travelers returning from Cuba with a rash, similarly to patients returning from other countries in which dengue fever, chikungunya fever, and Zika virus infection are endemic. Preventive measures, including advice to travelers on proper use of insect repellents, are critical for preventing CHIKV infection.

Acknowledgments
The authors thank the clinical staff at the Disease Control and Prevention Center, Tokyo, Japan, for their help in completing this study.

A grant from the National Center for Global Health and Medicine (27-6001) supported this work.

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Inactivation and Environmental Stability of Zika Virus

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DOI: http://dx.doi.org/10.3201/eid2209.160664

To the Editor: Zika virus is an emerging virus that has spread to most countries in Latin America and the Caribbean (*1*,*2*). It is transmitted by mosquitoes and through sexual intercourse (*3*). Most persons infected with Zika virus are asymptomatic or experience mild symptoms (*4*). However, in a pregnant woman, infection may cause severe pregnancy and birth complications, most notably microcephaly in children infected in utero (*5*–*7*). Zika virus infection might also be associated with an increased incidence of Guillain-Barré syndrome (*8*). Thus, the virus represents a threat to healthcare workers who manage infected patients or diagnostic samples and researchers who work with infectious virus in laboratories.

Working with Zika virus, a Biosafety Level 2 (BSL-2) pathogen in the European Union, except for the United Kingdom (where it is BSL-3), requires specific safety precautions (*9*). No inactivation data specific for Zika virus are available (*9*); consequently, disinfection guidelines are based on protocols to inactivate other flaviviruses. To gain experimental evidence regarding inactivation and disinfection for Zika virus, we determined its susceptibility to various disinfectants and inactivation methods.

To test susceptibilities, we determined the 50% tissue cell infectious dose per milliliter ($TCID_{50}$/mL) (*10*) of the Zika virus MR766 strain (*1*) before and after the virus was exposed to disinfectants or other inactivation procedures (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/9/16-0664-Techapp1.pdf). We then determined the effect of alcohol-based disinfectants on viral infectivity. Using Zika virus stock containing 2.5% fetal calf serum (FCS) mixed 3:10 (vol/vol) with indicated alcohols, we incubated the mixture for 1 minute and then used it for infection (Figure, panel A). All alcohols entirely inactivated

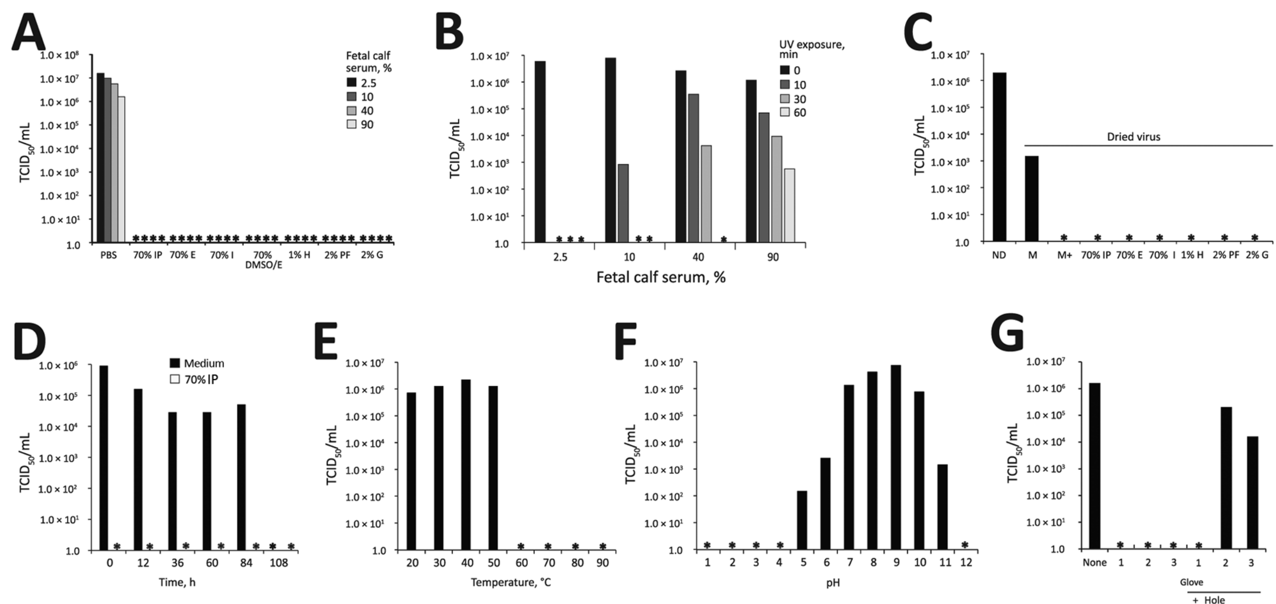

Figure. Inactivation and environmental stability of Zika virus. Asterisks (*) indicate lack of infection. A) Virus stocks containing 2.5%, 10%, 40%, or 90% fetal calf serum were incubated with alcohol-based disinfectants for 1 min. All disinfectants inactivated the virus. B) Virus stocks containing indicated concentrations of fetal calf serum were exposed to the ultraviolet (UV) light of a laminar flow hood. Higher concentrations of serum required more time to inactivate the virus. C) Virus stock was dried for 18 h and was then reconstituted in medium or the indicated disinfectants for 5 min or exposed to 10 min UV light before reconstitution. All disinfectants inactivated the virus. D) Virus was dried and incubated for indicated periods of time. Thereafter, dried virus was reconstituted in medium or 70% (vol/vol) isopropanol. Isopropanol inactivated the virus, but dried virus in medium remained infectious even after 84 h of incubation. E) Zika virus was incubated for 5 min at indicated temperatures. Temperatures ≥60°C inactivated the virus. F) Stocks were adjusted to indicated pH values and incubated for 10 min. pH levels ≤4 or >11 deactivated the virus. G) Finger tips of laboratory gloves were cut off, with or without introducing a hole by pinching with a needle, and put into medium. Glove tips were filled with virus stock and incubated for 90 min at room temperature. All gloves without needle holes were protective against transmission; 2 of 3 gloves with needle holes allowed virus transmission. For detailed experimental description, see online Technical Appendix (http://wwwnc.cdc.gov/EID/article/22/9/16-0664-Techapp1.pdf). DMSO, dimethyl sulfoxide; E, ethanol; G, glutaraldehyde; H, hypochlorite; I, incidin, IP, isopropanol; M, medium, M+, medium plus 10 min UV; ND, not dried; PBS, phosphate-buffered saline; PF, paraformaldehyde; $TCID_{50}$, 50% tissue culture infective dose; UV, ultraviolet.

the virus. Complete loss of infectivity was also observed after virus exposure to 1% hypochlorite (often used to inactivate virus in liquid wastes in BSL-2/3 laboratories), 2% paraformaldehyde (used to inactivate virus for subsequent flow cytometry), and 2% glutaraldehyde (often applied to fix virus for subsequent electron microscopy analysis) (Figure, panel A). Thus, routinely used disinfectants and inactivation procedures are sufficient to inactivate Zika virus in laboratory virus stocks. Next, we repeated these experiments in the presence of a high protein load using Zika virus preparations supplemented with FCS in increasing concentrations (10%, 40%, 90%), to mimic virus found in clinically relevant material. Again, all treatments entirely disrupted Zika virus infectivity (Figure, panel A).

Ultraviolet (UV) radiation inactivates viruses by chemically modifying the genome. We exposed 200 µL of Zika virus preparations containing increasing concentrations of serum to UV light of a laminar flow for up to 60 minutes. Exposure for 10 minutes entirely inactivated Zika virus in the presence of 2.5% FCS serum; increasing concentrations of serum reduced the antiviral effects of UV light (Figure, panel B). When Zika virus containing 90% serum was exposed for 60 min to UV light, infectivity was reduced by 99.95%; however, some residual infectivity was detected (Figure, panel B).

Next, we evaluated environmental stability by drying 100 µL of Zika virus stock for 18 hours. Thereafter, dried virus was reconstituted in the same volume of medium or disinfectants. Endpoint titrations showed that the reconstituted virus remained infectious, although $TCID_{50}$ was reduced by ≈3 orders of magnitude (Figure, panel C). All disinfectants and UV radiation entirely inactivated dried Zika virus (Figure, panel C). Additional experiments demonstrated that dried Zika virus remained infectious for >3 days (Figure, panel D) suggesting, for example, that dried droplets can be infectious, confirming that proper surface disinfection is essential.

We also assessed the environmental stability of Zika virus to heat and change in pH. The virus was stable at temperatures up to 50°C but lost all infectivity at temperatures

of ≥60°C (Figure, panel E). Thus, virus-contaminated materials such as surgical instruments can be decontaminated by heat. We also found that Zika virus infectivity was highest after adjusting the stock to a pH of ≈9 (Figure, panel F). In contrast, adjusting Zika virus to pH 12 or to ≤pH 4 abrogated infectivity (Figure, panel F).

Finally, we analyzed whether gloves routinely used in BSL-2 laboratories protect against Zika virus. For this, we cut off fingertips of nitrile and latex gloves, filled tips with a Zika virus suspension, and placed them into cell culture plates containing medium. Virus-containing fingertips were inserted in such a way that diffusion would only occur if the virus passed through the nitrile/latex barrier. As a control, we made a hole of <1 mm in the fingertips. All 3 tested gloves prevented virus diffusion (Figure, panel G). However, if glove integrity was disrupted by a pin, the virus passed through in 2 of 3 cases (Figure, panel G).

We demonstrated that Zika virus is destroyed by classical disinfectants and inactivation methods and that nitrile and latex gloves are protective. We also showed that UV light of a laminar flow hood inactivates Zika virus, but particularly if the virus is in a protein-rich environment, the exposure time range should be in hours rather than in minutes. Although we expected that Zika virus would be inactivated by alcohol and disinfectants, we conducted a thorough experimental verification to exclude uncertainties surrounding work with this emerging pathogen.

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ESBL-Producing Strain of Hypervirulent *Klebsiella pneumoniae* K2, France

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DOI: http://dx.doi.org/10.3201/eid2209.160681

To the Editor: *Klebsiella pneumoniae* is mainly responsible for hospital-acquired urinary tract infections, bacteremia, pneumonia and intra-abdominal infections. However, since the mid-1980s, *K. pneumoniae* has also been described as the cause of highly invasive community-acquired infections (*1,2*). The *K. pneumoniae* isolates associated with such infections are often hypermucoviscous and frequently belong to the capsular serotypes K1 or K2. Two of the most extensively studied genes associated with invasive infections are a mucoviscosity-associated gene A (*mag*A) in serotype K1 and a regulator of mucoid phenotype A (*rmp*A). These strains of hypervirulent *K. pneumoniae* (hvKP) are now circulating worldwide (*1*,*2*).

At the same time, a substantial increase of high-level antimicrobial resistance acquired by non-hvKP strains has also been observed. Clonal complexes of hvKP and multidrug-resistant (MDR) strains had been considered independent (*3*) until 2014, when extended-spectrum β-lactamase (ESBL)– or carbapenemase-producing hvKP were first identified in China (*4*). Here we report an ESBL-producing strain of hvKP isolated from a patient in France.

The patient was a 56-year-old woman, born in Algeria, who alternately resided in France and Algeria for several years without travel to any other country. She underwent liver transplant in 2007 for primary biliary cirrhosis. In 2012, she had a routine posttransplant liver biopsy indicating

Article DOI: http://dx.doi.org/10.3201/eid2209.160664

Inactivation and Environmental Stability of Zika Virus

Technical Appendix

Cells, Viruses, and Reagents

Vero E6 cells, used for propagation and infection of Zika virus (ZIKV), were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 2.5% inactivated fetal calf serum (FCS), 200 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, 1 mM sodium pyruvate, and nonessential amino acids (Sigma #M7145) at 37°C in a 5% CO_2–humidified incubator. ZIKV strain MR 766 was isolated in 1947 from a sentinel rhesus macaque.

Generation of ZIKV Stock

We infected 70% confluent Vero E6 cells in T175 cell culture flasks with ZIKV MR 766 for 2 h in 5 mL medium. Subsequently, 40 mL fresh medium was added, and the cells were cultured. Cytopathic effect (CPE) was monitored by light microscopy, and virus was harvested when 70% of the cells detached due to CPE. Supernatant was taken, centrifuged for 3 min at 1,300 rpm, before virus stocks were aliquoted and stored at –80 °C. The ZIKV stock used throughout this study had an infectious titer of 1.6×10^7 $TCID_{50}$/mL and a genome copy number of $\approx 1 \times 10^{10}$ /mL as assessed by quantitative reverse transcription PCR (RT-PCR) (RealStar Zika Virus RT-PCR Kit; Altona Diagnostics, Hamburg, Germany).

Effect of Alcohols, Disinfectants, and Other Chemicals on ZIKV Infection

To determine ZIKV sensitivity to disinfectants with different protein loads, we adjusted the virus stock to 2.5%, 10%, 40%, and 90% (v/v) FCS. Virus was then mixed 3/7 (v/v) with H_2O, isopropanol (VWR International #20842.330), ethanol (VWR International #20821.330), incidin (ECOLAB Healthcare #3021780), DMSO/ethanol (Merck #1029521000), 1% hypochlorite (PanReac AppliChem # 213322), and 2% paraformaldehyde (Merck #1040051000), and glutaraldehyde (PLANO #R1012) (v/v). Following a 1-min incubation, samples were serially diluted and used to inoculate VeroE6 cells. MTT assays in absence of virus were

performed at all times to check for cytotoxicity of the chemicals after dilution and inoculation of cells.

Effect of Ultraviolet (UV) Light on ZIKV Infection

To determine ZIKV sensitivity to UV light, we placed 100 μL of ZIKV stocks, supplemented with different concentrations of serum, into a reagent reservoir and exposed to bench UV light (HNS Puritec 15W G13 OFR. 200–260 nm, Osram) for indicated times. After irradiation samples were serially diluted, $TCID_{50}$ was determined.

Effect on Disinfectants of Dried ZIKV Solution

For ZIKV stability upon drying to be determined, 200 μL of virus stock solution was dried on a 24 cell culture plate for 18 h. The dried samples were then reconstituted in an equal volume of disinfectant or phosphate-buffered saline. One samples was UV-irradiated before reconstitution. Samples were then serially diluted, and the $TCID_{50}$ was determined.

Stability of ZIKV Infectivity over Time

To analyze virus infectivity over time, we mixed virus stock with 70% medium or isopropanol (v/v), and incubated the mixture at 37°C. At indicated time points, samples were taken and stored at –80 °C until they were thawed in parallel and analyzed as described.

Temperature sensitivity of ZIKV

The virus stock was incubated in 1.5-mL reaction tubes at indicated temperatures for 5 min. $TCID_{50}$ was then determined.

pH Sensitivity of ZIKV

Virus was mixed 1:1 (v/v) with medium to result in the indicated pH values during a 10-min room temperature incubation. Medium with different pH was generated using a pH-meter and confirmed by pH paper after mixing with virus. Samples were then titrated and used for inoculation.

Glove Protection against ZIKV

For this, we cut off the fingertips of nitrile gloves (Sempercare nitrile skin², Sempermed; and Peha-soft nitrile fino, Hartmann) and latex gloves (Sempercare premium latex, Sempermed), filled the tips with 100 μL of ZIKV suspension, and placed them into 24-well plates containing 500 μL medium. The ZIKV-containing fingertips were placed in a way that diffusion can only

occur when the virus passes through the nitrile/latex barrier. As a control, we inserted a hole of <1 mm in 1 fingertip by pinching it with a needle prior to loading with ZIKV. After 60 min, gloves were removed, and the medium below was serially diluted to determine $TCID_{50}$.

$TCID_{50}$ Endpoint Titration

We seeded 6,000 Vero E6 cells per well in 96-well plates in 100 μL medium and incubated the plates overnight. The next day, medium was taken and replaced with 180 μL fresh medium. For endpoint $TCID_{50}$ determination, ZIKV samples were serially diluted 10-fold, and 20 μL of each dilution was used for inoculation of Vero E6 cells. This endpoint titration resulted in final ZIKV dilutions of 10^1–10^9 fold on the cells in triplicates. Cells were then incubated and monitored for virus induced CPEs and plaque formation. $TCID_{50}$/mL was calculated according to Reed and Muench. The used ZIKV stock in this study had an infectious dose of 1.6×10^7 $TCID_{50}$/mL.