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LETTER TO THE EDITOR

Limit of Detection for Rapid Antigen Testing of the SARS-CoV-2 Omicron and Delta Variants of Concern Using Live-Virus Culture

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To bolster coronavirus disease 2019 (COVID-19) pandemic mitigation efforts, the U.S. Food and Drug Administration (FDA) issued an emergency use authorization (EUA) for easy-to-use rapid antigen (Ag) tests for the diagnosis and surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (1, 2). Unlike sensitive molecular tests that detect multiple SARS-CoV-2 genes, antigen tests target a singular yet genetically conserved nucleocapsid viral protein (3–6). As the pandemic continues, some hypothesized that the performance of available antigen tests may differ between SARS-CoV-2 variants. As of February 2022, the most recent SARS-CoV-2 strains declared variants of concern (VoC) by the U.S. Centers for Disease Control and Prevention are Omicron (strain B.1.1.529) and Delta (strain B.1.617.2) (7). Beyond striking amino acid mutations in the spike protein, Omicron also harbors P13L, Δ31–33, R203K, and G204R nucleocapsid mutations, while Delta strains carry D63G, R203M, and D377Y nucleocapsid mutations (8, 9). However, the limits of detection (LoDs) of many FDA EUA antigen tests were established with gamma-irradiated or heat-inactivated preparations of the USA WA1/2020 (WA1) strain (10) lacking these nucleocapsid mutations. This includes at-home lateral flow tests like the BinaxNOW COVID-19 Ag card (Abbott Diagnostics Scarborough, Inc., Scarborough, ME), the CareStart COVID-19 antigen home test (Access Bio, Inc., Somerset, NJ), and the GenBody Covid-19 Ag test (GenBody, Inc., Chungcheongnam-do, Republic of Korea) and also the LumiraDx SARS-CoV-2 Ag test (LumiraDx UK Ltd., Alloa, Great Britain), a microfluidic immunofluorescence assay for clinical laboratory testing (11–14). In the present study, we used cultured plaque-titered live Omicron, Delta, and WA1 viruses to assess differences in the LoDs with the BinaxNOW, CareStart, GenBody, and LumiraDx tests.

The titers of the Omicron lh01 (NCBI accession number OL719310), Delta (BEI Resources catalog number NR-55671, isolate hCoV-19/USA/MD-HP05285/2021; Johns Hopkins University), and WA1 (10) viruses were determined by a plaque assay (10) and further calibrated with the Abbott RealTime SARS-CoV-2 assay (Abbott Molecular, Inc., Des Plaines, IL) (15). The genomes of the Omicron, Delta, and WA1 viral stocks used in our analysis were also sequenced using the NEBNext ARTIC SARS-CoV-2 companion kit (New England BioLabs, Ipswich, MA) and MinION (Oxford Nanopore Technologies, Oxford, UK) technology (16–20), confirming the lack of mutation acquisition during propagation.

For LoD evaluation, 10-fold serial dilutions in phosphate-buffered saline (PBS) ranging from 2.5×10^4 to 2.5 PFU/mL were applied to swabs in 50-μL volumes and tested

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The authors declare a conflict of interest. LumiraDx provided instrumentation and diagnostic kits; Abbott provided diagnostic Ag kits; and Ginkgo Bioworks provided CareStart and Genbody antigen testing kits for the study. The BIDMC authors (J.E.K., A.C.) received support from Abbott Molecular unrelated to this study under a COVID-19 Diagnostics Evaluation Agreement. Abbott, LumiraDx, and Ginkgo Bioworks had no role in study design, manuscript preparation or decision to publish. All authors, no other conflicts of interest.

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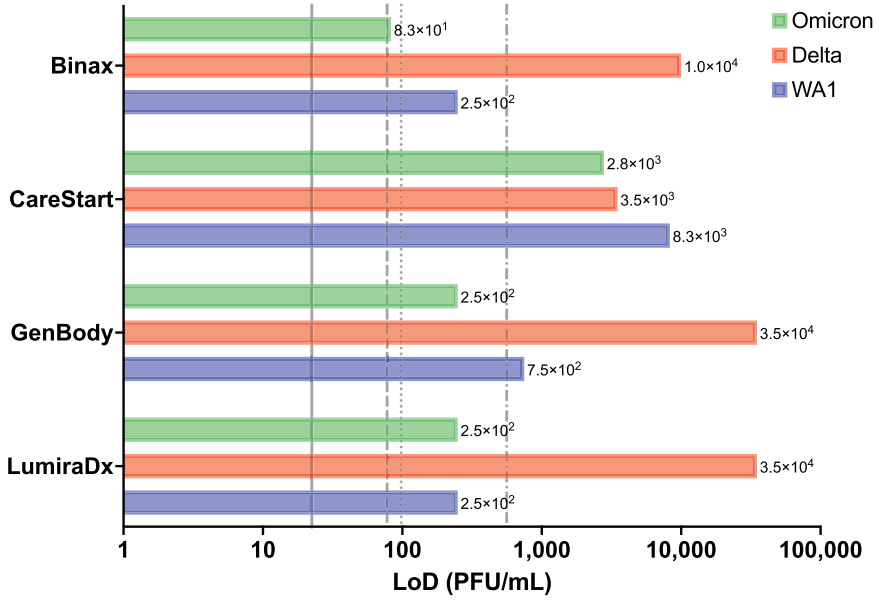

FIG 1 Limits of detection of antigen tests. Shown are the limits of detection (LoDs) in PFU per milliliter determined in our analysis (bars). Vertical lines reference the manufacturer-reported LoDs in the respective instructions for use (IFU) documents (11–14), converted from 50% tissue culture infective doses ($TCID_{50}$) per milliliter to PFU per milliliter by multiplying the $TCID_{50}$ per milliliter by 0.7, a standard conversion based on the Poisson distribution, for BinaxNOW (dotted line) (1.4×10^2 $TCID_{50}$/mL; 9.8×10^1 PFU/mL), CareStart (dashed and dotted line) (8.0×10^2 $TCID_{50}$/mL; 5.6×10^2 PFU/mL), GenBody (dashed line) (1.1×10^2 $TCID_{50}$/mL; 7.8×10^1 PFU/mL), and LumiraDx (solid line) (3.2×10^1 $TCID_{50}$/mL; 2.2×10^1 PFU/mL).

in triplicate according to the manufacturers' instructions (11–14). SteriPack sterile polyester spun nasal swabs (catalog number 60566REVA; LumiraDx UK Ltd., Alloa, Great Britain) and iClean foam swabs (catalog number CY-FS742; Supera, Houston, TX) were used with the LumiraDx test. After identifying the lowest 10-fold dilution with three replicate positive tests, we iteratively tested 3-fold dilutions around this concentration until identifying the lowest dilution (the LoD) in which at least 19 of 20 replicates ($\geq$95%) were positive.

We found that Omicron had a 95% LoD threshold similar to or lower than that of WA1 for all four tests (Fig. 1). In contrast, the LoDs were 40- to 140-fold higher for Delta than for WA1 for every test examined except for CareStart (Fig. 1). The equal detection of all three variants by CareStart and the only relatively modest increase in the ratio of PFU to genome copies per milliliter for Delta (Fig. 2) suggest that this represents a true reduction in analytical sensitivity for Delta rather than an artifact of enhanced plaquing efficiencies for Delta relative to antigen levels and associated levels of genome copies. We previously found that the CareStart and LumiraDx antigen tests were excellent in the detection of presumptively WA1-infected individuals (15). We expect, however, that the observed magnitude of the loss in Delta sensitivity could result in a $>$20% loss in the detection of potentially infectious individuals based on our previous examination of the effect of LoD on clinical sensitivity (21). Nevertheless, the most infectious individuals should still be detected.

Of note, our use of live virus, analyte volume, and swab type may explain the slight discrepancy with the manufacturers' determined LoDs. Our results for variant detection were also not completely consistent with similar reports, but these studies either fell short of the FDA's EUA requirement of 20 LoD replicates, examined tests unavailable in the United States, and/or tested gamma-irradiated or heat-killed virus, inactivation processes which may artifactually affect test performance (22–24). In summary, we demonstrate that the rapid antigen tests evaluated detect Omicron effectively. However, our unexpected findings of decreased detection of Delta virus suggest that antigen test performance needs to

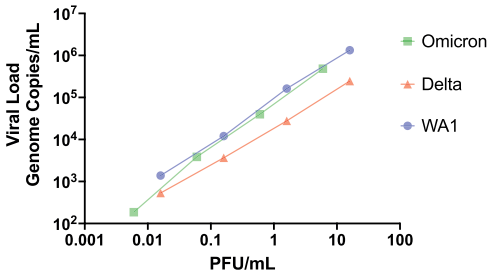

FIG 2 Correlation of PFU per milliliter and viral load in genome copies per milliliter. Stocks of each strain were serially diluted 10-fold in PBS and analyzed by PFU (10) and calibrated reverse transcription-quantitative PCR (RT-qPCR) assays (15). Both axes are on a $\log_{10}$ scale.

be reevaluated for emerging variants to ensure that they still meet the intended public health testing goals of the pandemic.

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