UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

ADRIANA AVILES, Individually and as Parent and Natural Guardian of N.A., N.A. and A.A., **STEPHANIE DENARO**, Individually and as Parent and Natural Guardian of D.D. and H.D., **CHRISTINE KALIKAZAROS,** Individually and as Parent and Natural Guardian of Y.K., **GAETANO LA MAZZA**, Individually and as Parent and Natural Guardian of R.L., **CRYSTAL LIA**, Individually and as Parent and Natural Guardian of F.L., and **CHILDREN'S HEALTH DEFENSE**, Plaintiffs, Against **BILL de BLASIO**, in his Official Capacity as Mayor of the City of New York, **DR. DAVID CHOKSHI**, in his Official Capacity of Health Commissioner of the City of New York, **NEW YORK CITY DEPARTMENT OF EDUCATION, RICHARD A. CARRANZA**, in his Official Capacity as Chancellor of the New York City Department of Education and **THE CITY OF NEW YORK**, Defendants.	**DECLARATION OF SIN HANG LEE, MD** Civil No.: 1:20−cv−09829−PGG

I, Sin Hang Lee, MD declare as follows:

1. I am a pathologist and research scientist based in Connecticut who has been developing DNA sequencing-based molecular tests to diagnose infectious diseases that are difficult to diagnose, such as Lyme disease and human papilloma virus (HPV) infection.

2. I have trained and taught in some of the world's most prestigious institutions and has published scores of scientific articles in peer-reviewed journals.

3. Exhibit 1 is a true and accurate copy of my current CV.

4. It is my professional opinion that the need for accurate coronavirus testing is imperative. It is especially critical in nursing homes and institutions caring for elderly patients, so that false-positive patients are not housed with true-positive patients. It's also essential to ensure that staff in direct contact with highly susceptible patients be infection-free.

5. I have been working diligently to overcome the roadblocks in coronavirus testing.

6. As early as March 2020, I wrote to the WHO and to Dr. Anthony Fauci at the National Institute of Allergies and Infectious Diseases of the National Institutes of Health (NIH) to explain why the current tests to detect SARS-CoV-2 RNA are generating false positives and negatives. The letter explained that a two-phased test with DNA sequencing would "guarantee no-false positive results" based on my research and published work from Japan. A true and correct copy of that letter is attached as Exhibit 2. The method I used was subsequently published in a peer-reviewed international journal based in Japan.

7. It is my opinion, one supported by the FDA, that questionable or false RT-qPCR test results can be investigated and resolved by Sanger sequencing, the testing method I developed.

8. Given the specificity of my letter, with its DNA sequencing electropherograms and its significance for preventing disease spread, it is almost unbelievable that as of the date of this declaration, I have received no response from either the WHO or NIH.

9. In April 2020, I reached out to the Connecticut Department of Public Health to receive patient samples for further validation testing. I re-tested 20 reference patient samples supplied by the Connecticut Department of Public Health on April 30, 2020, and found that 3 of 10 reference samples initially classified as positive for SARS-CoV-2 by RT-qPCR test were false positives and 2 of 10 reference samples initially classified as negative were found to

contain SARS-CoV-2 proven by DNA sequencing. These results were reported to the Connecticut Department of Public Health and published on July 17, 2020. Four days later on July 21, 2020, the Connecticut Department of Public Health also reported that a total of 90 out of 144 people tested between June 15 and July 17, many of whom were nursing home residents, received false-positive SARS-CoV-2 RT-qPCR test results because of a flaw in the test used at the state's public health laboratory. Gabrielle Fonrouge, *Connecticut lab finds 90 positive COVID-19 tests were false: report*, NEW YORK POST (Jul. 21, 2020), https://nypost.com/2020/07/21/connecticut-testing-lab-botches-dozens-of-coronavirus-tests/. Attached as Exhibit 3 is a true and correct link to that article.

10. There is ample evidence that the currently used RT-qPCR tests are inaccurate for determining if a patient's sample in fact contains SARS-CoV-2, the virus causing COVID-19. The inherent flaw of PCR for the detection of SARS-CoV-2 RNA is further discussed as follows.

11. PCR (polymerase chain reaction) is a chemical reaction used to duplicate a defined segment of DNA exponentially in the test tube. To detect or to analyze a segment of target DNA molecule in question is usually by DNA sequencing, the process of determining the orders of the nucleotides (As, Ts, Cs, and Gs), which link up as a chain in the target DNA molecule.

12. However, the current technology cannot analyze one or a few DNA molecules in the sample being tested. These DNA molecules must be amplified, or made larger in number by a duplicating process to reach a mass of identical molecules to be analyzed. This amplification process, commonly referred to as PCR, is what Kary Mullis discovered, and consists of multiplying sequentially and exponentially by doubling the target DNA segment present in a test tube. So, 2 becomes 4, then becomes 8, then 16, and so forth, using newly formed copies as the

templates to make more new copies of the same molecule continuously. In such a duplication manner, each molecule of DNA in the original sample can become more than 1 billion copies after 30 cycles of amplification, in theory.

13. As noted, PCR multiplies DNA. But the genetic material that comprises the genome of SARS-CoV-2, the virus causing COVID-19, is RNA that is much more labile or unstable than DNA. It must be converted to DNA in order to utilize the PCR process. This is accomplished by action of an enzyme called reverse transcriptase (RT) in the first of four steps involved in the process. RT thus allows a single strand of RNA to be reverse-transcribed into a complementary strand of DNA, cDNA in short. The process of RT acting on RNA, leading to cDNA amplification through PCR, is called RT-PCR, which should be distinguished from RT-qPCR or rRT-PCR, the method currently being used for SARS-CoV-2 PCR testing.

14. The principle of PCR is based on primer-initiated and template-directed enzymatic polymerization of nucleotides. That means that there must be a segment of single-stranded DNA (ssDNA) serving as the template to direct the nucleotide incorporation for the synthesis of the new ssDNA whose sequence is complementary to the template. It also means that the synthesis of the new ssDNA must start with a primer, which is an oligonucleotide of about 20 nucleotides long and complementary to a segment of the target template DNA, annealing to (attached to) the target DNA at one of the two beginning sites of the target DNA to be amplified. Without a primer, the enzyme, DNA polymerase, will not work. In other words, PCR begins with enzymatic primer extension. The enzyme, a DNA polymerase, works like a type writer adding the nucleotides (A, C, G or T bases) one by one to the 3’ end of the primer complementary to the sequence of the template in a 5’→3’ direction.

15. The primer/template annealing process of PCR is based on DNA/DNA hybridization by forming hydrogen bonds between all complementary base pairs of the primer and template. However, DNA/DNA hybridization or annealing can take place even if there is only a partial match in base pairs between a primer and an unintended DNA in the PCR mixture in the absence of a fully matched target DNA template. Under certain circumstances the DNA polymerase can amplify an unintended (undesirable) DNA with a pair of partially matching primers and generate unintended (undesirable) PCR products. If the PCR products are not further analyzed for confirmation, false-positive test results may be produced.

16. The endpoint of an RT-qPCR test is arbitrarily set by the test kit manufacturer, using a Ct number to divide cases into "positive" or "negative." The extraordinary implications of this simple decision to frame results of the PCR testing as the basis for the entire "case numbers" tracking upon which virtually all public health measures are being based is almost incomprehensible.

17. I agree with Dr. Michael Mina, assistant professor of epidemiology at the Harvard T. H. Chan School of Public Health, who is quoted in the New York Post article as saying that this oversimplified interpretation of PCR as positive or negative is "irresponsible." This relates to the following discussion of the amplification process, sometimes also referred to as cycles. Dr. Mina is quoted in the Harvard Magazine (8/3/20) as saying that Current PCR testing detects virus genome-related materials "long after the infected person has stopped transmitting the virus." He further states "That means *the results are virtually useless for public health efforts to contain the raging epidemic*." (emphasis added)

18. Tests with thresholds so high may detect not only live virus, but also simple genetic fragments, leftover from past infection that poses no risk for current exposure to others, or from other unrelated nucleic acids in the sample.

19. I agree with virologist Dr. Juliet Morrison that any test with a cycle threshold above 35 is too sensitive (in other words will read positive when the individual is not infectious).

20. I am of the opinion that it would be quite easy and simple to manipulate the number of positive results with this form of testing by the test kit manufacturers to please their customers whose business benefits from a high number of COVID-19 cases.

21. I believe that with varying numbers of cycles or amplifications being used in different states or even in different health systems in one state, it would be quite easy and simple to manipulate the number of positive results with this form of testing by simply changing the number of cycles to a higher number to produce the appearance of worsening or to a lower one to produce lower infection numbers. I agree with some experts who say that if over 40 amplifications be used, 100% of people tested might turn out to be positive.

22. I believe that the currently used RT-qPCR is a faulty diagnostic test, For example, an individual who gets a positive test result in a facility or area that is using a test setting the cutoff at 37 cycles might fly to another area where repeat testing is using a cutoff at 30 cycles and would likely get a negative test result. So, the same individual who “had COVID-19” in location one does not have it after flying to the second location.

23. This reveals the absurdity of the RT-qPCR based test.

24. To have children to take such an unreliable test is equally absurd.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Executed this 15th day of December, 2020 in Milford, Connecticut.

Signed

Sin Hang Lee, MD

EXHIBIT 1

CURRICULUM VITAE

NAME: SIN HANG LEE, M.D., F.R.C.P. (C), F.C.A.P.

OFFICE ADDRESS: Milford Molecular Diagnostics Laboratory
2044 Bridgeport Avenue,
Milford, CT 06460

PLACE OF BIRTH: HONG KONG

CITZENSHIP: NATURALIZED U.S. CITIZEN 1976

EDUCATION

Tongji University College of Medicine and
Wuhan Medical College (combined since 1952) Shanghai and Hankow,
Hubei, China 1951-56

HIGH PROFESSIONAL DEGREE

F.R.C.P. (C)
Royal College of Physicians and Surgeons of Canada 1966

POSTGRADUATE TRAINING and EXPERIENCE

Teaching assistant in microbiology, Sichuan Medical College and
Guiyang Medical College, China 1956-61

Demonstrator in pathology, University of Hong Kong,
School of Medicine, Hong Kong 1961-63

Rotating clinical intern, South Baltimore General Hospital,
Baltimore, MD 1963-64

Resident, Assistant Pathologist and Pathologist at New York Hospital,
Cornell Medical Center, New York, NY 1964-67

Pathology Fellow at Memorial Hospital for Cancer and Allied Diseases,
New York, NY 1967-68

Assistant Professor of Pathology, McGill University, Montreal, Canada 1968-71

Associate Professor of Pathology, Yale University, New Haven, CT 1971-73

Attending Pathologist at Hospital of St.Raphael and Associate Clinical Professor of Pathology, Yale University, New Haven, CT	1973-2003
Pathologist at Milford Hospital, Milford, CT	2004- 2015
Director, Milford Medical Laboratory, Inc. Milford, CT	2008- 2015
Director, Milford Medical Laboratory Molecular Diagnostic Section	2008- 2015
Director, Milford Molecular Diagnostics Laboratory	2015-

MEDICAL LICENSURE:

District of Columbia, New York and Connecticut (current), U.S.A.
Licentiate of the Medical Council of Canada
Certificate of full registration, General Medical Council, London, Great Britain

SPECIALTY BOARDS:

Diplomate, American Board of Pathology (AP)	1966
Certificated Specialist in General Pathology (AP and CP) Canada	1966

Expertise: General pathology, surgical pathology, clinical microbiology, and molecular diagnostics by PCR/direct DNA sequencing.

PUBLICATIONS:

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2. Lee, S.H. Chinese translation of a handbook, "Die Praxis der Resistenz-und Spiegelbestimmungen zur Antibiotischen Therapie" by H-J. Otte and W. Köhler Veb Gustav Fischer Verlag-Jena. 1958, Peoples Hygiene Publisher, Beijing, China, 1961.

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EXHIBIT 2

Sin Hang Lee, MD, F.R.C.P.(C)
Milford Molecular Diagnostics Laboratory
2044 Bridgeport Avenue
Milford, CT 06460
USA

March 22, 2020

Dr. Margaret Harris
The World Health Organization's coronavirus response team
harrism@who.int

Dr Eduardo Guerrero
WHO Regional Office for the Americas
guerrere@paho.org

Dr. Anthony S Fauci
af10r@nih.gov

Extremely sensitive, no false-positive tests needed for SARS-CoV-2

Dear Drs. Harris, Guerrero and Fauci:

It has been widely reported in the social media that the RT-qPCR test kits used to detect SARS-CoV-2 RNA in human specimens are generating many false positive results and are not sensitive enough to detect some real positive cases, especially during convalescence.

RT-qPCR is known to generate false positive results when used to detect influenza A virus [1] and MERS-CoV, [2] another Coronavirus.

Without a nested (two-round) PCR, a single round RT-PCR may miss real infections caused by SARS-CoV [3] and by SARS-CoV-2 [4].

The major technical flaw of RT-qPCR for molecular diagnosis is the limitation of the length of its DNA probe which is about 25 bases long or shorter. And hybridization is not an accurate method to determine nucleotide sequences, the foundation of all nucleic acid-based diagnostics.

This letter recommends that the WHO coronavirus response team adopt or develop a nested RT-qPCR protocol to generate a cDNA PCR amplicon to be used as the template for bi-directional sequencing. As demonstrated in this letter, nested RT-PCR is an extremely sensitive detection method and DNA sequencing will guarantee no-false positive results if all positive reports are accompanied by two-directional sequencing electropherograms, like an EKG for the diagnosis of Left Bundle Branch Block in a cardiologist’s consultation.

Based on information retrieved from the GenBank databases and available in the public domain, there is a unique 398-base segment in the SARS-CoV-2 nucleocapsid (N) gene which not only has a 100% match with that in the Wuhan seafood market pneumonia virus, but also contains four single-nucleotide mutations found in the viruses isolated from patients in the states of

California, Texas and Massachusetts of the U.S.A. This segment of the gene can be targeted for accurate molecular diagnosis.

The nucleotide sequence of this 398-base gene segment is copied from the GenBank and re-printed here with the 4 mutated bases typed in red color. Identification of these virus isolates each with a single-base mutation in this segment may be useful in tracing the immediate source of the pathogen among patients and carriers tested positive for SARS-CoV-2.

Severe acute respiratory syndrome coronavirus 2 SARS-CoV-2 RNA Isolated from throat swab of patient in cruise ship, Japan, 02-10-2020

Sequence ID: LC528233.1

Score	Expect	Identities	Gaps	Strand
736 bits(398)	0.0	398/398(100%)	0/398(0%)	Plus/Plus

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Query  1      CAATCCTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAA  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28728  CAATCCTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAA  28787

Query  61     AGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACG  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28788  AGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACG  28847

Query  121    TAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAG  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28848  TAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAG  28907

Query  181    AATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCA  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28908  AATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCA  28967

Query  241    GCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAAGAA  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28968  GCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAAGAA  29027

Query  301    ATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAA  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29028  ATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAA  29087

Query  361    TGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAA  398
              |||||||||||||||||||||||||||||||||||||
Sbjct  29088  TGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAA  29125
```

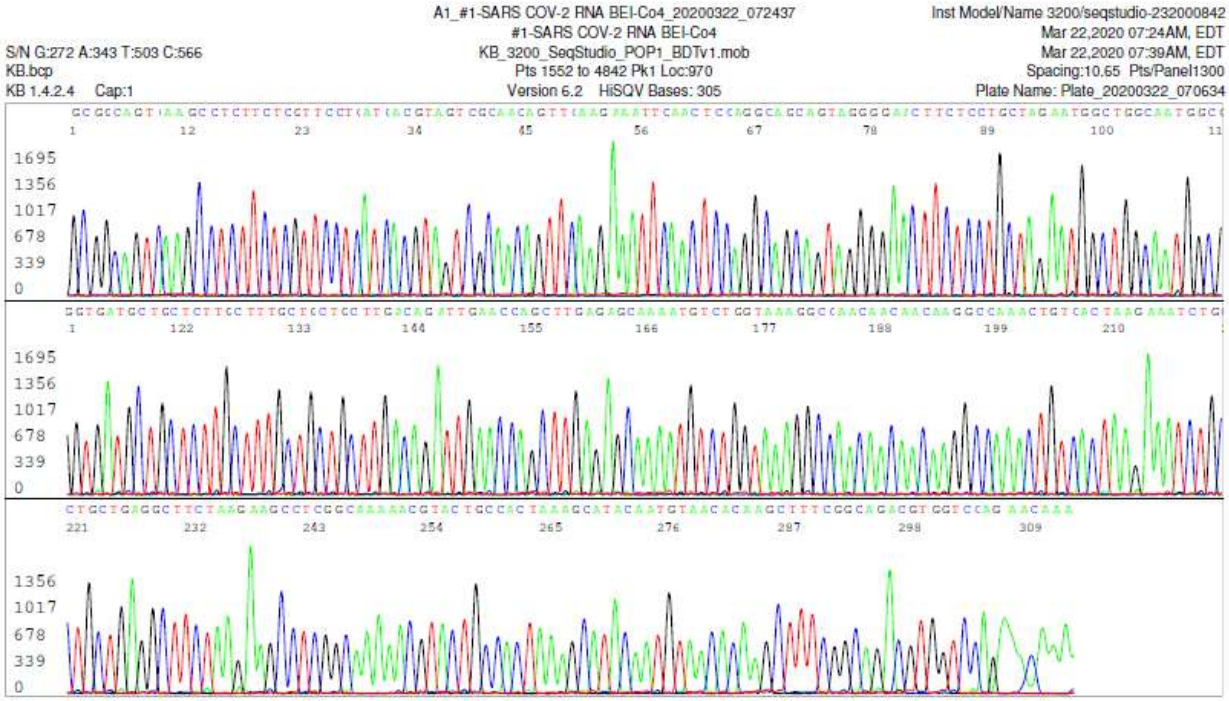
NOTE: This 398-base sequence is identical to that of the Wuhan seafood market pneumonia virus, isolated in December 2019, GenBank Sequence ID: NC_045512.2

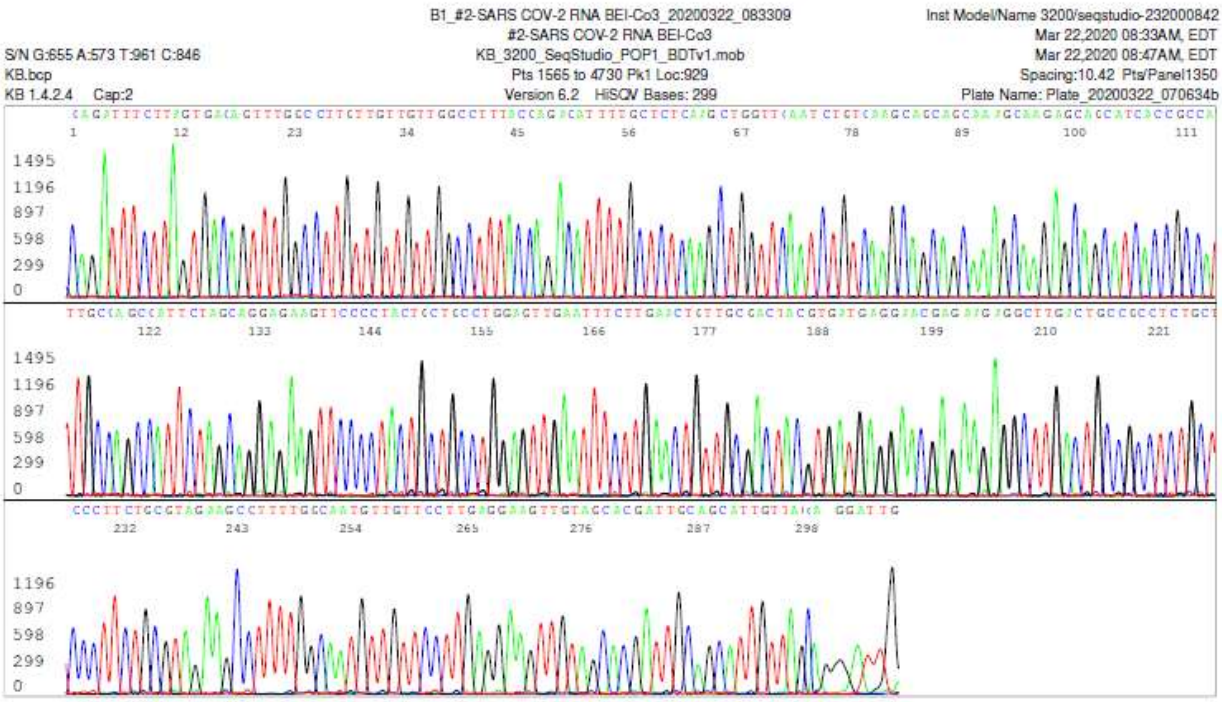
SARS CoV-2 isolates in the USA may have following single-base mutations in this segment at the positions typed in red (Sequences were retrieved from NCBI Databases).

29103 C>T Sputum of patient, TX, USA, 02-11-2020 Sequence ID: MT106054
28886 G>A Nasopharyngeal swab, CA, USA, 02-06-2020 Sequence ID: MT106052
28862 C>T Oropharyngeal swab, MA, USA, 01-29-2020 Sequence ID: MT039888
28792 A>T Nasopharyngeal swab, CA, USA, 01-23-2020 Sequence ID: MN994467

Left is an image of gel electrophoresis of the products of primary RT-PCR (upper half) and nested PCR (lower half) showing that nested PCR increases the sensitivity of RT-PCR at least 1,000 times in detecting SARS-CoV-2 RNA. The copy number of synthetic viral RNA added to each 25 µL primary RT-PCR mixture was calculated based on the analysis data supplied by BEI Resources, NIAID, NIH: Quantitative Synthetic RNA from SARS-Related Coronavirus 2, NR-52358. As demonstrated, this protocol can detect a single copy of viral RNA.

The 398-bp nested PCR amplicon shown in Lane 6 was used as the template for Sanger sequencing. The bi-directional sequences are pasted below.





Please inform your affiliated laboratories that we are now in position to assist them to resolve their questionable RT-qPCR test results with high Ct values (between 37 and 40) if they are able to send us 10 µL of the residual RNA extract kept at -80°C in dry ice package. We will perform a nested RT-PCR on each of received residual samples, and perform a bi-directional Sanger sequencing on all positive cases and report the results back to the sender.

Contact person is: Sin Hang Lee, MD email shlee01@snet.net

Sincerely,

Sin Hang Lee, MD, F.R.C.P.(C)

References

1. Martí NB, Del pozo ES, Casals AA, Garrote JI, Masferrer NM. False-positive results obtained by following a commonly used reverse transcription-PCR protocol for detection of influenza A virus. *J Clin Microbiol*. 2006;44(10):3845.
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EXHIBIT 3

NEWS

Connecticut lab finds 90 positive COVID-19 tests were false: report

By Gabrielle Fonrouge

July 21, 2020 | 1:38pm | Updated

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Sign up for our special edition newsletter to get a daily update on the coronavirus pandemic.

Nearly a hundred people in Connecticut who received positive coronavirus tests didn't actually have the disease, reports said.

A total of 90 out of 144 people tested between June 15 and July 17, many of whom are nursing home residents, received the false positives because of a flaw in the test used at the state's public health laboratory, NBC Connecticut reported.

State officials said the test manufacturer, the Food and Drug Administration and the Department of Public Health have all been notified about the snafu and immediate steps were taken to ensure patients were notified, the outlet reported.

"We have notified the healthcare facilities for everyone who received a false positive test result from our state laboratory," said Acting Commissioner Deidre S. Gifford from the state's department of social services.

"Accurate and timely testing for the novel coronavirus is one of the pillars supporting effective response to the COVID-19 pandemic."

Officials said the bad results came "from a widely-used laboratory testing platform that the state laboratory started using on June 15," the outlet reported.

Gifford said changes are already underway "to ensure the accuracy of future test results from this platform."

Moving forward, all positive coronavirus results will undergo further analysis from multiple scientists and if necessary, specimens will be retested using an alternate method, the outlet said.

Nursing home residents who received the false positive will be retested as soon as possible, the state's department of health said, according to the outlet.

FILED UNDER CONNECTICUT, CORONAVIRUS, LABORATORY, SAFETY TEST, 7/21/20