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Development of real-time PCR assays for the detection of *Moraxella macacae* associated with bloody nose syndrome in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques

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Abstract

Background—*Moraxella macacae* is a recently described bacterial pathogen that causes epistaxis or so-called bloody nose syndrome in captive macaques. The aim of this study was to develop specific molecular diagnostic assays for *M. macacae* and to determine their performance characteristics.

Methods—We developed six real-time PCR assays on the Roche LightCycler. The accuracy, precision, selectivity, and limit of detection (LOD) were determined for each assay, in addition to further validation by testing nasal swabs from macaques presenting with epistaxis at the Tulane National Primate Research Center.

Results—All assays exhibited 100% specificity and were highly sensitive with an LOD of 10 fg for chromosomal assays and 1 fg for the plasmid assay. Testing of nasal swabs from 10 symptomatic macaques confirmed the presence of *M. macacae* in these animals.

Conclusions—We developed several accurate, sensitive, and species-specific real-time PCR assays for the detection of *M. macacae* in captive macaques.

Keywords

epistaxis; molecular diagnostics; nonhuman primates; veterinary microbiology

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Introduction

Moraxella macacae is a recently described gram-negative, oxidase positive, aerobic bacterium within the family Moraxellaceae isolated from captive rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques with epistaxis or so-called bloody nose syndrome [4]. *M. macacae* cells are pleomorphic in size and shape, generally appearing as diplococci, but can appear as plump rods in pairs and in chains [4]. The whole genome of *M. macacae* has been sequenced and is estimated to be ~2.08 Mb [6]. The bacterium was also shown to contain a small mobilizable plasmid of unknown function [15]. Sequence analysis of its 16S rRNA [4] or whole genome [6] shows a >5% sequence divergence from other known moraxellae. Furthermore, phylogenetic analysis of the 16S rRNA sequence of *M. macacae* revealed that this species represents a novel fifth distinct clade within the family Moraxellaceae [6].

The genus *Moraxella* contains at least 14 recognized species isolated from a variety of terrestrial and aquatic mammalian hosts [5]. Of these species, the most important human pathogen is *M. catarrhalis*, which was once thought to be a commensal inhabitant of the upper respiratory tract, but is now recognized as an important pathogen that causes respiratory tract infection and childhood otitis media [14]. In addition to *M. macacae*, several other members of the genus are associated with animals, including *M. canis* (cats and dogs), *M. ovis* (sheep), *M. equi* (horses), *M. caprae* and *M. boevis* (goats), *M. caviae* (guinea pigs), and *M. cuniculi* (rabbits). *M. bovis* is a significant veterinary pathogen causing infectious bovine keratoconjunctivitis, a severe ocular disease affecting cattle worldwide [9]. And *M. osloensis* was recently found to be the cause of septic arthritis in a rhesus macaque [16]. Most other moraxellae are commensals on human or animal epithelia and have rarely, if ever, been associated with disease [5].

'Bloody nose syndrome' manifests as a mucohemorrhagic rhinitis and is commonly observed in captive non-human primates. The disease can spread easily among animals in close quarters, and thus, animals presenting with epistaxis should be promptly treated with penicillin to avoid further transmission. Outbreaks of epistaxis in non-human primates have occurred in both immunocompetent and immunocompromised macaques [2, 4, 13]. Such outbreaks, which are usually mild and self-limiting, occur primarily in winter and have been attributed to lower environmental humidity levels. Additionally, it has been observed that certain immunomodulatory drugs can render the host more susceptible to secondary bacterial invasion, thus increasing the incidence and severity of *Moraxella*-induced epistaxis [11]. Thus, in any experimental drug or vaccine studies using macaques in which there is the potential for the animal have a suppressed immune system response, it is important to carefully monitor the animals for the onset of epistaxis induced by *M. macacae*.

Detection of *M. macacae* thus far has been based on traditional microbiological and biochemical methods. Unfortunately, with the exception of beta-lactamase, *M. macacae* exhibits identical reactivity in the biochemical tests of the API NH panel (BioMérieux, Durham, NC, USA) as *M. catarrhalis*, the human pathogenic species [4]. In fact, previous descriptions of bloody nose syndrome in non-human primates in 1991 [13] and 2002 [2] were attributed to *M. (Branhamella) catarrhalis* based on colony morphology, Gram stain,

and biochemical analysis, but were most likely due to *M. macacae* [4]. Proper identification of *M. macacae* was ultimately only made by sequence analysis of its 16S rRNA gene [4].

We previously identified *M. macacae* as the etiologic agent of epistaxis in rhesus and cynomolgus macaques housed at the Tulane National Primate Research Center (TNPRC) and USAMRIID, respectively [4]. The infection appears to be quite common. In the previous 5 years at the TNPRC, 177 animals were recorded as presenting with epistaxis, some with multiple episodes, and more than 90% of those tested were positive for *Moraxella*. In this study, we developed several species-specific, rapid, real-time TaqMan-Minor Groove Binder (MGB) PCR assays, to both chromosomal and plasmid gene targets, for detecting *M. macacae*. Furthermore, the assays were used to test a subset of 10 nasal swabs from rhesus macaques presenting with epistaxis at TNPRC.

Materials and methods

Humane care guidelines

No animals were sacrificed during this study. Practices in the housing and care of animals conformed to the regulations and standards of the PHS Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals [12]. The Tulane National Primate Research Center (Animal Welfare Assurance # A4499-01) is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care-International. The Institutional Animal Care and Use Committee (IACUC) of the Tulane National Primate Research Center approved all animal-related protocols, including the anesthesia and nasal swab collection. All animals were kept on a 12-hour light/dark cycle and were fed LabDiet Fiber-Plus Monkey Diet (LabDiet, St. Louis, MO, USA). All animal procedures were overseen by university veterinarians and their staff. Nasal swabs were collected as part of routine clinical care for macaques that exhibited nose bleeding to assess infection and guide therapy. For nasal swab collection, monkeys were anesthetized with 10 mg/kg ketamine by intramuscular injection. This method is consistent with the recommendation of the American Veterinary Medical Association guidelines. Additional feeding enrichment and forage items were given as part of a comprehensive environmental enrichment program that also uses social housing to promote species-typical behavior.

Positive control DNA

The DNA used to develop and optimize the PCR assays was obtained from *M. macacae* 0408225, a strain isolated from a cynomolgus macaque at USAMRIID that demonstrated sneezing with serosanguinous nasal discharge. This strain has been characterized phenotypically and biochemically, and its genome has been sequenced [4, 6]. DNA from other *M. macacae* isolates was also used to validate the assays. These included CI24, a strain isolated from a rhesus macaque at TNPRC and 022479, a strain isolated from a cynomolgus macaque at USAMRIID that presented with epistaxis and nasal septum necrosis.

DNA extraction

DNA from culture isolates and brain–heart infusion (BHI) aliquots from nasal swabs was extracted using the BioRobot EZ1 Virus Mini Kit V 2.0 (Cat.# 955134; Qiagen, Valencia,

CA, USA) according to manufacturer's instructions. Total nucleic acid was eluted in 90 µl of AVE buffer and stored at -80°C. DNA quantity and quality was measured using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, Inc., Waltham, MA, USA).

PCR assay development and optimization

Primer pairs and TaqMan™-minor groove binder (MGB) probes (Cat.# 4316034; ThermoFisher Scientific, Inc., Waltham, MA, USA) were designed using Primer Express version 3.0 (PE3) (Applied Biosystems, Foster City, CA, USA) and AlleleID 7.82 (PREMIER BioSoft, Palo Alto, CA, USA). DNA sequence from the *M. macacae* and plasmid pMoma1 genomes (Gen-Bank accession numbers ANIN000000000 and ANIN01000000, respectively) was used to design primer and probes. BLAST alignments were performed for each gene sequence with the potential primer/probe combinations targeting non-homologous regions. The PE3 software was then implemented to design *M. macacae* 0408225-specific TaqMan™-MGB assays. In addition, individual gene targets were aligned with AlleleID using the integrated ClustalW2 algorithms with the Species-Specific Design option selected in the software. The design of *M. macacae* 0408225-specific assays by both software programs resulted in six primer/probe pairs targeting five chromosomal targets (*ompM35*, *uspA2*, *lpxA*, *copB*, and *hag*) and one plasmid target (*repA*). All real-time PCR assays were carried out in 20 µl volumes using the Roche LightCycler 2.0 or 480 (Roche, Indianapolis, IN, USA) with each reaction consisting of PCR buffer [50 mM Tris, pH 8.3; 25 µg/ml of bovine serum albumin, 50 mM MgCl₂] (Idaho Technology, Salt Lake City, UT, USA) and 0.2 mM dNTP mix (Cat.# 1774; Idaho Technology). Eight-tenths (0.8) unit of Platinum *Taq* DNA polymerase (ThermoFisher Scientific, Inc., Waltham, MA, USA) was added to each reaction. Thermal cycling for the LightCycler was performed as follows: one cycle at 95°C for 2 min, followed by 45 cycles of 95°C for 1 s, and 60°C for 20 s. A fluorescence reading was taken at the end of each 60°C step. Initial primer down selection was accomplished through a SYBR green I (Cat.# S9430; Sigma-Aldrich, St. Louis, MO, USA) assay screening, with SYBR Green I being substituted for a probe. Following primer down selection, the matching TaqMan-MGB probes were tested with optimum primer/probe concentrations being determined using an internal protocol. The final concentration selected resulted in the earliest C_q, lowest limit of detection (LOD), and highest end point fluorescence. For clinical specimen testing, C_q values of <40 were considered positive. The final assay conditions for each assay are summarized in Table 2.

Limit of detection determination

The LOD is defined as the lowest analyte concentration likely to be reliably distinguished from the blank and at which detection is feasible [1]. The LOD was established by running a total of 60 samples that consisted of two separate runs of 30 replicates each, performed on two different instruments. The lowest analyte concentration that produced a positive signal in 58/60 (97%; 95% success at 95% confidence based on binary sampling statistics) was considered the assay LOD.

Linearity testing

Genomic DNA of *M. macacae* 0408225 at 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg concentrations was prepared and run in triplicate with the optimized PCR conditions to generate a standard curve. Samples were run on the Roche LightCycler 480, and the LightCycler Data Analysis software version 3.5.3 was used to apply the standard curve to the results.

Cross-reactivity testing

All PCR assays were tested against the USAMRIID general bacterial DNA reference panel and human DNA (Table 3). This panel consisted of 65 organisms that included biothreat organisms; five *Moraxella* species; nearest genetic neighbors to *Moraxella* or biothreat organisms; organisms sharing an environmental or clinical niche with *Moraxella*, particularly respiratory pathogens, opportunists, and typical respiratory flora. In all cases, 100 pg of genomic DNA was used to determine whether the assays cross-reacted with nucleic acids from other organisms.

Sample collection for screening of rhesus macaques with epistaxis

Sterile rayon-tipped swabs (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used to collect nasal swab samples from the macaques. A total of 10 samples from rhesus macaques at the TNPRC exhibiting epistaxis were collected; an additional swab was collected from a normal healthy animal and was used as a negative control.

Sample processing and microbiology culture

Nasal swabs were processed for microbiological analysis by plating on 5% sheep blood agar and incubated overnight at 37°C. Suspect *Moraxella* colonies were isolated, observed by gram stain, and identified by the Remel™ catarrhalis disk test. Glycerol stocks were made from colony isolates that were identified as *Moraxella* positive and stored at -80°C. In addition, the swabs were immersed and stirred in 2.5 ml of BHI broth. One milliliter of the inoculated BHI broth was aliquoted into each of two cryovials labeled with animal ID and date and stored at 4°C. One sample from each animal was sent to USAMRIID for PCR testing. One clean swab was placed into BHI broth and that aliquot was also shipped to USAMRIID and used as a negative control. All PCR testing was performed blinded, and samples were unblinded only after all PCR results were obtained.

Results

Development and optimization of real-time PCR assays

Six real-time TaqMan-MGB PCR assays specific for *M. macacae* were developed and optimized. Five of the assays targeted genes (*copB*, *hag*, *lpxA*, *ompM35*, and *uspA2*) located on the bacterial chromosome. The sixth assay targeted the *repA* gene located on the plasmid pMoma1. The primers and probe sequences, amplicon sizes, and optimized assay conditions are listed in Tables 1 and 2.

Standard curves and LODs

Linearity was established using purified genomic DNA from *M. macacae* 0408225. Ten-fold serial dilutions from 100 pg to 10 fg concentrations were assayed in triplicate to establish linearity (Fig. 1A–F). The coefficients of determination (R^2) for the assays ranged from 0.9982 for the *copB* assay to 1 for the *repA* assay. Once linearity was established, 60 replicates were tested and the lowest analyte concentration that produced a positive signal in 97% (58 positives) samples established the LOD. All chromosomal assays had an LOD of 10 fg, whereas the plasmid *repA* assay was more sensitive with an LOD of 1 fg (Table 2).

Cross-reactivity testing

All six assays were tested against the general bacterial/eukaryote USAMRIID DNA reference panel for specificity (Table 3). Importantly, this panel contains five *Moraxella* species representing the nearest genetic neighbors to *M. macacae*. None of the assays showed cross-reactivity with any of the panel DNAs, and all were specific for their gene targets. As *M. macacae* has been commonly misidentified as *M. catarrhalis* in the past, it is important to note that none of the assays cross-reacted with DNA from *M. catarrhalis* or any other *Moraxella* species tested.

Non-human primate testing

Initial assay testing was conducted using clinical bacterial isolates from a naturally infected rhesus macaque at TNPRC (strain CI24) and from a cynomolgus macaque from USAMRIID that presented with epistaxis and nasal septum necrosis (strain 022479). All assays were strongly positive for both of these clinical isolates (data not shown). To evaluate our assays with genuine clinical specimens, we collected nasal swabs from monkeys presenting with bloody nose syndrome at the TNPRC. Swabs were processed for microbiological culture and were subsequently placed in 2.5 ml of BHI broth and shipped to USAMRIID for DNA extraction and PCR. A total of 11 samples were collected, blinded, and shipped to USAMRIID for testing. These included 10 samples from symptomatic animals (presenting with epistaxis) and one sample from an asymptomatic animal as a negative sample. Each sample was run in triplicate with all six assays, and results were provided to TNPRC at which time the samples were unblinded. PCR and culture results of all samples are summarized in Table 4. All 10 samples from the symptomatic animals were positive for all six assays, and the sample from the asymptomatic animal (JG46) was negative for all assays. Cq values ranged from a low (most concentrated) of 21.65 for the *repA* assay for animal JE83 to a high (least concentrated) of 38.00 for the *uspA2* assay for animal IJ92. Because one of the PCR assays targets the *repA* gene on the pMoma1 plasmid, we confirmed that all isolates from all 10 symptomatic animals contained this plasmid. All PCR-positive samples, except for one (KF26), also yielded a *Moraxella* sp. isolate upon culture. Biochemically, *M. macacae* is indistinguishable from *M. catarrhalis* using commercially available identification systems [4], thus we did not attempt to speciate the *Moraxella* isolates; however, morphology upon gram staining was consistent with *M. macacae* for all. In addition to *Moraxella*, some of the samples also grew other potential pathogens including *Staphylococcus aureus*, *Enterococcus* sp., and an unidentified gram-negative rod (UGNR).

(Table 4). Notably, cultures from the asymptomatic animal JG46 did not yield a *Moraxella* isolate, although it did grow an unidentified gram-negative rod.

Discussion

‘Bloody nose syndrome’ of unknown etiology in macaques has been known from at least the early 1970s [7]. During an outbreak of epistaxis in cynomolgus monkeys at a clinical research facility in England in 1976, *Neisseria catarrhalis*, along with several other pathogenic bacteria, was isolated from the monkey’s nasal mucosa [3]. Similarly, this same organism was isolated from animals during an outbreak at the Oregon Regional Primate Research Center in 1983 [8]. In both instances, however, the etiology of the condition remained uncertain. The first report definitively demonstrating the role of this bacterium in the etiology of epistaxis in non-human primates was from an outbreak in cynomolgus macaques at a quarantine facility at The Johns Hopkins University in 1991 [13]. At that time, the bacterium was taxonomically classified within the genus *Branhamella* (and previously as *Neisseria*). Incidentally, in that same year, the family Moraxellaceae was established to incorporate the genera *Moraxella*, *Acinetobacter*, *Psychrobacter*, and related organisms [10]. Thus, *Branhamella catarrhalis* became *Moraxella catarrhalis* and is now known to be a significant human pathogen that is a major cause of respiratory tract infections and childhood otitis media [14]. Previously in 2002, the TNPRC reported several rhesus macaques (33 animals from a colony of 968) presenting with bloody nose syndrome, all of which were positive for *M. catarrhalis* [2]. In retrospect, all of the previous cases of epistaxis in macaques were likely caused by *M. macacae*, which is distinguishable from the human pathogenic *M. catarrhalis* only by molecular-based methods. In fact, Embers et al. [4], using animal inoculation studies, showed that the human pathogenic *M. catarrhalis* only rarely colonized rhesus macaques and did not result in the induction of epistaxis in infected macaques.

Previously, proper identification of *M. macacae* has been made only by PCR and subsequent sequence analysis of its 16S rRNA gene or by whole genome sequencing [4, 6]. These methods are laborious, costly, and not suited for a routine diagnostic laboratory or for screening large numbers of animals. Thus, in this study, we developed several PCR assays to specifically detect *M. macacae* and used these assays to test several NHPs presenting with epistaxis at the TNPRC in 2014. Assays targeted chromosomal gene targets including conserved housekeeping genes and genes coding for structural proteins mined from the whole genome sequence of *M. macacae*. In addition, we included a gene target specific to the small conjugative plasmid pMoma1, which can be used to test for the presence of the plasmid. All assays used gene-specific primers and TaqMan-MGB probes and were run on the Roche LightCycler. All assays were specific for their respective gene targets and did not cross-react with any of the closely related or unrelated bacterial DNAs tested in Table 3. It is important to note that DNAs included in the cross-reactivity panel included multiple other species of *Moraxella* as well as bacteria that could occupy the same clinical niche (i.e., respiratory pathogens, opportunists, and typical respiratory flora). Furthermore, each of the chromosomal assays was highly sensitive, being able to detect 10 fg of purified target DNA. The plasmid *repA* assay was even more sensitive, being able to detect as little as 1 fg of purified target DNA. This difference in sensitivities is likely due to the pMoma1 plasmid

being a multicopy plasmid. Our analysis of the efficiencies of each assay and the genomic data from whole genome sequencing indicated that the pMoma1 plasmid was present in approximately 10 copies per chromosome (data not shown), which is consistent with the 10-fold differences in assay sensitivities observed.

The TNPRC has had periodic outbreaks of ‘bloody nose syndrome’ among their captive macaques for several years. Thus, to validate our assays with authentic clinical samples, we tested nasal swabs from 10 rhesus macaques that presented with epistaxis. To eliminate any subjective biases, all samples were tested blinded. All six assays correctly identified *M. macacae* in the samples, which were confirmed by culture isolation in all except for one sample (KF26; Table 4). In sample KF26, it is possible that there was a low level of bacteria present and the PCR assays were more sensitive than culture. Clearly, all six assays are not needed to test for the presence of *M. macacae* in clinical samples. Examination of the Cq values across all six assays for each clinical sample tested (see Table 4) reveals that the performance of each of the six assays was very consistent. Thus, a single assay could be used for initial screening of clinical samples for *M. macacae* with confirmation using a second assay. Additionally, the plasmid *repA* assay can be used to test isolates for the presence of the plasmid. Given that *M. macacae* and its plasmid pMoma1 have been only recently described, it will be of interest to determine how prevalent this plasmid is among *M. macacae* isolates. The plasmid has been detected in all *M. macacae* isolates and clinical samples tested to date [15].

A hemorrhagic syndrome in non-human primates can be due to several important viral diseases, including Ebola and Marburg hemorrhagic fevers caused by filoviruses and simian hemorrhagic fever caused by viruses within the family *Arteriviridae*. These viruses can lead to explosive disease outbreaks among captive NHPs and in some cases have zoonotic potential to infect humans. By contrast, respiratory disease caused by *M. macacae* is more benign and often self-limiting. As such, having the appropriate diagnostic tools to distinguish between these pathogens is of significant importance. In addition, the spread of this nuisance infection within a primate colony or research facility could compromise infectious disease research using non-human primates. Due to the ease of transmission, rapid and accurate identification of the causative agent will be critical to preventing spread throughout the colony.

In the current study, we developed and validated several rapid and specific real-time PCR assays for the detection of the bacterial pathogen *M. macacae*, the causal agent of ‘bloody nose syndrome’ in non-human primates. These are the first molecular diagnostic assays specifically developed for this bacterial pathogen. As such, these new tools should contribute to a better understanding of this pathogen and help to better diagnose epistaxis in monkeys caused by this bacterium.

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Fig. 1. Standard curves for the *Moraxella macacae* (A) *hag*, (B) *ompM35*, (C) *copB*, (D) *uspA2*, (E) *lpxA*, and (F) *repA* PCR assays.

Table 1Primers and probes used in the *Moraxella macacae* PCR assays

Forward/reverse/probe name	Primer/probe sequence	Target gene	Amplicon size
OMPM35-F806	5'-AAGCAACCACAGGACATGTTTATG-3'	<i>ompM53</i>	79 bp
OMPM35-R884	5'-GCACCATTGTACGTTTATCACTGA-3'	Outer membrane porin M35	
OMPM35-p831S-MGB	5'-AGGTTACTCTAAAGGTAATACAA – MGBNFQ-3'		
uspA2-334	5'-ATTGACAGCACAAACGATGGAT-3'	<i>uspA2</i>	76 bp
uspA2-409	5'-CCGCACGTTCAATGTCTTTTT-3'	Serum resistance factor	
uspA2-p363S-MGB	5'-AGTTGGCAATGTTGTGGCA – MGBNFQ-3'		
hag-F5596	5'-CAAGGTGCGATGGATATTGGT-3'	<i>hag</i>	67 bp
hag-R5662	5'-CATCGCTATCGGCAGATCCT-3'	Flagellin structural protein	
hag-p5618S-MGB	5'-ATCGTCAAAATCACAAGTGT – MGBNFQ-3'		
copB-F2061	5'-TGCAACTTATCGCCCAATTGAC-3'	<i>copB</i>	63 bp
copB-R2123	5'-TTCCCCCAAACCGACCAA-3'	Copper resistance factor	
copB-2084S-MGB	5'-AGCGTTACAGCATTACCG-3' – MGBNFQ-3'		
lpxA-F24	5'-CATTGATCCCAGTGCAAACATT-3'	<i>lpxA</i>	66 bp
lpxA-R89	5'-GCATGACCAATCACGCAAAA-3'	UDP-N-acetylglucosamine acyltransferase	
lpxA-p48S-MGB	5'-CCCATCTGTTAAAATT – MGBNFQ-3'		
REP-F106	5'-ATGAACCTTAGCCGTTACCAAGGTT-3'	<i>repA</i>	68 bp
REP-R173	5'-TCACCAGTTCTTTCGTGGTTTG-3'	Replicase (pMoma1)	
REP-p132S-MGB	5'-TCAAAGCGAGGTTAAAA – MGBNFQ-3'		

Table 2

Optimized reaction conditions used for each PCR assay

Final concentrations and reaction conditions						
Reaction components	<i>copB</i>	<i>hag</i>	<i>lpxA</i>	<i>ompM35</i>	<i>uspA2</i>	<i>repA</i>
10× PCR Buffer w/MgCl ₂	4 mmol/l	5 mmol/l	7 mmol/l	7 mmol/l	6 mmol/l	5 mmol/l
Primers	0.8 μmol/l	0.9 μmol/l	0.7 μmol/l	0.7 μmol/l	0.8 μmol/l	0.7 μmol/l
Cycles	45	45	45	45	45	45
PCR product size	63 bp	67 bp	66 bp	79 bp	76 bp	68 bp
Limit of detection	10 fg/rxn.	10 fg/rxn.	10 fg/rxn.	10 fg/rxn.	10 fg/rxn.	1 fg/rxn.
Reaction efficiencies, %	93.4	95.0	96.8	94.4	95.0	98.6

Table 3

List of the DNAs used for general cross-reactivity and near-neighbor testing

Organism name	ATCC#
<i>Acinetobacter baumannii</i>	19606
<i>Actinomyces naeslundii</i>	12104D
<i>Actinobacillus pleuropneumoniae</i>	27088D
<i>Alcaligenes xylosoxidans</i>	27061
<i>Bacillus anthracis</i> Ames	NA
<i>Bacillus cereus</i>	19637
<i>Bacillus (Geobacillus) staerothermophilus</i>	7953
<i>Bacillus subtilis</i> var <i>niger</i>	NA
<i>Bacillus thuringiensis</i>	35646
<i>Bacteroides fragilis</i>	25285D
<i>Bartonella henselae</i>	49882D
<i>Bifidobacterium infantis</i>	15697D
<i>Bordetella bronchiseptica</i>	10580
<i>Bordetella pertussis</i>	9797D
<i>Borrelia burgdorferi</i>	35210D
<i>Brucella melitensis</i>	NA
<i>Budvicia aquatica</i>	35567
<i>Burkholderia mallei</i>	NA
<i>Campylobacter jejuni</i>	33560D
<i>Candida albicans</i>	10231D
<i>Chryseobacterium meningosepticum</i>	33958D
<i>Clostridium botulinum</i> type A	19397
<i>Clostridium difficile</i> <i>perfringens</i>	9689D
<i>Clostridium perfringens</i>	13124
<i>Comamonas (Delftia) acidovorans</i>	15668
<i>Corynebacterium diphtheriae</i>	700971D
<i>Coxiella burnetii</i>	NA
<i>Deinococcus radiodurans</i>	13939D
<i>Enterobacter aerogenes</i>	15038D
<i>Enterobacter (Pantoea) agglomerans</i>	29904
<i>Enterococcus faecalis</i>	700802D
<i>Escherichia coli</i>	10798D
<i>Francisella tularensis</i>	NA
<i>Haemophilus influenzae</i>	51907D
Human DNA	NA
<i>Klebsiella pneumoniae</i>	700721D
<i>Legionella pneumophila</i>	33152D
<i>Listeria monocytogenes</i>	15313
<i>Moraxella atlantae</i>	29525

Organism name	ATCC#
<i>Moraxella canis</i>	51391
<i>Moraxella catarrhalis</i>	25240
<i>Moraxella lincolnii</i>	51388
<i>Moraxella nonliquefaciens</i>	19975
<i>Mycoplasma pneumoniae</i>	15531D
<i>Neisseria meningitidis</i>	53415D
<i>Pasteurella multocida</i>	43137
<i>Porphyromonas gingivalis</i>	33277D
<i>Proteus mirabilis</i>	7002
<i>Pseudomonas aeruginosa</i>	17933D
<i>Rhizobium radiobacter</i>	33970D
<i>Salmonella enterica</i> serovar Paratyphi A	9150D
<i>Serratia marcescens</i>	13880
<i>Shigella flexneri</i>	12022
<i>Staphylococcus aureus</i>	29247
<i>Staphylococcus epidermidis</i>	12228D
<i>Stenotrophomonas maltophilia</i>	13637
<i>Streptococcus pneumoniae</i>	33400
<i>Streptococcus pyogenes</i>	12344D
<i>Ureaplasma urealyticum</i>	700970D
Vaccinia virus R1352	NA
<i>Vibrio cholerae</i>	51394D
<i>Yersinia enterocolitica</i>	9610
<i>Yersinia pestis</i> (CO92; PW)	NA
<i>Yersinia pseudotuberculosis</i>	6904

All assays were analyzed with 100 pg of DNA from this panel to ensure specificity.

Table 4

Results of oral swabs collected from non-human primates presenting with epistaxis at the Tulane National Primate Research Center

Primate#	Date of collection	<i>copB</i> (Cq)	<i>hag</i> (Cq)	<i>lpxA</i> (Cq)	<i>ompM35</i> (Cq)	<i>uspA2</i> (Cq)	<i>repA</i> (Cq)	Culture results ²
JE83	3/5/2014	POS (30.44)	POS (30.74)	POS (31.24)	POS (31.52)	POS (30.75)	POS (26.92)	<i>Moraxella</i> sp.
JG46 ¹	3/6/2014	NEG	NEG	NEG	NEG	NEG	NEG	UGNR
IT2b	3/10/2014	POS (25.53)	POS (27.39)	POS (27.91)	POS (28.19)	POS (26.80)	POS (24.17)	<i>Moraxella</i> sp.
IT3b	3/10/2014	POS (27.78)	POS (29.66)	POS (29.98)	POS (30.30)	POS (29.42)	POS (26.60)	<i>Moraxella</i> sp.
IJ92	3/17/2014	POS (35.53)	POS (36.49)	POS (37.08)	POS (37.77)	POS (38.00)	POS (32.51)	<i>Moraxella</i> sp.
HV48	3/27/2014	POS (25.00)	POS (26.82)	POS (27.37)	POS (27.55)	POS (26.18)	POS (24.19)	<i>Moraxella</i> sp.
KF26	3/28/2014	POS (24.42)	POS (25.96)	POS (26.59)	POS (26.94)	POS (25.51)	POS (23.27)	<i>Staphylococcus aureus</i> , UGNR
IC87	3/31/2014	POS (25.95)	POS (27.62)	POS (28.32)	POS (28.58)	POS (27.08)	POS (24.79)	<i>Moraxella</i> sp.
JV14	4/9/2014	POS (24.86)	POS (25.52)	POS (25.93)	POS (26.20)	POS (24.59)	POS (22.46)	<i>Moraxella</i> sp., <i>Staphylococcus aureus</i> , <i>Enterococcus</i> sp.
KF25	4/9/2014	POS (28.93)	POS (30.46)	POS (30.99)	POS (31.60)	POS (30.05)	POS (27.75)	<i>Moraxella</i> sp.
JE83	4/10/2014	POS (24.89)	POS (25.81)	POS (26.27)	POS (26.61)	POS (25.76)	POS (21.65)	<i>Moraxella</i> sp., <i>Staphylococcus aureus</i>

UGNR, unidentified gram-negative rod.

¹ Normal healthy monkey used as a negative control.² Normal oral flora organisms were observed in all samples in addition to the potential pathogens listed.