ELISA-BASED COLORIMETRIC DETECTION OF RICKETTSIA TSUTSUGAMUSHI DNA FROM PATIENT SERA BY NESTED POLYMERASE CHAIN REACTION

Gow Jen Shieh, Huei Ling Chen, Hour Young Chen, Gong Ren Wang and Chi Byi Horng

Division of Virology, Department of Health, National Institute of Preventive Medicine, 161 Kun-Yang Street, Nan-Kang, Taipei, Taiwan

Abstract. A rapid diagnostic system for scrub typhus was established using colorimetric detection of nested polymerase chain reaction (PCR). This system relied on binding the amplified DNA via a sequence in one of oligodeoxyribonucleotide to the DNA-binding protein GCN4 coated on the well of a micotiter dish. The primer pairs used for the nested PCR were designed on the basis of the homologous nucleotide sequence of the gene that encodes the 56 kDa antigen of serovariants. With this colorimetric PCR, diagnosis can be performed easily from serum samples of patients before the antibody titer increases or in the early stage of the disease. Furthermore, these positive results are able to be confirmed by pathogenic isolation.

INTRODUCTION

Rickettsia tsutsugamushi is a very small coccobacillus and an obligate intracellular parasite of human beings transmitted via chigger mites. The antigenic types of this rickettsia referred to as Gilliam, Karp and Kato strains are commonly recognized, and thus frequently called the prototype strains. Yet, additional antigenic types such as Shimokoshi (Tamura et al, 1984), Kawasaki (Yamamoto et al, 1986) Kuroki (Ohashi et al, 1990; Yamamoto et al, 1989) and Boyong (Kim et al, 1993) were isolated in the last decade. These variants are distinguishable from each other by serological cross-tests with strain-specific polyclonal or monoclonal antibodies. The serotype variation of the rickettsia depend on the antigenicity of an immunodominant 56 kDa major protein, which was demonstrated to be a type-specific antigen, located on the rickettsial outer membrane (Murata et al, 1986; Ohashi et al, 1992).

The immunofluorescence assay (IFA) technique has been widely employed for its clinical identification. However, the process of cultivating and isolating the antigenic *R. tsutsugamushi* for IFA is quite complicated and time consuming. Besides, the diagnostic method is somewhat of limited used in the early stage of the illness, when the serum antibody titer of the patient happens to be too low to

Correspondence: Tel: 886-2-7856671; Fax: 886-2-7837779

measure (Sugita et al, 1993).

It has been reported that PCR can be used to detect the DNA sequence corresponding to this antigenic 56 kDa protein from blood clots (Furuya et al, 1992, 1993; Kawamori et al, 1993; Sugita et al 1993). Although the sensitivity of the PCR is excellent, the procedures for detection of the products of PCR are not well suited to routine equipment in many laboratories and to mass screening, as they generally require gel electrophoresis, Southern blot or specific DNA microplate hybridization. We describe here a nested colorimetric PCR, which is an ELISA-based test, is applicable to the detection of Rickettsia DNA from serum of patients in the early stage of the illness.

MATERIALS AND METHODS

Bacterial strains and media

R. tsutsugamushi Gillam, Karp and Kato, courtesy of Okinawa University, were propagated respectively in L929 cells as positive controls for the PCR.

Flasks (with 150 cm² culture surface) with fully grown L929 cells were infected with suspensions of *R. tsutsugamushi* at 5 moi. After 1 hour at 37°C, the monolayer was washed with minimal essential medium containing 2% fetal calf serum in an atmosphere of 5% CO₂ and 95% air (Chen *et al*,

1992). The infected monolayer were harvested when greater than 70% of the cell were infected. The isolation of *R. tsutsugamushi* from lymphocytes and cell cultures were performed as described by Shieh *et al* (1995).

Clinical samples

Serum for the PCR was taken obtained from a 40 year old farmer who was having skin rash, fever, and chill, symtoms typical of the acute phase of scrub typhus. The lgGAM antibody titer to R. tsutsugamushi is 40 at first bleeding, 5 days after onset. After tetracycline treatment, the symptoms of this patient subsided and he went home. Therefore the second bleeding for the confirmational IFA could not be done and the case remains dubious. The detection of antibody titer against R. tsutsugamushi were performed according to Chen et al (1992).

Furthermore, sera were obtained from another four patients with low IFA titers or without secondary bleeding. For comparison, serum sample was also obtained from a patient with Q fever, which showed a titer for phase II antigen up to 2640.

For PCR amplification, template DNA was isolated from culture suspension of each of Gilliam, Karp and Kato strains or from patient's sera. $300 \,\mu l$ of the specimens were incubated with lysozyme at 1 mg/ml in 1 × TE buffer (50 mM Tris-HCl, pH 9.0, 1mM EDTA) and 0.5% SDS for 30 minutes at 37°C. Following digestion with protease K at 2 mg/ml, for 30 minutes at 55°C, the DNA was purified by repeated phenol-chloroform extraction and then followed by ethanol precipitation. The DNA pellets were resuspended in $10 \,\mu l$ of $1 \times TE$ buffer (pH 7.5).

Polymerase chain reaction

Two pairs of primers were synthesized for the nested PCR. In the first PCR amplification, a pair of 5'-AGTACTATTAAATATTAGTATAC-3' (501-523, primer a) and 5'-TAAAGTCAATACC-AGCATAATTCTT-3' (1207-1186, primer b) were synthesized to generate 707 bp DNA fragments. In the second case, 5'-biotin-ATTGTCATACCC-GCAGCCAATGT-3' (673-688, primer c) and 5'-GGATGACTCATTGTTGTCGTTGCCGTTTTCAG-3' (875-853, primer d) were the pair for a 203 bp

DNA fragment instead, plus a 5'-biotin label and a 3'-GCN4 protein binding site (5'-GGATGACT-CATT-3').

100 µl of PCR mixture contained 50 mM KCl, 10 mM tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM MgCl₂, 250 uM each dNTP, 5 uM of primers, 1.25 U of Taq DNA polymerase (Promega) and 2 µl of DNA template. The target DNA was first amplified by PCR with oligo primers a and b. The mixture was denatured at 94°C for 1 minute 30 seconds, annealed at 55°C for 1 minute 30 seconds, and then chain was extended at 70°C for 1 minute 30 seconds in a thermal cycler. (Perkin-Elmer Cetus). This cycle was repeated 30 times. For the second PCR amplification, 5 µl of the above PCR product were amplified by a similar PCR cycle but with oligo primers c and d. The mixture was denatured at 94°C for 1 minute, annealed at 37°C for 1 minute, and then extended at 70°C for 1 minute. The cycle was repeated for 30 times,

Colorimetric detection of PCR product

The colorimetric assay of the PCR product was carried out according to Kemp et al (1990). In brief, it relies on the binding of the amplified DNA to its binding protein GCN4 from Saccharomyces cerevisiae, which is precoated on the wells of the microtiter dish (AMRAD Corporation, Ltd, Kew, Australia). The DNA products of the second PCR contained a 3'-GCN4-recognition site, could be captured by binding to GCN4 immobilized in wells of microtiter dish (Kemp et al, 1990). On the other hand, the products of PCR contained 5'-biotin label which allowed detection with avidin conjugated to horseradish peroxidase, followed by reaction with chromogenic substrate as a standard ELISA reaction.

RESULTS

The specific primers for DNA amplification used in this study were selected on the basis of the homology with the nucleotide sequence of a gene encoding the antigenic 56 kDa protein in seven sero-variants (Gilliam, Karp, Kato, Shimokoshi, Kato, Kawasaki and Boryong) of R. tsutsugamushi (Fig 1). Agarose gel electrophoresis showed that amplification of DNA of expected size was ob-

	501 550	551
Kurokı	AGTACTATTA AATATTAGTA TACTAAATAA TACTTTTTTTC ATATAAA	
Boyong		
Karp		
Kato		
Gilliam		
Kawasaki		
Shimokoshi	AGTACTATTA NATATTAGTG TACTANATAN TAGTTTTTTG ATATANAGT	
	THE THE THE THE TENT OF THE TE	. AAGTTAGTG TGGCTAAATA ATTAGTTTAG AATGGTTACC ACTAAAAAAT
	601	
Kuroki	AAATTTAATT CTTTTAAGGA GATTAGAATG AAAAAAATTA TGTTAATTGC	651 700
Boyong	AAATTTAATT CTTTTAAGGA GATTAGAATG AAAAAAATTA TGTTAATTGC	TAGTGCAATG TCTGCGTTGT CGTTGCCGTT TTCAGCTAGT GCGATAGAAT
Karp	AAATTIAATT CTTTTAAGGA GATTAGAATG AAAAAAATTA TGTTAATTGC	IAGIGCAATG TCTGCGTTGT CGTTGCCGTT TTCAGCTACT CCCAMACAA
Kato	ABATTTAATT CTTTTAAGGA GATTAGAATG AAAAAAATTA TGTTAATTGC	INGIGUARIG TOTGOGTTGT CGTTGCCATT TTCACCTACT CC.LT.C.L.
Gilliam	AAATTTAATT CTTTTAAGGA GATTAGAATG AAAAAAATTA TGTTAATTGC	TAGIGCAATG TCTGCATTGT CATTGCCGTT TTCAGCTACT CCCATAGAA
Kawasaki	AAATITAATT CTTTTAAGGA GATTAGAATG AAAAAAATTA TGTTAATTGC AAATITAATT CTTTTAAGGA GATTAGAATG AAAAAAATTA TGTTAATTGC	TAGTGCAATG TCTGCATTGT CATTGCCGTT TTCACCTACT CCAATAGAA
Shimokoshi	ABATTTAATT CTTTTAACCA CATTACAATC AAAAAAATTA TGTTAATTGC	INGIGERATE TETECETTET CETTECCETT TTCACCTACT CCCATACAA
***************************************	AAATTTAATT CTTTTAAGGA GATTAGAATG AGAAAAATTA TGTTAATTGC	TAGCGCAATG TCTGCCTTGT CATTGCCGTT TTCAGCTAAT GCGATAGAGT
	701 750	——————————————————————————————————————
Kuroki	TGGAGGATGA AGTAGGATTA GAGTGTGGTC CTTATGCTAA AGTTGGAGTT	751
Boyong	TGGAGGATGA AGTAGGATTA GAGTGTGGTC CTTATGCTAA AGTTGGAGTT	GTTGGAGGAA TGATTACTGG TGCAGAATCT ACTCCCTTGG ATTACK
Karp	TGGGGGA AGAAGGATTA GAGTGTGGTC CTTATGCTAA AGTTGGAGTT	
Kato	TGGGGGATGA AGGAGGATTA GAGTGTGGTC CTTATGCTAA AGTTGGAGTC	
Gilliam	TGGGTGAGGA AGGAGGATTA GAGTGTGGTC CTTACGGTAA AGTTGGAATC	
Kawasaki	TGGGGGATGA AGGAGTATTA GAGTGTGGTC CTTATGCTAA AATTGGAGTT	GIIOGAGGAA IGAITACTEG TGCAGAATCT ACTCCCTTCC ATTCAACTC
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Kuroki	TTCTGAGGGA AAAAAACATT TGTCATTAAC AACTGGACTG CCATTTGGTG	851 900
Boyong	TTCTGAGGGA AAAAAACATT TGTCATTAAC AACTGGACTG CCATTTGGTG	GTACATTAGC TGCGGGTATG ACAATTGCAC CAGGATTTAG AGCAGAGCTA
Karp	TGCTGAAGGC AAAAACACT TGTCATTAAC AAATGGGCTG CCATTTGGTG	GTACATTAGC TGCGGGTATG ACAATTGCAC CAGGATTTAG AGCAGAGCTA
Kato	TGCTGGTGGC AAAAAACAAT TGCCATTAAC AACCTCGATG CCATTTGGTG	GAACGTTGGC TGCAGGTATG ACAATCGCTC CAGGATTTAG AGCAGAGATA
Gilliam	TTCTGAGGGA AAAAAACATT TGTCATTAAC AACTGGACTG CCATTTGGTG	GTACATTAGC TGCAGGTATG ACAATCGCGC CAGGATTTAG AGCAGAGCTA
Kawasaki	TGTTGATTGC AAAAAACATT TGTCATTAAC AACTATGCTG CCATTTGGTG	GTACATTAGC TGCGGGTATG ACAATTGCAC CAGGATTTAG AGCAGAGCTA
Shimokoshi	TTCTGAAGGT AAGAAACATT TGCCGTTAAT AAAAGGGATG CCATTTGGTG	GCACATTGGC TGCAGGTATG ACAATTGCTC CAGGGTTTAG AGCAGAGCTA
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Kuroki	GGTGTTATGT ACCTTAGAAA TATAAGCGCT GAGGTTGAAG TAGGTAAAGC	CGAGGTAGAT TCTAAAGGTG AGATAAAGGC AGATTCTGGA GGTGGGACAG
Boyong	GGTGTTATGT ACCTTAGAAA TATAAGCGCT GAGGTTGAAG TAGGTAAAGG	CGAGGTAGAT TCTAAAGGTG AGATAAAGGC AGATTCTGGA GGTGGGACAG
Karp	GGTGTTATGT ACCTTACAAA TATAACTGCT CAGGTTGAAG AAGGTAAAGT	TAAGGCAGAT TCTGTAGGTG AGACAAAGGC AGATTCTGTA GGTGGGAAAG
Kato	GGGGTTATGT ACCTTGCGAA TGTAAAAGCA GAGGTGGAAT CAGG	
Gilliam	GGTGTTATGT ACCTTAGAAA TATAAGCGCT GAGGTTGAAG TAGGTGAAGT	TAAAACTGGC TCTGATGCTG ATATTAGATCTGGTGCAG AGGTAAAGGC AAGGTAGATT CTAAAGGTGA GATAAAGGCA GATTCTGGAG
Kawasaki	GGGGTTATGT ACCTTAGAAA TATAAACGCT GAGGTTGAAT TAGGTGAAGG	
Shimokoshi	AGTGCCATGT ACCTTATGAA CGTAAAAGCA GAGGTAGAGT TAGGGAAAAT	TAAAACAGGT TCTGGTGCTG CTAATGCTGCT ATTGATACAG
	TAGGAAAA	GGGTTCTGAT GCTAATACTG GTAC TACTGCT GATGCAAGTG
	1001 1050	1051
Kuroki	ATGCTCCTAT ACGTAAGCCG TTTAAACTTA CACCACCTCA GCCTACTATG	ATGCCTATAA GTATAGCTGA TCGTGACTTT GGGATTGATA TTCCTAA.CA
Boyong	ATGCTCCTAT ACGTAAGCCG TTTAAACTTA CACCACCTCA GCCTACTATG	AGCCCTATAA GTATAGCTGA TCGTGACTTT GGGATTGATA TTCCTAA.CA
Karp	ATGCTCCTAT ACGTAAGCGG TTTAAACTTA CACCTCCTCA GCCTACTATA	ATGCCTATAA GTATAGCTGA TCGTGACTTT GGGATTGATA TTCCTAACCA
Kato	ATTCTCCTAT GCCTCAGCGG TATAAACTTA CACCACCTCA GCCTACTATA	
Gilliam	GGTGGGACAG ATACTCGCGG TTTAAACTTA CACCACCTCA CCTACTGATA	ATGCCTATAA GTATTGCGGA TCGTGACCTT GGGGTTGATA TTCCTAACGT ATGCCTATAA GTATAGCTGA TCGTGATGTG GGGGTTGATA CTGATATTCT
Kawasaki	GCGCTCCTAT ACGTAAACGG TTTAAACTTA CACCTCCTCA ACCTACTATA	ATGCCTATAA GTATAGCGGA TCGTGATGTG GGGGTTGATA CTGACATTCT
Shimokoshi	CTGGTGTTAT ACGTAAGCAT AAAAAACTTA CACCACCTCA ACCTAATATA	ATGCCTATAA GTATAGCGGA TCGTGATTTG GGAGTTGATA CTGACATTCT
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	1101	1161
Kuroki	TACCTCAGGC GCAAGC GCAAGCTGCA CAGCCTC.CG CTTAATGATC	1151 1200
Boyong	TACCTCAGGC GCAACG GCAAGCTGCA CAGCCTC.CG CTTAATGATC	AGAAGCGTGC TGCAGCTAGG ATCGCTTGGT TAAAGAATTG TGCTGGTATT
Karp	GACCTCAGCA GCAAGC ACAAGCCG CAGCCTCAGG CTTAATGATG	AGAAGCGTGC TGCAGCTAGG ATCGCTTGGT TAAAGAATTG TGCTGGTATT
Kato	ACCTCAAGGA GGAGCTAATC ACCTGGGTGA TAACCTTGGT GCTAATGATA	AGCAACGTGC TGCAGCTAGG ATCGCTTGGT TAAAGAATTG TGCTGGTATT
Gilliam	TGCTCAAGCT GCTGCTGGGCAACCACAG. CTTACTGTTG	TTCGGCGTGC TGACGATAGG ATCACTTGGT TGAAGAATTA TGCTGGTGTT
Kawasaki	TGCTCAGG C TGCTGTTGGA CAACAACAG. CTTACTGTTG	AGCGGGCTGC TGCAGATAGG ATTGCTTGGT TGAAGAATTA TGCTGGTATT
Shimokoshi	AGCAGGACAA GGAAATGTTG	AGCAGCGGGC TGAAGATAGG ATTGCTTGGT TGAAGAATTA TGCTGGTATT
	AAIGITG	ATGTGCGTGC TGCTGCTAGG ATTGCTTGGT TGAAGAATTA TGCTGGTATT
	1201 1250	
Kuroki	1250	
	GACTATATGG TGAAGGATCC TAATAATCC TGGGGATA TGATGGTAAA	
Boyong	GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA	
Boyong Karp	GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA	
Karp	GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTATATGGG TAAAAAACCC TAATGATCCT AATGGGCCTA TGGTTATAAA	
Karp Kato	GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTATAGGG TAAAAAACCC TAATGATCCT AATGGGCCTA TGGTTATAAA GACTATATGG TTCCGGATCC TAATGATCCT CAGGCTA GAATTGTAAA	
Karp Kato Gilliam	GACTANATGG TBAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTANATGG TBAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTANATGG TAAAAAACCC TAATGATCCT AATGGGCCTA TGGTTATAAA GACTANATGG TCCCAGATCC TAATAATCCT CAGGCTA GAATTGTAAA GACTANATGG TCCCAGATCC TCAGAATCCT	
Karp Kato Gilliam Kawasaki	GACTATATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTATAATGG TGAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTATAGGG TAAAAAACCC TAATGATCCT AATGGGCCTA TGGTTATAAA GACTATATGG TTCCAGATCC TAATAATCCT CAGGCTA GAATTGTAAA GACTATATGG TCCCAGATCC TAGAATCCT	
Karp Kato Gilliam	GACTANATGG TBAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTANATGG TBAAGGATCC TAATAATCCTGGGCATA TGATGGTAAA GACTANATGG TAAAAAACCC TAATGATCCT AATGGGCCTA TGGTTATAAA GACTANATGG TCCCAGATCC TAATAATCCT CAGGCTA GAATTGTAAA GACTANATGG TCCCAGATCC TCAGAATCCT	

Fig 1-Alignment showing the positions and nucleotide sequences of primers (a, b, c and d, as indicated by bold letter and underline) from the 56 kDa protein corresponding gene of the seven strains. The DNA sequence data were run on a micro VAXII (Devereux et al, 1984) with UWGCG SEQUENCE ANALYSIS SOFTWARE (University of Wisconsin Genetic Computer Group). The program Stringsearch was used to search the individual sequence of the seven strains; whereas Pileup were performed to alignment the nucleotide sequences.

served from Gilliam, Karp and Kato strains tested (Fig 2). This 203 bp DNA fragment was also generated from the serum sample of a 40 year old patient (Fig 3, lanes 2, 3 and 4); no DNA amplification was observed from the patient with Q fever (Fig 3, lane 1). These results indicate that the primers we describe here are specific to R. tsutsu-

gamushi, within the limits of the testing done.

The various volumes of the PCR product amplified from the serum of the 40 year old patient, are shown in Fig 4. Samples 1-5 were analyzed by the colorimetric assay. The color reaction was clearly visualized within 30 minutes of incubation with

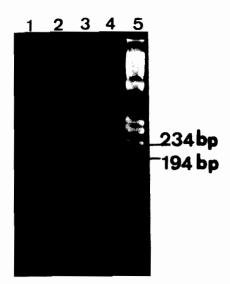


Fig 2-Agarose gel electrophoresis (1.5%) of amplified DNA by nested PCR with primers a, b, c and d for strains Gilliam (lane 2), Karp (lane 3), Kato (lane 4) and uninfected cell (lane 1). Lane 5 contained a DNA size marker (HT, Biotechnology Ltd), and the size fragments were 1353 bp, 1078 bp, 872 bp, 603 bp, 310 bp, 281 bp, 271 bp, 234 bp, 194 bp, 118 bp and 72 bp, only 234 and 194 bp are indicated.)

the substrate, and the optical density of the reaction at 450 nm was measured. Five μl (5 ng) or PCR products could be detected by this colorimetric assay (Fig 4, sample 5). However up to $20\,\mu l$ (20 ng) of PCR product could be observed by agarose gel electrophoresis (Fig 3, lane 2).

There was negligible background from a PCR mix incubated with a serum sample from a Q fever patient and without substrate DNA (Fig 4, samples Q and 6). Furthermore, with template DNA from cell cultures of Gilliam, Karp and Kato strains, ODs of 0.836, 0.929 and 0.592 were obtained, respectively (Fig 4, samples G, Kp and Kt).

Serum samples with low antibody titers obtained from four suspected patients were also examined by colorimetric PCR in early stage of onset. All four samples were positive (Table 1). These results were then confirmed by pathogenic isolation with cell cultures.

DISCUSSION

The symptom of R. tsutsugamushi disease ap-

pear after 1 to 2 week-latent period. Because the antibody titer against *R. tsutsugamushi* is not high enough to be detected at the initial stage of hospitalization, the timely serodiagnosis of this disease is difficult sometimes. Here we have presented a nested colorimetric PCR for direct diagnosis of serum samples from suspected patients in the acute phase of the illness.



Fig 3-Agarose gel electrophoresis (1.5%) of amplified DNA by nested PCR with primers a, b, c and d with template DNA from the patient of suspected with scrub typhus. Lanes 2, 3 and 4 are with 20, 15 and 10 µl of the template DNA; lane 1, with 20 µl of the template DNA from a patient with Q fever. Lane 5, DNA size marker the same as Fig 2, lane 5.

The application of PCR is expected to be good for the diagnosis of this disease (Furuya et al, 1992, 1993; Kawamori, et al, 1993). The specific primers for DNA amplification were selected on the basis of the nucleotide sequence of a gene encoding the 56 kDa protein of R. tsutsugamushi, which has been suggested as being specific and sensitive for PCR detection. Although the primers used were practical for diagnosis of the strains isolated from Japan, in our cases, an additional lower molecular weight band was also seen (Shieh et al, 1995).

Primers a, b, c and d we selected have high homology with known serovariants including the

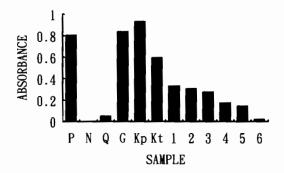


Fig 4-Use of the colorimetric PCR to detect the specific DNA of R. tsutsugamushi. Sample G, Kp and Kt: 25 μl of the PCR product from the cell culture of Gilliam, Karp and Kato strains. Sample Q: 25 μl of the PCR products from derived from the serum of the patients with Q fever. Sample 1-6: 25, 20, 15, 10, 5 and 0 μl of PCR products derived from the serum of the 40 year old patient, respectively. Sample P and N: positive and negative control of the colorimetric reaction.

Boyong strain isolated from Korea. The specific 203 bp DNA fragments of Gilliam, Karp and Kato strains were amplified by PCR, but sequences of Q fever were not. DNA isolated from other rickettsial pathogens such as R. typhi, R. porowazekii, R. conorii and Coxiella burnettii (data not shown) were not amplified by PCR. These indicate that the

Table 1
Diagnosis of R. tsutsugamushi patients by antibody titers, colorimetric PCR (CPCR) and pathogenic isolation (PI).

Patient No.	Days of illness	Titers	CPCR	ΡI
35	6	0	0.408	+b
73	5	40	0.815	+
100	4	320	1.074	+
101	5	0	0.628	+
Controlc		nd^d	0.000	nd

a) Antibody titers of patient sera against *R. tsutsugamushi* protostrains. The titers were expressed as the highest dilution of the serum. Antigens were prepared from infected L929 cell culture.

primers we used are specific for the diagnosis of tsutsugamuchi disease.

As many as 295 patients may have suffered from R. tsutsugamushi infection in Taiwan in 1994 alone. Nevertheless, it remains unknown whether there are serovarient strains in Taiwan. Therefore it is important for pathogen identification to be done. Here we present a colorimetric PCR method which could specificly detect the pathogens from sera of suspected patients with low IFA titers.

An enzyme-linked immunosorbent assay (ELISA) microplate hybridization method for R. tsutsugamushi detection has been reported (Sugita et al, 1993). This assay is quite sensitive, highly specific and reliable, but the protocol is time consuming since the PCR products obtained from whole blood sample had to be passaged through a sephadex G-50 column, the specific DNA probe had to be immobilized on the microplate, and the hybridization had to be carried out in the microplate wells for performing the enzymatic color reaction. Here we have described the application of a novel detection system for serum, which is an ELISAbased test, which can be readily adapted to diagnostic laboratories. The routine detection of R. tsutsugamushi from serum of suspected patients by this colorimatric PCR method concomitantly with IFA and pathogenic isolation are in progress.

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b) Pathogen isolation with positive results.

c) Serum from healthy donor.

d) Not done.

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