New recommended policies for pathogen surveillance testing of researchers and improved stewardship of diagnostic DNA

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Abstract

The SARS-CoV-2 pandemic has brought about the unprecedented expansion of highly sensitive molecular diagnostics as a primary infection control strategy. At the same time, many laboratories have shifted focus to SARS-CoV-2 research and diagnostic development, leading to large-scale production of nucleic acids that can interfere with these tests. We have identified multiple instances, in independent laboratories, in which nucleic acids generated in research settings are suspected to have caused researchers to test positive for SARS-CoV-2 in surveillance testing. In some cases, the affected individuals did not work directly with these nucleic acids, but were exposed via a contaminated surface or object. Though researchers have long been vigilant of DNA contaminants, the transfer of these contaminants in SARS-CoV-2 testing samples results in anomalous test results. The impact of these stretches into the public sphere, placing additional burdens on public health resources, placing affected researchers and their contacts in quarantine, and carrying the potential to trigger shutdowns of classrooms and workplaces. We report our observations as a call for increased stewardship over nucleic acids with the potential to impact both use and development of diagnostics. These experiences highlight a neglected aspect of the test, trace, isolate public health strategy for managing COVID-19: we cannot easily identify and diagnose an erroneous test result. To prevent undue personal and economic strain and maintain faith in the testing process, we propose: a test, isolate, verify, and trace approach to COVID-19 diagnosis in research and clinical diagnostic workplaces.

A case study in SARS-CoV-2 nucleic acid researcher contamination

Following a state-imposed stay at home order, a back-to-work plan for a research institute called for community-wide SARS-CoV-2 surveillance testing using self-administered nasal swabs. This testing program identified an epidemiologically linked

cluster of SARS-CoV-2 positive researchers in one laboratory. Additional positive tests from researchers in this group were reported in the three months following the initial results. In total, five members of this group tested positive, with two individuals testing positive on two separate occasions (Figure 1A). In all cases, university and state health departments were notified. In accordance with state and CDC guidelines, these researchers and their close contacts completed a 10-14 day isolation period without further incident.

Several aspects of these incidents were inconsistent with SARS-CoV-2 infection and epidemiology. In all cases, the affected researchers and their contacts did not present with any clinical manifestations of SARS-CoV-2 infection. All close contacts of affected researchers tested negative. Subsequent tests of affected researchers by other diagnostic laboratories were negative. We investigated these anomalous test results.

The researchers all worked in a laboratory that was developing SARS-CoV-2 molecular diagnostics, investigating loop-mediated isothermal amplification (LAMP) of reverse transcribed non-infectious, and non-hazardous SARS-CoV-2 sequences. This laboratory did not work with infectious virus or patient samples. The target sequence included the CDC “N2” locus that is now widely used in RT-qPCR diagnostics (*1*) (Figure 1B-C). It is also the sole viral locus used in the initial community surveillance tests that were administered to the researchers. The diagnostic labs that produced the negative follow-up testing results employed molecular tests that detect different genomic loci of SARS-CoV-2.

To determine whether amplified DNA products were widely present in the laboratory that could affect test results, we collected swabs from various surfaces and equipment throughout the laboratory, eluted any captured DNA and performed qPCR using the CDC N2 primer/probe set. The qPCR did not include reverse transcriptase and therefore only detected amplified cDNA, not viral RNA. Although the researchers had

followed standard practices for working with amplified nucleic acids, including physical separation of pre- and post-amplification workspaces, we found that nearly every surface had detectable quantities of N2 amplicon (Figure 1D). The highest levels of DNA were found in the workspaces used for SARS-CoV-2 LAMP reactions, on shared equipment used for analyzing these products, and the refrigerators and freezers used to store these products. Common areas, sinks, and door handles in the laboratory were also positive. Contaminated surfaces were identified in four separate rooms. Following the discovery of extensive laboratory contamination, work using the N2 amplicon ceased. Repeated efforts to decontaminate the laboratory were met with limited success (Figure 1E-F). This highlights that complete removal of contaminating DNA may be quite difficult. Indeed, three of the positive test results occurred after these widespread cleaning procedures were implemented.

DNA that can confound SARS-CoV-2 tests can come from many sources, including amplified sequences and plasmid DNA. The issue of contamination in this laboratory was likely exacerbated by the use of LAMP reactions, which generate concatenated copies of the amplified target sequence (*2, 3*). A single molecule of LAMP product captured in a diagnostic assay is likely sufficient to produce a positive RT-qPCR result. As a contaminant, LAMP products are more likely to persist in the environment due to their high molecular weight and structure.

We later learned that anomalous SARS-CoV-2 test results among researchers were not limited to a single laboratory. At a second research institute, nine researchers in three separate research groups tested positive for SARS-CoV-2 (Figure 1G). These researchers shared common spaces and equipment, including thermocyclers, benches, and centrifuges. For all researchers, follow-up testing yielded no confirmation of infection. Similarly, none of the researchers or their close contacts exhibited symptoms of COVID-19. Sampling of the laboratory space revealed that there was also widespread

N2 amplicon contamination in the space (Figure 1H), which was likely generated during the course of development of SARS-CoV-2 diagnostics. This contamination was found in multiple rooms across two floors of the building.

This widespread presence of amplified SARS-CoV-2 DNA products in both research settings had impacts that extended beyond the research teams that generated the amplicons. Several of the researchers who tested positive were not involved in SARS-CoV-2 research. They likely unknowingly interacted with contaminated equipment or surfaces. Amplicons were also identified on researchers' personal items and had spread into the home of at least one researcher. A contaminated doormat deposited amplicons onto the shoes of a researcher's spouse who was never physically present in the laboratory.

The rapid spread and persistence of contaminating DNA throughout a laboratory and beyond raises important issues regarding the stewardship of nucleic acids that can confound test results. It is possible that other researchers, janitorial, security, and maintenance staff, and those involved in the disposal of laboratory waste were all unknowingly exposed. It also highlights the possibility of contaminating DNA in public spaces outside the laboratory. The current test, trace, isolate procedures do not include a mechanism for verifying positive test results or allowing people to exit isolation if they received an erroneous test result. While these anomalous positives tests constitute a very small number relative to the total number of tests performed at these institutions, these cases serve as a powerful reminder that non-infectious material can still result in significant public health implications.

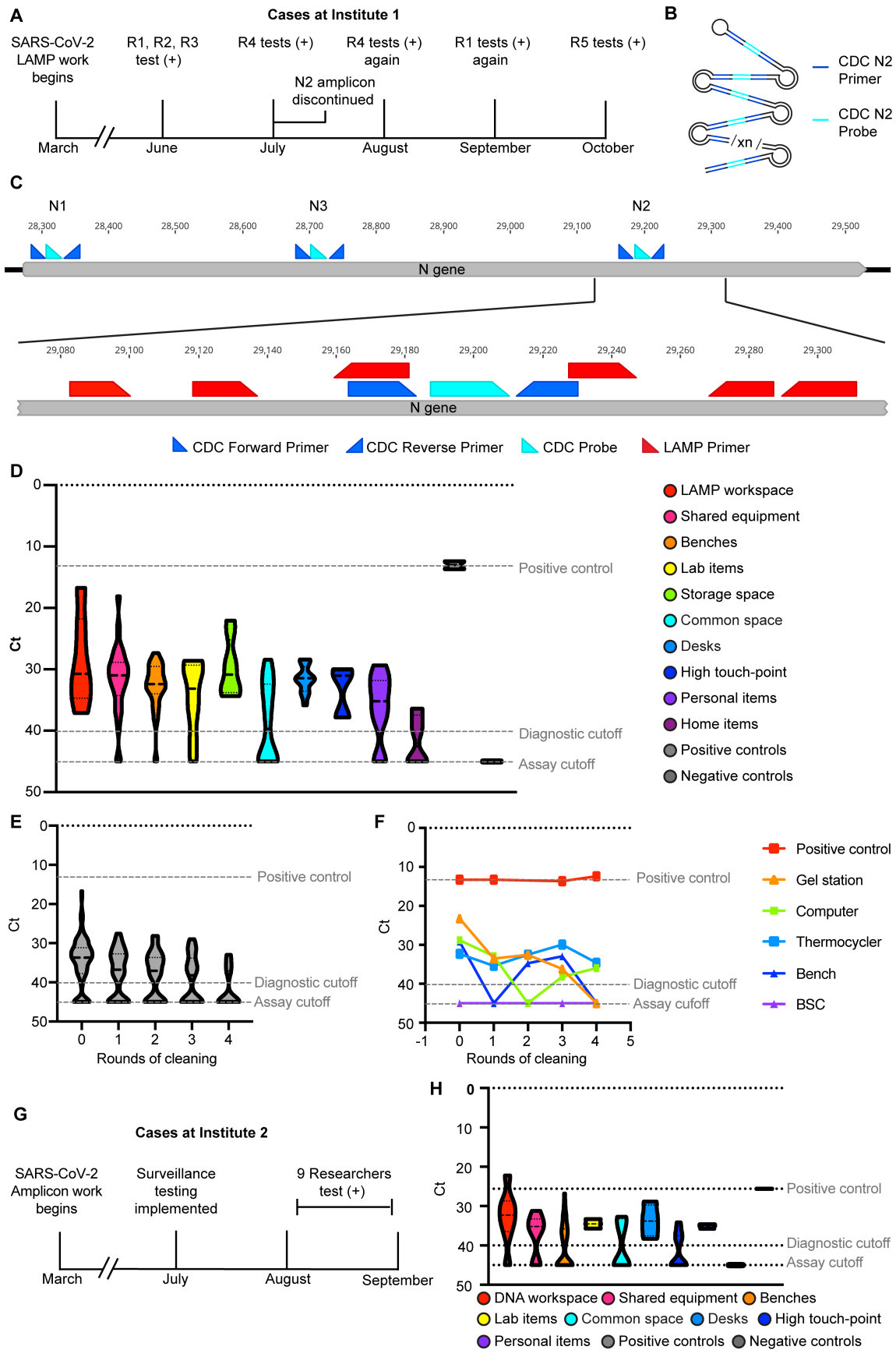
A
Cases at Institute 1
SARS-CoV-2 LAMP work begins
R1, R2, R3 test (+)
R4 tests (+)
R4 tests (+) again
R1 tests (+) again
R5 tests (+)
N2 amplicon discontinued
March
June
July
August
September
October
B
CDC N2 Primer
CDC N2 Probe
/xn/
C
N1
N3
N2
N gene
CDC Forward Primer
CDC Reverse Primer
CDC Probe
LAMP Primer
D
Ct
Positive control
Diagnostic cutoff
Assay cutoff
LAMP workspace
Shared equipment
Benches
Lab items
Storage space
Common space
Desks
High touch-point
Personal items
Home items
Positive controls
Negative controls
E
Rounds of cleaning
F
Positive control
Gel station
Computer
Thermocycler
Bench
BSC
Assay cufoff
G
Cases at Institute 2
SARS-CoV-2 Amplicon work begins
Surveillance testing implemented
9 Researchers test (+)
H
DNA workspace

Figure 1. SARS-CoV-2 researcher and laboratory amplicon contamination. (A) Timeline of positive tests and SARS-CoV-2 research at institute 1. R1, R2, R3, R4, R5: Researchers 1-5. (B) Schematic of the LAMP product produced in this laboratory highlighting the diagnostic RT-qPCR primer/probe annealing sites. (C) Schematic of SARS-CoV-2 N gene with diagnostic and research (LAMP) primer binding sites annotated. (D) Laboratory contamination with SARS-CoV-2 amplicons. Surfaces and equipment throughout the laboratory space were sampled with dry cotton swabs. Swabs were eluted in TE buffer and the eluate was analyzed using a qPCR assay with CDC N2 primer/probe. (E) Swabs were collected before and after multiple rounds of cleaning. Data shown are for all surfaces tested. 0: before cleaning. (F) Select surfaces over successive rounds of cleaning. BSC: biosafety cabinet. (G) Timeline of positive tests at institute 2. (H) Laboratory contamination at institute 2. For all graphs: Positive control: dsDNA containing the N2 target sequence. Negative control: clean cotton swabs processed alongside other swabs. Y-axis (Ct value) is inverted for ease of reading. Diagnostic cutoff: threshold for calling a positive test. Assay cutoff: assay was run for 45 cycles. Negative values were set at 45 for visualization purposes.

Living and researching in the age of widespread nucleic acid testing

The SARS-CoV-2 pandemic has brought about an era during which, for the first time in history, humans are subject to recurrent, population-scale nucleic acid testing. It has also prompted laboratories around the world to research SARS-CoV-2 biology and improved nucleic acid diagnostics. Therefore, nucleic acids with the capacity to trigger positive diagnostic test results are likely present at nearly every research institute and many clinical laboratories. These cases highlight the efficiency of test, trace, isolate public health programs. We have uncovered a neglected aspect of this strategy: verification. It is currently impossible to undo an erroneous test result. The pandemic will likely continue for years and testing will only increase in capacity and its coverage of the population. Anomalous results will scale with testing. To ensure trust and broad support for this public health strategy we must develop a framework to verify positive results and limit undue personal and economic consequences.

We are at the forefront of a new public health paradigm. Overtly healthy individuals are being tested at large scale for communicable disease to protect at risk individuals and improve the overall health of the community. Wastewater surveillance has been implemented as an early warning system to detect community spread that has

fallen outside the framework of individual testing(*4, 5*). This is only the beginning. Similar or more widespread measures may be implemented for other pathogens in the future. Increased use of viral DNA constructs in laboratories could produce false positives in in wastewater samples as well, especially in communities that have a high concentration of academic and industrial research laboratories. We have the opportunity and obligation to design our processes to preserve the integrity of the diagnostic tests, while ensuring that important work focused on responding to this crisis continues unabated. Thus, uptake and compliance will require that any steps taken do not impose undue burdens on researchers, environmental health and safety, or institutes.

Recommendations to prevent research related positive tests

In order to improve the stewardship of nucleic acids that can compromise testing, we have devised a series of common-sense recommendations for researchers. These nucleic acids include not only amplicons from LAMP, RPA, PCR, or other methods, but also plasmids and any other nucleic acid containing SARS-CoV-2 sequences. Steps to prevent laboratory contamination (engineering controls, good work practices, and proper PPE), as well as a plan for how to respond to a positive test result and how to verify it should be coordinated in advance of the initiation of research. When designing experiments, attempts should be made to limit the likelihood that a contaminant in the lab would interfere with all approved tests. In the laboratory described here, amplicons did not contain sequences that are detected by the N1 or N3 primer-probe sets and these were used in subsequent diagnostic tests that returned negative results.

Whenever feasible, unique nucleotide substitutions or “watermarks” should be introduced to distinguish laboratory products from circulating pathogens. These watermarks would ideally prevent detection by diagnostic tests (*e.g.* nucleotide substitutions in qPCR primer or probe annealing sites). Watermarks have been used in

engineered microorganisms to differentiate recombinant viruses from circulating viruses (*6, 7*). Whenever possible, controls that prevent carryover contamination, such as the dUTP/Uracil N-glycosylase system should also be used (*8*).

Laboratories should declare and post notices if they produce problematic nucleic acids and indicate their presence to those who handle waste streams. This would help direct those with positive test results to the resources for verification. Rigorous engineering controls and standard operating procedures for working with these DNA products, including proper use of fume hoods or biosafety cabinets when working with amplified nucleic acids, should be in place (*9*). These procedures are typically focused on maintaining sample purity; now, attention must be paid to the handling of amplified DNA to prevent contamination of the researcher and environment. Special care should be taken with waste to prevent contamination of the environment and of those handling the waste. Laboratory hygiene is critical: testing surfaces, tracing sources of contamination, and cleaning of equipment and surfaces in this laboratory has reduced the likelihood that additional laboratory personnel will test positive for SARS-CoV-2. Finally, for DNA species that can interfere with testing, the traditional view of laboratory contaminants needs to change. These are no longer merely a problem of contaminated experiments. They can put in question one's health status, cause unnecessary isolations and quarantines, impose significant stress, impact businesses and schools, and skew wastewater or similar sentinel testing programs.

A strategy for the inevitable anomalous tests

A positive RT-qPCR result in a SARS-CoV-2 test triggers a public health response that, at present, does not address or rectify anomalous results. The positive individual must isolate for a minimum of 10 days and close contacts must quarantine for up to 24 days; moreover, it is currently recommended that the individual not receive a follow-up

diagnostic test for three months given the long residence time of residual SARS-CoV-2 RNA in infected patients (*10*). By not testing these individuals, we could miss testing individuals infected with COVID-19. This policy was critical in the early days of the pandemic response with limited testing capacity. Now that it is clear that the pandemic will continue, possibly for years, testing policies must evolve. Anomalous positives will occur and will scale with increased testing. The United States is currently performing more than one million tests per day with the goal of increasing testing to 10,000,000 tests/day (*11*). Popularized cases of anomalous positives may erode trust in this vital public health measure. Erroneously diagnosed individuals may believe that they are immune and engage in more risky actions and would go without testing for months following a positive result. This outcome is far worse than the expense of performing a verification test. For asymptomatic individuals in research settings with no known SARS-CoV-2 exposures, who test positive in surveillance testing (not seeking testing for medical reasons), we propose a policy of test, isolate, verify, trace. The verification step will depend on whether laboratory DNA contamination is suspected and the specific nucleic acids being used by each research laboratory. It should be put in place prior to the commencement of working with these products, or prior to initiation of testing at the institution. This step could be as simple as duplicate testing in which one sample is tested without reverse transcriptase to determine whether contaminating DNA is present, although further verification may be needed to rule out a case of authentic infection along with DNA contamination. We believe that this may prevent undue burdens on the individual and public health infrastructure and will help maintain public confidence in the process. Individuals who are determined to have anomalous positive test should be allowed to be retested whenever necessary so that true infections are not missed in these individuals.

Concluding remarks

Research involving SARS-CoV-2 nucleic acids is being performed across cities, states and countries. Each has its own department that is dedicated to public health and these departments make their own policies to best meet the needs of the communities they serve. Our recommendations are general and are intended to start a dialog and collaboration between researchers and public health agencies.

PCR-based testing is the current “gold-standard” for SARS-CoV-2 testing, and these tests are highly sensitive and accurate. Even in these reported instances, the tests are performing as intended to detect SARS-CoV-2 sequences. We emphasize that research-produced nucleic acids triggering a SARS-CoV-2 positive diagnostic test result is a rare circumstance. The general public and most scientists need not worry. For those engaged in research that generates nucleic acids with the capacity to interfere with testing or surveillance, we have a responsibility to not contaminate our environments in ways that will impede life saving public health initiatives, SARS-CoV-2 or otherwise. The general public will, however, benefit from open discussion of how to deal with anomalous positive test results. We propose the test, isolate, verify, trace strategy to maintain trust in the process and ensure that no one is unfairly burdened with its consequences.

A note on researcher tests results: Test results from individual researchers were volunteered and directly communicated. The Institutional Review Board of the Harvard Faculty of Medicine determined that this did not constitute human subjects research.

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