITC)-anti-human IgG conjugate for 30 min at 37°C. For Virion slides the conjugate (lot 396318.02; Virion) was diluted 1:10; the conjugates for MRL-BA and MRL-Vero slides (goat anti-human IgG FITC; Tago Immunologicals, Biosource International, USA) and for Bion and in-house slides (Fluoline G; bioMérieux, Switzerland) were diluted 1:100. After incubation for 30 min the slides were washed and examined with a fluorescence microscope. The endpoint titer was defined as the dilution that still presented a specific fluorescence.

Statistical Calculations. The optimal cut-off for the commercial Vero cell-associated IFA evaluated was chosen according to the balance desired between sensitivity and specificity (11).

Results

The comparison of commercial with in-house slides for the determination of IgG titers to *Bartonella henselae* by IFA is shown in Table 1. Among the 58 individuals from the rural region, titers of ≥ 1:256 were detected with the agar-derived MRL-BA and Virion slides in 26 (44.8%) and 30 (51.7%) sera and titers of < 1:64 in six (10.3%) and seven (12%) sera, respectively. In contrast, when employing Bion, MRL-Vero, and in-house slides with cell-associated *Bartonella henselae,* titers of ≥ 1:256 were found in two (3.4%), three (5.1%), and two (3.4%) sera and titers of < 1:64 in 20 (34.5%), 25 (43.1%), and 24 (41.5%) sera, respectively. With Bion slides 97% of the titers were within one $\log_2$ dilution of the corresponding titers obtained with the in-house system; with MRL-Vero slides all titers were within one $\log_2$ dilution and 72.4% were identical to those of the in-house system. However, with the two commercial slides with agar-derived *Bartonella henselae,* 60.3% of all sera had titer differences of two or more $\log_2$ dilutions compared to titers obtained with the in-house IFA.

Thirty-three of the 58 individuals were cat owners. Using any slides with cell-associated *Bartonella henselae,* cat owners more frequently had titers of > 1:64 than persons without cats ($p < 0.05$,

Table 2: Comparison of immunoglobulin G titers in paired sera from four patients with cat scratch disease. Intervals between sera a and b were 42, 21, 24, and 14 days in patients 1, 2, 3, and 4, respectively.

		IgG titer				
		MRL-BA	Virion	Bion	MRL-Vero	In-house
Patient 1	a	1:128	1:256	1:512	1:512	1:256
	b	1:256	1:256	1:2048	1:1024	1:1024
Patient 2	a	1:64	nd	1:128	1:512	1:128
	b	1:128	nd	1:512	1:512	1:512
Patient 3	a	1:64	1:128	1: < 64	1:128	1:64
	b	1:128	1:512	1:256	1:1024	1:1024
Patient 4	a	1:128	1:128	1:64	1:128	1:128
	b	1:256	1:256	1:256	1:1024	1:512

Fisher's exact test; data not shown). With MRL-BA and Virion slides, the median titers were 1:256 in cat owners and 1:128 in persons without cats; titers of 1:512 were more frequent in cat owners ($p < 0.05$, Fisher's exact test). Since a rise of IgG is a good marker for an ongoing infection, we included four pairs of sera in this comparison (Table 2). The slides with blood agar-derived *Bartonella henselae* showed in only one patient a four-fold titer rise in paired sera and a higher titer than the corresponding median titer of cat owners. The Bion and MRL-Vero slides showed higher titers than the median titers of cat owners in all four patients.

The diagnostic value of the commercial Vero cell-associated MRL slides was further evaluated. Table 3 shows the IgG titers of 26 patients (including the first sera of the 4 patients from Table 2) with CSD and controls. All control patients with lymphadenopathy without suspected CSD had titers of < 1:64, except one who had a titer of 1:128. Fourteen of 220 blood donors had titers of 1:256 and one a titer of 1:512. Blood donors from the mixed urban/rural area more frequently had IgG titers (50.8%) than blood donors from the urban area (37%) ($p < 0.05$, Fisher's exact test). For the serodiagnosis of CSD in our mixed urban/rural population, the IgG titer of 1:256 had a sensitivity of 84.6% and a specificity of 93.4%.

Discussion

In the present comparison of commercial IFA slides, those containing cell-associated *Bartonella henselae* revealed lower IgG titers than slides containing blood agar-derived bacilli. Four patients included in this comparison had IgG titers to *Bartonella henselae* similar to those of the normal rural population when the agar-derived slides were used. We therefore excluded these slides from consideration for replacement of our in-house system. The Vero cell-associated MRL slides showed in 72% the same titer as the in-house slides and were further evaluated for detection of IgG to *Bartonella henselae.*

Sera from patients with and without CSD and from blood donors from two different geograph-

Table 3: Immunoglobulin G titers to *Bartonella henselae* of patients with cat scratch disease (CSD), control patients, urban blood donors, and mixed urban/rural blood donors using the MRL-Vero slides. When a titer of 1:512 is used as the cut-off, sensitivity is 61.5% and specificity 99.6%; with 1:256 as the cut-off, sensitivity is 84.6% and specificity 93.4%; with 1:128 as the cut-off, sensitivity is 100% and specificity 83.4%.

IgG titer	Patients with CSD (n = 26)	Patients without CSD (n = 20)	Urban blood donors (n = 100)	Urban/rural blood donors (n = 120)
1: < 64		19	63	59
1:64			19	40
1:128	4	1	14	10
1:256	6		4	10
1:512	10			1
1:1024	6			

ical regions were included in this evaluation. To achieve a specificity of over 90%, the titer of 1:256 was chosen as the cut-off. The titer of 1:128 would have had a specificity below 85% but a sensitivity of 100%. Others have found that a cut-off titer of 1:100 with a cell-associated system is not sensitive enough (40.6%) to confirm a CSD diagnosis; the low sensitivity was attributed to blood sampling before an antibody increase had occurred (12). However, the positive predictive value of a titer of 1:100 with the same IFA was only 68.2% (13). A cell-associated IFA with the new *Bartonella henselae* serovariant "Marseille" revealed in three of 100 healthy blood donors a titer of 1:200, which was considered the cut-off titer (14). In a previous study, 11 patients with a *Bartonella henselae* infection (proven by PCR or characteristic histopathological findings) had titers of 1:256 or higher with the in-house IFA (15). In a further study with the in-house system, titers of 1:512 or higher were found in 20 of 20 children presenting with subacute lymphadenopathy and a history of recent cat scratch (10). In three of these CSD patients, the initial titers were only 1:128 and 1:256, but within three weeks a rise to 1:1024 was noted. By contrast, in only one of the 332 controls (including 43 persons from households of the patients) was a titer of 1:512 found (10).

Our most important observation was the difference in IgG titers obtained with the various commercial slides, depending on the antigen preparation. It was recently shown in one patient that titers to *Bartonella henselae* differed greatly, depending on the antigen preparation for the IFA: the patient had a titer of 1:12,800 against a sheep blood agar strain and a titer of 1:200 against the same strain cultivated on endothelial cells (14). A similar discrepancy was described for IgG antibodies against *Bartonella quintana* grown on sheep blood agar and human epithelial cells (16). The first IFA to detect IgG to *Bartonella henselae* was based on cocultivation with Vero cells to inhibit autoagglutination of *Bartonella henselae* (5). The present study showed that cocultivation improves the specific detection of IgG to *Bartonella henselae*. The reasons for this are unknown, but antigenic proteins could perhaps change during cocultivation. Such changes are suggested during phase variation (17). The intracellular location of *Bartonella henselae* in the two commercial cell-associated slides (MRL-Vero and Bion) can be assumed because the specific fluorescence had the same granular aspect as shown previously with our in-house slides (9).

Besides the antigen preparation of *Bartonella henselae*, epidemiological parameters affect the serodiagnosis of *Bartonella henselae* infection (18). In all IFA systems serum samples of people reporting cat contact had higher titers than sera of persons without cat contact. In line with our previous study of the in-house system (10), we also found by means of the MRL-Vero slides a higher seroprevalence in blood donors from the mixed urban/rural population than in those from the city. This could be explained by close cat contact in rural regions. Low IgG titers could relate to unapparent or past infection with *Bartonella henselae*. Finally, serodiagnosis can be influenced by cross-reacting antibodies against other bacterial antigens, e.g., *Bartonella* with *Coxiella* (19) and other α-2 protobacteria (20).

We conclude that, using the MRL-Vero slides, a titer of 1:256 is compatible with CSD in our mixed urban/rural population. For routine purposes, the MRL-Vero slides can replace our in-house system. Until tests to detect IgM to *Bartonella henselae* have been thoroughly evaluated, patients with clinical signs and symptoms of CSD plus low specific IgG titers should have paired sera drawn in intervals of three to four weeks in order to detect a possibly significant antibody rise. We believe that serology may replace traditional diagnostic criteria for CSD (8, 10), but histology and PCR may still be necessary in atypical clinical situations.

Acknowledgements

The authors thank V. Kaspar for technical assistance; J. Zihler, general practitioner, Unteriberg, Switzerland, for supplying sera from the rural population; K. Giger, Kantonsspital Aarau, Switzerland, for supplying sera of blood donors from the mixed rural/urban area; and, P. Schenker, Blutspendezentrum SRK Zurich, Switzerland, for supplying the sera of blood donors from the urban population.

References

1. Adal KA, Cockerell CJ, Petri WA: Cat scratch disease, bacillary angiomatosis, and other infections due to *Rochalimaea*. New England Journal of Medicine 1994, 330: 1509–1515.
2. Dolan MJ, Wong MT, Regnery RL, Jorgensen JH, Garcia M, Peters J, Drehner D: Syndrome of *Rochalimaea henselae* adenitis suggesting cat scratch disease. Annals of Internal Medicine 1993, 118: 331–336.
3. Anderson B, Sims K, Regnery R, Robinson L, Schmidt MJ, Goral S, Hager C, Edwards K: Detection of

Rochalimaea henselae DNA in specimens from cat scratch disease patients by PCR. Journal of Clinical Microbiology 1994, 32: 942–948.
4. Bergmans AMC, Groothedde JW, Schellekens JFP, van Embden JDA, Ossewaarde JM, Schouls LM: Etiology of cat scratch disease: comparison of polymerase chain reaction detection of *Bartonella* (formerly *Rochalimaea*) and *Afipia felis* DNA with serology and skin tests. Journal of Infectious Diseases 1995, 171: 916–923.
5. Regnery RL, Olson JG, Perkins BA, Bibb W: Serological response to *"Rochalimaea henselae"* antigen in suspected cat-scratch disease. Lancet 1992, 339: 1443–1445.
6. Zangwill KM, Hamilton DH, Perkins BA, Regnery RL, Plikaytis BD, Hadler JL, Cartter ML, Wenger JD: Cat scratch disease in Connecticut. Epidemiology, risk factors, and evaluation of a new diagnostic test. New England Journal of Medicine 1993, 329: 8–13.
7. Itin PH, Flückiger R, Zbinden R, Frei R: Recurrent pyogenic granuloma with satellitosis – a localized variant of bacillary angiomatosis? Dermatology 1994, 189: 409–412.
8. Dalton MJ, Robinson LE, Cooper J, Regnery RL, Olson JG, Childs JE: Use of *Bartonella* antigens for serologic diagnosis of cat-scratch disease at a national referral center. Archives of Internal Medicine 1995, 155: 1670–1676.
9. Zbinden R, Höchli M, Nadal D: Intracellular location of *Bartonella henselae* cocultivated with Vero cells and used for an indirect fluorescent-antibody test. Clinical and Diagnostic Laboratory Immunology 1995, 2: 693–695.
10. Nadal D, Zbinden R: Serology to *Bartonella (Rochalimaea) henselae* may replace traditional diagnostic criteria for cat-scratch disease. European Journal of Pediatrics 1995, 154: 906–908.
11. Rossing RG, Hatcher WE: A graphic method for the evaluation of diagnostic tests. Methods of Information in Medicine 1980, 19: 149–156.
12. Dupon M, Savin de Larclause A-M, Brouqui P, Drancourt M, Raoult D, de Mascarel A, Lacut J-Y: Evaluation of serological response to *Bartonella henselae, Bartonella quintana* and *Afipia felis* antigens in 64 patients with suspected cat-scratch disease. Scandinavian Journal of Infectious Diseases 1996, 28: 361–366.
13. Raoult D, Dupont HT, Enea-Mutillod M: Positive predictive value of *Rochalimaea henselae* antibodies in the diagnosis of cat-scratch disease. Clinical Infectious Diseases 1994, 19: 355.
14. Drancourt M, Birtles R, Chaumentin G, Vandenesch F, Etienne J, Raoult D: New serotype of *Bartonella henselae* in endocarditis and cat-scratch disease. Lancet 1996, 347: 441–443.
15. Goldenberger D, Zbinden R, Perschil I, Altwegg M: Nachweis von *Bartonella (Rochalimaea) henselae/B. quintana* mittels Polymerase-Kettenreaktion (PCR). Schweizerische Medizinische Wochenschrift 1996, 126: 207–213.
16. Drancourt M, Mainardi JL, Brouqui P, Vandenesch F, Carta A, Lehnert F, Etienne J, Goldstein F, Acar J, Raoult D: *Bartonella (Rochalimaea) quintana* endocarditis in three homeless men. New England Journal of Medicine 1995, 332: 419–423.
17. Anderson BE, Neuman MA: *Bartonella* spp. as emerging human pathogens. Clinical Microbiology Reviews 1997, 10: 203–219.
18. Amerein MP, de Briel D, Jaulhac B, Meyer P, Monteil H, Piemont Y: Diagnostic value of the indirect immunofluorescence assay in cat scratch disease with *Bartonella henselae* and *Afipia felis* antigens. Clinical and Diagnostic Laboratory Immunology 1996, 3: 200–204.
19. La Scola B, Raoult D: Serological cross-reactions between *Bartonella quintana, Bartonella henselae,* and *Coxiella burnetii.* Journal of Clinical Microbiology 1996, 34: 2270–2274.
20. Engbaek K, Koch C: Immunoelectrophoretic characterization and cross-reactivity of *Rochalimaea henselae, Rochalimaea quintana* and *Afipia felis.* Acta Pathologica, Microbiologica, et Immunologica Scandinavica 1994, 102: 931–942.