L.F., C.I., and T.C. conceived the study; S.E.W., K.G., H.G., S.D., B.B., C.S., and J.O. performed experiments; A.I., J.F., H.S., B.G., and A.K. contributed isolates and reagents; L.F., B.B., C.I., and T.C. analyzed the data; and T.C. and L.F. wrote the manuscript, which all authors approved.

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Specificity of Dengue NS1 Antigen in Differential Diagnosis of Dengue and Zika Virus Infection

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To the Editor: Circulation of new arboviruses of the genus *Flavivirus* poses a major problem for differential diagnosis. Zika virus, a mosquitoborne virus of the family *Flaviviridae*, is closely related to other arboviruses circulating in the Americas, including dengue, yellow fever, Saint Louis encephalitis, and West Nile viruses (*1,2*). Serologic cross-reactivity between these arboviruses is common; thus, to ensure optimal patient care and accurate epidemiologic surveillance, an effective differential diagnosis is required in regions with active transmission of dengue virus and circulation of Zika virus (*2–4*).

Cross-reactivity between flaviviruses has been reported in antibody assays and in tests for Dengue nonstructural 1 glycoprotein (NS1) antigen. Gyurech et al. (*5*) reported false-positive test results for dengue NS1 antigen in a patient with acute Zika virus infection. Of the 3 NS1 tests used in that study, only the SD Bioline Dengue Duo (Standard Diagnostics, Inc., Gyeonggi-do, South Korea) showed positive results for 3 of 4 sequential serum samples from the patient.

Cross-reactivity in NS1 dengue tests (ELISA and immunochromatographic) using serum samples from patients with acute Zika virus infection would have medically significant consequences. We therefore conducted a retrospective analysis of the differential diagnosis for dengue and Zika virus infections since the beginning of the Zika virus outbreak in French Guiana, a department of France on the northeast coast of South America.

French Guiana is subject to endemoepidemic circulation of dengue and experienced a large outbreak of chikungunya in 2014. We conducted our study from December 17, 2015 (the time of biologic confirmation of the first case of Zika virus disease in French Guiana), through March 2, 2016. During that time, the incidence of dengue virus infection in French Guiana was low, and only 1 sporadic case was confirmed. We studied clinical samples collected during this period from all patients with suspected arbovirus infection.

Table. RT-PCR results for 65 persons with clinical samples tested during the acute phase of Zika virus infection, French Guiana, December 17, 2015–March 2, 2016*

Days from symptom onset to sample collection	No. cases	RT-PCR results, by clinical sample					
		Serum only		Urine only		Serum and urine	
		No. samples	C_t, mean ± SD	No. samples	C_t, mean ± SD	No. samples	C_t, mean ± SD
0	8	5	31.8 ± 2.8	1	34.6†	2	Serum: 28.6 ± 10.45; urine: 28.9 ± 11.3
1	12	4	29.9 ± 4.6	2	36.5 ± 0.3	6	Serum: 36.0 ± 1.7; urine: 33.6 ± 4.4
2	20	13	32.8 ± 3.0	1	33.7†	6	Serum: 34.2 ± 3.6; urine: 33.0 ± 1.4
3	15	8	33.6 ± 3.6	2	34.2 ± 1.5	5	Serum:33.6 ± 2.8; urine: 34.1 ± 1.6
4	7	5	31.7 ± 2.4	1	28.9†	1	Serum:32†; urine: 32†
5	3	0	NA	1	31.7†	2	Serum: 35.1 ± 0.8; urine: 32.9 ± 3.9
Total	65	35	32.5 ± 3.1	8	33.8 ± 2.6	22	Serum: 33.6 ± 3.8; urine: 32.9 ± 3.8

*C_t, cycle threshold; NA, not applicable; RT-PCR, reverse transcription PCR.
†SD not determined.

Samples were analyzed for the differential diagnosis of dengue, chikungunya, and Zika virus disease. Zika virus diagnosis was conducted by the National Reference Centre for Arboviruses (NRC) at the Institut Pasteur of French Guiana, in Cayenne, according to the real-time reverse transcription PCR (rRT-PCR) protocol described by Lanciotti et al. (3). Dengue diagnosis was routinely performed by all medical diagnostic laboratories using various rRT-PCR techniques or dengue NS1 test kits. We used the same SD Bioline Dengue Duo test used by Gyurech et al. (5); this test was performed at the laboratory of the Centre Hospitalier de l'Ouest-Guyanais in Saint-Laurent du Maroni, French Guiana. We also used the Platelia Dengue NS1 Ag kit (Bio-Rad, Marnes-la-Coquette, France); the assay was performed at NRC.

Since Zika virus first appeared in French Guiana, the NRC has investigated 270 samples collected 0–5 days after fever onset for molecular diagnosis of Zika virus and dengue NS1. Of the 270 suspected patients, 65 were confirmed positive for acute Zika virus infection by rRT-PCR of serum, urine, or both. The mean cycle thresholds (± SDs) were 33.0 (± 3.4) for serum samples and 33.2 (± 3.5) for urine samples (Table). Of the 65 acute-phase Zika virus–positive serum samples, 36 were also tested with the Platelia Dengue NS1 test, 21 were tested with the SD Bioline Dengue Duo test, and 8 were tested with both tests; none of the results were positive. Of the 205 Zika virus–negative samples, 204 were also negative for dengue NS1; only 1 patient had a positive dengue NS1 test result, and the infection was confirmed by molecular investigations to be a case of acute dengue-1 disease.

This retrospective analysis of dengue and Zika virus diagnoses indicates that no false-positive dengue NS1 test results occurred among samples with acute-phase Zika virus infection. Indeed, samples from all 65 patients with rRT-PCR–confirmed acute Zika virus infection were negative by both dengue NS1 tests. Zika virus is closely related to dengue virus, and during the acute phase of disease, Zika

virus might release NS1 into patients' serum; however, this putatively released nonstructural protein does not appear to cross-react with the dengue NS1 tests used in our study. No Zika NS1 antigen assay currently exists, and acute-phase release of Zika NS1 has not been verified. If a Zika virus NS1 test is developed, it should be evaluated for cross-reactivity with serum from patients with acute dengue infection. The false-positive result reported by Gyurech et al. (5) for dengue NS1 antigen in a patient with acute Zika virus infection requires further investigation. Little is known about false-positive NS1 tests. Zika virus might show cross-reactivity with other flaviviruses and possibly cytomegalovirus, and hematologic disorders might cause NS1 positivity (6,7).

The co-circulation of Zika virus and dengue virus in the Americas is causing a health emergency. Our findings show that dengue NS1 antigen assays are still entirely appropriate for dengue surveillance, even during the epidemic circulation of Zika virus.

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Vibrio cholerae O1 Imported from Iraq to Kuwait, 2015

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To the Editor: The etiologic agent of the sixth pandemic of cholera was classical biotype of *Vibrio cholerae* O1. The ongoing seventh pandemic is caused by El Tor biotype. The biotypes are differentiated by phenotypic and genotypic characteristics. However, this differentiation blurred when *V. cholerae* O1 strains were detected in Matlab, Bangladesh, in 2006, in which characteristics were mixed. Genetically, the differences occurred in *tcpA*, which encodes the major adherence antigen *rstR* that regulates site-specific recombination of CTXφ phage and *ctxB* that encodes the B subunit of cholera toxin. These genes had the characteristics of classical biotype in Matlab variants of El Tor strains. Later, various types of El Tor variants were reported in Southeast Asia, Africa, and Haiti. Differentiating features also occur in repeat toxin A gene (*rtxA*), chromosomal location of CTXφ, the number of heptad repeats in *ToxR* binding region, and the occurrence of vibrio seventh pandemic islands I and II (*1,2*).

Kuwait is free of endemic cholera, but imported cases occur there (*3*). Cholera is endemic to neighboring Iraq. An outbreak caused by *V. cholerae* O1 Inaba serotype started in Iraq in September 2015 (*4*). However, a full characterization of the strain is lacking. A thorough characterization of the strain assumes urgency in light of the spread of variants. We characterized isolates from 2 recent cholera cases imported to Kuwait from Iraq.

The first case was in a 19-year-old Kuwaiti man who visited Najaf and Karbala in Iraq in September 2015; the second case was in a 52-year-old Kuwaiti woman who visited the same 2 locations in October 2015. Both had watery diarrhea 3–4 times daily and vomiting; they returned to Kuwait and were admitted to Al Amiri Hospital (Sharq, Kuwait). They gave histories of drinking local water in Iraq, had moderate dehydration, and were treated with intravenous rehydration solution and a single doxycycline dose (500 mg). Diarrhea resolved after 2–3 days.

Fecal specimens collected at admission from both patients grew yellow colonies on thiosulfate bile salt sucrose agar (Eiken, Tokyo, Japan); these colonies were confirmed as *V. cholerae* O1 Inaba serotype by biochemical reactions and agglutination with specific antiserum (Denka Seiken, Tokyo, Japan). The woman's isolate was designated as Kuwait 36 and the man's as Kuwait 37. The isolates were positive for chicken cell agglutination and Voges-Proskauer tests and were polymyxin B resistant, characteristics of El Tor biotype. The isolates were resistant to nalidixic acid but susceptible to ciprofloxacin, norfloxacin, ofloxacin, tetracycline, meropenem, ampicillin, ceftriaxone, trimethoprim/sulfamethoxazole, chloramphenicol, erythromycin, azithromycin, streptomycin, neomycin, and gentamicin by disk diffusion test. Tetracycline susceptibility confirmed favorable response to doxycycline.

We studied the genotype of *ctxB* using a double-mismatch amplification mutation PCR (i.e., mismatches in both primers). PCR with classical *ctxB*-specific primers ctxBF4/ctxBRvCla yielded an amplicon of 191 bp, but not with Haitian *ctxB* specific primers ctxBF3/ctxBRvCla, indicating that the isolates had a *ctxB* of classical biotype (genotype 1) (*5,6*). Mismatch amplification mutation assay PCR (MAMA-PCR, i.e., mismatch in only 1 primer) with Haitian-specific *tcpA* primers tcpAF2/tcpARev produced an amplicon of 167 bp but not with El Tor *tcpA*–specific primers tcpAF1/tcpAElRev, suggesting these isolates had the Haitian variant *tcpA* (*2*). MAMA-PCR for *rtxA* with El Tor–specific primer pair rtxAF/rtxAR1 yielded a 187-bp amplicon but no amplicon for Haitian variant primer pair rtxAF/rtxAR2, suggesting the occurrence of *rtxA* of El Tor variety (*2*). The isolates possessed El Tor type *rstR* because they produced a 500-bp amplicon with primer pair rstR2/rstA3R (*7*). The isolates were positive for *rstC*, a repeat sequence activator found in El Tor biotype, because they yielded an amplicon of 238 bp with primer pair rstC1/rstC2 (*8*). *rstB* is required for CTXφ phage integration. The Haitian strain has a GTA deletion at positions 77–79. MAMA-PCR with primer pair rstB F1/rstB R1 produced a 160-bp amplicon, suggesting the absence of deletion in El Tor type *rstB* (*2*). The isolates had CTXφ integrated in the large chromosome with RS element downstream because they produced a 766-bp amplicon with CII F/CII R primers