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BRIEF REPORT

Laboratory Evaluation Links Some False-Positive COVID-19 Antigen Test Results Observed in a Field Study to a Specific Lot of Test Strips

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During a household-transmission field study using COVID-19 antigen rapid diagnostic tests (Ag-RDT), a common test strip lot was identified among 3 participants with false-positive results. In blinded laboratory evaluation, this lot exhibited a significantly higher false-positive rate than other lots. Because a positive Ag-RDT result often prompts action, reducing lot-specific false positives can maintain confidence and actionability of true-positive Ag-RDT results.

Keywords. COVID-19; diagnostics; false positive; faulty lot; lateral flow test; Quidel QuickVue.

Antigen rapid diagnostic tests (Ag-RDTs) are increasingly used for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Usage of at-home Ag-RDTs in the United States (US) has increased nearly 4-fold among those with self-reported coronavirus disease 2019 (COVID-19)–like illness between the period of Delta (23 August–11 December 2021) to Omicron (19 December 2021–12 March 2022) variant predominance [1]. Ag-RDTs are also used widely for test-to-enter events and serial screening testing in schools and workplaces; for example, in May 2022 [2], the California Department of Public Health began recommending Ag-RDTs as the primary test for COVID-19 in schools [3].

Ag-RDTs typically have very high specificity; of the 51 Ag-RDTs currently authorized for at-home use in the US as of 2 September 2022, all are required to demonstrate false-positive rates of ≤2% [4]. However, with widespread use imperfect specificity can result in many false-positive results, and at low prevalence of infection, these false positives can represent a large fraction of or even dominate among all positive results [5].

As part of a COVID-19 household-transmission field study in Southern California initiated in November 2021, participants performed a daily at-home nasal swab Ag-RDT (Quidel QuickVue At-Home OTC COVID-19 Test) and self-collected saliva, anterior nares swab, and oropharyngeal swabs for reverse-transcription quantitative polymerase chain reaction (RT-qPCR) testing [6]. This test was selected for the field study because it was one of the first Ag-RDTs to be granted US Food and Drug Administration Emergency Use Authorization [7] and is widely in use in the US and internationally.

In January 2022, interim analysis of the field study showed a string of 24 Ag-RDT positive results from participants who had corresponding negative results in all 3 specimen types tested by RT-qPCR, causing an elevated clinical false-positive rate (Figure 1*A*). Further investigation revealed a common Ag-RDT strip lot number (152000) among 3 participants with false-positive results. We then investigated the technical false-positive rate of Ag-RDT test strip lot 152000, and other lots acquired for use in the field study, in a controlled laboratory setting.

METHODS

Participant Consent Statement

The Ag-RDT field study [6] was approved by the California Institute of Technology Institutional Review Board under protocol number 20-1026. All adult participants in the study provided written informed consent and all minors provided verbal assent accompanied by written parental permission.

Laboratory Evaluation of Ag-RDT Test Strips

We created contrived specimens using heat-inactivated SARS-CoV-2 particles (BEI, catalog number NR-52286, lot 70034991) spiked into commercial SARS-CoV-2–negative human nasal fluid (Lee BioSolutions, catalog number 991-13-P, lot 03f4044 and catalog number 991-13-P-PreC, lot 09F3280) at concentrations above and below the inferred limit of detection (LOD) for this assay (7×10^6 copies/mL) [6] and applied them to 2 lots of test strips (152194 and 152532) that did not yield any false-positive results among participants in the field study. Contrived specimens with SARS-CoV-2–negative human nasal fluid alone were also applied to 4 Ag-RDT strip lots (152194, 152532, 000202, as well as 152000, the lot common to participants with observed clinical false-positive

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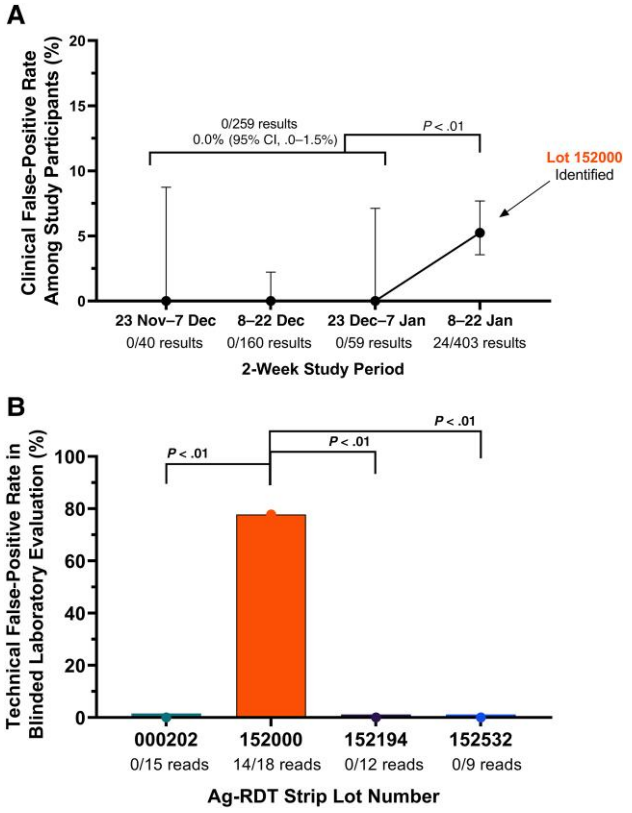

Figure 1. Clinical false-positive rate of antigen rapid diagnostic tests (Ag-RDTs) among participants in a coronavirus disease 2019 (COVID-19) household-transmission field study and subsequent laboratory evaluation of technical false-positive rates among Ag-RDT strip lots. *A,* The biweekly clinical false-positive rate for nasal swab Ag-RDT, defined as a positive Ag-RDT at the same timepoint as negative results by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) in saliva, nasal swabs, and oropharyngeal swab specimens. The proportions displayed below each month represent the number of clinical false-positive results over the total number of false-positive and true-negative Ag-RDT results in the field study during each period. Error bars represent 95% confidence interval (CI). *B,* Laboratory evaluation of the technical false-positive rate for 4 Ag-RDT strip lots was performed using severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–negative human nasal fluid (see Methods). The proportion of technical false-positive reads to all reads by readers blinded to experimental conditions is displayed below each lot number. *P* values were obtained using an upper-tailed Fisher exact test. Additional details are provided in Supplementary Table 1.

results). The order of contrived specimens and Ag-RDT strip lots was randomized by the operator.

Contrived specimens (20 µL) were pipetted onto the swab that came with each Ag-RDT, and the swab was placed into the Ag-RDT tube containing buffer. Manufacturer instructions were then followed exactly [8], by mixing the swab in the buffer for 1 minute, removing the swab, then placing an Ag-RDT strip in the tube and incubating at room temperature for 10 minutes. The result was then interpreted within 5 minutes by 3 readers blinded to the experimental conditions and test strip lot numbers; each trial with a single test strip therefore resulted in 3 independent reads. Readers were provided with the manufacturer instructions for result interpretation [8] and no additional guidance. Readers were unable to see the interpretations of other readers.

Statistical Methods

Clinical false-positive results were defined as positive Ag-RDT results reported by a study participant, at the same timepoint when saliva, nasal swab, and oropharyngeal swab specimens collected by the same participant all resulted negative by high-analytical RT-qPCR testing. The clinical false-positive rate was calculated as the number of clinical false-positive Ag-RDT results over all timepoints with false-positive and true-negative Ag-RDT results, using RT-qPCR as the reference standard. The clinical false-positive rate was binned by 2-week periods for visualization (Figure 1*A*).

Technical false-positive Ag-RDT results were defined as reads interpreted as positive when contrived specimen containing only SARS-CoV-2–negative nasal fluid was tested. The technical false-positive rate was calculated as the number of technical false-positive reads over all reads originating from specimen containing only SARS-CoV-2–negative nasal fluid. The technical false-positive rate was grouped by Ag-RDT strip lot (Figure 1*B*).

The 95% confidence interval (CI) of both the clinical and technical false-positive rate was calculated using the method described in the Clinical and Laboratory Standards Institute EP12-A2 document [9]. Statistical testing was performed to assess differences in the clinical false-positive rates between time periods in the field study (Figure 1*A*), and to compare the technical false-positive rates between Ag-RDT strip lots in the laboratory evaluation (Figure 1*B*); for all analyses we used the Fisher exact test, implemented in Python 3.8.8.

RESULTS

A significantly elevated clinical false-positive rate was observed among participants in a field study of a COVID-19 Ag-RDT, compared with what had previously been observed in the study ($P < .01$, upper-tailed Fisher exact test; Figure 1*A*). The elevated false-positive rate prompted the identification of a common Ag-RDT strip lot (152000) among 3 participants with multiple, daily clinical false-positive results. We then sought to evaluate the technical false-positive rate of this lot and other lots acquired for use in the field study, through laboratory evaluation.

To confirm that this Ag-RDT could be performed and produce expected results in a laboratory setting, we created contrived specimens with and without SARS-CoV-2 particles. Contrived specimens were applied to 2 Ag-RDT strip lots that had not yielded clinical false-positive results in the field study. Positive reads were expected when nasal fluid with viral concentrations above the LOD were applied to Ag-RDT strips, and negative reads were expected when viral concentrations were below the inferred LOD, and when only

SARS-CoV-2–negative nasal fluid (without any viral particles) was applied. Contrived specimens with SARS-CoV-2 concentrations between 1.0×10^7 and 1.5×10^7 copies/mL (above the inferred LOD of the Ag-RDT) were interpreted by readers as positive in 8 of 9 reads (3 independent trials each with 3 reads, 1 from each reader); contrived specimens with viral concentrations between 2.0×10^6 and 4.1×10^6 copies/mL (below the inferred LOD of the Ag-RDT) were interpreted by readers as negative in all 6 reads (Supplementary Table 1). These results confirmed that the Ag-RDT used in the field study yields expected positive and negative results with contrived specimens in a laboratory setting.

To assess the technical false-positive rate of different lots, SARS-CoV-2–negative human nasal fluid (without the addition of viral particles) was applied to Ag-RDT strips from 4 lots: 152194, 152532, 000202, and 152000 (the lot that produced clinical false positives among 3 different participants) (Figure 1*B*). No false-positive reads were reported for any trial performed on lots 152194, 152532, or 000202. However, 14 of 18 reads from lot 152000 were interpreted by readers as positive, yielding a technical false-positive rate of 77% (95% CI, 55%–91%); 1 read from this lot was interpreted as invalid. Furthermore, at least 1 reader interpreted a positive result for every trial with a lot 152000 test strip (Supplementary Table 1). The false-positive rate of Ag-RDT strip lot 152000 on laboratory evaluation was significantly higher than the false-positive rate observed for the other 3 test strip lots analyzed ($P < .01$, upper-tailed Fisher exact test).

DISCUSSION

In a field study of a COVID-19 Ag-RDT in Southern California, a specific lot of test strips was found to be common among 3 participants (from 3 different households) with false-positive Ag-RDT results. These participants had negative test results in 3 paired high-analytical-sensitivity RT-qPCR assays (saliva, nasal swab, and oropharyngeal swab) that were collected at the same timepoint. Laboratory evaluation confirmed that when SARS-CoV-2–negative nasal fluid was tested with this specific lot of Ag-RDT strips, readers blinded to randomized test conditions and strip lot numbers consistently interpreted results as positive. The laboratory evaluation supports that this lot was likely yielding false-positive results when in use by participants in our field study.

At-home Ag-RDTs are known to have low clinical sensitivity [6, 10, 11] and are likely to produce false-negative results. The low clinical sensitivity of Ag-RDTs is due to both their low-analytical sensitivity (high limits of detection) and, in the US, their authorized use exclusively with nasal swab specimens, which are not always representative of the patient infection status, especially early in the infection [6, 12–16]. The Centers for Disease Control and Prevention (CDC) has recognized the lack of clinical sensitivity of Ag-RDTs and in September 2022 updated recommendations to Ag-RDT testing protocols to repeat testing 24–48 hours later [17].

False positives are less frequent. The manufacturer of the Quidel QuickVue At-Home OTC COVID-19 Test, which was not involved in the design or execution of this study, reports a 99.2 negative percent agreement [8], and Ag-RDTs generally have >97% clinical specificity in field evaluations [11, 18]. By late 2020, the CDC recommended a confirmatory nucleic acid amplification test for Ag-RDT–positive results in cases with low pretest probability [19]. However as of April 2022, a single positive result now typically prompts immediate action from individuals, their close contacts, and healthcare personnel [20]. Notably, the Emergency Use Authorization for the Quidel QuickVue At-Home OTC COVID-19 Test [21] encourages individuals who test positive to self-isolate and contact their healthcare provider for follow-up care, which may include additional testing. Therefore, false-positive results can prompt unnecessary isolation and quarantine, needless treatment, consumption of additional testing resources, and diversion of contact tracing efforts from true-positive cases [22]. Further, false-positive results undermine trust in positive Ag-RDT results, such that isolation, treatment, additional testing, and contact tracing may not be initiated when it is appropriate.

False-positive Ag-RDT results are not unique to the current COVID-19 pandemic. The Quidel QuickVue Influenza A + B Test, another Ag-RDT that uses nasal swab specimens, is reported by the manufacturer to have >97 negative percent agreement [23], but during the 2009 influenza A(H1N1) pandemic, the clinical performance of the test resulted in a 62.2 negative percent agreement against RT-PCR [24].

COVID-19 Ag-RDT false-positive results have been reported in a number of contexts [18, 25, 26]. In a recent evaluation of the Quidel QuickVue At-Home OTC COVID-19 test in a college community [27], 8 of 11 participants with positive Ag-RDT results were found to be negative on RT-PCR testing within 24 hours. No definitive cause for these false-positive results was identified.

False-positive results may occur due to a variety of reasons [22, 25, 28–30], including user error, invalid test conditions, improper storage or manufacturing errors that affect reagent chemistry, or off-target binding of human or microbial material (including viruses other than SARS-CoV-2); for example, infection of human rhinovirus A has produced false-positive results in SARS-CoV-2 Ag-RDTs [26]. However, both we and others [31, 32] have found false-positive Ag-RDT results traceable to specific lots. Importantly, the overall false-positive rate observed among participants in our field study was 2.8% (95% CI, 2.1%–3.9%) [6]; monitoring only an overall false-positive rate across lots could mask specific lots with higher false-positive rates.

Lot issues can arise during manufacturing and transportation or can be due to storage conditions after distribution [33, 34]. In our study, Ag-RDTs were stored at room temperature and the mild winter climate in Southern California ensured that temperatures were stable during shipment as well. Here, we demonstrate through a controlled laboratory evaluation that false-positive results captured in a field study of Ag-RDTs were not due to operator error but were lot specific. Therefore, efforts to monitor for lot-dependent false positives (and whether they originate from issues at the manufacturer or distributor/retailer level) can increase the confidence and actionability of positive Ag-RDT results.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Data availability. The data underlying the results presented in the study are available at CaltechDATA at: https://doi.org/10.22002/fmz6a-0x036.

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