DNA Amplification for the Diagnosis of Cat-Scratch Disease in Small-Quantity Clinical Specimens

Boaz Avidor, PhD,[1] Merav Varon, BsC,[1] Sylvia Marmor, MD,[2] Beatriz Lifschitz-Mercer, MD,[2] Yehudith Kletter, PhD,[1] Moshe Ephros, MD,[3] and Michael Giladi, MD[1]

Key Words: Cat-scratch disease; Polymerase chain reaction; *Bartonella henselae*

Abstract

Diagnosis of cat-scratch disease (CSD) by polymerase chain reaction (PCR) of lymph node fine-needle aspiration (FNA) and primary lesion specimens can be difficult owing to the minute amount of available material. A PCR assay specifically suited to test these specimens was developed. First, small-quantity (10 µL) samples were prepared from 17 CSD-positive and 16 CSD-negative specimens, and DNA extraction and amplification from these samples were compared using 3 methods. Sensitivity and specificity of PCR were 100% using material collected on glass microscope slides and by using Qiagen (Hilden, Germany) columns for DNA extraction. Then, this method was used to test 11 archival glass microscope slides of FNA (7 malignant neoplasms, 4 undiagnosed lymphadenitis) and 2 primary lesion specimens. Two of the 4 lymphadenitis samples and the 2 primary lesion specimens were PCR positive. The technique presented could facilitate CSD diagnosis from a wider range of clinical samples.

Cat-scratch disease (CSD) usually manifests as a subacute regional lymphadenitis following a cat scratch or a bite. *Bartonella henselae* is the major causative agent.[1-3] A typical disease course begins with a primary lesion at the site of inoculation, which develops within 3 to 10 days into a papule or a pustule. Usually 7 to 14 days after infection, regional lymphadenopathy occurs proximal to the primary lesion and may proceed to suppuration in 10% to 15% of cases. In most patients, CSD resolves spontaneously within several months.[4-7] In about 10%, atypical CSD occurs, and patients may experience severe morbidity and complications.[5,8-10]

The diagnosis of CSD can be difficult owing to the limitations of available confirmatory tests. Culture of *B henselae* from affected lymph nodes has low sensitivity.[11,12] Skin testing lacks standardization and is not licensed for routine use. Warthin-Starry silver impregnation stain and the Brown-Hopps tissue Gram stain have low sensitivity, while the histopathologic examination is nonspecific.[5,13] Serologic assays are not readily available and have relatively low sensitivity.[14-19]

Polymerase chain reaction (PCR) is a valuable tool for CSD diagnosis.[1,20-25] Avidor et al[20] showed that a PCR assay using the amplification of a portion of the citrate synthase encoding gene followed by *Taq*I restriction digest of the amplified product is a sensitive tool (94%) for the detection of *B henselae* DNA in tissue biopsy specimens and pus aspirates from lymph nodes of patients with CSD. Although highly sensitive, this method requires relatively large amounts of clinical material that is not always available, particularly if attempts are made to avoid an operative procedure such as excisional biopsy. Alternatively, obtaining tissue from affected lymph nodes by fine-needle aspiration (FNA) is a much more attractive procedure since it is simple and

© American Society of Clinical Pathologists

minimally invasive, and it is used widely for cytologic examination of affected lymph nodes. However, the aspirates obtained by this method usually are of minute quantity, and we often have found FNA samples to be insufficient for DNA extraction and amplification. In fact, Demers et al[12] performed FNAs on 39 lymph nodes from patients with CSD, but they could not test these aspirates by PCR owing to an insufficient amount of material obtained by this technique. The same problem also applies for material derived from primary CSD lesions that often is too small a volume for PCR processing.

In the present study, we developed a PCR method for the amplification of *B henselae* DNA from small-quantity clinical specimens, particularly from FNA samples of affected lymph nodes and from primary lesion specimens. To do so, a "model" of small-quantity specimens was prepared by dividing minute amounts of material, from pus or lymph node tissue samples from patients with or without CSD, into aliquots. These specimens were used to choose the optimal method for DNA extraction and amplification. The developed method then was used to test FNA and primary lesion specimens.

Materials and Methods

Bacterial Strain

Bartonella henselae strain BhTA-2 was used to prepare DNA for a positive control in all PCR assays. Bacterial isolation, culture, and DNA preparation have been described previously.[20]

Patients and Specimens

A total of 33 samples from 17 patients with CSD and 16 patients without CSD were used to prepare small-quantity (FNA-like) specimens. Description of patients and specimens are summarized in **Table 1**. All patients with CSD had history of cat contact and had regional lymphadenopathy. Diagnosis was confirmed by PCR of *B henselae* citrate synthase gene as previously described.[20] Serum samples were available for 12 patients with CSD; of these samples, 11 were positive for specific anti–*B henselae* IgM and/or IgG antibodies. Samples from patients without CSD all were PCR-negative, and the majority of patients had other definite diagnoses. Serum samples were available for 14 of these patients, and all were negative for *B henselae* antibodies. Specimens, including tissue biopsy specimens or pus aspirates, were stored at –80°C until used.

Preparation of Small-Quantity Specimens

Specimens were thawed and homogenized to uniform suspensions. Pus was homogenized with a disposable polypropylene homogenizer driven by a cordless motor (Pellet Pestle Mixer, Kontes, Vineland, NJ). Tissue samples were dispersed with a porcelain tissue homogenizer in PCR diluent (a 10-mmol/L concentration of tris[hydroxymethyl] aminomethane-hydrochloride buffer, pH 8.0; a 10-mmol/L concentration of sodium chloride; and a 1-mmol/L concentration of EDTA). Three 10-µL aliquots from each suspension were processed by 3 methods: One aliquot was dotted on an IsoCode Stix device (Shleicher and Schuell, Keene, NH), which is a paper-based matrix designed for collection and processing of small-quantity samples before amplification for genetic screening applications. The second was spotted onto glass microscope slides, and the third was pipetted into 1.5-mL microtubes. IsoCode Stix devices and slides were air dried at room temperature and stored for later use. The microtubes were processed immediately.

DNA Extraction From IsoCode Stix Devices

DNA was extracted from the IsoCode sticks as recommended by the manufacturer. Briefly, the tip of the paper stick containing the dotted sample was placed into a 1.5-mL microtube and washed with 500 µL of distilled water, followed by incubation in 50 µL of distilled water for 30 minutes at 95°C to elute the bound DNA. Purified DNA was stored at –20°C until used for PCR amplification.

DNA Extraction From Glass Microscope Slides and Microtubes

DNA was extracted from specimens on slides and in microtubes with the QIAmp Blood Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. Briefly, slides were moistened with a drop of phosphate-buffered saline (PBS), and the cytologic material was scraped with the edge of a clean glass slide into a microtube containing 180 µL of PBS. The sample was digested for 10 minutes at 70°C with proteinase K and lysis buffer, followed by the addition of ethanol. The lysate was purified on a QIAmp spin column, and the DNA was eluted with 50 µL of preheated elution buffer (supplied by the manufacturer) after a 5-minute preincubation at 70°C. Specimens in the microtubes were suspended in 190 µL of PBS, and the DNA was extracted as described in the preceding section. DNA samples extracted from slides and microtubes were stored at –20°C until used for PCR.

Polymerase Chain Reaction

Three PCR assays were used in the present study: (1) PCR/citrate synthase (CS) amplifies a 379-base-pair (bp) fragment of the *gltA* gene (encoding citrate synthase) of *B henselae*. Primers and the cycling parameters have been described previously.[20] (2) PCR/16S ribosomal RNA (rRNA) amplifies a portion of the 16S rRNA gene with the *Bartonella*-specific primers, 12B and 24E, as described by

© American Society of Clinical Pathologists

Table 1
Clinical and Laboratory Characteristics for 17 Patients With and 16 Patients Without Cat-Scratch Disease (CSD)*

Patient No./Sex/Age (y)	Cat Contact	Location of Lesion	Specimen Type	PCR†	Anti–*Bartonella henselae* IgG	Anti–*Bartonella henselae* IgM	Histopathologic Features	Diagnosis
1/F/42	Yes	Axillary	Tissue	+	+	–	Necrotizing granulomatous lymphadenitis	CSD
2/M/1	Yes	Submandibular	Pus	+	–	–	ND	CSD
3/M/68	Yes	Axillary	Pus	+	+	–	ND	CSD
4/M/23	Yes	Axillary	Pus	+	+	+	Necrotizing lymphadenitis	CSD
5/M/51	Yes	Axillary and epitrochlear	Pus	+	–	+	Reactive lymphadenitis	CSD
6/M/30	Yes	Submandibular	Tissue	+	–	+	Necrotizing granulomatous lymphadenitis	CSD
7/M/11	Yes	Supraclavicular	Pus	+	ND	ND	ND	CSD
8/F/6	Yes	Axillary	Pus	+	ND	ND	ND	CSD
9/F/28	Yes	Axillary	Pus	+	+	–	Necrotizing lymphadenitis	CSD
10/M/10	Yes	NR	Pus	+	+	+	ND	CSD
11/M/15	Yes	Cervical	Pus	+	ND	ND	Reactive lymphadenitis	CSD
12/M/NR	Yes	NR	Pus	+	ND	ND	ND	CSD
13/M/6	Yes	Inguinal	Pus	+	ND	ND	ND	CSD
14/M/12	Yes	Axillary and epitrochlear	Pus	+	+	+	ND	CSD
15/M/40	Yes	Cervical	Pus	+	+	–	Necrotizing granulomatous lymphadenitis	CSD
16/F/6	Yes	Cervical	Pus	+	+	+	ND	CSD
17/M/14	Yes	Axillary	Pus	+	–	+	ND	CSD
18/M/66	No	Submandibular	Pus	–	–	–	ND	*Salmonella* lymphadenitis
19/F/2	Yes	NR	Tissue	–	–	–	Reactive lymphadenitis	Unknown
20/M/2	Yes	Cervical	Pus	–	–	–	ND	Unknown
21/M/1.5	Yes	Cervical	Tissue	–	–	–	Necrotizing granulomatous lymphadenitis	Unknown
22/F/NR	No	Cervical	Pus	–	–	–	Reactive lymphadenitis	Unknown
23/M/6.5	Yes	Submandibular	Pus	–	–	–	ND	*Staphylococcus aureus* lymphadenitis
24/M/5	Yes	Leg	Pus	–	–	–	ND	Abscess with osteomyelitis
25/M/26	No	Supraclavicular	Tissue	–	–	–	Reactive lymphadenitis	Unknown
26/F/15	No	Cervical	Tissue	–	–	–	Infected bronchial cyst	Infected bronchial cyst
27/NR/24	No	Cervical	Tissue	–	–	–	Hodgkin lymphoma	Hodgkin lymphoma
28/F/21	Yes	Cervical	Pus	–	–	–	ND	Unknown
29/F/25	Yes	Generalized	Tissue	–	–	–	Small cell carcinoma	Small cell carcinoma
30/M/50	Yes	Breast	Pus	–	–	–	ND	Breast abscess
31/F/21	No	Axillary	Pus	–	–	–	ND	MTB
32/F/2	Yes	Cervical	Pus	–	ND	ND	ND	Unknown
33/M/5	Yes	Axillary	Pus	–	ND	ND	ND	*S aureus* lymphadenitis

MTB, *Mycobacterium tuberculosis;* ND, not done; NR, not reported; PCR, polymerase chain reaction; +, positive; –, negative.
* The clinical manifestation for patients 24 and 30 was abscess; for all others, it was lymphadenitis.
† As previously described.[20]

Relman et al.[26] Cycling conditions included 3 minutes at 95°C, followed by 40 cycles of 1 minute at 94°C, 1 minute at 55°C, and 2 minutes at 72°C. This was followed by an extension step of 10 minutes at 72°C. A product size of 277 bp is expected. (3) PCR/beta-globin amplifies a 268-bp fragment of the human beta-globin gene with primers PCO4 and GH20, described by Greer et al.[27] Cycling conditions were the same as for PCR/16S rRNA. PCR beta-globin was performed in all test specimens to establish the presence of amplifiable DNA and to exclude the presence of inhibitory factors of the PCR reaction in these samples.

All PCR reactions were performed in a 100-µL reaction volume with a programmable thermal cycler with a heat bonnet (PTC-100, MJ Research, Watertown, MA). A hot-start procedure was applied in all PCR reactions by placing the reaction tubes, which were prepared on ice, directly in the thermocycler preheated to 95°C. A standard PCR mixture consisted of the following: 10 µL of the appropriate DNA template, 40 pmol of each primer, a 400-µmol/L concentration of each deoxynucleoside triphosphate, 1 U of *Taq* DNA polymerase, and 10 µL of 10× *Taq* buffer (both from Advanced Biotechnologies, Leatherhead, England)

 © American Society of Clinical Pathologists

containing a 15-mmol/L concentration of magnesium chloride. The PCR/16S rRNA 10× buffer contained a 25-mmol/L concentration of magnesium chloride. To avoid contamination, preparation of amplification mixtures, DNA extractions, and analyses of PCR products were performed in 3 separate rooms.

Analyses of PCR Products

One tenth of the PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide, and photographed. A 1-kilobase (kb) DNA ladder (Gibco BRL Life Technologies, Gaithersburg, MD) was used as the DNA size marker. Restriction fragment length polymorphism analysis was applied to 30 µL of the PCR/CS and PCR/16S rRNA products using *Taq*I and *Dde*I restriction enzymes, respectively. All digested products were electrophoresed on 12% polyacrylamide gel with a 1-kb ladder standard. After digestion, PCR/CS expected bands were 170, 138, and 71 bp, and the PCR/16S rRNA bands were 178 and 99 bp.

Enzyme Immunoassay

Testing for the presence of anti–*B henselae* IgG and IgM antibodies was performed by enzyme immunoassay (EIA) as described previously.[28] The optical density (OD) readings were determined with an automated enzyme-linked immunosorbent assay reader (LP400, Rosys Anthos, Salzburg, Austria). A serum specimen was considered positive if the mean OD reading was equal to or greater than 3 SDs above the mean OD reading for serum samples from healthy people in Israel, as determined in our laboratory (data not shown).

Archival FNA Slides

Eleven historic lymph node FNA samples from 11 patients were retrieved from the department of pathology at our hospital. All samples were stored air dried and unstained on glass microscope slides. Seven slides were diagnosed as malignant disease, 3 as nonspecific (reactive) lymphadenitis, and 1 as granulomatous lymphadenitis. Patient and specimen data are given in **Table 2**.

Primary Lesion Specimens

Lesions were pricked lightly with a scalpel tip. The lesions were squeezed, and the exudate was smeared onto a glass microscope slide that was air dried and stored at room temperature until used.

Results

PCR Performed on DNA Extracted From IsoCode Stix Devices

DNA was extracted from a total of 25 small-quantity specimens (12 CSD-positive and 13 CSD-negative) that were spotted on IsoCode Stix devices. Extracts from the sticks were amplified by PCR/CS, PCR/16S rRNA, or both to detect *B henselae* DNA. The results are summarized in **Table 3**. PCR/CS was performed on 8 CSD and 4 non-CSD samples. Two (25%) of 8 CSD samples were positive, and 2 other CSD samples were negative. The remaining 4 CSD and all non-CSD samples were nonspecifically amplified, resulting in a PCR product that was not digested by *Taq*I restriction enzyme or digested with a band pattern different from the expected. Typical results are given in **Image 1A**. The Image shows digestion by *Taq*I and polyacrylamide gel electrophoresis of PCR/CS amplification products. Three bands, 170, 138, and 71 bp, characteristic of *Taq*I cleavage of *B henselae* DNA, are clearly demonstrated in the CSD-positive sample (lane 1). The undigested band

Table 2
Clinical Characteristics and Polymerase Chain Reaction (PCR) Results for 11 Archival Fine-Needle Aspiration Specimens

				PCR Results	
Patient No./ Sex/Age (y)	Location of Lymph Nodes	Histopathologic Diagnosis	Storage Time (wk)	Citrate Synthase	beta-Globin
1/NR/NR	Cervical	Adenocarcinoma	NR	Negative	Positive
2/M/79	Inguinal	Non-Hodgkin lymphoma	26	Negative	Positive
3/M/83	Cervical	Non-Hodgkin lymphoma	6	Negative	Positive
4/M/51	Inguinal	Malignant melanoma	16	Negative	Positive
5/F/73	Axillary	Hodgkin lymphoma	8	Negative	Positive
6/M/53	Inguinal	Non-Hodgkin lymphoma	18	Negative	Positive
7/M/93	Supraclavicular	Bronchoalveolar carcinoma	20	Negative	Positive
8/M/43	Inguinal	Granulomatous lymphadenitis	8	Positive	Positive
9/M/52	Submandibular	Reactive lymphadenitis	20	Negative	Positive
10/F/70	Supraclavicular	Reactive lymphadenitis	8	Negative	Positive
11/M/19	Cervical	Reactive lymphadenitis	2	Positive	Positive

NR, not reported.

© American Society of Clinical Pathologists

Table 3
Results of PCR Amplification of DNA Extracted From Small-Quantity Specimens*

		DNA Extracted From			
		IsoCode Stix		Microtubes	Glass Slides
Patient No.	CSD	PCR/CS	PCR/16S rRNA	PCR/CS	PCR/CS
1	Yes	Negative	Negative	Positive	Positive
2	Yes	Positive	Positive	Positive	Positive
3	Yes	Negative	Negative	Positive	Positive
4	Yes	Nonspecific	Negative	Positive	Positive
5	Yes	Nonspecific	Positive	Positive	Positive
6	Yes	Positive	Positive	Positive	Positive
7	Yes	Nonspecific	Positive	Positive	Positive
8	Yes	Nonspecific	Positive	Positive	Positive
9	Yes	ND	Negative	Positive	Positive
10	Yes	ND	Positive	Positive	Positive
11	Yes	ND	Positive	Positive	Positive
12	Yes	ND	Negative	Positive	Positive
13	Yes	ND	ND	Positive	Positive
14	Yes	ND	ND	Positive	Positive
15	Yes	ND	ND	Positive	Positive
16	Yes	ND	ND	Positive	Positive
17	Yes	ND	ND	Positive	Positive
18	No	Nonspecific	Negative	Negative	Negative
19	No	Nonspecific	Negative	Negative	Negative
20	No	Nonspecific	Negative	Negative	Negative
21	No	Nonspecific	Negative	Negative	Negative
22	No	ND	Negative	Negative	Negative
23	No	ND	Negative	Negative	Negative
24	No	ND	Negative	Negative	ND
25	No	ND	Negative	Negative	Negative
26	No	ND	Negative	ND	Negative
27	No	ND	Negative	Negative	ND
28	No	ND	Negative	Negative	Negative
29	No	ND	Negative	Negative	Negative
30	No	ND	Negative	Negative	Negative
31	No	ND	ND	Negative	Negative
32	No	ND	ND	Negative	Negative
33	No	ND	ND	Negative	Negative

CS, citrate synthase; CSD, cat-scratch disease; ND, not done; PCR, polymerase chain reaction; rRNA, ribosomal RNA.
* IsoCode Stix, Shleicher and Schuell, Keene, NH.

present in the CSD-negative sample (lane 2) suggests nonspecific amplification. PCR/16S rRNA was performed on 12 CSD and 13 non-CSD specimens. Of 12 CSD samples, 7 (58%) were positive, and all non-CSD samples were negative. Typical analysis by polyacrylamide gel electrophoresis of PCR/16S rRNA amplification products digested with the *Dde*I restriction enzyme is shown in **Image 1B**. The 178- and 99-bp bands expected, characteristic of *B henselae* DNA, are visualized in the CSD-positive samples (lanes 3, 4, and 5). Of the 8 CSD-positive specimens tested by both PCR/CS and PCR/16S rRNA, 5 were positive by PCR/16S rRNA and only 2 by PCR/CS (Table 3).

All samples eluted from the IsoCode Stix were positive by PCR/beta-globin (data not shown), indicating that genomic DNA was eluted from the sticks and excluding the presence of inhibitory factors of PCR amplification in these samples.

PCR Performed on DNA Extracted From Microtubes and Glass Microscope Slides

DNA from 17 CSD-positive specimens was extracted from both microtubes and glass microscope slides by the QIAmp spin columns and tested by PCR/CS (Table 3). All CSD specimens were PCR/CS positive (100%). DNA was extracted from 15 CSD-negative specimens from microtubes and from 14 negative samples from glass microscope slides. All these samples were negative by PCR/CS and positive by PCR/beta-globin (data for PCR/beta-globin not shown). Typical analyses by *Taq*I digestion and polyacrylamide gel electrophoresis of PCR/CS products amplified from DNA samples extracted from microtubes and glass microscope slides are shown in **Image 2A** and **Image 2B**, respectively.

Detection of *B henselae* DNA in Archival FNA Specimens

We assayed 11 archival glass-slide FNA specimens. DNA was extracted by QIAmp spin columns and tested by

© American Society of Clinical Pathologists

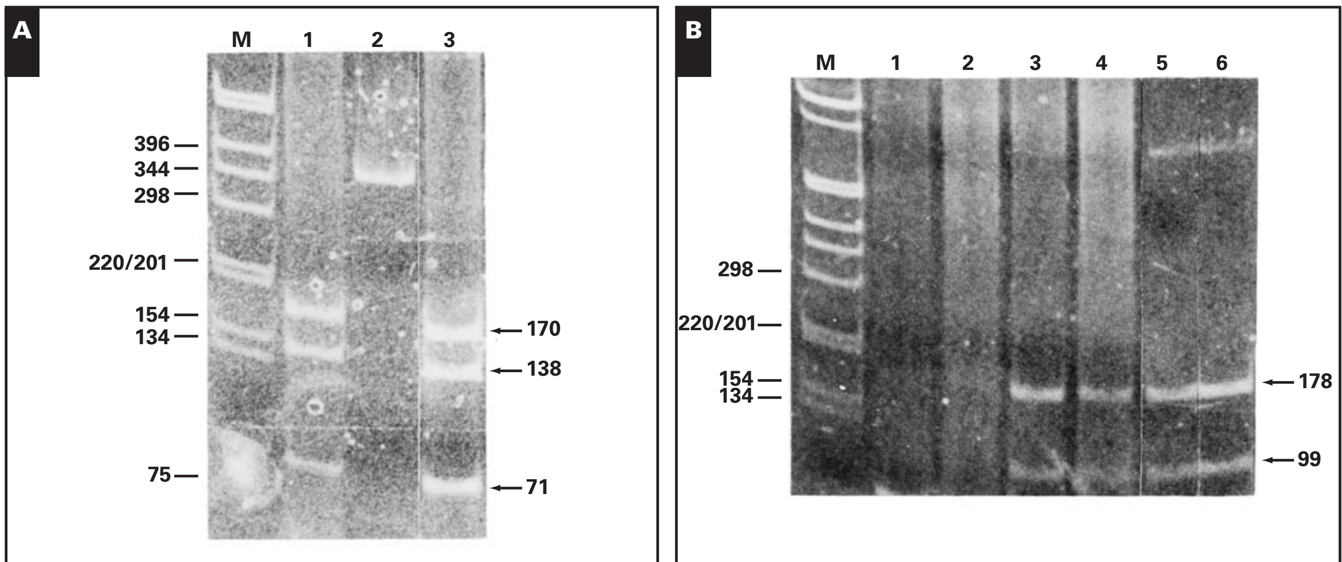


Image 1 Analyses of polymerase chain reaction (PCR) products amplified from samples extracted from IsoCode Stix devices (Shleicher and Schuell, Keene, NH). **A**, Digestion with *Taq*I restriction enzyme and polyacrylamide gel electrophoresis of PCR/citrate synthase products. Lane 1, cat-scratch disease (CSD)-positive sample; lane 2, CSD-negative sample; lane 3, *Bartonella henselae* DNA. **B**, Digestion with *Dde*I and polyacrylamide gel electrophoresis of PCR/16S ribosomal RNA products. Lanes 1 and 2, CSD-negative samples; lanes 3-5, CSD-positive samples; lane 6, *B henselae* DNA. Lane M, molecular size markers (in base pairs). Arrows and numbers indicate the sizes (in base pairs) of the DNA bands.

PCR/CS and PCR/beta-globin. Descriptions of samples and PCR results are summarized in Table 2. All 11 samples were positive by PCR/beta-globin, indicating that sufficient genomic DNA was extracted from the glass slides. Seven specimens with a definite histopathologic diagnosis of malignant neoplasm (patients 1-7) were negative by PCR/CS. Two specimens (patients 8 and 11), were PCR/CS positive. **Image 3A** shows polyacrylamide gel

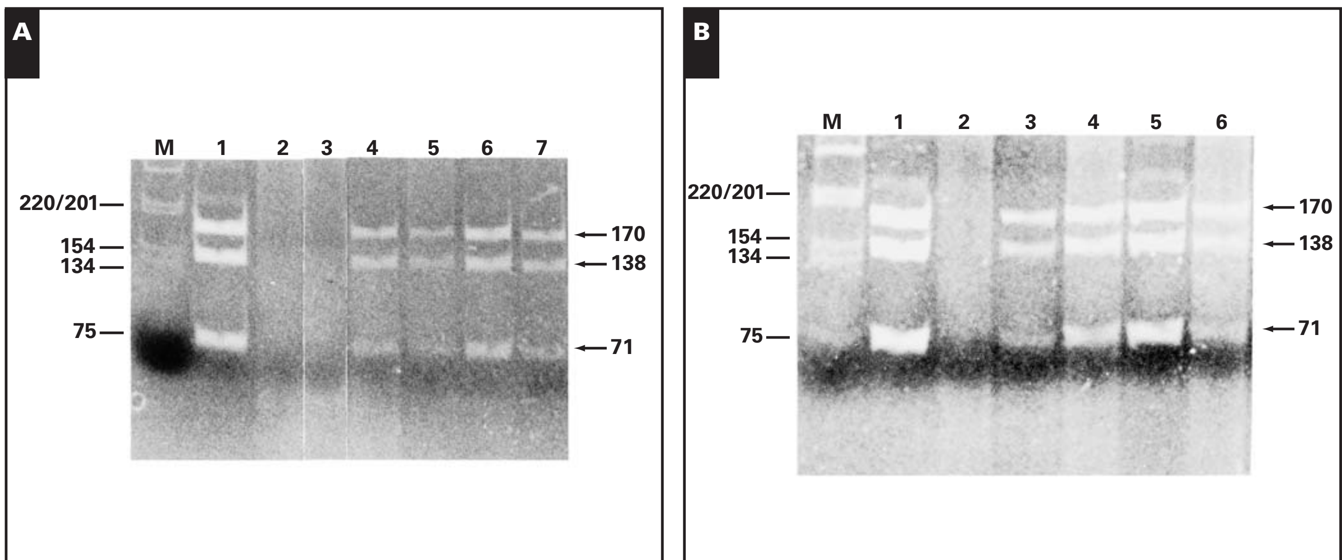


Image 2 Analyses of polymerase chain reaction (PCR)/citrate synthase products amplified from samples extracted from microtubes and glass microscope slides. PCR products were digested with *Taq*I and analyzed by polyacrylamide gel electrophoresis. **A**, Samples extracted from microtubes. Lane 1, *Bartonella henselae* DNA; lanes 2 and 3, cat-scratch disease (CSD)-negative samples; lanes 4-7, CSD-positive samples. **B**, Samples extracted from glass microscope slides. Lane 1, *B henselae* DNA; lane 2, CSD-negative sample; lanes 3-6, CSD-positive samples. Lane M, molecular size markers (in base pairs). Arrows and numbers indicate the sizes (in base pairs) of DNA bands.

© American Society of Clinical Pathologists

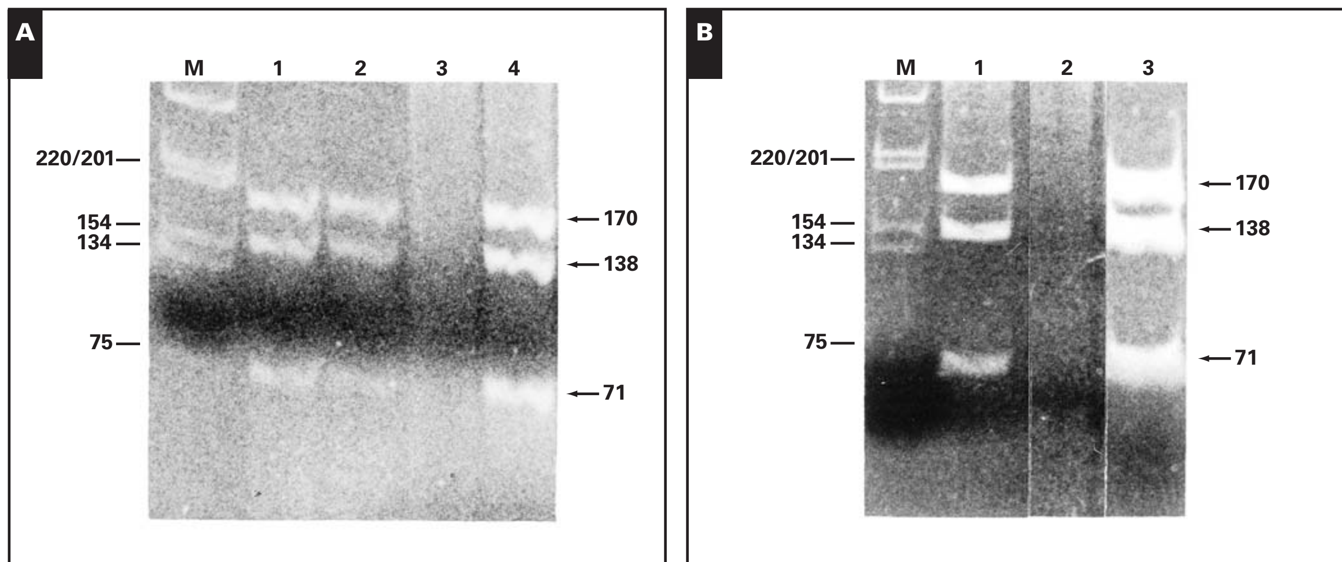


Image 3 Analyses of polymerase chain reaction (PCR)/citrate synthase products amplified from archival fine-needle aspiration (FNA) specimens and from a primary lesion sample. Amplification products were digested with *Taq*I and analyzed by polyacrylamide gel electrophoresis. **A**, Archival FNA specimens. Lanes 1 and 2, samples from cat-scratch disease (CSD)-positive patients; lane 3, sample from a patient with cancer; lane 4, *Bartonella henselae* DNA. **B**, A primary lesion specimen. Lane 1, a primary lesion specimen from a patient with CSD; lane 2, pus specimen aspirated from a lymph node of a CSD-negative patient; lane 3, *B henselae* DNA. Lane M, molecular size markers (in base pairs). Arrows and numbers indicate the sizes (in base pairs) of DNA bands.

electrophoresis analysis of the PCR/CS products from these 2 patients. The characteristic bands (170, 138, and 71 bp) of *Taq*I digestion of the *B henselae* amplification products are clearly visualized in both specimens (lanes 1 and 2).

The FNA specimen from patient 8 was obtained from a lymph node from a 43-year-old, obese, diabetic man admitted for evaluation of a large, tender inguinal mass. Serologic test results for cytomegalovirus, Epstein-Barr virus, and *Toxoplasma* species were negative. Ultrasound examination of the inguinal region revealed 6 solid masses, ranging in diameter from 1.5 to 6.3 cm, consistent with enlarged lymph nodes. Cytologic examination of FNA material obtained from an inguinal lymph node revealed granulomatous lymphadenitis. Amoxicillin/clavulanic acid was started with slow resolution of the symptoms. The patient was discharged from the hospital without a definite diagnosis. Four months later, PCR/CS was performed retrospectively on the archival FNA slide, and results were positive. Owing to this result, the patient was followed up. His lymphadenopathy has resolved completely. He had never been scratched or bitten by a cat, but stray cats and kittens frequently inhabit his backyard, and he owned a dog and a few puppies. A serum sample that had been obtained at the time of admission was positive by EIA for anti–*B henselae* IgM and IgG antibodies. A second sample at follow-up, 4 months later, demonstrated the disappearance of IgM and an increase of the IgG titer, consistent with acute *B henselae* infection.

Patient 11 was a 19-year-old man with a left-sided, hard, tender cervical mass, 5 × 4.5 cm. Several weeks earlier, he had been scratched on his neck by a kitten. Although CSD serologic test results were negative, the clinical picture was consistent with CSD. FNA of the cervical mass was performed, showing reactive lymphadenitis by cytologic examination. The patient was treated with doxycycline for 10 days. The lymph node slowly disappeared over a period of 3 months. Results of PCR/CS performed retrospectively on the FNA slide were positive, suggestive of CSD.

Detection of *B henselae* DNA in Primary Lesion Specimens

Two primary lesion specimens, collected and stored on glass microscope slides, were tested.

Case 1

A 9-year-old previously healthy girl was examined because of a 1-week history of left-sided, tender, axillary lymphadenitis and fever. She owned several kittens and often was scratched by them. Physical examination also revealed 2 pustular lesions 4 and 6 mm in diameter on the posterior aspect of the left forearm **Image 4**. DNA was extracted by a QIAmp spin column and assayed by PCR/CS. Analysis of the amplified product by *Taq*I digestion and polyacrylamide gel electrophoresis is shown in **Image 3B**. A typical band pattern of *B henselae* DNA is clearly visualized (Image 3B,

 © American Society of Clinical Pathologists

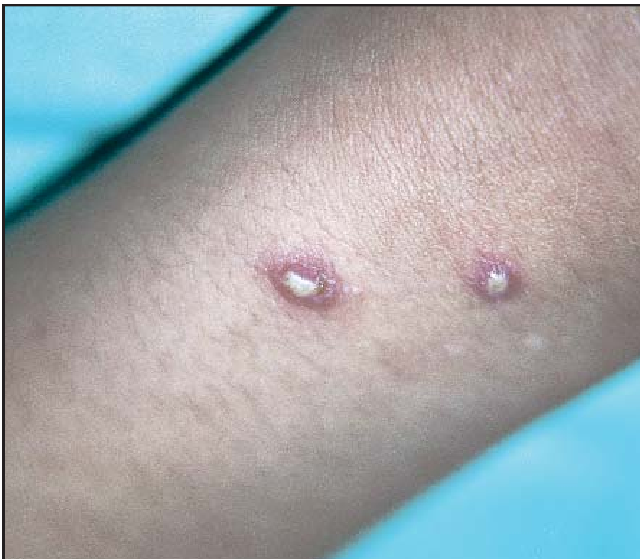
lane 1). The patient was examined 4 weeks after the initial examination, and pus was aspirated from her axillary node **❚Image 5❚**. The result of PCR performed on the pus sample by our routine method[20] was positive (data not shown). The patient also was positive by EIA for anti–*B henselae* IgG but negative for anti–*B henselae* IgM. However, a substantial increase of the IgG titer was demonstrated in a second serum specimen (obtained 2 weeks later), consistent with acute CSD infection.

Case 2

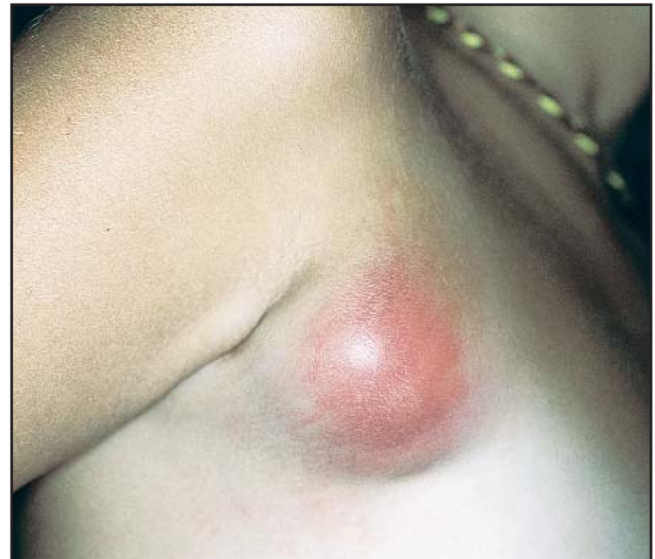
The second patient was a 55-year-old woman with a 2-week history of tender supraclavicular lymphadenitis. She owned several cats and a kitten that she hugged and kissed, and she often was scratched by them. Physical examination revealed an inflammatory papule on the upper part of the left side of the chest, about 3 cm below the clavicle, consistent with a primary inoculation lesion of CSD **❚Image 6❚**. The result of PCR/CS of the exudate from the lesion was positive for *B henselae* DNA (data not presented). The anti–*B henselae* IgG titer was negative on the first serum sample and intermediate on the second sample obtained 3 weeks later.

Discussion

The present study was undertaken to develop an assay specifically designed for PCR detection of *B henselae* DNA in small-quantity clinical samples, particularly from FNA and primary lesion specimens. In doing so, we sought a method by which the FNA material could be used optimally for PCR diagnosis of CSD. We first constructed a model of FNA-like specimens consisting of a series of small-quantity (10-µL) samples from CSD and non-CSD specimens that previously were tested by PCR.[20] These samples were spotted onto IsoCode Stix devices and glass microscope slides and pipetted into microtubes. The IsoCode Stix devices have been used successfully for processing small-quantity specimens of blood, saliva, urine, feces, and other biologic fluids before PCR analysis for HLA typing, forensics, and paternity applications.[29,30] Our results, however, showed that processing of pus and tissue samples by the IsoCode devices for PCR detection of *B henselae* DNA has low sensitivity of 25% to 58%. Since all samples that were processed by the IsoCode devices tested positive by PCR/beta-globin, indicating the presence of amplifiable genomic DNA, we speculate that *B henselae* DNA, which constitutes only a minute fraction of the entire DNA present in the specimen, is probably not sufficiently eluted from these devices and, thus, is poorly detected by PCR. In addition, many of the samples processed by the IsoCode devices were nonspecifically amplified by PCR/CS. This phenomenon was observed in both CSD-positive and CSD-negative specimens. We suspect that the proprietary detergent present in the paper-based matrix is responsible for a lower annealing temperature of the PCR/CS primers, resulting in nonspecific priming and eventually reducing the sensitivity and specificity of the PCR detection. This effect, however, was not seen with the PCR/16S rRNA primers for which a



❚Image 4❚ Two primary pustular lesions found on the posterior aspect of the left forearm of the patient with cat-scratch disease described in case 1 (see text).



❚Image 5❚ Lymphadenitis of the axillary lymph node of the patient with cat-scratch disease in case 1 (see text), 4 weeks after initial examination. Pus aspirated from this node was assayed by polymerase chain reaction, as described.

© American Society of Clinical Pathologists

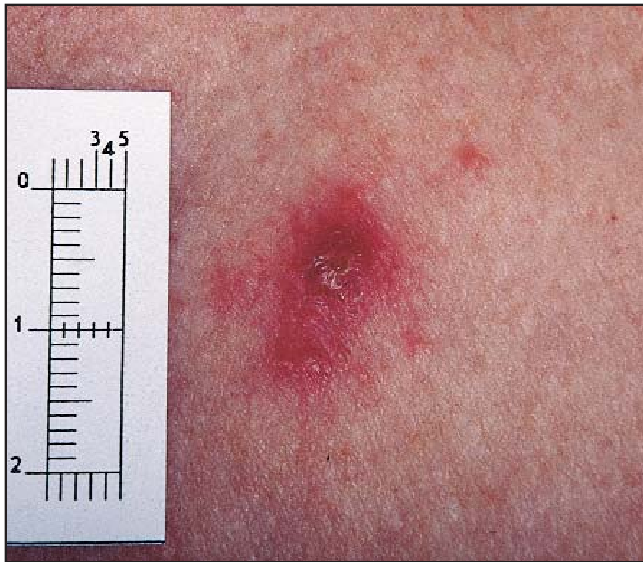


Image 6 Inflammatory papular primary lesion on the upper part of the left side of the chest, below the clavicle, of the patient with cat-scratch disease described in case 2 (see text). The scale is in centimeters.

higher annealing temperature was used. We also found that crude extracts of pus and tissue specimens solubilized by 0.5% polysorbate 20 or a 4-mol/L concentration of urea are similarly nonspecifically amplified by the PCR/CS assay (unpublished data).

The use of the QIAmp Blood Kit for processing small-quantity specimens in microtubes and on glass microscope slides was efficient and reliable, resulting in detection by PCR/CS with a sensitivity and a specificity of 100%. It should be noted that samples were extracted with AL lysis buffer (included in the QIAmp kit) and not with ATL, also supplied in the same kit. The latter resulted in nonspecific amplification of the same samples (data not shown) and, therefore, should be avoided. The company does not reveal the composition of these 2 buffers; therefore, we cannot determine the nature of this phenomenon.

The use of glass microscope slides for collecting FNA and primary lesion specimens for PCR testing of CSD is simple and convenient. The samples can be stored at room temperature and shipped by regular mail, thus avoiding high costs and the logistics associated with shipping on ice. The use of stored slides allows retrospective diagnosis when CSD was not suspected initially and fresh material has not been retained, provided that archival slides are available. This was clearly demonstrated in the present study in which CSD was diagnosed retrospectively by PCR in 2 of the archival FNA specimens.

In the present study, we used air-dried (unfixed and unstained) slide specimens since reports have shown that cytologic stains can inhibit the PCR reaction.[31,32] Our experience, though, with long-term storage of such specimens is limited. It has been shown, however, that unstained archival glass slides containing bone marrow material can be stored for up to 13 years at room temperature and can be used successfully for PCR diagnosis of hematologic malignant neoplasms.[33]

Our results show that DNA is efficiently extracted from microtubes and glass microscope slides and is detected equally by PCR. However, scraping of slides for DNA extraction can be potentially hazardous and should be performed cautiously in a laminar flow hood. When potentially dangerous (biohazardous) specimens are collected and processed for PCR, such as FNA samples from patients with AIDS, the use of microtubes is recommended.

The ease and safety of obtaining FNA specimens for cytologic examination and PCR testing make this procedure a prime first-line diagnostic tool. Data presented herein show that FNA can be a valuable source of clinical material for PCR diagnosis of CSD and that testing of such samples should be attempted more frequently. In fact, during the year 2000, the cytopathologic unit in our hospital received more than 100 lymph node FNA samples, of which 75% were diagnosed as reactive lymphadenitis in the absence of any other diagnosis. We have tested only 3 specimens with reactive lymphadenitis, of which 1 was CSD-positive. It is likely that more patients with CSD are undiagnosed or not specifically diagnosed.

Data about PCR testing of primary lesion specimens for the diagnosis of CSD is limited, although primary lesions are an attractive source of clinical material for early diagnosis of CSD. Primary lesions are present in 61% to 93% of patients with CSD and usually develop during the early stages of the disease.[5,34] In addition, Margileth et al[35] demonstrated, by using histologic staining, that substantial amounts of bacteria can be found in papules and pustules of patients with CSD. Our data show that *B henselae* DNA can be found in both pustular and papular primary lesions of patients with CSD. However, since only 2 cases were tested, further studies are required to determine the importance of this approach.

Data presented imply that PCR diagnosis of CSD from FNA and primary lesion specimens can be minimally invasive and highly accurate, thus precluding the necessity for excisional biopsies. The modifications presented, together with the standard PCR method previously developed for testing larger volumes of tissue and pus specimens,[20] suggest a greater role for PCR diagnosis of CSD from a larger spectrum of clinical samples.

From the [1]Bernard Pridan Laboratory for Molecular Biology of Infectious Diseases and the [2]Department of Pathology, Tel-Aviv Sourasky Medical Center, Tel-Aviv; and the [3]Department of Pediatrics, Carmel Medical Center, Haifa, Israel.

 © American Society of Clinical Pathologists

Address reprint requests to Dr Avidor: Bernard Pridan Laboratory for Molecular Biology of Infectious Diseases, Ichilov Hospital, Tel-Aviv Sourasky Medical Center, 6 Weizman St, Tel-Aviv 64239, Israel.

Acknowledgment: We thank Ofra Avidor for excellent technical assistance.

References

1. Bergmans AMC, Groothedde J-W, Schellekens JFP, et al. Etiology of cat scratch disease: comparison of polymerase chain reaction detection of *Bartonella* (formerly *Rochalimaea*) and *Afipia felis* DNA with serology and skin tests. *J Infect Dis*. 1995;171:916-923.
2. Dolan MJ, Wong MT, Regnery RL, et al. Syndrome of *Rochalimaea henselae* adenitis suggesting cat scratch disease. *Ann Intern Med*. 1993;118:331-336.
3. Regnery RL, Olson JG, Perkins BA, et al. Serological response to "*Rochalimaea henselae*" antigen in suspected cat-scratch disease. *Lancet*. 1992;339:1443-1445.
4. Bass JW, Vincet JM, Person DA. The expanding spectrum of *Bartonella* infections, II: cat-scratch disease. *Pediatr Infect Dis J*. 1997;16:163-179.
5. Carithers HA. Cat scratch disease: an overview of a study of 200 patients. *Am J Dis Child*. 1985;139:1124-1133.
6. Moriatry RA, Margileth AM. Cat scratch disease. *Infect Dis Clin North Am*. 1987;1:575-590.
7. Schwartzman, WA. Infections due to *Rochalimaea*: the expanding clinical spectrum. *Clin Infect Dis*. 1992;15:893-902.
8. Margileth AM. Cat scratch disease and nontuberculous mycobacterial disease: diagnostic usefulness of PPD-Batty, PPD-T and cat scratch skin test antigens. *Ann Allergy*. 1992;68:149-154.
9. Marra CM. Neurological complications of *Bartonella henselae* infection. *Curr Opin Neurol*. 1995;8:164-169.
10. Waldvogel K, Regnery RL, Anderson BE, et al. Disseminated cat-scratch disease: detection of *Rochalimaea henselae* in affected tissue. *Eur J Pediatr*. 1994;153:23-27.
11. Clarridge JE III, Raich TJ, Pirwani D, et al. Strategy to detect and identify *Bartonella* species in routine clinical laboratory yields *Bartonella henselae* from human immunodeficiency virus–positive patient and unique *Bartonella* strain from his cat. *J Clin Microbiol*. 1995;33:2107-2113.
12. Demers DM, Bass JW, Vincent JM, et al. Cat-scratch disease in Hawaii: etiology and seroepidemiology. *J Pediatr*. 1995; 127:23-26.
13. Min KW, Reed JA, Welch DF, et al. Morphologically variable bacilli of cat scratch disease are identified by immunocytochemical labeling with antibodies to *Rochalimaea henselae*. *Am J Clin Pathol*. 1994;101:607-610.
14. Bergmans AM, Peeters MF, Schellekens JF, et al. Pitfalls and fallacies of cat scratch serology: evaluation of *Bartonella henselae*–based indirect fluorescence assay and enzyme-linked immunoassay. *J Clin Microbiol*. 1997;35:1931-1937.
15. Drancourt M, Birtles R, Chaumentin G, et al. New serotype of *Bartonella henselae* in endocarditis and cat scratch disease. *Lancet*. 1996;347:441-443.
16. Dupon M, Savin De Larclause A-M, Brouqui P, et al. Evaluation of serological response to *Bartonella henselae, Bartonella quintana* and *Afipia felis* in 64 patients with suspected cat-scratch disease. *Scand J Infect Dis*. 1996;28:361-366.
17. La Scola B, Raoult D. Serological cross-reactions between *Bartonella quintana, Bartonella henselae*, and *Coxiella burnetii*. *J Clin Microbiol*. 1996;34:2270-2274.
18. Not T, Canciani M, Buratti E, et al. Serologic response to *Bartonella henselae* in patients with cat scratch disease and in sick and healthy children. *Acta Paediatr*. 1999;88:284-289.
19. Szelc-Kelly CM, Goral S, Perez-Perez GI, et al. Serologic responses to *Bartonella* and *Afipia* antigens in patients with cat scratch disease. *Pediatrics*. 1995;96:1137-1142.
20. Avidor B, Kletter Y, Abulafia S, et al. Molecular diagnosis of cat scratch disease: a two-step approach. *J Clin Microbiol*. 1997;35:1924-1930.
21. Anderson B, Slims K, Regenery R, et al. Detection of *Rochalimaea henselae* DNA in specimens from cat scratch disease patients by PCR. *J Clin Microbiol*. 1994;32:942-948.
22. Gottlieb T, Atkins BL, Robson JM. Cat scratch disease diagnosed by polymerase chain reaction in a patient with suspected tuberculous lymphadenitis. *Med J Aust*. 1999; 170:168-170.
23. Sander AM, Posselt NB, Ruess M. Detection of *Bartonella henselae* DNA by two different PCR assays and determination of the genotypes of strains involved in histologically defined cat scratch disease. *J Clin Microbiol*. 1999;37:993-997.
24. Mouritsen CL, Litwin CM, Maiese RL, et al. Rapid polymerase chain reaction–based detection of the causative agent of cat scratch disease (*Bartonella henselae*) in formalin-fixed, paraffin-embedded samples. *Hum Pathol*. 1997;28:820-826.
25. Scott MA, McCurley TL, Vnencak-Jones CL. Cat-scratch disease: detection of *Bartonella henselae* DNA in archival biopsies from patients with clinically, serologically, and histologically defined disease. *Am J Pathol*. 1996;149:2161-2167.
26. Relman DA, Loutit JS, Schmidt TM, et al. The agent of bacillary angiomatosis: an approach to the identification of uncultured pathogens. *N Engl J Med*. 1990;323:1573-1580.
27. Greer CE, Peterson SL, Kiviat NB, et al. PCR amplification from paraffin-embedded tissues: effects of fixative and fixation time. *Am J Clin Pathol*. 1991;95:117-124.
28. Welch DF, Hensel DM, Pickett DA, et al. Bacteremia due to *Rochalimaea henselae* in a child: practical identification of isolates in the clinical laboratory. *J Clin Microbiol*. 1993; 31:2381-2386.
29. Kline MC, Duewer DL, Newall P, et al. Interlaboratory evaluation of short repeat triplex CTT. *J Forensic Sci*. 1997;42:897-906.
30. Roy R, Middendorf LR. Infrared fluorescent detection of D1S80 alleles from blood and body fluid collected on IsoCode devices. *Biotechniques*. 1997;23:942-945.
31. Burton MP, Schneider BG, Brown R, et al. Comparison of histologic stains for use in PCR analysis of microdissected, paraffin-embedded tissues. *Biotechniques*. 1998;24:86-92.
32. Chen T-J, Lane MA, Clark DP. Inhibitors of polymerase chain reaction in Papanicolaou stain. *Acta Cytol*. 1996;40:873-877.
33. Fey MF, Pilkington SP, Summers C, et al. Molecular diagnosis of haematological disorders using DNA from stored bone marrow slides. *Br J Haematol*. 1987;67:489-492.
34. Margileth AM. Cat scratch disease update. *Am J Dis Child*. 1984;139:1124-1133.
35. Margileth AM, Wear DJ, Hadfield TL, et al. Cat-scratch disease: bacteria in skin at the primary inoculation site. *JAMA*. 1984;252:928-931.

© American Society of Clinical Pathologists