Article Summary Line: Production contamination issues at multiple oligo-nucleotide synthesizing companies delayed the rapid laboratory response to SARS-CoV-2 emergence in multiple laboratories in several European countries.

Running title: Contamination SARS-CoV-2 molecular diagnostics

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Contamination issues related to the rapid, uniform and ubiquitous implementation of SARS-CoV-2 molecular diagnostics across Europe delayed the laboratory response to COVID-19 at national levels

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Abstract – 49 words

The emergence of SARS-CoV-2 created an exceptional situation, in which numerous European laboratories simultaneously implemented SARS-CoV-2 diagnostics. European laboratories signaled in February 2020 that commercial primer and probe batches for SARS-CoV-2 detection were contaminated with synthetic control material, leading to delays of regional roll-out of testing in various countries.

Text – 803 words

Timely and reliable laboratory diagnosis is crucial for clinical care and to inform public health responses in the ongoing SARS-CoV-2 pandemic (1). The European laboratory response to the emergence of SARS-CoV-2 appeared rapid at the country level with 38 laboratories in 24 EU/EEA countries having molecular testing already available by January 29th 2020 and an expected complete coverage of all EU/EEA countries mid-February (1). The first protocol for molecular detection, with a focus on E- and RdRp-gene targets, was available on January 13th 2020 (2, 3) and shared rapidly. Towards the end of January 2020, signals came from the European laboratory field that commercial, custom-made primer and probe batches for SARS-CoV-2 detection might be contaminated with synthetic control material for the E-gene target. This observation was disclosed within the expert laboratory network for emerging viruses EVD-LabNet (4) on February 5th 2020 and resulted in an alert

and advice to perform a second target confirmation by ECDC on its website (5). A call for more detailed information was send out to assess the extent of the situation.

Ten laboratories from eight European countries reported PCR-template contamination in commercially ordered primer and probe batches which led to SARS-CoV-2 RT-PCR signals in their no-template controls (NTC), and provided detailed information. Five additional laboratories (including addition of ninth affected country) indicated to have received contaminated material but did not provide details. Materials were ordered in the period from January 13th to February 28th from eight companies offering custom nucleic acid synthesis. Delivery of contaminated oligonucleotides was reported in the period January 22th to February 28th for six different companies, including those that initially delivered contamination-free oligonucleotides till January 21st (Fig.1). The contamination issues concerned primer and probe batches for both the E- and the RdRp-gene targets as well as batches for non-related targets received on the same day. Others reported sporadic contamination. The extent of the contamination varied strongly with reported Ct values ranging from 23 to 39. The laboratories systematically excluded other, own laboratory-related, potential sources of contamination (data not shown). None of the ten laboratories ordered long synthetic DNA polymers themselves.

Six laboratories indicated a delayed implementation of SARS-CoV-2 diagnostics. Three were central laboratories responsible for roll-out of diagnostic capability to regional and hospital laboratories within their country, which was therefore delayed by 7 to 14 days. Three laboratories indicated a delay in molecular test implementation of 2 to 7 days in their own facilities (Fig. 1C). One laboratory described a delay in final negative result reporting for one suspected patient in a tense period where the country had no cases yet.

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

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

Companies that produce custom synthetic nucleotides need to be aware of these potential problems that might only appear in extreme situations like the massive laboratory response to SARS-CoV-2 at the end of January/beginning of February 2020 in Europe that was uniform and based on few available protocols (2). In normal circumstances the common practice of synthesis of primers, probes and long nucleic acids would not necessarily pose a significant problem since different nucleic acids are randomly ordered and produced. However in an emergency response scenario as described here this common practice had consequences for an efficient laboratory and public health response. Comparison of ordered nucleic acids against sequence data-bases, might inform the synthesis set-up at companies. This could be combined with the already existing protocol for nucleic acids synthesizing companies regarding synthesis of high-risk pathogens (6). Other measures might include separate production facilities for long and short nucleic acids. The necessity for this was highlighted

by a sixteenth laboratory that failed to order their primers and probes through a company's explicit routing to avoid contamination with popular PCR targets. E-gene contaminated primers and probes were received at the end of March 2020.

This letter provides a warning for oligonucleotides manufacturers and diagnostic laboratories alike to remain vigilant for contamination issues in popular RT-PCR reagents to avoid delays in crucial laboratory responses now and in future outbreak events.

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References

1. 5.1.2e 5.1.2e, 5.1.2e et al. Laboratory readiness and response for novel coronavirus (2019-nCoV) in expert laboratories in 30 EU/EEA countries, January 2020. Euro Surveill. 2020 Feb;25(6).

2. [redacted], [redacted], [redacted] et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3).
3. WHO. Novel Coronavirus. (2019-nCoV) technical guidance: laboratory guidance. 2020 [cited 03-03-2020]; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance
4. EVD-LabNet. EVD-LabNet. 2020 [cited 05.03.2020]; Available from: https://www.evd-labnet.eu/
5. ECDC. Questions and answers regarding laboratory topics on SARS-CoV-2. 2020 [cited 04-05-2020]; Available from: https://www.ecdc.europa.eu/en/all-topics-z/coronavirus/threats-and-outbreaks/covid-19/laboratory-support/questions
6. IGSC. International Gene Synthesis consortium. 2020 [cited 29-03-2020]; Available from: https://genesynthesisconsortium.org/

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Figure 1. Schematic representation of the timeline and extent of product contamination issues in ten European laboratories

A) Contamination status of commercially ordered primers and probes for molecular detection of SARS-CoV-2 based on Corman *et al.* over time for ten European laboratories. Red dotted line indicates starting date of European laboratories receiving contaminated commercial primers and probes. Letters A – H are unique identifiers for the eight companies that produced the materials. B) Timeline of simultaneous hallmark events in the SARS-CoV-2 outbreak. C) Delay of implementation of SARS-CoV-2 diagnostic test in own laboratories and delay of national or regional roll-out schemes per laboratory. Laboratories that indicated no delay had access to non-contaminated material from previous orders or cooperated with another laboratory.