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19 Delayed Laboratory Response to COVID- 20 19 Caused by Molecular Diagnostic 21 Contamination

22 [Q1. Title has been edited for brevity and EID style. Subtitles and sentences are not used.
 23 Your title was a sentence and much too long for a research letter. Titles must be as
 24 general (common language) as possible. OK?]

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18 (10)(2e) [Q2. Journal style is to list

19 main affiliations only. We do not list departments, etc.

20 Affiliations correct?]

21 ¹These authors contributed equally to this article.

22 The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) created an
 23 exceptional situation in which numerous laboratories in Europe simultaneously implemented SARS-
 24 CoV-2 diagnostics. These laboratories reported in February 2020 that commercial primer and probe
 25 batches for SARS-CoV-2 detection were contaminated with synthetic control material, leading to
 26 delays of regional roll-out of testing in various countries.

27 Timely and reliable laboratory diagnosis is crucial for clinical care and to inform public
 28 health responses in the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
 29 pandemic (*1*). The laboratory response in Europe to emergence of SARS-CoV-2 appeared rapid
 30 at the country level; 38 laboratories in 24 European Union/European Economic Area countries
 31 had molecular testing already available by January 29, 2020 and an expected complete coverage
 32 of all European Union/European Economic Area countries by mid-February (*1*).

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1 The first protocol for molecular detection, with a focus on envelope (E) and RNA-
2 dependent RNA polymerase gene targets, was available on January 13th, 2020 (2,3) and shared
3 rapidly. Toward the end of January 2020, reports from laboratories in Europe indicated that
4 commercial, custom-made primer and probe batches for SARS-CoV-2 detection might be
5 contaminated with synthetic control material for the E gene target. This observation was
6 disclosed within the expert laboratory network for Emerging Viral Diseases–LabNet (4) on
7 February 5, 2020, and resulted in an alert and advice to perform a second target confirmation by
8 European Centre for Disease Prevention and Control (ECDC) on its website (5). A call for more
9 detailed information was send out to assess the extent of the situation.

10 Ten laboratories from 8 countries in Europe reported PCR template contamination in
11 commercially ordered primer and probe batches, which led to SARS-CoV-2 reverse transcription
12 PCR (RT-PCR) signals in their no-template controls, and provided detailed information. Five
13 additional laboratories (including addition of the ninth affected country) indicated that they
14 received contaminated material but did not provide details.

15 Materials were ordered during January 13–February 28 from 8 companies offering
16 custom nucleic acid synthesis. Delivery of contaminated oligonucleotides was reported during
17 January 22–February 28 for 6 companies, including those that initially delivered contamination-
18 free oligonucleotides until January 21 (Figure). The contamination issues concerned primer and
19 probe batches for the E and the RNA-dependent RNA polymerase gene targets, as well as
20 batches for nonrelated targets received on the same day. Others reported sporadic contamination.
21 The extent of contamination varied strongly; reported cycle threshold values ranged from 23 to
22 39. The laboratories systematically excluded other, own laboratory-related, potential sources of
23 contamination. None of the 10 laboratories ordered long synthetic DNA polymers.

24 Six laboratories indicated a delayed implementation of SARS-CoV-2 diagnostics. Three
25 were central laboratories responsible for roll-out of diagnostic capability to regional and hospital
26 laboratories within their country, which was therefore delayed by 7–14 days. Three laboratories
27 indicated a delay in molecular test implementation of 2–7 days in their own facilities (Figure,
28 panel C). One laboratory described a delay in final negative result reporting for 1 suspected
29 patient during a tense period in which the country did not have any cases.

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1 The companies involved were informed. Some offered new batches free of charge, started
2 to screen their products postproduction, or stopped production of long oligonucleotides. Others
3 did not respond or denied that a problem existed. One company decontaminated its production
4 facility.

5 The emergence of SARS-CoV-2 created an exceptional situation that demanded a rapid
6 implementation of RT-PCRs. We hypothesize that the combined simultaneous and huge demand
7 across Europe for primers, probes, and controls, related to the protocol of Corman et al. (2),
8 might have led to production of primers and probes contaminated with synthetic controls. Initial
9 limited access to positive controls (1) might have led to orders of long synthetic DNA polymers
10 spanning SARS-CoV-2 RT-PCR target genes. In combination with extensive and simultaneous
11 ordering of associated primers and probes, this ordering resulted in synthesis on the same
12 production line within a short time span or in close proximity within some companies.

13 Companies that produce custom synthetic nucleotides need to be aware of these potential
14 problems that might only appear in extreme situations, such as the massive laboratory response
15 to SARS-CoV-2 at the end of January/beginning of February 2020 in Europe that was uniform
16 and based on few available protocols (2). In normal circumstances, the common practice of
17 synthesis of primers, probes, and long nucleic acids would not necessarily pose a major problem
18 because different nucleic acids are randomly ordered and produced. However, in an emergency
19 response scenario as described here, this common practice had consequences for an efficient
20 laboratory and public health response.

21 Comparison of ordered nucleic acids against sequence databases might inform the
22 synthesis set-up at companies. This comparison could be combined with the already existing
23 protocol for nucleic acid-synthesizing companies regarding synthesis of high-risk pathogens (6).
24 Other measures might include separate production facilities for long and short nucleic acids. The
25 necessity for this change was highlighted by a sixteenth laboratory that failed to order their
26 primers and probes through explicit routing of a company to avoid contamination with popular
27 PCR targets. E gene-contaminated primers and probes were received at the end of March 2020.

28 This report provides a warning to manufacturers of oligonucleotides and diagnostic
29 laboratories alike to remain vigilant for contamination issues in popular RT-PCR reagents. This

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1 vigilance will help avoid delays in crucial laboratory responses now and in future outbreak
 2 events.

3 Acknowledgments

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 5 technical assistance in the laboratory.

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 9 through the Reacting (REsearch and ACTion Targeting emerging infectious diseases) initiative.

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11 Dr (10)(2e) is a research associate at the National Institute for Public Health and the Environment,
 12 Bilthoven, the Netherlands. Her primary research interests include (re)emerging viral infections and (inter)national
 13 laboratory preparedness and response activities.

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6 **[Author: The Figure is OK. We can make any required changes (style) in it.]**

7 **Figure.** Timeline and extent of product and molecular diagnostic contamination issues in 10 laboratories
 8 in Europe during delayed laboratory response to COVID-19. A) Contamination status of commercially
 9 ordered primers and probes for molecular detection of SARS-CoV-2 based on Corman et al (2). Red
 10 vertical dotted line indicates starting date of laboratories in Europe receiving contaminated commercial
 11 primers and probes. A–H are unique identifiers for the 8 companies that produced the materials. B)
 12 Timeline of simultaneous hallmark events in the SARS-CoV-2 outbreak. C) Delay of implementation of
 13 SARS-CoV-2 diagnostic test in laboratories and delay of national or regional roll-out schemes per
 14 laboratory. Laboratories that indicated no delay had access to noncontaminated material from previous
 15 orders or cooperated with another laboratory. COVID-19, coronavirus disease; E, envelope; EVAg,
 16 European Virus Archive Global; GISAI; Global Initiative on Sharing All Influenza Data; ID, identification;
 17 PHEIC, public health emergency of international concern; SARS-CoV, severe acute respiratory syndrome
 18 coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health
 19 Organization.