6.4 nmol/L and that showed an H243Y mutant type codon. The role of such a polymorphism appears unclear. We found no association between the 3-bp indel in G7 and in vitro dhART susceptibility because mutants were regularly distributed in highly susceptible isolates and in isolates having a diminished susceptibility.

For our samples obtained during 2004–2006, the geometric mean IC$_{50}$ value for dhART was very close to values found in Cameroon during 1997–1998 (mean dhART IC$_{50}$ = 1.11 nmol/L) (9), in Senegal in 2001 (mean artemether IC$_{50}$ = 1.3 nmol/L) (5), and in Republic of Congo during 2005–2006 (mean dhART IC$_{50}$ = 1.02 nmol/L) (10). Ringwald et al. observed a narrower range of IC$_{50}$8, but their series included only 65 samples (9). Previous comparisons between ACs suggested that dhART is 1.7 times more potent than artemether against *P. falciparum* (9). Thus, the highest IC$_{50}$ value for artemether observed by Jambou et al. in Senegal (44.7 nmol/L) (5) is comparable to the highest IC$_{50}$ value for dhART in our series (31.8 nmol/L). The resistance levels of ACs are still undefined. For artemether, Jambou et al. used a threshold of 30 nmol/L to evaluate the association between the S769N mutation and in vitro susceptibility. The presence of ATPase6 S769N was not associated with diminished in vitro susceptibility in our series. Conversely, the only S769N mutant that we observed was found in a fully susceptible isolate. Thus, we confirmed that polymorphism exists in this gene in positions 769 and 243, but we did not prove an association between these point mutations and resistance to ACs. Similarly, our results did not support the hypothesis of an association between the 3-bp indel in G7 and resistance to ACs.

ACs, considered the most important class of antimalarial drugs, merit close surveillance for susceptibility. Continued monitoring of the efficacy of their associated partner drugs also appears to be essential.

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**Real-time PCR for Francisella tularensis Types A and B**

To the Editor: Francisella tularensis, the etiologic agent of tularemia, is highly infectious and considered a potential bioweapon (1–3). Although 4 subspecies of *F. tularensis* are recognized, most cases of tularemia are due to infection by subsp. *tularensis* (type A) or holarctica (type B). North America is the only region where both type A and type B cause human disease. Subspecies novicida is also found in North America, but it is of reduced virulence. Disease incidence attributable to either type A or type B is
essentially unknown because the traditional method for classification of these subspecies is glycerol fermentation, which requires culture recovery (4). F. tularensis is fastidious and slow growing, with isolates recovered in a small percentage of cases.

We developed real-time TaqMan PCR assays for classification of F. tularensis type A and type B after F. tularensis is identified by culture or, in the absence of culture, by a PCR method such as the F. tularensis multigene TaqMan assay (5). The type A TaqMan assay targets pdpD, which is present in type A, almost entirely absent from type B, and contains a 144-bp insert in novicida (6,7) (F: 5′-GAGACATCAATGAAGACCAATCCTTT-3′; R: 5′-CCAGAGATTACTATTCCGGTTGGT-3′; probe: 5′-TAATTTTTCATCGTCTACGGCAGCATATCAATCCT-3′). The type B assay targets a junction between ISFtu2 and a flanking 3′ region (GenBank AY06) (F: 5′-CCTGTACCTTTATTTGGCTACTGAGAACT-3′; R: 5′-CATTGGCAAGATTCGTTATTTCCGGTTGGT-3′; probe: 5′-ACCTAGTTCAACC“T”GCAGGACATCTACGGCAGCATATCAATCCT-3′). In type A and novicida, ISFtu2 is absent from this position (8). Oligonucleotides were designed with Primer Express version 2.0 (Applied Biosystems, Foster City, CA, USA). Probes were synthesized with a 5′ 6-carboxy-fluorescein reporter and an internal quencher (either BHQ1 [type A] or QSY-7 [type B]) at the nucleotide position indicated by the quotation marks.

Assays were optimized by using 1 ng of type A (strain SchuS4) or type B (strain LVS) DNA on the LightCycler 1.2 (Roche Applied Science, Indianapolis, IN, USA). Optimized concentrations (20 µL final volume) were 1× LightCycler Fast Start DNA Master Hybridization Probe mix (Roche), 750 nmol/L primers, 200 nmol/L probe, 5 mmol/L MgCl2, and 0.5 U uracil-DNA glycosylase. PCR conditions were 50°C for 2 min, 95°C for 10 min, 45 cycles of 95°C for 10 s, and 65°C for 30 s, then 45°C for 5 min. Cycle threshold (Ct) values were calculated by using the second derivative maximum method with the y-axis at F1/F3 (LightCycler software version 3.5).

Sensitivity of each assay was assessed by using 10-fold serial dilutions (100,000 to 1 genomic equivalents [GE]) of SchuS4 or LVS DNA. Testing was performed in triplicate, with a reproducible detection limit of 10 GE for both assays. Specificity of each assay was tested with 1 ng of DNA from a panel of 62 Francisella isolates (online Appendix Table, available from http://www.cdc.gov/neid/2006-0629_appT.htm) and 22 non-Francisella isolates (Acinetobacter, Bacillus, Brucella, Corynebacterium, Enterobacter, Enterococcus, Escherichia, Haemophilus, Klebsiella, Legionella, Proteus, Pseudomonas, Serratia, Staphylococcus, Streptococcus, and Yersinia species). Isolates were grown, DNA purified, and quantified as previously described (5). Specificity was also evaluated with DNA (2 µL) extracted as previously described from Francisella-like tick endosymbionts of Dermacentor variabilis and Francisella-like soil bacteria (online Appendix) (9,10). The type A assay recognized all type A isolates with an average Ct value of 17.9 (n = 19). The type B assay detected all type B strains with an average Ct value of 17.1 (n = 21). Neither assay displayed cross-reactivity with F. tularensis isolates (Acinetobacter, Bacillus, Brucella, Corynebacterium, Enterobacter, Enterococcus, Escherichia, Haemophilus, Klebsiella, Legionella, Proteus, Pseudomonas, Serratia, Staphylococcus, Streptococcus, and Yersinia species). Isolates were grown, DNA purified, and quantified as previously described (5). Specificity was also evaluated with DNA (2 µL) extracted as previously described from Francisella-like tick endosymbionts of Dermacentor variabilis and Francisella-like soil bacteria (online Appendix) (9,10). The type A assay recognized all type A isolates with an average Ct value of 17.9 (n = 19). The type B assay detected all type B strains with an average Ct value of 17.1 (n = 21). Neither assay displayed cross-reactivity with F. tularensis subsp. novicida (n = 7), F. philomiragia (n = 15), Francisella-like tick endosymbionts (n = 3), Francisella-like soil bacteria (n = 7) (Appendix), or non-Francisella spp. (n = 22).

To evaluate the ability of the type A and type B TaqMan assays, in conjunction with the multigene assay, to identify F. tularensis and classify subspecies in primary specimens, human, animal, and tick samples were tested (Table) available from DNA was extracted from 200 µL fluid, 25 mg liver, and 10 mg spleen or lung by using the QIAamp DNA MiniKit (Qiagen, Valencia, CA, USA) and 1 µL tested. Multigene PCR conditions were as described (5).

The multigene and subspecies-specific PCR assays accurately identified and classified F. tularensis in all specimens positive by standard diagnostic methods (Table). In addition, the type A and type B assays provided subspecies information for positive specimens in which an isolate was not recovered for glycerol fermentation testing (Table). All specimens negative by standard diagnostic methods tested negative by PCR. These preliminary results suggest that a F. tularensis PCR identification method, in combination with the type A and type B assays, provides the capability to identify F. tularensis and determine subspecies in the absence of culture.

We describe real-time PCR assays capable of classifying F. tularensis type A and type B and distinguishing these subspecies from the less virulent subsp. novicida. These assays are designed for use after F. tularensis has been identified by culture or by PCR. Supplemental use of these assays will allow laboratories to actively subtype F. tularensis isolates and primary specimens, thus providing subspecies information for a higher percentage of tularemia cases. Improved subspecies information will further understanding of the disease incidence and geographic distribution of F. tularensis type A and type B in North America.

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### Table. Comparison of standard diagnostic methods with the multitarget *Francisella tularensis* TaqMan assay and type A and type B assays using primary specimens

| Specimen                  | Source       | *F. tularensis* identified* | Subspecies identification† | Multitarget *F. tularensis* TaqMan assay‡ | Type A assay‡ (C<sub>r</sub> value)§ | Type B assay‡ (C<sub>r</sub> value)§ |
|---------------------------|--------------|----------------------------|----------------------------|-------------------------------------------|--------------------------------------|--------------------------------------|
| Lymph node aspirate       | Human        | +                          | A                          | + + + +                                    | 31 –                                 | –                                    |
| Bronchial wash            | Human        | +                          | A                          | + + + +                                    | 34 –                                 | –                                    |
| Upper lung                | Human        | +                          | A                          | + + + +                                    | 20 +                                 | –                                    |
| Lower lung                | Human        | +                          | A                          | + + + +                                    | 26 –                                 | –                                    |
| Liver                     | Human        | +                          | A                          | + + + +                                    | 29 –                                 | –                                    |
| Spleen                    | Human        | +                          | A                          | + + + +                                    | 31 –                                 | –                                    |
| Pleural fluid             | Human        | +                          | B                          | + + + +                                    | 36 –                                 | –                                    |
| Blood                     | Human        | +                          | –                          | + + + +                                    | 38 –                                 | –                                    |
| Spleen                    | Human        | –                          | –                          | –                                          | –                                    | –                                    |
| Liver                     | Human        | –                          | –                          | –                                          | –                                    | –                                    |
| Cerebrospinal fluid       | Human        | –                          | –                          | –                                          | –                                    | –                                    |
| Blood                     | Human        | –                          | –                          | –                                          | –                                    | –                                    |
| Liver/spleen              | Tamarin      | +                          | A                          | + + + +                                    | 28 –                                 | –                                    |
| Tissue                    | Tick‖        | +                          | A                          | + + + +                                    | 26 –                                 | –                                    |
| Tissue                    | Tick‖        | +                          | A                          | + + + +                                    | 33 –                                 | –                                    |
| Blood                     | Prairie dog  | +                          | B                          | + + + +                                    | 30 –                                 | –                                    |
| Blood                     | Prairie dog  | +                          | B                          | + + + +                                    | 27 –                                 | –                                    |
| Spleen                    | Prairie dog  | +                          | B                          | + + + +                                    | 21 –                                 | –                                    |
| Spleen                    | Prairie dog  | +                          | B                          | + + + +                                    | 31 –                                 | –                                    |
| Spleen                    | Prairie dog  | –                          | –                          | –                                          | –                                    | –                                    |
| Liver                     | Cat          | –                          | –                          | –                                          | –                                    | –                                    |
| Liver                     | Rat          | –                          | –                          | –                                          | –                                    | –                                    |
| Spleen                    | Rat          | –                          | –                          | –                                          | –                                    | –                                    |
| Spleen                    | Squirrel     | –                          | –                          | –                                          | –                                    | –                                    |

*F. tularensis* infection identified by culture, direct fluorescent antibody testing, or serologic testing.
†Subspecies was determined by glycerol fermentation when an isolate was recovered.
‡ = positive result, 17°C < C<sub>r</sub> < 58; – = negative result, no fluorescence detected after 45 cycles of amplification.
§, C<sub>r</sub> cycle threshold.
‖Tick species tested were *Haemaphysalis leporispalustris* and *Dermacentor andersoni*.

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