

Original Article

Development of a new real-time PCR system for simultaneous detection of bacteria and fungi in pathological samples

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Abstract: A novel system for simultaneous detection of pathogenic bacteria and fungi in pathological samples was developed using a real-time polymerase chain reaction (PCR) system. This system, designated the “multi-microbial real-time PCR”, has the potential to simultaneously detect 68 bacterial and 9 fungal species in a 96-well plate format. All probe-primer sets were designed to produce amplicons smaller than 210 bp using formalin-fixed paraffin-embedded samples as input. The specificity and sensitivity of each probe-primer set were tested against DNA extracted from pure cultures of specific pathogens. The multi-microbial real-time PCR system revealed profiles of microorganism infection in lung samples collected at autopsy from 10 patients with acquired immunodeficiency syndrome. *Staphylococcus aureus* was the most common microbe detected (n=8), but with low copy numbers. High copy numbers of *Pseudomonas aeruginosa* were detected in the lung samples with abscess (n=6). *Enterococcus faecium* (n=6), *Elizabethkingia meningoseptica* (n=4), and *Candida albicans* (n=4) were also frequently detected. In addition, a latent infection of *Mycobacterium tuberculosis* was detected in one case of pneumonia. In conclusion, this multi-microbial real-time PCR system can be useful for detecting bacteria and fungi in pathological specimens from patients with uncertain diagnoses.

Keywords: Real-time PCR, bacteria, fungi, detection, pathological sample, FFPE sample

Introduction

In pathological samples, bacteria and fungi are detectable using a conventional microscope. Hematoxylin and eosin staining and special staining techniques, such as periodic acid-Schiff staining, Gram staining, silver staining, and immunohistochemistry, are useful tools for characterizing microbes; however, these stains are not sufficiently specific to identify individual species. In clinical samples, conventional culture is required to identify microbial species, a technique that is not available for formalin-fixed paraffin-embedded (FFPE) samples.

Polymerase chain reaction (PCR) is widely used to identify pathogens from clinical samples. Real-time PCR can be used to both to accurately quantify microbial DNA and to identify species of pathogen using specific primers. Several

papers describe real-time PCR systems capable of identifying bacterial or fungal DNA that are the most frequent causative agents of sepsis [1] or acute gastroenteritis [2, 3]. However, the number of bacterial species that can be detected in a sample is limited by the fluorescence wavelength, and some rare, but important, infectious species were not included in these systems. Microarray can be used to simultaneously identify microbial species from samples with large numbers of bacteria or fungi, but its sensitivity is much lower than real-time PCR, even if amplified DNA is used for the assay [4]. Current sequencing technologies are remarkably advanced, and next generation sequencing (NGS) can analyze highly complex populations of sequences quickly, enabling massively parallel analyses [5]. Despite its promise, however, the high cost of equipment and the necessary time investment has pre-

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vented NGS from being useful in clinical laboratories. We previously established the multi-virus real-time PCR system to detect viruses in pathological specimens from patients with uncertain diagnoses [6]. This system is able to simultaneously detect more than one hundred human pathogenic viruses using a multiplex Taqman real-time PCR system. In addition, all probe-primer sets were designed to produce amplicons of less than 210 bp from viral genomes in FFPE samples.

In present study, we established a novel system for simultaneous detection of pathogenic bacteria and fungi in pathological samples using real-time PCR. We designate this system the “multi-microbial real-time PCR” system as it is analogous in design to the multivirus real-time PCR system we previously developed. The multi-microbial real-time PCR system could simultaneously assay for 68 bacterial species and 9 fungal species, all common human pathogens, in a 96-well plate. The specificity and sensitivity of each probe-primer set was estimated and confirmed by testing against standard lab strains. Using this system, we quantified the bacteria and fungi present in FFPE samples of infectious diseases. Finally, we investigated lung specimens from 10 cadavers with acquired immunodeficiency syndrome (AIDS) to reveal the profile of microbial infection in each case.

Materials and methods

Probe and primer sets

A total of 68 bacteria and 9 fungi were chosen as targets (**Table 1**). The choice of bacterial strains was made based on associations with human diseases and prevalence among humans. Probe-primer sets for each target were designed using Primer Express 2.0 (Applied Biosystems, Foster City, CA; **Table S1**). For some species, the designs of probe-primer sets published elsewhere were employed in our system. To detect short DNA fragments extracted from FFPE samples, primers were designed so that amplicons would be less than 210 bp. Probes and primers were synthesized by Sigma Genosys (Sigma-Aldrich, St. Louis, MO). All probes were labeled with 6-carboxy fluorescein (FAM) and 6-carboxytetramethylrhodamine (TAMRA). The sensitivity of each probe-primer set was confirmed by detection of at least 10

copies of a positive control PCR amplicon using conventional TaqMan real-time PCR (Applied Biosystems).

Establishment of the multi-microbial real-time PCR system

A TaqMan real-time PCR protocol was designed to detect specific bacterial and fungal species in a 96-well plate format. A Quantitect Probe PCR kit (Qiagen, Hilden, Germany), MicroAmp Optical 96-Well Reaction Plates (Applied Biosystems), and MicroAmp Optical Adhesive Film (Applied Biosystems) were used as 2× master mix, 96-well plates, and adhesive film, respectively. Each well contained a probe-primer set (**Table S1**), and each 96-well plate contained each probe-primer set for simultaneous detection of the 68 bacteria and 9 fungi listed in **Table 1**. To estimate microbial quantities, nine wells (A1-A9) from each plate contained a mixture of the glutathione S-transferase gene probe-primer set with dilutions of control plasmids (10^1 - 10^8 copies) to generate a standard curve [6]. To enable routine use of the system, 10 µl/well of 2× probe-primer mix was stored in 96-well plates at -20°C. DNA samples (50 ng per well) were added to the 2× master mix, and 10 µl were added to each well of a reaction plate and mixed with the 2× probe-primer mix. Real-time PCR was performed in an ABI PRISM 7900HT (Applied Biosystems), an Mx3005P (Stratagene, La Jolla, CA), or a 7500 real-time PCR system (Applied Biosystems). The PCR conditions were 95°C for 15 min, followed by 40 cycles of 94°C for 15 sec and 60°C for 1 min. Microbial quantities were calculated based on the standard curves generated from the plasmids in wells A1-A9.

Gene expression image

A gene expression image was produced with TreeView and Cluster software by Michael Eisen, University of California at Berkeley [7].

Positive control DNA samples

The control DNA samples were provided by the DNA Bank of the Riken Bio Resource Center (Tsukuba, Ibaraki, Japan) or purchased from the American Type Culture Collection (ATCC, Manassas, VA). Some control DNAs were also kindly provided by researchers in the National Institute of Infectious Diseases (**Table S2**).

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Clinical samples

The study protocol was approved by the Institutional Review Board, National Institute of Infectious Diseases, Japan (Approval No. 569). Pathological samples were collected from anonymized samples stored in our department. Lung tissues were taken at autopsy from 10 patients with AIDS. All lung tissue samples were immediately frozen and stored at -80°C. Nine patients were male and one was female. The mean age of the patients was 41.8 years (range: 26-62 years). No patients had received highly active anti-retroviral therapy. The CD4 count of each patient was below 4 counts/ μ l.

DNA extraction

Frozen samples were homogenized with a Multi-Beads Shocker (Yasui Kikai, Tokyo, Japan) in TEN buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 100 mM NaCl) with 100 ng/ml proteinase K and 0.1% sodium dodecyl sulfate. DNA was extracted from the homogenized tissues using the phenol-chloroform method. A QIAamp DNA FFPE tissue kit (Qiagen) was used for FFPE samples.

Histology and immunohistochemistry

In FFPE sections, bacteria/fungi were characterized by their morphology in hematoxylin-eosin stain, periodic acid-Schiff stain, acid-fast stain, Grocott's methenamine silver stain, and/or by immunohistochemistry. Immunohistochemistry was performed using primary antibodies specific to the bacteria/fungi listed in **Table 2**.

Results

Establishment and validation of multi-microbial real-time PCR

To validate the sensitivity and specificity of each probe and primer set used in the multi-microbial real-time PCR system, we examined DNA samples extracted from control bacteria and fungi (**Figure 1** and **Table S2**). Each probe and primer set amplified a gene fragment from the specific target microbe. The specificity of each probe-primer set was calculated by dividing the copy number of the target microbe in the positive control by the sum of all control samples. With only 9 exceptions, the probe-primer sets demonstrated more than 95% specificity. Of the probe-primer sets with low

specificity, some had amplified DNA from closely related bacterial families. For example, the probe-primer set for *Mycobacterium avium* complex (MAC) amplified gene fragments from *nocardia asteroides* and some members of the Mycobacteriaceae family, including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycobacterium kansasii*, and *Mycobacterium avium*, but not *Mycobacterium chelonae*. A probe-primer set for *Mycobacterium kansasii* also reacted with MAC. The probe-primer set for *Neisseria gonorrhoeae* also amplified a gene fragment of *Neisseria meningitidis*. The probe-primer set for *Providencia* had low specificity (21%) because of cross-reactions with the enterobacteriaceae family, including *Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis*, and *Morganella morganii*; however, these probe-primer sets failed to detect *Providencia* spp. *Yersinia pestis*, *Yersinia enterocolitica*, and *Citrobacter freundii* were not amplified with the probe-primer set for *Providencia*, although they are members of the Enterobacteriaceae family. The probe-primer set for *Clostridium tetani* cross-reacted with *Helicobacter cinaedi*. The set for *Rickettsia prowazekii* cross-reacted with *Rickettsia japonica*. The set for *Burkholderia cepacia* cross-reacted with *Burkholderia mallei*, although the set for *B.mallei* did not react with *B.cepacia*. Similarly, the set for *E.coli* cross-reacted with *Shigella* spp, although a set for *Shigella* spp. did not react with *E.coli*. All other probe-primer sets demonstrated high specificity (>95%), and no, or very low cross-reaction with off-target bacteria/fungi controls. Some FFPE samples from a mouse model of bacterial infection were positive for bacteria other than the target bacteria (**Table S2**). The multi-microbial real-time PCR system also detected human internal control genes, such as glyceraldehyde-3-phosphate dehydrogenase and beta-actin (**Tables S1** and **S2**), which were tested to determine if there was an inhibitory effect in FFPE samples on the PCR.

Detection of bacteria and fungi in FFPE samples

To evaluate if the multi-microbial real-time PCR method is able to detect microbes in pathological samples, FFPE samples from patients with infectious diseases were tested (**Table 2**; **Figure 2**). The multi-microbial real-time PCR detected at least 7 bacteria/fungi from the FFPE samples, including *Cryptococcus* sp, *Aspergillus* sp, *M.tuberculosis*, *Listeria monocy-*

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Table 1. List of Target Bacteria and Fungi

Bacteria
Firmicutes
Bacillales: <i>Staphylococcus aureus</i> , <i>Bacillus anthracis</i> , <i>Listeria monocytogenes</i> .
Lactobacillales: <i>Streptococcus pyogenes</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus mutans</i> , <i>Streptococcus sobrinus</i> , <i>Streptococcus sanguinis</i> , <i>Streptococcus oralis</i> , <i>Streptococcus salivaris</i> , <i>Streptococcus pneumoniae</i> , <i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i> .
Clostridiales: <i>Clostridium tetani</i> , <i>Clostridium difficile</i> , <i>Peptostreptococcus anaerobius</i> .
Actinobacteria
Actinomycetes: <i>Propionibacterium acnes</i> .
Corynebacteriales: <i>Corynebacterium diphtheriae</i> , <i>Mycobacterium tuberculosis</i> , <i>Mycobacterium laprae</i> , <i>Mycobacterium chelonae</i> , <i>Mycobacterium kansasii</i> , <i>Mycobacterium avium</i> complex, <i>Nocardia asteroides</i> .
Bacteroides
<i>Bacteroides fragilis</i> , <i>Elizabethkingia meningoseptica</i> .
Proteobacteria
Francisella tularensis, <i>Stenotrophomonas maltophilia</i> , <i>Legionella pneumophila</i> , <i>Aeromonas hydrophila</i> , <i>Haemophilus influenzae</i> .
Campylobacteriales: <i>Campylobacter jejuni</i> , <i>Helicobacter cinaedi</i> , <i>Helicobacter pylori</i> .
Rickettsiales: <i>Rickettsia prowazekii</i> , <i>Rickettsia japonica</i> , <i>Orientia tsutsugamushi</i> .
Rhizobiales: <i>Bartonella henselae</i> , <i>Brucella</i> .
Burkholderiales: <i>Bordetella pertussis</i> , <i>Burkholderia mallei</i> , <i>Burkholderia cepacia</i> .
Neisseriales: <i>Neisseria gonorrhoeae</i> , <i>Neisseria meningitidis</i> .
Pseudomonadales: <i>Moraxella catarrhalis</i> , <i>Pseudomonas aeruginosa</i> , <i>Acinetobacter baumannii</i> .
Vibrionales: <i>Vibrio cholerae</i> , <i>Vibrio parahaemolyticus</i> , <i>Vibrio vulnificus</i> .
Enterobacteriales: <i>Escherichia coli</i> , <i>Salmonella enterica</i> , <i>Shigella</i> , <i>Klebsiella pneumoniae</i> , <i>Yersinia pestis</i> , <i>Yersinia enterocolitica</i> , <i>Citrobacter freundii</i> , <i>Proteus mirabilis</i> , <i>Morganella morganii</i> , <i>Providencia</i> .
Tenericutes
<i>Mycoplasma pneumoniae</i> .
Fusobacteria
<i>Fusobacterium nucleatum</i> .
Spirochaetes
<i>Leptospira interrogans</i> , <i>Treponema pallidum</i> .
Chlamydiae
<i>Chlamydia psittaci</i> , <i>Chlamydia trachomatis</i> , <i>Chlamydia pneumoniae</i> .
Fungi
<i>Aspergillus fumigatus</i> , <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Cryptococcus</i> , <i>Candida albicans</i> , <i>Histoplasma</i> , <i>Trichosporon</i> , <i>Mucor</i> , <i>Coccidioides</i> .

togenes, and *Treponema pallidum*. The presence of these microbes in the FFPE samples was confirmed by immunohistochemistry or additional methods (Figure 2). The copy number of each microbe was also determined by a single, real-time PCR specific to each microbe. Copy numbers calculated using our multi-microbial real-time PCR were comparable to those determined by the individual real-time PCR (Table 2). In addition, six microbes were detected in FFPE samples from mouse models (Table 2, Table S2). Together, the multi-microbial real-time PCR successfully detected DNA from at least 13 microbial species in FFPE samples.

Detection of bacteria and fungi in the lungs of AIDS patient autopsy samples

Using the multi-microbial real-time PCR, we investigated the presence of bacteria and fungi

in 10 lung samples from autopsies of AIDS patients. The multi-microbial real-time PCR detected 17 bacterial DNA and one fungal DNA in 9 cases (Table 3). The multi-microbial real-time PCR also revealed copy numbers of each bacterium. The most commonly detected species was *Staphylococcus aureus* (n=8), but copy numbers were low in all cases. All samples positive for *Pseudomonas aeruginosa* (n=6) showed high bacterial copy numbers. *Enterococcus faecalis* (n=6), *Elizabethkingia meningoseptica* (n=4) and *Candida albicans* (n=4) were occasionally detected. In some cases, the microbes detected in the samples correlated with the patients' clinical diagnoses. For example, a high copy number of MAC was detected in a clinical case of atypical mycobacterial disease (Case No. 5). In Case No. 9, *P.aeruginosa* and *E.faecalis* were detected in lung abscesses by our system, while *P.aeruginosa* had been

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Table 2. Detection of bacteria and fungi in FFPE samples

Sample No.	Human/ mouse	Organ	Autopsy/ biopsy	Confirmed microbe	Copy number in MMRP	Copy number by single real-time PCR	Other microbes detected in MMRP (copy No.)	Methods of confirmation (antibody)
1	Clinical sample	Lymph node	Autopsy	Cryptococcus	2,918	5,404	E.meningoseptica (52), P.acnes (52), S.maltophilia (15), Providencia (15), S.aureus (12)	IHC (novocastra: NCL-CN)
2	Clinical sample	Paranasal sinus	Biopsy	A.fumigatus	1,732	2,329	P.acnes (58), E.meningoseptica (26), S.maltophilia (25), S.aureus (15)	IHC (LSBio: LS-C121391)
3	Clinical sample	Skin	Autopsy	M.tuberculosis	37	17	E.meningoseptica (42), S.maltophilia (20), H.cinaedi (18), P.acnes (14)	IHC (LSBio: LS-C72963)
4	Clinical sample	Heart	Autopsy	L.monocytogenes	1,997	699	S.aureus (30), P.acnes (30), E.meningoseptica (20)	IHC (In-house)
5	Clinical sample	Pharynx	Biopsy	T.pallidum	3,888	481,841	See Table S2	IHC (Abcam: ab20923)
6	Clinical sample	Tongue	Autopsy	P.acnes	65	193	S.maltophilia (16)	Gram
7	Clinical sample	Nasal mucosa	Autopsy	C.albicans	22	75	E.meningoseptica (60), S.aureus (34), P.acnes (14), S.maltophilia (12)	PAS, Grocott
8	Mouse model	Liver	-	S.pneumoniae	214	1,474	See Table S2	Injected with isolated microbe
9	Mouse model	Liver	-	C.tetani	92	1,329	See Table S2	Injected with isolated microbe
10	Mouse model	Liver	-	C.difficile	286	118	C.tetani (1446), Trichosporon (88), E.meningoseptica (84), P.acnes (47), R.japonica (35), B.pertussis (24), P.anaerobius (18), B.cepacia (18), S.maltophilia (16), C.freudii (14), C.albicans (12)	Injected with isolated microbe
11	Mouse model	Liver	-	H.cinaedi	827	216	See Table S2	Injected with isolated microbe
12	Mouse model	Liver	-	M.pneumoniae	144,418	592,381	See Table S2	Injected with isolated microbe
13	Mouse model	Liver	-	Y.pestis	315,300	238,439	E.meningoseptica (34), M.leprae (29), P.acnes (20)	Injected with isolated microbe

IHC: immunohistochemistry, PAS: Periodic acid-Schiff stain, MMRP: multi-microbial real-time PCR; A.fumigatus: Aspergillus fumigatus, B.cepacia: Burkholderia cepacia, B.pertussis: Bordetella pertussis, C.albicans: Candida albicans, C.freudii: Citrobacter freundii, C.tetani: Clostridium tetani, E.meningoseptica: Elizabethkingia meningoseptica, H.cinaedi: Helicobacter cinaedi, L.monocytogenes: Listeria monocytogenes, M.leprae: Mycobacterium leprae, M.tuberculosis: Mycobacterium tuberculosis, P.anaerobius: Peptostreptococcus anaerobius, P.acnes: Propionibacterium acnes, R.japonica: Rickettsia japonica, S.aureus: Staphylococcus aureus, S.maltophilia: Stenotrophomonas maltophilia, Y.pestis: Yersinia pestis.

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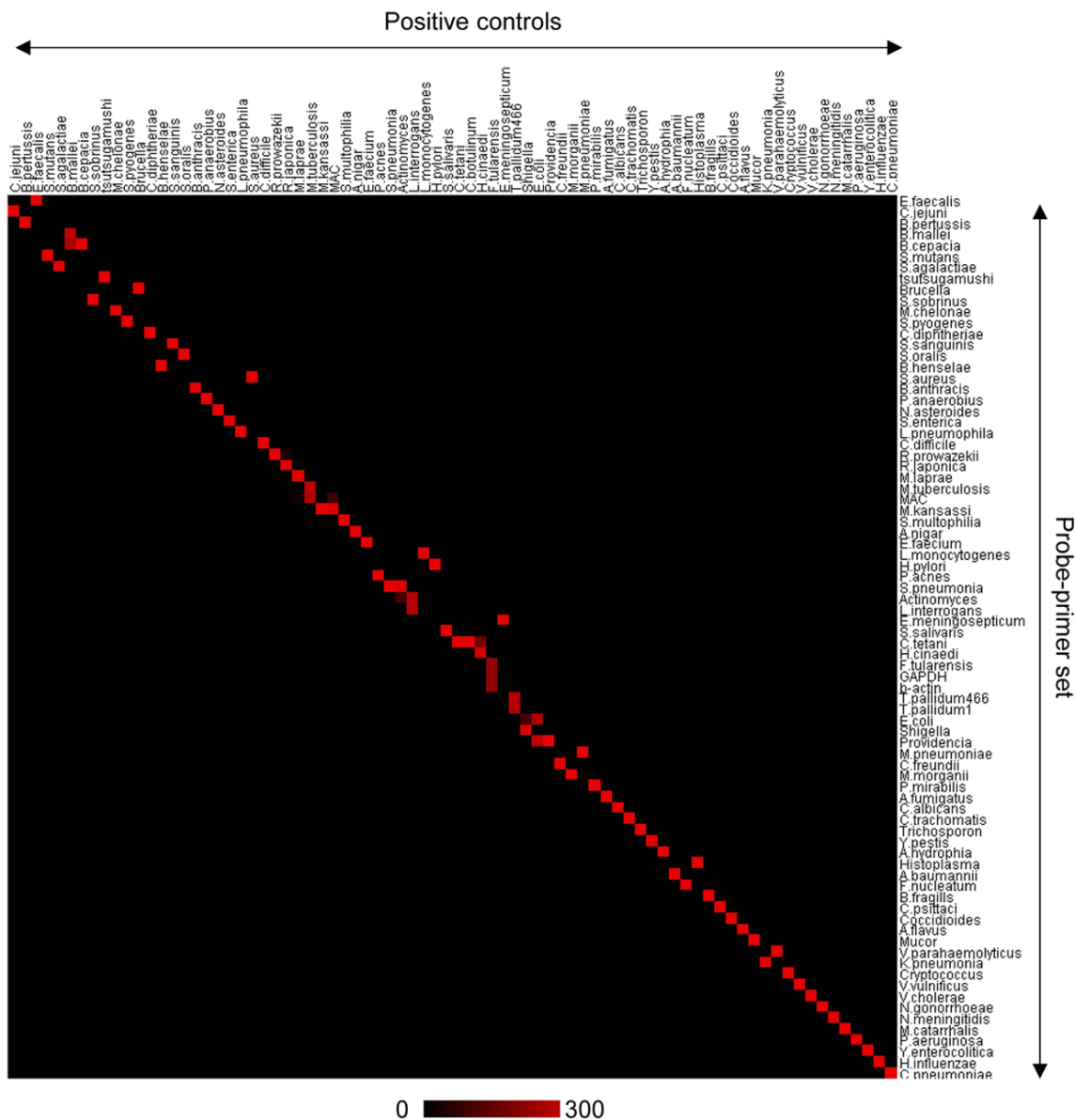


Figure 1. Validation of the multi-microbial real-time PCR assay. A gene expression image produced using TreeView software based on the results of the multi-microbial real-time PCR for control samples is shown. A horizontal line shows each probe-primer set and a vertical line is one sample of the positive control. A scale bar indicates copy number of color. Details of positive controls are provided in [Table S2](#).

cultivated from patient sputum culture. Case No. 6 was from a patient with sepsis, the cause of which had been unclear in his lifetime; however, our system detected a high copy number of *S.aureus* from lung samples. *E.coli*, *K.pneumoniae*, and *P.aeruginosa* were detected in case No. 7, a patient whose lung showed organized pneumonia.

Discussion

In the present study, we developed a new real-time PCR system, designated the “multi-micro-

bial real-time PCR”, with the potential to detect 68 bacterial and 9 fungal species simultaneously, even in FFPE samples. This system detected human pathogenic bacteria and fungi in the lungs of diseased AIDS patients who had not received any anti-retroviral therapy. The sensitivity and specificity of the multi-microbial real-time PCR system are equivalent to those of standard real-time PCR systems. Moreover, once the system is established, it follows simple protocols and is completed rapidly, requiring only 2 hours obtaining results. The system

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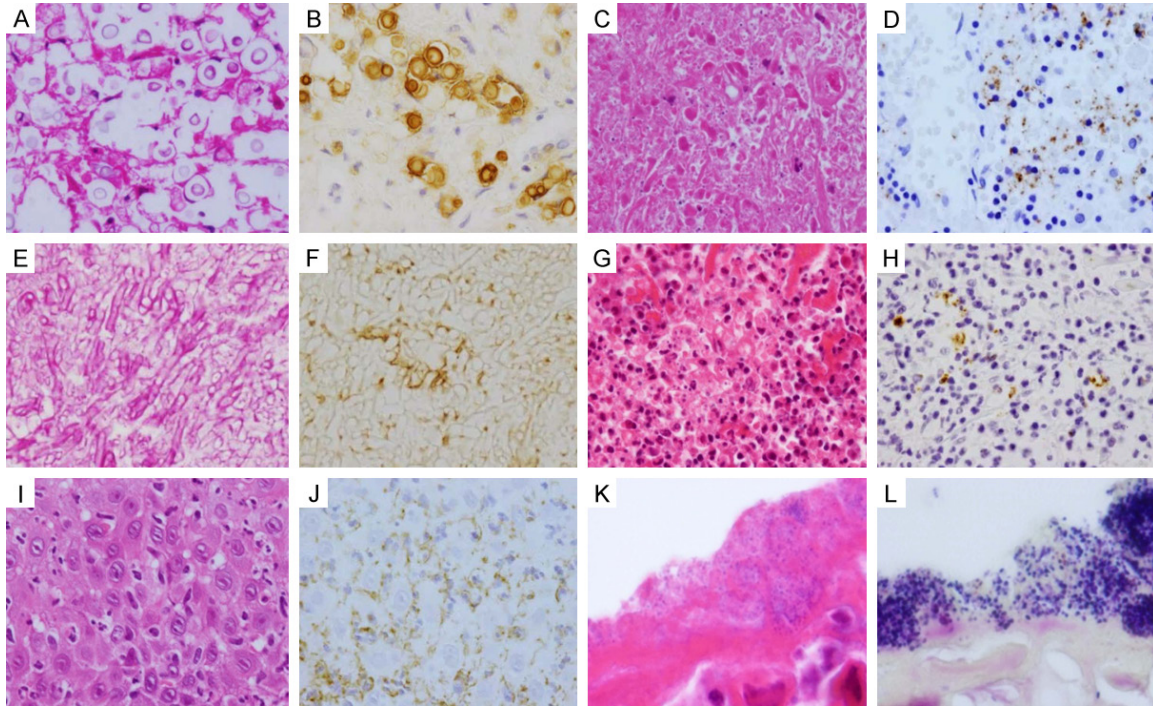


Figure 2. Immunohistochemical and pathological detection of bacteria and fungi in formalin-fixed paraffin-embedded (FFPE) samples. The presence of microbes in FFPE samples identified as containing *Cryptococcus sp* (A and B), *Mycobacterium tuberculosis* (C and D), *Aspergillus* (E and F), *Listeria* (G and H), and *Treponema pallidum* (I and J) were confirmed by immunohistochemistry using specific antibodies to each microbe. *Propionibacterium acnes* (K and L) was confirmed by Gram stain.

is flexible, in that new probe-primer sets can be incorporated; thus, methods for detecting new species of microbe can be established quickly.

To detect DNA sequences in FFPE samples, PCR amplicons should be less than 300 bp, because DNA is usually fragmented by formalin fixation [8, 9]. Many probe-primer sets were designed specifically for this study, because there are few reports describing real-time PCR for detection of bacterial/fungal genomes in FFPE samples. The 16S rRNA gene is encoded by genomes of almost all bacteria, and variable regions in this gene have been identified. Thus, while there are some known shortcomings, using the 16S rRNA sequence is a promising strategy to identify bacterial pathogens [10]. The 23S rRNA gene has more sequence variations between bacterial species than the 16S [11]. More recently, the locus between the 16S and 23S regions, the so-called internal transcribed region, has been targeted, because it contains greater variability than either 16S or 23S rRNA, allowing even better discrimination of bacterial species [12]. In the present study, we designed probe-primer sets targeting main-

ly 16S rRNA, 23S rRNA, and the internal transcribed regions. High specificities were achieved in the cross-reaction analysis, with few exceptions. Generally, to categorize a bacterial strain, it is necessary to amplify and sequence more than 1 kbp of the 16S rRNA region [10]. The use of amplicons shorter than 210 bp may be the main reason for the cross-reactions observed in our system. High copy numbers in target bacteria will assist identification of microbes in cases where cross-reaction is suspected. Although there is some room to improve the specificity of the probe-primer sets for reducing the cross-reactivities, the system as currently designed will function as a system for preliminary screening.

Systems for simultaneous molecular detection of bacteria and fungi DNA in blood samples have been previously reported [13-15]. Among them, Septifast® is a commercialized detection system that can detect 25 pathogens in clinical samples using real-time PCR [16]. Septifast® is useful for screening blood samples for bacterial and fungal infections [17-20]. However, there is no report describing the abil-

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Table 3. Detection of bacteria/fungi in the lung of AIDS autopsies

Case No.	Clinical diagnosis	Pathological diagnosis	Detected bacteria by multibacteria real-time PCR (Copy number)
1	Toxoplasma encephalitis, PCP	Blonchial pneumonia, Adrenal CMV infection, Epididymis small abscess	P.aeruginosa (4476), S.aureus (171), C.albicans (66), F.nucleatum (56), S.maltophilia (26)
2	PCP, SLE	PCP, CMV pneumonia, SLE	S.aureus (44), S.maltophilia (41), P.acnes (20), E.faecium (14)
3	No Data	No Data	P.aeruginosa (20406), E.faecalis (174), E.meningoseptica (55), C.albicans (42), S.maltophilia (23)
4	CMV infection, Pulmonary edema, DIC	Systemic CMV infection	Not detected
5	CMV retinitis, CMV colitis, Atypical mycobacterial disease, Chronic bronchial pneumonia, Kidney disarfaction, Liver mass	Atypical mycobacterial disease, ML, CMV infection	MAC (18721), P.aeruginosa (6746), M.kansasii (4468), S.aureus (167), S.maltophilia (97), Providencia (42), E.meningoseptica (33), M.morganii (32), C.albicans (30), P.acnes (22), E.faecalis (10)
6	ML, Cryptococcus meningitis, CMV retinitis, Aspiration pneumonitis	ML, Cryptococcus meningitis, CMV infection, sepsis	S.aureus (38515), E.faecalis (70)
7	PML, Aspiration pneumonitis, Hemophilia A, Oral candidiasis, Chronic hepatitis C	PML, Organized pneumonia	E.coli (7217224), Providencia (95254), K.pneumoniae (87912), P.aeruginosa (5784), E.faecalis (352), S.aureus (36), E.meningoseptica (31)
8	PCP, CMV pneumonia	Severe pneumonia (PCP and CMV pneumonia)	M.tuberculosis (125), E.meningosepticum (36), S.aureus (21)
9	Kidney disfunction induced drug, ML, Lung abscess (P.aeruginosa), KS, CMV infection, Amoeba liver abscess, adrenal disarfaction	ML, CMV infection, Lung abscess, KS	P.aeruginosa (5891), E.faecalis (3423), S.aureus (45), B.fragilis (26)
10	No Data	No Data	P.aeruginosa (727389), Providencia (65430), E.faecalis (4093), S.oralis (749), E.faecium (734), C.albicans (202), P.acnes (70), S.maltophilia (52), S.aureus (20)

CMV: cytomegalovirus, KS: Kaposi's sarcoma, ML: malignant lymphoma, PCP: Pneumocystis pneumonia, PML: progressive multifocal leukoencephalopathy, SLE: systemic lupus erythematosus; B.fragilis: Bacteroides fragilis, C.albicans: Candida albicans, E.coli: Escherichia coli, E.faecalis: Enterococcus faecalis E.faecium: Enterococcus faecium, E.meningoseptica: Elizabethkingia meningoseptica, F.nucleatum: Fusobacterium nucleatum, K.pneumoniae: Klebsiella pneumoniae, M.kansasii: Mycobacterium kansasii, M.morganii: Morganella morganii, M.tuberculosis: Mycobacterium tuberculosis, MAC: Mycobacterium avium complex, P.acnes: Propionibacterium acnes, P.aeruginosa: Pseudomonas aeruginosa, S.aureus: Staphylococcus aureus, S.maltophilia: Stenotrophomonas maltophilia, S.oralis: Streptococcus oralis.

ity of Septifast® to detect bacterial DNA in FFPE samples. Our multi-microbial real-time PCR can detect a greater number of bacterial/fungal species than Septifast®, and can detect DNA from pathogens even in FFPE samples in which DNA had degraded into fragments of less than 300 bp. This provides a large advantage in screening for pathogens in pathological samples. Generally, FFPE samples at autopsy were fixed in formalin for a longer time than FFPE biopsy samples, suggesting a greater degradation of DNA. Our system identified microbial DNA not only in biopsy FFPE samples, but also in autopsy FFPE samples. Our system provides a useful tool for safe detection of pathogens in FFPE samples, even after long periods of storage. In addition to FFPE samples, blood and other fluid samples, such as cerebral spinal fluid, pharyngeal swab, throat washing, and pleural and peritoneal effusions may be also suitable for our system. Various types of samples will be tested by our system to confirm its suitability in the future.

Using the multi-microbial real-time PCR, we detected 17 bacterial and one fungal species

from 10 lung samples from autopsies of AIDS patients. Basically, a pathogen detected with a high copy number in Septifast® system is correlated with the severity of sepsis [21]. On the other hand, when small quantities of bacteria are detected, it is difficult to determine whether they are associated with disease as a pathogen or a colonization event. A series of studies using bacterial DNA sequencing approaches have shown that healthy lungs harbor a unique steady-state microbiota, which is very different from other organs, such as the skin and gut [22]. Slight alterations to the microbiota in the lung could underlie susceptibility to lung disease. Additionally, the detection of bacteria may be complicated by previous treatments with antibiotics. Thus, when bacteria are detected in low copy numbers, we need to consider the whole picture, including the copy numbers of other bacteria, the patient's history of antibiotics usage, and the condition of host's immunity.

We have established a multi-microbial real-time PCR system with the potential to detect 68 bacteria and 9 fungi simultaneously from

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FFPE samples and preserved frozen samples from cadavers. Further research is required to confirm the utility of the system to probe additional patient samples, such as blood and other fluids. This system may provide a powerful tool for screening clinical samples for the presence of bacteria and fungi, particularly pathological samples, including FFPE samples from patients with uncertain diagnoses. In addition, this system is inherently flexible, allowing for addition of additional species of microbe on an as-needed basis. Use of this method in combination with a previously developed multivirus real-time PCR system will be considered for generation of a real-time PCR system detecting almost all human pathogens, which will compensate for some of the disadvantages of next generation sequencing-based assays.

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Disclosure of conflict of interest

None.

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Table S1. List of probe and primer sequences

No	Name of microbe	Well	Sequence of forward primer (5'-3')	Sequence of reverse primer (5'-3')	Sequence of probe (5'-3')	Target gene	GenBank	Start	Stop	Size of amplicon	Reference
1	Staphylococcus aureus	A10	TGGCGGCGTGCCTA-ATA	CGCTAACATCAGAGA-AGCAAGCT	ATGCAAGTCGAGCGAACG-GACGAG	16S ribosomal RNA	AB681714	536	603	68	This study (J Appl Microbiol. 2012 112: 823-30)
2	Bacillus anthracis	B10	CAGGCTTAGATTGGT-CAGCAA	CCGTGGTATGTTGAATGCT-CAT	CGTGAATTCTCATT-GCTCCTGGATCCG	pXO2	AF188935	56511	56584	74	This study (Biotechnol Lett. 2007 29: 1939-46)
3	Listeria monocytogenes	B11	TCCATAGCACCACCAG-CATCT	ATCCGCGTGTTCCTTTTCGA	CGCCTGCAAGTCCTAAGAC-GCCA	listeriolysin O (hly) gene	U25443	177	242	66	This study (J Appl Microbiol. 2012 112: 823-30)
4	Streptococcus pyogenes	A11	GGRACAG-TACCCAAAATGTAGGA	TCTTGAGCTCTTTGTCG-GTRTAG	CGTGACCAAAAAGGCG-GCATGC	sdaB gene	AB180951	427	500	74	This study (Ann Clin Microbiol Antimicrob. 2011 10: 33)
5	Streptococcus agalactiae	A12	GGAACTCTAGTG-GCTGGTGAT	ACTTGTGGAGTTGTCACCT-GATCAG	TTATTTTCACCAGCTG-TATTAGAAGTACA	cAMP factor (cfb) gene	EF694027	31	110	80	This study (Gynecol Obstet Invest. 2010 70: 250-5)
6	Streptococcus mutans	B1	CAGCGATAAGA-CAGCCTATGCTAA	TGGTTTACCCGTTTGACTG-GTT	CCGATTACCGTCTTTGAAC-CGCACA	gtfD gene	D88653	1146	1221	76	This study (Diagn Microbiol Infect Dis. 2004 48: 195-9)
7	Streptococcus sobrinus	B2	CAGATGCTGGTAAT-GTCCTTGGT	TCGGCAGACATGACATCTTGA	ACAGCGAAGTCTTGGTATC-GGTTTCGTTTC	gtfT gene	D13928	2377	2452	76	This study (Diagn Microbiol Infect Dis. 2004 48: 195-9)
8	Streptococcus sanguinis	B3	CAAAATTGTTG-CAAATCCAAAGG	GCTATCGCTCCCTGTCTTTGA	CAAAGAAAGATCGCTTGC-CAGAACCG	gtfP gene	AB056712	626	700	75	This study (Diagn Microbiol Infect Dis. 2004 48: 195-9)
9	Streptococcus oralis	B4	AATCAATACTATC-GCCCTGTGCTT	AGCGATTGCGGCACCTTCT	AGATGGCATTCCCGCTATC-TAACTGACG	gtfR gene	AB025228	5204	5286	83	This study (J Periodont Res 2013 48: 517-26)
10	Streptococcus salivarius	B5	CAC GCC ATG CTG GAA GTG	GCG ATG AGC CAA GCT GAA G	TTA GCT GCT GCG TAG ACT TCG TCT	gtfP gene	Z17279	48	114	67	Int J Peadiatr Dent. 2009 19: 406-11, PLoS One. 2012 7: e32169
11	Streptococcus pneumoniae	B6	GCTTATGGGCGC-CAAGTCTA	CAAAGCTTCAAAGCAGCCTC-TA	CTCAAGTTGGAAACCAC-GAGTAAGAGTGATGAA	ply gene	AE008539	2785	2862	78	J Clin Microbiol. 2007 45: 2460-6
12	Enterococcus faecalis	B7	TGATGCCCGC-GTTCATTTA	CGTCTTGTAAATTTTCAC-GATCAA	AAACCAATCGGCGAAA-CAACGTCTG	groESL gene	AY328531	1362	1439	78	This study (J Clin Microbiol. 2005 43: 235-41)
13	Enterococcus faecium	B8	GTATCAAGATCGTT-GTTCGTGCTT	CTGATCCTTCATAACCAGC-GTTT	AGAAGAACCAATTCGT-CAAATCGCTG	groESL gene	AF417582	1618	1692	75	This study (J Clin Microbiol. 2005 43: 235-41)
14	Actinomyces	F7	AAGAACCTTAC-CAGGGCTTGACA	GAGCTGACGACAACCATGCA	TTGGGCCGGTTCACAGGTG	16S ribosomal RNA	M33912	737	836	100	This study (Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 106: 443-9)
15	Propionibacterium acnes	C2	GCGTGAGTGACGGTA-ATGGGTA	TTCCG ACGCGATCAACCA	AGCGTTGTCCGGATT-TATTGGGCG	16S rRNA	KM099489	441	571	131	J. Clin. Microbiol. 2002 40: 198-204
16	Clostridium tetani	B12	AGCCACCATACTGTA-AGGGTCTAGA	TAATGAAGATGGTGGGTTA-AAATCTTC	TGCCGGAAGGTAT-GAATTTGGGACA	tetanus toxin	X06214	389	516	128	This study (J Clin Microbiol. 2005 43: 4342-8)

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17	<i>Clostridium difficile</i>	C1	AAGGGTATTGCTC-TACTGGCATT	CCTCATGGTCTTCAGAA-CAAGCT	TGGGCGTGTTTTTTG-GCAATATATCCTCA	tcdC gene	HQ596359	369	450	82	This study (J Clin Microbiol. 2002 40: 3470-5)
18	<i>Peptostreptococcus anaerobius</i>	E8	ACACCATGGGAGTCG-GAAAC	ACGCCACCTTCGACGACTT	CCGAAGCCGATTATCCAAC-CGCA	16S ribosomal RNA gene	L04168	1381	1450	70	This study (Microbiology. 2003 149: 1719-27)
19	<i>Mycoplasma pneumoniae</i>	F11	GCCACACCAATGC-CATCA	GAGGGAGGAAAAGCTC-GTGTT	CCGCCTTAACCCCGT-GAACGT	P1gene	M18639	328	395	68	This study (J Clin Microbiol. 2009 47: 2269-71)
20	<i>Corynebacterium diphtheriae</i>	B9	TTCTCCTGGCGTG-TACTTTGATC	CACCGCGGGAAGGAATG	CCGAGCGTCCACTG-CACTCCG	rpo B gene	BX248355	61792	61877	86	This study (Diagn Microbiol Infect Dis. 2012 73: 111-20)
21	<i>Mycobacterium tuberculosis</i>	F2	TCGACGCGATCGAG-CAA	GGATAACGTCTTTCAGGTC-GAGTAC	ATCTGGACCCGCCAA-CAAGAAGG	IS986	X52471	939	1007	69	This study (J Clin Microbiol. 2012 50: 646-50)
22	<i>Mycobacterium laprae</i>	F3	CGTTCTGGTTCGCATG-GTT	CCCTGGCGATAGCCGTAGT	ATGGCGACGGCCTACCTG-GT	pra gene	X65546	836	899	64	This study (Med Microbiol Immunol. 2004 193: 189-93)
23	<i>Mycobacterium chelonae</i>	F4	GGTTACTCGCTTGGT-GAATATGTTT	CGACGTTTTGCCGACTACCT	AAATCCTGTCCACCCC-GTGGGA	ITS1 gene	AM709729	121	190	70	This study (J Clin Microbiol. 2009 47: 1497-502)
24	<i>Mycobacterium kansasii</i>	F5	GGCGAACGGGTGAG-TAACA	ACCCAGTTTCCCAGGCTTATC	TGGGCAATCTGCCCTGCAC	16 SrRNA gene	X15916	59	124	66	This study (J Mol Diagn. 2009 11: 42-8)
25	<i>Mycobacterium avium complex</i>	F6	GCATCGAGAGCCGAT-GAAG	GGATCAATGCTCGGTTGACA	ACGTGGGAGGCTGC-GATATGC	24S rRNA gene	X74494	337	407	71	This study (J Clin Microbiol. 2000 38: 4080-5)
26	<i>Nocardia asteroides</i>	F8	GCCTTCGGGTTGTA-AACCTCTT	GCCGGTGCTTCTTACAG-GTA	ACAGGGACGAAGCG-CAAGTGACG	16S rRNA gene	AF430019	382	452	71	This study (J Clin Microbiol. 2010 48: 503-11)
27	<i>Fusobacterium nucleatum</i>	E10	CAAGCGGTGGAG-CATGTG	CTAAGATGTCAAACGCTG-GTAAGG	TTAATTCGACGCAACGC-GAGGA	16S rRNA gene	JQ001893	572	638	67	This study (J Clin Periodontol. 2006 33: 427-33)
28	<i>Leptospira interrogans</i>	F10	ACGATCTCTCAATCTA-ACGGAGAATAC	GGTCCGGTTCCTGTGAAAG	TTTTACCTGGACGGTC-GCTGGTCAA	lfb1 gene	HQ328633	165	239	75	This study (J Clin Microbiol. 2011 49: 2154-60)
29	<i>Treponema pallidum</i>	F9	GAAGCAGTCGAGGGT-GCAGTA	CGCTGTCAACCATGAAGGAA	CGTTGGCGGATCGCGCG	47kDa antigen gene	M88769	466	526	61	This study (Sex Transm Infect. 2011 87: 479-85)
29	<i>Treponema pallidum</i>	H3	CAGG-TAGAAGGGAGGGC-TAGTACA	CAGACACAGCACTC-GTCTTCAAC	CGGAGGTGAACTCCGTATT-GAAGTCGG	pol A gene	U57757	1574	1649	76	This study (J Clin Microbiol. 2010 48: 497-502)
30	<i>Chlamydia psittaci</i>	G3	GGGAAGGTGCTTCAG-GAGATC	TCCTGCGCGGATGCTAAT	TCCTTGCGCTACTTGGTGT-GACGC	ompA gene	AB468956	188	258	71	This study (Vet J. 2009 181: 145-50)
31	<i>Chlamydia trachomatis</i>	G4	AACGACATTTCTTGCT-GCAAAG	TCAGGACATTTTCGGATAGG	TTACCCATGAAATCCCTCGT-GATATA	23S ribosomal RNA gene	M19487	1441	1521	81	This study (J Med Microbiol. 2006 55: 1667-74)
32	<i>Chlamydia pneumoniae</i>	G5	ACTTGGTGCACGC-TATTAGC	GCATCTACTTTTAAAGATACG-GTCGAA	CGTGCTGGATTTTACG-GAGACTATG	ompA gene	AF131889	160	236	77	This study (J Clin Microbiol. 2003 41: 592-600)

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33	<i>Bacteroides fragilis</i>	E9	CACTTGACTGTTGTAG-ATAAAGC	CATCTTCATTGCAGCATTATCC	TGTGCTTGCTCCAGTC-GTCTATG	leuB gene	CR626927	3844464	3844598	135	J. Clin. Microbiol. 2013 51: 1593
34	<i>Elizabethkingia meningoseptica</i>	E2	CCAGCAGCCGCG-GTAAT	CGGACCCCTTTAAACCCAATA-AAT	CGGAGGGTGCAAGCGT-TATCC	16 SrRNA gene	HM748601	432	495	64	This study
35	<i>Campylobacter jejuni</i>	D3	TGCTTCTTACTTGTG-TGGCTTT	TGCTTACAAGTCT-GAATTTGG	CAAAGCATAGTATCTCG-CAATGTTGAT	hipOgene	Z36940	1964	2040	77	This study (BMC Microbiol. 2011 11: 113)
36	<i>Helicobacter cinaedi</i>	E12	GAATACGTTCCCGG-TCTTGT	TCCCGACTTAAGGCGAATACA	CTCACCCGCCGTACACCAT	16S rRNA gene	AF426158	1309	1377	69	This study (BMC Infect Dis. 2006 6: 86)
37	<i>Helicobacter pylori</i>	F1	TTCCATAGGCTATAAT-GTGATCCAAA	GCGCATGTCTTCGGTTAAAAA	AGGGCCTATGCCTACCCCT-GCGA	phosphoglucosamine mutase (glmM) gene	JN390578	78	153	76	This study (J. Clin. Microbiol. 2012 50: 3233-7)
38	<i>Rickettsia prowazekii</i>	F12	ATGAGCAGAATGCTTC-TACTTCAACA	CCAGTGCTAATACATG-CAAAAGGAT	CGGATTGCTGGCTCATCAG-GAGCT	gltA gene	M17149	785	862	78	This study (Am J Trop Med Hyg. 2005 73: 1083-5)
39	<i>Rickettsia japonica</i>	G1	CCGAATTGCCG-GCTCAT	GTGAGGCAATACCCGTGCTAA	CGGAGCTAACCCTTTT-GCTTGT	gltA gene	AY743327732	732	793	62	This study (Jpn J Infect Dis. 2010 63: 353-4)
40	<i>Orientia tsutsugamushi</i>	G2	AACTGATTT-TATTCAAATAATGCT-GCT	TATGCCTGAGTAAGATACRT-GAATRGAATT	TGGGTAGCTTTGGTGGAC-CGATGTTAATCT	47kDa outer membrane protein gene	L31934	630	747	118	Am J Trop Med Hyg. 2004 70: 351-6
41	<i>Bartonella henselae</i>	C12	AGATGAT-GATCCCAAGCCTTCTG-GCG	GGATRAAYRGWAAACCT-TYMYCGG	CCACCGTGGGCTTT-GAAAAACGCT	ITS region	L35101	321	421	101	Emerg Infect Dis. 2005 11: 1894-8
42	<i>Brucella</i>	D10	CGCCAAGATGTG-CAACGA	GGTCGCACCGGCTTTCTA	AAATCGCGGCAACCG-GCCTC	chromosome2	NC_010740	308676	308734	59	This study (J Clin Microbiol. 2010 48: 697-702)
43	<i>Bordetella pertussis</i>	C7	GGCATCGACCCAC-CAA	GGTTGTATTCGTCACAGTT-GAGT	TCGGGCGCGCTG-TACCCATCT	IS481 gene	AB473880	966	1034	69	This study (BMC Res Notes. 2011 4: 11)
44	<i>Burkholderia mallei</i>	D12	CGAGCGCATCG-TACTCGTA	CAAGTCGTGGATGCGCATTA	5'CTgAAACgCgCagCg3'MGB	YD repeat protein	CP000525.1	267228	267300	73	PLoS One. 2010 5: e15413
45	<i>Burkholderia cepacia</i>	E1	TCAAGAAGAACGAC-GAGGTGATC	CGGCGGCGACACCTT	AAACCCGCGTGAAGGTC-GTCAAGA	rec A gene	D90120	914	984	71	This study (J Clin Microbiol. 2001 39: 4247-55)
46	<i>Neisseria gonorrhoeae</i>	C3	AACGCCACGACG-GTATGC	CCGCTGAAACCGGAAAATC	TTTCCGTGCGTTAC-GATTCCCCC	porA pseudogene	AJ223446	662	725	64	This study (Sex Transm Infect. 2010 86: 207-11)
47	<i>Neisseria meningitidis</i>	C4	TGTTTACCCCTGATT-TACAAGGATT	GGCTCACAGCTTGGGTTTTTC	AGCGAAGGCTTACATG-GTTTCCACATCC	sodC gene	AJ001313	1879	1954	76	This study (PLoS One. 2011 6: e19361)
48	<i>Francisella tularensis</i>	D8	CCATATCACTGGCTTT-GCTAGACTAGT	TGTTGGCAAAGCTA-AAGAGTCTAAA	AAATTATAAAAC-CAAACCCAGACCTTCAAAC-CACA	hypothetical protein	CP003048	453602	453708	107	This study
49	<i>Stenotrophomonas maltophilia</i>	E11	GCC-GAAAGCCC AAGGTTT	CGACTTTCGTCTCGCCTTA	CGCAACGTTAATCGGCG-CAGG	23S rRNA gene	AF273255	1278	1354	77	This study (J Appl Microbiol. 2011 111: 1185-93)
50	<i>Legionella pneumophila</i>	D11	TGCCAAGTGGTTG-CAATACAA	CTCGACAGTGACTGTATC-CGATTT	ATCAATGCTGGAATGGTGT-TAAACCCGG	mip gene	AM051115	408	487	80	This study (Eur J Clin Microbiol Infect Dis. 2012 31: 2017-28)

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51	<i>Moraxella catarrhalis</i>	C5	GGTGAGTGCCGCTTT-TACAAC	TGTATCGCCTGCCAAGACAA	TGCTTTTGACAGCTGTTAGC-CAGCCTAAG	copB outer membrane protein gene	U69982	49	121	73	This study (J Clin Microbiol. 2003 41: 1386-90)
52	<i>Pseudomonas aeruginosa</i>	D9	AGCCTTCTG-GTCCCTTAC	CCTAATGAACCCCAAGTGTATA-AGTTTG	TGAAGTACGCGTCCG-CAACGGTT	oprL gene	Z50191	131	202	72	This study (J Clin Microbiol. 1997 35: 1295-9)
53	<i>Acinetobacter baumannii</i>	E7	GCTAATGCTGGCGTAA-CAGTTACTC	TTTACC GCCATTGTTGTGTTG	TGCTTGGTTACACTTTT-C AAGACAG	omp gene	AY485227	253	330	78	This study (J Clin Microbiol. 2012 50: 1412-4)
54	<i>Aeromonas hydrophila</i>	D7	TGGAACCACACCTTC-GTCATC	TGTAACGCTTGTCCCACTG-GTA	CCGTACAAGGACAAGGC-GAGCAGC	hemolysin gene	C0000462	467593	467668	76	This study (Forensic Science International. 2012 222: 11-26)
55	<i>Vibrio cholerae</i>	D4	TCCGGAG-CATAGAGCTTGGA	TCGATGATCTTGAGCATTCC	TCATCATGCACCGCC-GGGTTG	ctxA gene	HM042644	558	637	80	This study (J Clin Microbiol 1992 30: 2118-21)
56	<i>Vibrio parahaemolyticus</i>	D5	ACTCAACACAAGAAGA-GATCGACAA	GATGAGCGGTTGATGTCCAA	CGCTCGCGTTCACGAAAC-CGT	thermolabile hemolysin	CP006005.1	682659	682866	208	Appl. Environ. Microbiol. 2007 73: 5840-7
57	<i>Vibrio vulnificus</i>	D6	TGCGGGTGGTTCG-GTTAA	TTCTTCTTATCTAG-GCCCCAACT	AGCTGTCACGGCAGTTG-GAACC	vvhA gene	M34670	1833	1905	73	This study (J Clin Microbiol. 1998 36: 2887-92)
58	<i>Haemophilus influenzae</i>	C6	AGCGGCTTG-TAGTTCCTCTAACA	CAACAGAGTATCCGC-CAAAAGTT	CGATGCTGCAGGCAATG-GTGCT	omp P6	M19391	118	191	74	This study (Diagn Microbiol Infect Dis. 2009 64: 366-73)
59	<i>Escherichia coli</i>	C8	CGGCGTGGTGTAGAG-CATT	GCAGTCTTACTTCCAT-GATTTCTTTAAC	CGCTGCGATGGATCCCGG	beta-D-glucuronidase (uid A gene)	U00096.3	1695532	1695601	70	This study (water research 2009 43: 3019-28)
60	<i>Salmonella enterica</i>	C9	AATGGCGGCGAAT-TACGA	CGGATCCCTTTGCGAATAAC	CAGTAATGGTATCTGCT-GAAGTTGAGGA	InvA gene	U43272	1732	1799	68	This study (J Appl Microbiol. 2012 112: 823-30)
61	<i>Shigella</i>	C10	AACTGCCTGTGTTTCG-GTCTTCT	GATTATCACCG-CATCTGGGATAA	TGCAGCACCTTCCCTTG-GCCCA	ipaH gene	AE005674	749519	749587	69	This study (J Appl Microbiol. 2012 112: 823-30)
62	<i>Klebsiella pneumoniae</i>	C11	CGGGCGTAGCGC-GTAA	GATACCCGCATTACAT-TAAACAG	CCC GG CATGGATCGTTC-CGA	mdh gene	AM051115	140	201	62	This study (Plos One 2008 3: e3701)
63	<i>Yersinia pestis</i>	D1	TTTCCATCCTGAGAAG-TAAATGTTAAGTA	GGAACCACTAGCACATCTGT-TAACTTT	TGGGATCACCCGCG-GCATCT	caf1 gene	CP001595	67282	67361	80	This study (J Forensic Sci. 2006 51: 548-8)
64	<i>Yersinia enterocolitica</i>	D2	TCACGGAAAGGTTA-AGTCATCTGTAT	TGCCCGTATGCCATTG	TGATGGGTCAAGTCAAGT-CAAGTAAGACG	ail gene	AJ605740	9405	9477	73	This study (Appl Environ Microbiol. 2008 74: 6060-7)
65	<i>Citrobacter freundii</i>	E3	TGGCACGAGCGCTT-TAATC	TGAAGTGGCGGAATAACG	AGCATGGCCGGAGCTGT-CATC	cfa gene	U09771	92	152	61	This study (Curr Microbiol. 2005 51: 229-32)
66	<i>Proteus mirabilis</i>	E4	TGTGGCGGGTACTA-ATGCAA	CGCCTCTAACATGCGGTACA	CACAGTTACCCCCGG-TATTTGGAA	ureC gene	M31834	2406	2472	67	This study (Curr Microbiol. 2012 65: 44-53)
67	<i>Morganella morganii</i>	E5	GTGAAATCCCC-GGGCTTAA	ACCCCTCTACAAGACTC-TAGCT	CCGGAATTGCATCT-GATACTGG	16S rRNA gene	DQ358146	524	592	69	This study (Lett Appl Microbiol. 2012 54: 292-8)
68	<i>Providencia</i>	E6	GGCGG CAGGCCTAA-CAC	CGTCAGCGAGAAGCAAGCT	TGCAAGTCGAGCCGTAA-CAGGG	16s RNA gene	AY994312	31	91	61	This study (Jpn J Infect Dis. 2012 65: 545-7)
69	<i>Aspergillus fumigatus</i>	G6	GCCGAAGACCCCAA-CATG	CGGAACCAAGAGATCCGGTGT	ACGCTGTTCTGAAAGTATG-CAGTCTG	ITS1 gene	JQ080317	95	191	97	This study (J Clin Microbiol. 2009 47: 379-84)
70	<i>Aspergillus nigar</i>	G7	ACCCAACACGAA-CACTGTCT	CAAGAGATCCATTGTT-GAAAGTTTTAAC	AAAGCTGCAGTCTGAGTT-GATTGAATGC	ITS 1/2	GU256739	102	185	84	This study (J Clin Microbiol. 2011 49: 4150-7)

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71	Aspergillus flavus	G8	CCGTGTTTACTG- TACCTTAGTTGCTT	TACAATCAACTCAGACTTCAC- TAGATCAGA	CGCCCCCGGAGACACCA	ITS1 gene	HQ026744	37	168	132	This study (J Clin Microbiol. 2011 49: 4150-7)
72	Cryptococ- cus	G9	GCCCTTAAATGTGT- TAGTGGGAAGGT	CCATAGGCCAGCGAAACT	TTACCTGTGAGCCCGGC- GTAAT	ITS2 gene	AJ876523	391	459	69	This study (Diagn Microbiol Infect Dis. 2009 65: 69-72)
73	Candida albicans	G10	GGTTTGGTGTGAG- CAATACGA	AAGCGATCCCGCCTTACC	TGGGTTTGCTTGAAAGACG- GTAG	5.8S ribo- somal RNA	EF065152	96	161	66	This study (Eur J Clin Microbiol Infect Dis. 2012 31: 2237-45)
74	Histoplasma	G11	CAGCCAGCCAGGAG- CAAT	CCCCCAATTATCGTTCACTGA	TGGTGCGAGAGGATG- CAAGGTT	GAPDH gene	AF273703	652	717	66	This study (J Clin Microbiol. 2011 49: 3204-8)
75	Trichospor- on	G12	TGGCAG- GCTTGGGTCTGTA	AGGCTCACCAGCACT- CATACTTG	CGGTGAGCATACTAG- GAGCTGCAAAG	intergenic spacer (IGS) 1 region	AB439003	345	415	71	This study (Microbiol Immunol. 2011 55: 483-8)
76	Mucor	H1	CGACTAGAGA- TTGGGCGTGT	CCCCCAGAACCCAAAAA	TATGACTCGCTCAGCATCT- TAGCG	18S ribo- somal RNA gene	AF113440	1003	1077	75	This study (J Clin Microbiol. 2011 49: 2151-3)
77	Coccidioi- des	H2	CTGGACAATGCCAT- GCTATACTG	TGTGTCCCCACGCATCTGTA	AAGGCCACACGTCCTT- GTCG	RS hypothet- ical protein mRNA	XM_001245957	1262	1332	71	This study (Med Mycol. 2010 48: 466-9)
78	Beta-actin	H4	TGAGCGCGGCTA- CAGCTT	TCCTTAATGTCACGCACGATTT	ACCACCACGGCCGAGCGG	beta-actin	NM_001101	655	714	60	Clin Cancer Res. 2006 12: 29-33
79	Human GAPDH-DNA	H5	GCTCCCTCTTTCTTTG- CAGCAAT	TACCATGAGTCTTCCAC- GATAC	TCCTGCACCACCAACTGCT- TAGCACC	hGAPDH- DNA	NW_001838050.1	1055926	1056029	104	J Infect Dis. 2006 193: 773-82
80	Control plasmid	A1-9	AGAGCGTGCAGAGA- TTTCAATG	TGCAATTCTCGAAACAC- CGTAT	TTGAAGGAGCGGTTTTG- GATATT	GST	U13857	518	587	70	J Med Virol. 2011 83: 322-30

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Table S2. Validation of the sensitivity and specificity of each probe and primer set in the multi-microbial real-time PCR system

No.	1	2	3	4	5	6	7	8	9	10
Bacteria name	S.aureus	B.anthraxis	L.monocytogenes	S.pyogenes	S.agalactiae	S.mutans	S.sobrinus	S.sanguinis	S.oralis	S.salivaris
Sample	S.aureus subsp. aureus (JCM 2151) DNA	B.anthraxis BA103 DNA	Listeria infected mouse FFPE liver sample	S.pyogenes (JCM 5674T) DNA	S.agalactiae (JCM 5671T) DNA	S.mutans (ATCC25175) cell pellet	S.sobrinus MT8245 cell pellet	Streptococcus sanguinis (JCM 5708) DNA	S.oralis (ATCC35037) cell pellet	S.salivaris HT9R cell pellet
1	S.aureus	169993	0	0	0	0	0	0	0	0
2	B.anthraxis	0	22258	0	0	0	0	0	0	0
3	L.monocytogenes	0	0	3183	0	0	0	0	0	0
4	S.pyogenes	0	0	0	477	0	0	0	0	0
5	S.agalactiae	0	0	0	0	3752	0	0	0	0
6	S.mutans	0	0	0	0	0	57532	0	0	0
7	S.sobrinus	0	0	0	0	0	0	216526	0	0
8	S.sanguinis	0	0	0	0	0	0	0	208600	111
9	S.oralis	0	0	0	0	0	0	0	0	115200
10	S.salivaris	0	0	0	0	0	0	0	0	0
11	S.pneumoniae	0	0	0	0	0	0	0	0	9137
12	E.faecalis	0	0	0	0	0	0	0	0	0
13	E.faecium	0	0	0	0	0	0	0	0	0
14	C.tetani	0	0	0	0	0	0	0	0	0
15	C.difficile	0	0	0	0	0	0	0	0	0
16	P.anaerobius	0	0	0	0	0	0	0	0	0
17	Actinomyces	0	0	0	0	0	0	0	0	0
18	P.acnes	0	0	20	0	0	0	0	0	0
19	C.diphtheriae	0	0	0	0	0	0	0	0	0
20	M.tuberculosis	0	0	0	0	0	0	0	0	0
21	M.laprae	0	0	0	0	0	0	0	0	0
22	M.chelonae	0	0	0	0	0	0	0	0	0
23	M.kansasii	0	0	0	0	0	0	0	0	0
24	MAC	0	0	0	0	0	0	0	0	0
25	N.asteroides	0	18	0	0	0	0	0	0	0
26	B.fragilis	0	0	0	0	0	0	0	0	0
27	E.meningoseptica	0	0	22	18	0	0	0	0	0
28	C.jejuni	0	0	0	0	0	0	0	0	0
29	H.cinaedi	0	0	0	0	0	0	0	0	96
30	H.pylori	0	0	0	0	0	0	0	0	0
31	R.prowazekii	0	0	0	0	0	0	0	0	0
32	R.japonica	0	0	0	0	0	0	0	0	0
33	Tsutsugamushi	0	0	0	0	0	0	0	0	0
34	B.henselae	0	0	0	0	0	0	0	0	0
35	Brucella	0	0	0	0	0	0	0	0	0
36	B.pertussis	0	0	0	0	0	0	0	0	0
37	B.mallei	0	0	0	0	0	0	0	0	0

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38	B.cepacia	0	0	0	0	0	0	0	0	0	0
39	N.gonorrhoeae	0	0	0	0	0	0	0	0	0	0
40	N.meningitidis	0	0	0	0	0	0	0	0	0	0
41	F.tularensis	0	0	0	0	0	0	0	0	0	0
42	S.maltophilia	0	0	0	0	0	0	0	0	0	0
43	L.pneumophila	0	0	0	0	0	0	0	0	0	0
44	M.catarrhalis	0	0	0	0	0	0	0	0	0	0
45	P.aeruginosa	0	0	0	0	0	0	0	0	0	0
46	A.baumannii	0	0	0	0	0	0	0	0	0	0
47	A.hydrophila	0	0	0	0	0	0	0	0	0	0
48	V.cholerae	0	0	0	0	0	0	0	0	0	0
49	V.parahaemolyticus	27	0	0	0	0	0	0	0	0	0
50	V.vulnificus	0	0	0	0	0	0	0	0	0	0
51	H.influenzae	0	0	0	0	0	0	0	0	0	0
52	E.coli	0	0	0	0	26	21	23	0	0	0
53	S.enterica	0	0	0	0	0	0	0	0	0	0
54	Shigella	0	0	0	0	0	0	0	0	0	0
55	K.pneumoniae	0	0	0	0	0	0	0	0	0	0
56	Y.pestis	0	0	0	0	0	0	0	0	0	0
57	Y.enterocolitica	0	0	0	0	0	0	0	0	0	0
58	C.freundii	0	0	0	0	0	0	0	0	0	0
59	P.mirabilis	0	0	0	0	0	0	0	0	0	0
60	M.morganii	0	0	0	0	0	0	0	0	0	0
61	Providencia	0	13	0	0	0	0	0	0	0	0
62	M.pneumoniae	0	0	0	0	0	0	0	0	0	0
63	F.nucleatum	0	0	0	0	0	0	0	0	0	0
64	L.interrogans	0	0	0	0	0	0	0	0	0	0
65	T.pallidum466	0	0	0	0	0	0	0	0	0	0
65	T.pallidum1	0	0	0	0	0	0	0	0	0	0
66	C.psittaci	0	0	0	0	0	0	0	0	0	0
67	C.trachomatis	0	0	0	0	0	0	0	0	0	0
68	C.pneumoniae	0	0	0	0	0	0	0	0	0	0
69	A.fumigatus	0	0	0	0	0	0	0	0	0	0
70	A.nigar	0	0	0	0	0	0	0	0	0	0
71	A.flavus	0	0	0	0	0	0	0	0	0	0
72	Cryptococcus	0	0	0	0	0	0	0	0	0	0
73	C.albicans	0	0	0	0	0	0	0	0	0	0
74	Histoplasma	0	0	0	0	0	0	0	0	0	0
75	Trichosporon	0	0	0	0	0	0	0	0	0	0
76	Mucor	0	0	0	0	0	0	0	0	0	0
77	Coccidioides	0	0	0	0	0	0	0	0	0	0
78	b-actin	0	0	0	54	0	0	0	0	0	0
79	GAPDH	0	0	0	0	0	0	15	0	0	0

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No.	11	12	13	14	15	16	17	18	19	20
Bacteria name	S.pneumoniae	E.faecalis	E.faecium	C.tetani	C.difficile	P.anaerobius	Actinomyces	P.acnes	C.diphtheriae	M.tuberculosis
Sample	S.pneumoniae infected mouse FFPE liver sample	E.faecalis (MRY04-402) DNA	E.faecium (MRY04-403) DNA	C.tetani infected mouse FFPE liver sample	C.difficile (JCM 1296T) DNA	P.anaerobius (JCM 6477) DNA	A.israelii (JCM 12964T) DNA	P.acnes (JCM 6425) DNA	C.diphtheriae (JCM 1310) DNA	M.tuberculosis H37RV DNA
1	S.aureus	0	0	0	0	0	5	0	0	0
2	B.anthraxis	0	0	0	0	0	0	0	0	0
3	L.monocytogenes	0	0	0	0	0	0	0	0	0
4	S.pyogenes	0	0	0	0	0	0	0	0	0
5	S.agalactiae	0	0	0	0	0	0	0	0	0
6	S.mutans	0	0	0	0	0	0	0	0	0
7	S.sobrinus	0	0	0	0	0	0	0	0	0
8	S.sanguinis	0	0	0	0	0	0	0	0	0
9	S.oralis	0	0	0	0	0	0	0	0	0
10	S.salivaris	0	0	0	0	0	0	0	0	0
11	S.pneumoniae	215	0	0	0	0	6	0	0	0
12	E.faecalis	0	4760	0	0	0	0	0	0	0
13	E.faecium	0	0	13179	0	0	0	0	0	0
14	C.tetani	0	0	0	92	0	0	0	0	0
15	C.difficile	0	0	0	0	16282	0	0	0	0
16	P.anaerobius	0	0	0	0	0	168084	0	0	0
17	Actinomyces	0	0	0	0	0	0	3952	0	0
18	P.acnes	0	0	0	0	0	3	134900	27	0
19	C.diphtheriae	0	0	0	0	0	0	0	1493000	0
20	M.tuberculosis	0	0	0	7	0	0	0	0	23650000
21	M.laprae	0	0	0	0	0	0	0	0	0
22	M.chelonae	0	0	0	0	0	0	0	0	0
23	M.kansasii	0	0	0	0	0	0	0	0	0
24	MAC	0	0	0	17	0	0	0	0	3336000
25	N.asteroides	0	0	0	0	0	23	0	0	0
26	B.fragilis	0	0	0	0	0	0	0	0	0
27	E.meningoseptica	13	0	0	24	0	0	0	0	0
28	C.jejuni	0	0	0	0	0	0	0	0	0
29	H.cinaedi	0	0	0	0	0	0	0	0	0
30	H.pylori	0	0	0	0	0	0	0	0	0
31	R.prowazekii	0	0	0	0	0	0	0	0	0
32	R.japonica	0	0	0	0	0	0	0	0	0
33	Tsutsugamushi	0	0	0	0	0	0	0	0	0
34	B.henselae	0	0	0	0	0	0	0	0	0
35	Brucella	0	0	0	0	0	0	0	0	0
36	B.pertussis	0	0	0	0	0	0	0	0	0
37	B.mallei	0	0	0	0	0	0	0	0	0

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38	B.cepacia	0	0	0	0	0	0	0	0	0	0
39	N.gonorrhoeae	0	0	0	0	0	0	0	0	0	0
40	N.meningitidis	0	0	0	0	0	0	0	0	0	0
41	F.tularensis	0	0	0	0	0	0	0	0	0	0
42	S.maltophilia	0	0	0	0	0	0	0	0	0	0
43	L.pneumophila	0	0	0	0	0	0	0	0	0	0
44	M.catarrhalis	0	0	0	0	0	0	0	0	0	0
45	P.aeruginosa	0	0	0	0	0	0	0	0	0	0
46	A.baumannii	0	0	0	6	0	0	0	0	0	0
47	A.hydrophia	0	0	0	0	0	0	0	0	0	0
48	V.cholerae	0	0	0	0	0	0	0	0	0	0
49	V.parahaemolyticus	0	0	0	0	0	0	0	0	0	0
50	V.vulnificus	0	0	0	0	0	0	0	0	0	0
51	H.influenzae	0	0	0	0	0	0	0	0	0	0
52	E.coli	0	0	0	0	84	10	0	26	0	0
53	S.enterica	0	0	0	0	0	0	0	0	0	0
54	Shigella	0	0	0	0	0	0	0	0	0	0
55	K.pneumonia	0	0	0	0	0	0	0	0	0	0
56	Y.pestis	0	0	0	0	0	0	0	0	0	0
57	Y.enterocolitica	0	0	0	0	0	0	0	0	0	0
58	C.freundii	0	0	0	0	0	0	0	0	0	0
59	P.mirabilis	0	0	0	0	0	0	0	0	0	0
60	M.morganii	0	0	0	0	0	0	0	0	0	0
61	Providencia	0	0	0	0	0	0	0	0	0	0
62	M.pneumoniae	0	0	0	0	0	0	0	0	0	0
63	F.nucleatum	0	0	0	0	0	0	0	0	0	0
64	L.interrogans	0	0	0	0	0	0	0	0	0	0
65	T.pallidum466	0	0	0	0	0	0	0	0	0	0
65	T.pallidum1	0	0	0	0	0	0	0	0	0	0
66	C.psittaci	0	0	0	0	0	0	0	0	0	0
67	C.trachomatis	0	0	0	0	0	0	0	0	0	0
68	C.pneumoniae	0	0	0	0	0	0	0	0	0	0
69	A.fumigatus	0	0	0	0	0	0	0	0	0	0
70	A.nigar	0	0	0	0	0	0	0	0	0	0
71	A.flavus	0	0	0	0	0	0	0	0	0	0
72	Cryptococcus	0	0	0	0	0	0	0	0	0	0
73	C.albicans	0	0	0	6	0	0	0	0	0	0
74	Histoplasma	0	0	0	0	0	0	0	0	0	0
75	Trichosporon	0	0	0	0	0	0	0	0	0	0
76	Mucor	0	0	0	0	0	0	0	0	0	0
77	Coccidioides	0	0	0	0	0	0	0	0	0	0
78	b-actin	5880	0	0	3430	65	0	0	0	0	0
79	GAPDH	0	0	0	0	0	0	0	0	0	0

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No.		21	22	23	24	25	26	27	28	29	30
Bacteria name		M.laprae	M.chelonae	M.kansasii	MAC	N.asteroides	B.fragills	E.meningosepticum	C.jejuni	H.cinaedi	H.pylori
Sample		M.laprae Thai-53 DNA	M.chelonae type 090312 DNA	M.kansasii KK21-01 DNA	M.avium JATA 51-01 DNA	N.asteroides (JCM 3384T) DNA	B.fragills (JCM 11019) DNA	E.meningoseptica (JCM 21065) DNA	C.jejuni subsp.Jejuni (JCM2013) DNA	H.cinaedi 1296 strain infected mouse FFPE liver sample	H.pylori (JCM 12095) DNA
1	S.aureus	0	0	0	0	18	0	0	0	0	0
2	B.anthraxis	0	0	0	0	0	0	0	0	0	0
3	L.monocytogenes	0	0	0	0	0	0	0	0	0	0
4	S.pyogenes	0	0	0	0	0	0	0	0	0	0
5	S.agalactiae	0	0	0	0	0	0	0	0	0	0
6	S.mutans	0	0	0	0	0	0	0	0	0	0
7	S.sobrinus	0	0	0	0	0	0	0	0	0	0
8	S.sanguinis	0	0	0	0	0	0	0	0	0	0
9	S.oralis	0	0	0	0	0	0	0	0	0	0
10	S.salivaris	0	0	0	0	0	0	0	0	0	0
11	S.pneumoniae	0	0	0	0	0	0	0	0	0	0
12	E.faecalis	0	0	0	0	0	0	0	0	0	0
13	E.faecium	0	0	0	0	0	0	0	0	0	0
14	C.tetani	0	0	0	0	0	0	0	0	748	0
15	C.difficile	0	0	0	0	0	0	0	0	21	0
16	P.anaerobius	0	0	0	0	0	0	0	0	9	0
17	Actinomyces	0	0	0	0	0	0	0	0	0	0
18	P.acnes	0	0	23	0	0	0	0	0	32	5
19	C.diphtheriae	0	0	0	0	0	0	0	0	0	0
20	M.tuberculosis	133	0	0	0	0	0	0	0	0	0
21	M.laprae	73022	0	0	0	0	0	0	0	0	0
22	M.chelonae	0	878050	0	0	0	0	0	0	0	0
23	M.kansasii	0	0	102208	109000	0	0	0	0	0	0
24	MAC	567158	0	140892	1016000	126	0	0	0	0	0
25	N.asteroides	0	0	0	0	10790	0	0	0	0	0
26	B.fragills	0	0	0	0	0	147426	0	0	0	0
27	E.meningoseptica	59	0	0	0	20	0	271535	0	33	0
28	C.jejuni	0	0	0	0	0	0	0	9378	0	0
29	H.cinaedi	0	0	0	0	0	0	0	0	828	8
30	H.pylori	0	0	0	0	0	0	0	0	0	5745
31	R.prowazekii	0	0	0	0	0	0	0	0	0	0
32	R.japonica	0	0	0	0	0	0	0	0	6	0
33	Tsutsugamushi	0	0	0	0	0	0	0	0	0	0
34	B.henselae	0	0	0	0	0	0	0	0	0	0
35	Brucella	0	0	0	0	0	0	0	0	0	0
36	B.pertussis	0	0	0	0	0	0	0	0	0	0
37	B.mallei	0	0	0	0	0	0	0	0	0	0

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38	B.cepacia	0	0	0	0	0	0	0	0	0	0
39	N.gonorrhoeae	0	0	0	0	0	0	0	0	0	0
40	N.meningitidis	0	0	0	0	0	0	0	0	0	0
41	F.tularensis	0	0	0	0	0	0	0	0	0	0
42	S.maltophilia	34	0	0	22	13	0	0	0	19	0
43	L.pneumophila	0	0	0	0	0	0	0	0	9	0
44	M.catarrhalis	0	0	0	0	0	0	0	0	0	0
45	P.aeruginosa	0	0	0	0	0	0	0	0	0	0
46	A.baumannii	0	0	0	0	0	0	0	0	0	0
47	A.hydrophia	0	0	0	0	0	0	0	0	0	0
48	V.cholerae	0	0	0	0	0	0	0	0	0	0
49	V.parahaemolyticus	0	0	0	0	0	0	0	0	0	0
50	V.vulnificus	0	0	0	0	0	0	0	0	0	0
51	H.influenzae	0	0	0	0	0	0	0	0	0	0
52	E.coli	0	0	0	0	0	34	0	0	0	0
53	S.enterica	0	0	0	0	0	0	0	0	0	0
54	Shigella	0	0	0	0	0	0	0	0	0	0
55	K.pneumonia	0	0	0	0	0	0	0	0	0	0
56	Y.pestis	0	0	0	0	0	0	0	0	0	0
57	Y.enterocolitica	0	0	0	0	0	0	0	0	0	0
58	C.freundii	0	0	0	0	0	0	0	0	21	0
59	P.mirabilis	0	0	0	0	0	0	0	0	0	0
60	M.morganii	0	0	0	0	0	0	0	0	0	0
61	Providencia	0	0	0	0	0	0	0	0	0	0
62	M.pneumoniae	0	0	0	0	0	0	0	0	0	0
63	F.nucleatum	0	0	0	0	0	0	0	0	0	0
64	L.interrogans	0	0	0	0	0	0	0	0	0	0
65	T.pallidum466	0	0	0	0	0	0	0	0	0	0
65	T.pallidum1	0	0	0	0	0	0	0	0	0	0
66	C.psittaci	0	0	0	0	0	0	0	0	0	0
67	C.trachomatis	0	0	0	0	0	0	0	0	0	0
68	C.pneumoniae	0	0	0	0	0	0	0	0	0	0
69	A.fumigatus	0	0	0	0	0	0	0	0	6	0
70	A.nigar	0	0	0	0	0	0	0	0	0	0
71	A.flavus	0	0	0	0	0	0	0	0	0	0
72	Cryptococcus	0	0	0	0	0	0	0	0	0	0
73	C.albicans	0	0	0	0	0	0	0	0	0	0
74	Histoplasma	0	0	0	0	0	0	0	0	0	0
75	Trichosporon	0	0	0	0	0	0	0	0	21	0
76	Mucor	0	0	0	0	0	0	0	0	0	0
77	Coccidioides	0	0	0	0	0	0	0	0	0	0
78	b-actin	0	0	0	0	0	0	0	0	15571	0
79	GAPDH	0	19	0	0	0	0	0	0	0	0

New real-time PCR system for bacteria and fungi

No.		31	32	33	34	35	36	37	38	39	40
Bacteria name		R.prowazekii	R.japonica	Tsutsugamushi	B.henselae	Brucella	B.pertussis	B.mallei	B.cepacia	N.gonorrhoeae	N.meningitidis
Sample		R.typhi DNA	R.japonica DNA	O.tsutsugamushi Kuroki DNA	B.henselae (ATCC 49882D-5) DNA	B.abortus S99 strain DNA	B.pertussis tohama DNA	B.mallei DNA	B.cepacia DNA	N.gonorrhoeae (Zopf) Trevisan (ATCC 700825D-5) DNA	N.meningitidis DNA
1	S.aureus	26	6	0	0	0	0	0	0	0	0
2	B.anthraxis	0	0	0	0	0	0	0	0	0	0
3	L.monocytogenes	0	0	0	0	0	0	0	0	0	0
4	S.pyogenes	0	0	0	0	0	0	0	0	0	0
5	S.agalactiae	0	0	0	0	0	0	0	0	0	0
6	S.mutans	0	0	0	0	0	0	0	0	0	0
7	S.sobrinus	0	0	0	0	0	0	0	0	0	0
8	S.sanguinis	0	0	0	0	0	0	0	0	0	0
9	S.oralis	0	0	0	0	0	0	0	0	0	0
10	S.salivaris	0	0	0	0	0	0	0	0	0	0
11	S.pneumoniae	0	0	0	0	0	0	0	0	0	0
12	E.faecalis	0	0	0	0	0	0	0	0	0	0
13	E.faecium	0	0	0	0	0	0	0	0	0	0
14	C.tetani	0	0	0	0	0	0	0	0	0	0
15	C.difficile	0	0	0	0	0	0	0	0	0	0
16	P.anaerobius	0	0	0	0	0	0	0	0	0	0
17	Actinomyces	0	0	0	0	0	0	0	0	0	0
18	P.acnes	26	0	0	35	10	0	0	0	0	0
19	C.diphtheriae	0	0	0	0	0	0	0	0	0	0
20	M.tuberculosis	0	0	0	0	0	0	0	0	0	0
21	M.laprae	0	0	0	0	0	0	0	0	0	0
22	M.chelonae	0	0	0	0	0	0	0	0	0	0
23	M.kansasii	0	0	0	0	0	0	0	0	0	0
24	MAC	0	0	0	0	0	0	0	0	0	0
25	N.asteroides	0	7	0	0	0	0	0	0	0	0
26	B.fragilis	0	0	0	0	0	0	0	0	0	0
27	E.meningoseptica	21	0	11	0	0	0	0	0	0	0
28	C.jejuni	0	0	0	0	0	0	0	0	0	0
29	H.cinaedi	0	0	0	0	0	0	0	0	0	0
30	H.pylori	0	0	0	0	0	0	0	0	0	0
31	R.prowazekii	481	118	0	0	0	0	0	0	0	0
32	R.japonica	0	4448	0	0	0	0	0	0	0	0
33	Tsutsugamushi	0	0	5162	0	0	0	0	0	0	0
34	B.henselae	0	0	0	459	0	0	0	0	0	0
35	Brucella	0	0	0	0	44	0	0	0	0	0
36	B.pertussis	0	0	0	0	0	444016	0	0	0	0
37	B.mallei	0	0	0	0	0	0	55	0	0	0

New real-time PCR system for bacteria and fungi

38	B.cepacia	0	0	0	0	0	0	7235	4210	0	0
39	N.gonorrhoeae	0	0	0	0	0	0	0	0	54922	2825
40	N.meningitidis	0	0	0	0	0	0	0	0	0	869245
41	F.tularensis	0	0	0	0	0	0	0	0	0	0
42	S.maltophilia	15	0	0	0	0	0	0	0	0	0
43	L.pneumophila	0	0	0	0	0	0	0	0	0	0
44	M.catarrhalis	0	0	0	0	0	0	0	0	0	0
45	P.aeruginosa	0	0	0	0	0	0	0	0	0	12
46	A.baumannii	0	0	0	0	0	0	0	0	0	0
47	A.hydrophia	0	0	0	0	0	0	0	0	0	0
48	V.cholerae	0	0	0	0	0	0	0	0	0	0
49	V.parahaemolyticus	0	0	0	0	0	0	0	0	0	0
50	V.vulnificus	0	0	0	0	0	0	0	0	0	0
51	H.influenzae	0	0	0	0	0	0	0	0	0	0
52	E.coli	0	0	0	30	0	0	19	0	0	10
53	S.enterica	0	0	0	0	0	0	0	0	0	0
54	Shigella	0	0	0	0	0	0	0	0	0	0
55	K.pneumonia	0	0	0	0	0	0	0	0	0	0
56	Y.pestis	0	0	0	0	0	0	0	0	0	0
57	Y.enterocolitica	0	0	0	0	0	0	0	0	0	0
58	C.freundii	0	0	0	0	0	0	0	0	0	0
59	P.mirabilis	0	0	0	0	0	0	0	0	0	0
60	M.morganii	0	0	0	0	0	0	0	0	0	0
61	Providencia	0	0	0	17	0	0	13	0	21	0
62	M.pneumoniae	0	0	0	0	0	0	0	0	0	0
63	F.nucleatum	0	0	0	0	0	0	0	0	0	0
64	L.interrogans	0	0	0	0	0	0	0	0	0	0
65	T.pallidum466	0	0	0	0	0	0	0	0	0	0
65	T.pallidum1	0	0	0	0	0	0	0	0	0	0
66	C.psittaci	0	0	0	0	0	0	0	0	0	0
67	C.trachomatis	0	0	0	0	0	0	0	0	0	0
68	C.pneumoniae	0	0	0	0	0	0	0	0	0	0
69	A.fumigatus	0	0	0	0	0	0	0	0	0	0
70	A.nigar	0	0	0	0	0	0	0	0	0	0
71	A.flavus	0	0	0	0	0	0	0	0	0	0
72	Cryptococcus	0	0	0	0	0	0	0	0	0	0
73	C.albicans	0	0	0	0	0	0	0	0	0	0
74	Histoplasma	0	0	0	0	0	0	0	0	0	0
75	Trichosporon	0	0	0	0	0	0	0	0	0	0
76	Mucor	0	0	0	0	0	0	0	0	0	0
77	Coccidioides	0	0	0	0	0	0	0	0	0	0
78	b-actin	0	0	0	0	0	0	0	0	0	0
79	GAPDH	0	0	0	0	0	0	0	0	0	0

New real-time PCR system for bacteria and fungi

No.		41	42	43	44	45	46	47	48	49	50
	Bacteria name	F.tularensis	S.malto- philia	L.pneumophila	M.catarrhalis	P.aeruginosa	A.baumannii	A.hydrophilia	V.cholerae	V.paraha- emolyticus	V.vulnificus
	Sample	F.tularensis clinical pus- tule	S.malto- philia (JCM 1975T) DNA	L.pneumophila serum type 1 80- 045 DNA	M.catarrhalis DNA	P.aeruginosa (MRY06-352) DNA	A.baumannii (MRY09- 642) DNA	A.hydrophilia (ATCC7966) cell pellet	V.cholerae N16961cell pellet	V.paraha- emolyticus (RIMD2210633) cell pellet	V.vulnificus (ATCC27562) cell pellet
1	S.aureus	0	0	0	0	0	0	0	0	0	0
2	B.anthraxis	0	0	0	0	0	0	0	0	0	0
3	L.monocytogenes	0	0	0	0	0	0	0	0	0	0
4	S.pyogenes	0	0	0	0	0	0	0	0	0	0
5	S.agalactiae	0	0	0	0	0	0	0	0	0	0
6	S.mutans	0	0	0	0	0	0	0	0	0	0
7	S.sobrinus	0	0	0	0	0	0	0	0	0	0
8	S.sanguinis	0	0	0	0	0	0	0	0	0	0
9	S.oralis	0	0	0	0	0	0	0	0	0	0
10	S.salivaris	0	0	0	0	0	0	0	0	0	0
11	S.pneumoniae	0	0	0	0	0	0	0	0	0	0
12	E.faecalis	0	0	0	0	0	0	0	0	0	0
13	E.faecium	0	0	0	0	0	0	0	0	0	0
14	C.tetani	0	0	0	0	0	0	0	0	0	0
15	C.difficile	0	0	0	0	0	0	0	0	0	0
16	P.anaerobius	0	0	0	0	0	0	0	0	0	0
17	Actinomyces	0	0	0	0	0	0	0	0	0	0
18	P.acnes	0	0	24	0	0	0	0	0	0	0
19	C.diphtheriae	0	0	0	0	0	0	0	0	0	0
20	M.tuberculosis	0	0	0	0	0	0	0	0	0	0
21	M.laprae	0	0	0	0	0	0	0	0	0	0
22	M.chelonae	0	0	0	0	0	0	0	0	0	0
23	M.kansasii	0	0	0	0	0	0	0	0	0	0
24	MAC	0	0	0	0	0	0	0	0	0	0
25	N.asteroides	0	0	22	0	0	0	0	0	0	0
26	B.fragilis	0	0	0	0	0	0	0	0	0	0
27	E.meningoseptica	0	0	0	0	0	0	78	0	21	0
28	C.jejuni	0	0	0	0	0	0	0	0	0	0
29	H.cinaedi	0	0	0	0	0	0	0	0	0	0
30	H.pylori	0	0	0	0	0	0	0	0	0	0
31	R.prowazekii	0	0	0	0	0	0	0	0	0	0
32	R.japonica	0	0	0	0	0	0	0	0	0	0
33	Tsutsugamushi	0	0	0	0	0	0	0	0	0	0
34	B.henselae	0	0	0	0	0	0	0	0	0	0
35	Brucella	0	0	0	0	0	0	0	0	0	0
36	B.pertussis	0	0	0	0	0	0	0	0	0	0
37	B.mallei	0	0	0	0	0	0	0	0	0	0

New real-time PCR system for bacteria and fungi

38	B.cepacia	0	0	0	0	0	0	0	0	0	0
39	N.gonorrhoeae	0	0	0	0	0	0	0	0	0	0
40	N.meningitidis	0	0	0	0	0	0	0	0	0	0
41	F.tularensis	3780	0	0	0	0	0	0	0	0	0
42	S.maltophilia	0	14278	0	0	0	0	0	0	0	0
43	L.pneumophila	0	0	287980	0	0	0	0	0	0	0
44	M.catarrhalis	0	0	0	1010000	0	0	0	0	0	0
45	P.aeruginosa	0	0	0	15	581200	0	0	0	0	0
46	A.baumannii	0	0	0	0	0	337700	0	0	0	0
47	A.hydrophia	0	0	0	0	0	0	3517666	0	0	0
48	V.cholerae	0	0	0	0	0	0	38	2310000	0	0
49	V.parahaemolyticus	0	0	0	0	0	0	0	0	1243794	0
50	V.vulnificus	0	0	0	0	0	0	23	0	0	702647
51	H.influenzae	0	0	0	0	0	0	0	0	0	0
52	E.coli	0	0	65	0	45	0	0	0	70	22
53	S.enterica	0	0	0	0	0	0	0	0	0	0
54	Shigella	0	0	0	0	0	0	0	0	0	0
55	K.pneumonia	0	0	0	0	0	0	0	0	0	0
56	Y.pestis	0	0	0	0	0	0	0	0	0	0
57	Y.enterocolitica	0	0	0	0	0	0	0	0	0	0
58	C.freundii	0	0	0	0	0	0	0	0	0	0
59	P.mirabilis	0	0	0	0	0	0	0	0	0	0
60	M.morganii	0	0	0	0	0	0	0	0	0	0
61	Providencia	0	0	0	0	0	0	0	0	0	0
62	M.pneumoniae	0	0	0	0	0	0	0	0	0	0
63	F.nucleatum	0	0	0	0	0	0	21	0	0	0
64	L.interrogans	0	0	0	0	0	0	0	0	0	0
65	T.pallidum466	0	0	0	0	0	0	0	0	0	0
65	T.pallidum1	0	0	0	0	0	0	0	0	0	0
66	C.psittaci	0	0	0	0	0	0	0	0	0	0
67	C.trachomatis	0	0	0	0	0	0	0	0	0	0
68	C.pneumoniae	0	0	0	0	0	0	0	0	0	0
69	A.fumigatus	0	0	0	0	0	0	0	0	0	0
70	A.nigar	0	0	0	0	0	0	0	0	0	0
71	A.flavus	0	0	0	0	0	0	0	0	0	0
72	Cryptococcus	0	0	0	0	0	0	0	0	0	0
73	C.albicans	0	0	0	0	0	0	0	0	0	0
74	Histoplasma	0	0	0	0	0	0	0	0	0	0
75	Trichosporon	0	0	0	0	0	0	0	0	0	0
76	Mucor	0	0	0	0	0	0	0	0	0	0
77	Coccidioides	0	0	0	0	0	0	0	0	0	0
78	b-actin	524200	0	0	0	0	0	0	0	33	0
79	GAPDH	261700	0	0	0	0	0	0	0	0	0

New real-time PCR system for bacteria and fungi

No.	51	52	53	54	55	56	57	58	59	60
Bacteria name	H.influenzae	E.coli	S.enterica	Shigella	K.pneumonia	Y.pestis	Y.entero- colitica	C.freundii	P.mirabilis	M.morganii
Sample	H.influenzae (ATCC 51907D) DNA	E.coli JM109 cell pellet	S.enteritidis DNA	S.dyscen- teriae Type1DNA	K.pneumonia (MRY06-359) DNA	Y.pestis Bry- ans DNA	Y.entero- colitica DNA	C.freundii (JCM 1657T) DNA	P.mirabilis (JCM 1669T) DNA	M.morganii subsp. Mor- ganii (JCM 1672T) DNA
1	S.aureus	0	0	0	0	0	0	0	0	0
2	B.anthraxis	0	0	0	0	0	0	0	0	0
3	L.monocytogenes	0	0	0	0	0	0	0	0	0
4	S.pyogenes	0	0	0	0	0	0	0	0	0
5	S.agalactiae	0	0	0	0	0	0	0	0	0
6	S.mutans	0	0	0	0	0	0	0	0	0
7	S.sobrinus	0	0	0	0	0	0	0	0	0
8	S.sanguinis	0	0	0	0	0	0	0	0	0
9	S.oralis	0	0	0	0	0	0	16	0	0
10	S.salivaris	0	0	0	0	0	0	0	0	0
11	S.pneumoniae	0	0	0	0	0	0	0	0	0
12	E.faecalis	0	0	0	0	0	0	0	0	0
13	E.faecium	0	49	0	0	0	0	0	0	0
14	C.tetani	0	0	0	0	0	0	0	0	0
15	C.difficile	0	0	0	0	0	0	0	0	0
16	P.anaerobius	0	0	0	0	0	0	0	0	0
17	Actinomyces	0	0	0	0	0	0	0	0	0
18	P.acnes	0	0	0	0	21	0	6	0	20
19	C.diphtheriae	0	0	0	0	0	0	0	0	0
20	M.tuberculosis	0	0	0	0	0	0	0	0	0
21	M.laprae	0	0	0	0	0	0	0	0	0
22	M.chelonae	0	0	0	0	0	0	0	0	0
23	M.kansasii	0	0	0	0	0	0	0	0	0
24	MAC	0	0	0	0	0	0	0	0	0
25	N.asteroides	0	0	22	0	0	0	0	0	0
26	B.fragilis	0	0	0	0	0	0	0	0	0
27	E.meningoseptica	0	0	0	0	0	0	0	28	9
28	C.jejuni	0	0	0	0	0	0	0	0	0
29	H.cinaedi	0	31	0	0	0	0	0	0	0
30	H.pylori	0	0	0	0	0	0	0	0	0
31	R.prowazekii	0	0	0	0	0	0	0	0	0
32	R.japonica	0	0	0	0	0	0	0	0	0
33	Tsutsugamushi	0	0	0	0	0	0	0	0	0
34	B.henselae	0	0	0	0	0	0	0	0	0
35	Brucella	0	0	0	0	0	0	0	0	0
36	B.pertussis	0	0	0	0	0	0	0	0	0
37	B.mallei	0	0	0	0	0	0	0	0	0

New real-time PCR system for bacteria and fungi

38	B.cepacia	0	0	0	0	0	0	0	0	0	0
39	N.gonorrhoeae	0	0	0	0	0	0	0	0	0	0
40	N.meningitidis	0	0	0	0	0	0	0	0	0	0
41	F.tularensis	0	0	0	0	0	0	0	0	0	0
42	S.maltophilia	0	0	0	0	0	6	0	0	0	0
43	L.pneumophila	0	0	0	0	0	0	0	0	0	0
44	M.catarrhalis	0	0	0	0	0	0	0	0	0	0
45	P.aeruginosa	0	0	0	0	0	0	0	0	0	0
46	A.baumannii	0	0	0	0	0	0	0	0	0	0
47	A.hydrophia	0	0	0	0	0	0	0	0	0	0
48	V.cholerae	0	0	0	0	0	0	0	0	0	0
49	V.parahaemolyticus	0	0	0	0	0	0	0	0	0	0
50	V.vulnificus	0	0	0	0	0	0	0	0	0	0
51	H.influenzae	468	0	0	0	0	0	0	0	0	0
52	E.coli	0	5959318	0	3000215	18	0	0	0	29	19
53	S.enterica	0	0	8859	0	0	0	0	0	0	0
54	Shigella	0	0	0	7579209	0	0	0	0	0	0
55	K.pneumonia	0	0	0	0	43427	0	0	0	0	0
56	Y.pestis	0	0	0	0	0	420000000	14	0	0	0
57	Y.enterocolitica	0	0	0	0	0	0	1007417	0	0	0
58	C.freundii	0	0	0	0	0	0	0	2881	0	0
59	P.mirabilis	0	0	0	0	0	0	0	0	7136	0
60	M.morganii	0	0	0	0	0	0	0	0	0	40227
61	Providencia	0	2858817	139	6755	348	0	0	0	7234	15798
62	M.pneumoniae	0	0	0	0	0	0	0	0	0	0
63	F.nucleatum	0	0	0	0	0	0	0	0	0	0
64	L.interrogans	0	0	0	0	0	0	0	0	0	0
65	T.pallidum466	0	0	0	0	0	0	0	0	0	0
65	T.pallidum1	0	0	0	0	0	0	0	0	0	0
66	C.psittaci	0	0	0	0	0	0	0	0	0	0
67	C.trachomatis	0	0	0	0	0	0	0	0	0	0
68	C.pneumoniae	0	0	0	0	0	0	0	0	0	0
69	A.fumigatus	0	0	0	0	0	0	0	0	0	0
70	A.nigar	0	0	0	0	0	0	0	0	0	0
71	A.flavus	0	0	0	0	0	0	0	0	0	0
72	Cryptococcus	0	0	0	0	0	0	0	0	0	0
73	C.albicans	0	0	0	0	0	0	0	0	0	0
74	Histoplasma	0	0	0	0	0	0	0	0	0	0
75	Trichosporon	0	0	0	0	0	0	0	0	0	0
76	Mucor	0	0	0	0	0	0	0	0	0	0
77	Coccidioides	0	0	0	0	0	0	0	0	0	0
78	b-actin	0	0	0	0	0	14	0	0	0	0
79	GAPDH	0	0	0	0	0	0	0	0	0	0

New real-time PCR system for bacteria and fungi

No.	61	62	63	64	65	66	67	68	69	70
Bacteria name	Providencia	M.pneumoniae	F.nucleatum	L.interrogans	T.pallidum466	C.psittaci	C.trachomatis	C.pneumoniae	A.fumigatus	A.nigar
Sample	P.rettgeri (JCM 1675T) DNA	M.pneumoniae infected mouse FFPE liver sample	F.nucleatum cell pelet	L.interrogans serovar Manilae strain UP-MMC-NIID DNA	T.pallidum clinical FFPE sample	C.psittaci Budgeriger-1 DNA	C.trachomatis type D DNA	C.pneumoniae TW183 DNA	A.fumigatus (MYA-3626) DNA	A.nigar (JCM 22282) DNA
1	S.aureus	0	0	0	0	9	25	0	0	13
2	B.anthraxis	0	0	0	0	0	0	0	0	0
3	L.monocytogenes	0	0	0	0	0	0	0	0	0
4	S.pyogenes	0	0	0	0	0	0	0	0	0
5	S.agalactiae	0	0	0	0	0	0	0	0	0
6	S.mutans	0	0	0	0	0	0	0	0	0
7	S.sobrinus	0	0	0	0	0	0	0	0	0
8	S.sanguinis	0	0	0	0	0	0	0	0	0
9	S.oralis	9	0	16	0	0	0	0	0	0
10	S.salivaris	0	0	0	0	0	0	0	0	0
11	S.pneumoniae	0	0	0	0	0	0	0	0	0
12	E.faecalis	0	0	0	0	0	0	0	0	0
13	E.faecium	0	0	0	0	0	0	0	0	0
14	C.tetani	0	0	0	0	0	0	0	0	0
15	C.difficile	0	0	0	0	0	0	0	0	0
16	P.anaerobius	0	0	0	0	0	0	0	0	0
17	Actinomyces	0	0	0	48030	0	0	0	0	0
18	P.acnes	0	0	0	0	0	0	0	7	0
19	C.diphtheriae	0	0	0	0	0	0	0	0	0
20	M.tuberculosis	0	0	0	0	0	0	0	0	0
21	M.laprae	0	0	0	0	0	0	0	0	0
22	M.chelonae	0	0	0	0	0	0	0	0	0
23	M.kansasii	0	0	34	0	0	0	0	0	4
24	MAC	0	0	0	0	0	0	0	0	0
25	N.asteroides	0	0	0	0	0	0	0	0	0
26	B.fragilis	0	0	0	0	28	0	0	0	0
27	E.meningoseptica	0	10	0	0	35	0	0	12	0
28	C.jejuni	0	0	0	0	0	0	0	0	0
29	H.cinaedi	0	0	0	0	0	0	0	0	0
30	H.pylori	0	0	0	0	0	0	0	0	0
31	R.prowazekii	0	0	0	0	0	0	0	0	0
32	R.japonica	0	0	0	0	0	0	0	0	0
33	Tsutsugamushi	0	0	0	0	0	0	0	0	0
34	B.henselae	0	0	0	0	0	0	0	0	0
35	Brucella	0	0	0	0	0	0	0	0	0
36	B.pertussis	0	0	0	0	0	0	0	0	0

New real-time PCR system for bacteria and fungi

37	B.mallei	0	0	0	0	0	0	0	0	0	0
38	B.cepacia	0	0	0	0	0	0	0	0	0	0
39	N.gonorrhoeae	0	0	0	0	0	0	0	0	0	0
40	N.meningitidis	0	0	0	0	0	0	0	0	0	0
41	F.tularensis	0	0	0	0	0	0	0	0	0	0
42	S.maltophilia	0	0	0	0	0	0	18	0	0	187
43	L.pneumophila	0	0	0	0	0	0	0	0	0	0
44	M.catarrhalis	0	0	0	0	0	0	0	0	0	0
45	P.aeruginosa	0	0	0	0	0	0	0	0	0	0
46	A.baumannii	0	0	0	0	0	0	0	0	0	0
47	A.hydrophila	0	0	0	0	0	0	0	0	0	0
48	V.cholerae	0	0	0	0	0	0	0	0	0	0
49	V.parahaemolyticus	0	0	0	0	0	0	0	0	0	0
50	V.vulnificus	0	0	0	0	0	0	0	0	0	0
51	H.influenzae	0	0	0	0	0	0	0	0	0	0
52	E.coli	0	0	0	0	0	0	0	0	0	0
53	S.enterica	0	0	0	0	0	0	0	0	0	0
54	Shigella	0	0	0	0	0	0	0	0	0	0
55	K.pneumonia	0	0	0	0	0	0	0	0	0	0
56	Y.pestis	0	0	0	0	0	0	0	0	0	0
57	Y.enterocolitica	0	0	0	0	0	0	0	0	0	0
58	C.freundii	0	0	0	0	0	0	0	0	0	0
59	P.mirabilis	0	0	0	0	0	0	0	0	0	0
60	M.morganii	0	0	0	0	0	0	0	0	0	0
61	Providencia	772346	0	0	0	26	0	0	0	0	0
62	M.pneumoniae	0	144418	0	0	0	0	0	0	0	0
63	F.nucleatum	0	0	1946000	0	77	0	0	0	0	0
64	L.interrogans	0	0	0	1492000	0	0	0	0	0	0
65	T.pallidum466	0	0	0	0	1571	0	0	0	0	0
65	T.pallidum1	0	0	0	0	3888	0	0	0	0	0
66	C.psittaci	0	0	0	0	0	5269	0	0	0	0
67	C.trachomatis	0	0	0	0	0	0	135800	0	0	0
68	C.pneumoniae	0	0	0	0	0	0	0	5006	0	0
69	A.fumigatus	0	0	0	0	0	0	0	0	3368	0
70	A.nigar	0	0	0	0	0	0	0	0	0	7663
71	A.flavus	0	0	0	0	0	0	0	0	0	0
72	Cryptococcus	0	0	0	0	0	0	0	0	0	0
73	C.albicans	0	0	0	0	0	0	0	0	0	0
74	Histoplasma	0	0	0	0	0	0	0	0	0	0
75	Trichosporon	0	0	0	0	0	0	0	0	0	0
76	Mucor	0	0	0	0	0	0	0	0	0	0
77	Coccidioides	0	0	0	0	0	0	0	0	0	0
78	b-actin	0	2890	0	0	29416	0	0	0	0	0
79	GAPDH	0	0	0	0	6021	0	0	0	0	0

New real-time PCR system for bacteria and fungi

No.	71	72	73	74	75	76	77		
Bacteria name	A.flavus	Cryptococcus	C.albicans	Histoplasma	Trichosporon	Mucor	Coccidioides		
Sample	A.flavus (ATCC 204304) DNA	C.neoformans H99 (ATCC 208821) DNA	C.albicans (ATCC90028) DNA	H.capsulatum infected mouse FFPE sample	T.asahii (JCM 1809) DNA	Rhizopus sp. Clinical isolate DNA	C.posadasii clinical isolate DNA	Specificity*	
1	S.aureus	21	13	10	10	9	0	21	100%
2	B.anthraxis	0	0	0	0	0	0	0	100%
3	L.monocytogenes	0	0	0	0	0	0	0	100%
4	S.pyogenes	0	0	0	0	0	0	0	100%
5	S.agalactiae	0	0	0	0	0	0	0	100%
6	S.mutans	0	0	0	0	0	0	0	100%
7	S.sobrinus	0	0	0	0	0	0	0	100%
8	S.sanguinis	0	0	0	0	0	0	0	100%
9	S.oralis	6	0	0	0	0	0	0	100%
10	S.salivaris	0	0	0	0	0	0	0	100%
11	S.pneumoniae	0	0	0	0	0	0	0	97%
12	E.faecalis	0	0	0	0	0	0	0	100%
13	E.faecium	0	0	0	0	0	0	0	100%
14	C.tetani	0	0	0	0	0	0	0	89%
15	C.difficile	0	0	0	0	0	0	0	100%
16	P.anaerobius	0	0	0	0	0	0	0	100%
17	Actinomyces	0	0	0	0	0	0	0	91%
18	P.acnes	0	0	13	24	0	0	0	100%
19	C.diphtheriae	0	0	0	0	0	0	0	100%
20	M.tuberculosis	0	0	0	0	0	0	0	100%
21	M.laprae	0	0	0	0	0	0	0	100%
22	M.chelonae	0	0	0	0	0	0	0	100%
23	M.kansasii	0	0	0	0	0	0	0	52%
24	MAC	0	0	0	0	0	0	0	66%
25	N.asteroides	0	0	0	0	0	0	0	99%
26	B.fragilis	0	0	0	0	5	0	0	100%
27	E.meningoseptica	10	12	0	0	0	14	0	100%
28	C.jejuni	0	0	0	0	0	0	0	100%
29	H.cinaedi	0	0	0	0	0	0	0	86%
30	H.pylori	0	0	0	0	0	0	0	100%
31	R.prowazekii	0	0	0	0	0	0	0	80%
32	R.japonica	0	0	0	0	0	0	0	100%
33	Tsutsugamushi	0	0	0	0	0	0	0	100%
34	B.henselae	0	0	0	0	0	0	0	100%
35	Bruceella	0	0	0	0	0	0	0	100%
36	B.pertussis	0	0	0	0	0	0	0	100%
37	B.mallei	0	0	0	0	0	0	0	100%

New real-time PCR system for bacteria and fungi

38	B.cepacia	0	0	0	0	0	0	63%
39	N.gonorrhoeae	0	0	0	0	0	0	95%
40	N.meningitidis	0	0	0	0	0	0	100%
41	F.tularensis	0	0	0	0	0	0	100%
42	S.maltophilia	0	0	21	0	7	0	98%
43	L.pneumophila	0	0	0	0	0	0	100%
44	M.catarrhalis	0	0	0	0	0	0	100%
45	P.aeruginosa	0	0	0	0	0	0	100%
46	A.baumannii	0	0	0	0	0	0	100%
47	A.hydrophila	0	0	0	0	0	0	100%
48	V.cholerae	0	0	0	0	0	0	100%
49	V.parahaemolyticus	0	0	0	0	0	0	100%
50	V.vulnificus	0	0	0	0	0	0	100%
51	H.influenzae	0	0	0	0	0	0	100%
52	E.coli	0	0	0	0	0	0	67%
53	S.enterica	0	0	0	0	0	0	100%
54	Shigella	0	0	0	0	0	0	100%
55	K.pneumonia	0	0	0	0	0	0	100%
56	Y.pestis	0	0	0	0	0	0	100%
57	Y.enterocolitica	0	0	0	0	0	0	100%
58	C.freundii	0	0	0	0	0	0	99%
59	P.mirabilis	0	0	0	0	0	0	100%
60	M.morganii	0	0	0	0	0	0	100%
61	Providencia	0	0	0	0	0	0	21%
62	M.pneumoniae	0	0	0	0	0	0	100%
63	F.nucleatum	0	0	0	0	0	0	100%
64	L.interrogans	0	0	0	0	0	0	100%
65	T.pallidum466	0	0	0	0	0	0	100%
65	T.pallidum1	0	0	0	0	0	0	100%
66	C.psittaci	0	0	0	0	0	0	100%
67	C.trachomatis	0	0	0	0	0	0	100%
68	C.pneumoniae	0	0	0	0	0	0	100%
69	A.fumigatus	0	0	0	0	0	0	100%
70	A.nigar	0	0	0	0	0	0	100%
71	A.flavus	62	0	0	0	0	0	100%
72	Cryptococcus	0	94023	0	0	0	0	100%
73	C.albicans	0	0	141100	0	0	0	100%
74	Histoplasma	0	0	0	102	0	0	100%
75	Trichosporon	0	0	0	0	170013	0	100%
76	Mucor	52	0	0	0	0	1222	96%
77	Coccidioides	0	0	0	0	0	826	100%
78	b-actin	0	0	0	0	0	0	-
79	GAPDH	0	0	0	0	0	0	-

*Specificity of each probe-primer set was calculated by a copy number of the target microbe in the positive control divided by a sum of copy numbers in all control samples.