

Contamination of reagents used for molecular detection of SARS-CoV-2 risks compromising diagnostic assays

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Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases

Interim guidance

19 March 2020 | COVID-19: Laboratory and diagnosis



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Overview

The purpose of this document is to provide interim guidance to laboratories and stakeholders involved in laboratory testing of patients who meet the definition of suspected case of pneumonia associated with a novel coronavirus identified in Wuhan, China.

Previous versions:

[Laboratory testing of 2019 novel coronavirus \(2019-nCoV\) in suspected human cases: interim guidance, 17 January 2020](#)

[Other resources for laboratories](#)

Summary table of available protocols in this document

Institute	Gene targets
China CDC, China	ORF1ab and N
Institut Pasteur, Paris, France	Two targets in RdRP
US CDC, USA	Three targets in N gene
National Institute of Infectious Diseases, Japan	Pancorona and multiple targets, Spike protein
Charité, Germany	RdRP, E, N
HKU, Hong Kong SAR	ORF1b-nsp14, N
National Institute of Health, Thailand	N

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Analytical sensitivity and efficiency comparisons of SARS-COV-2 qRT-PCR assays

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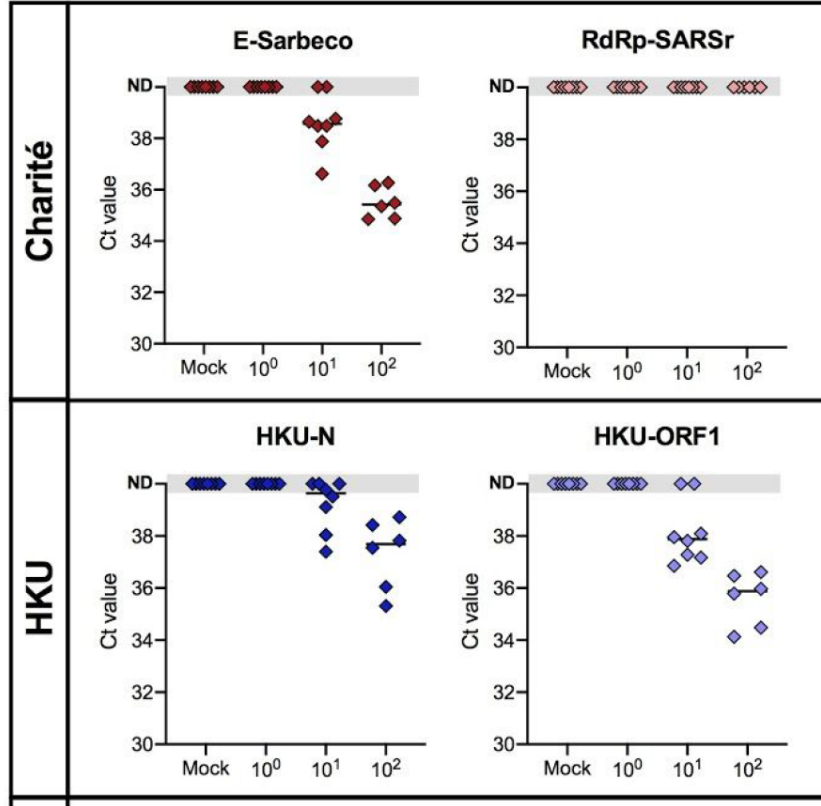
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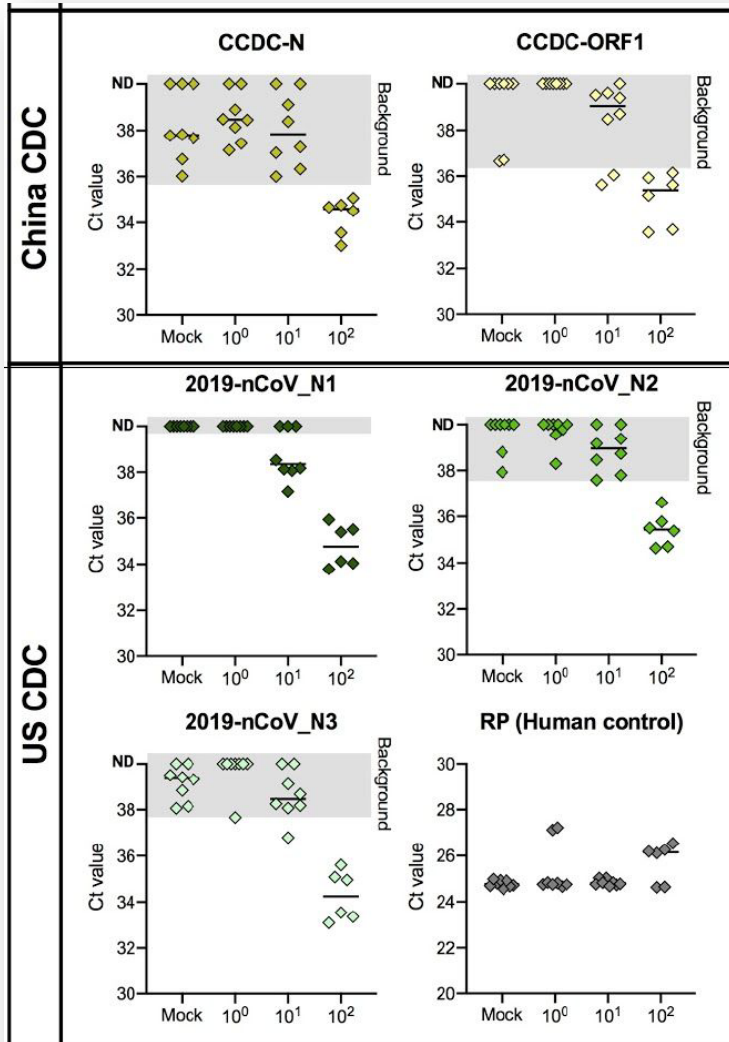
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Detection of virus at low concentrations and false positives

To determine the lower limit of detection and the occurrence of false positive or inconclusive detections, we tested the primer-probe sets using SARS-CoV-2 RNA spiked into RNA extracted from pooled nasopharyngeal swabs from respiratory disease patients during 2017 (pre-COVID-19). Our mock clinical samples demonstrated that many of the primer-probe sets cross-reacted with non-SARS-CoV-2 nucleic acid, which may lead to inconclusive or false positive results (**Fig. 3**).

RAPID COMMUNICATION

Rapid establishment of laboratory diagnostics for the novel coronavirus SARS-CoV-2 in Bavaria, Germany, February 2020

Regina Konrad^{1,2}, Ute Eberle^{2,3}, Alexandra Dangel^{1,2}, Bianca Treis³, Anja Berger⁴, Katja Bengs¹, Volker Fingerle³, Bernhard Liebl^{4,5}, Nikolaus Ackermann^{2,3}, Andreas Sing^{1,2,5}

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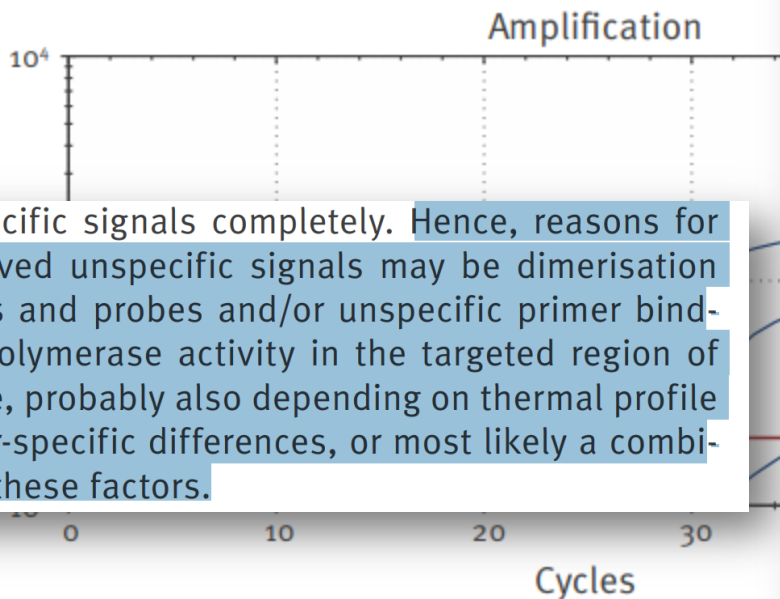
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<https://doi.org/10.2807/1560-7917.ES.2020.25.9.2000173>

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FIGURE 2

Example image of real-time RT-PCR curves of the E gene assay with unspecific signals at late cycles, Bavaria, February 2020



the unspecific signals completely. Hence, reasons for the observed unspecific signals may be dimerisation of primers and probes and/or unspecific primer binding and polymerase activity in the targeted region of the E gene, probably also depending on thermal profile and cyler-specific differences, or most likely a combination of these factors.

Contamination as a reason for unspecific signals was ruled out, as stringent prevention measures were taken, e.g. strict separation of working areas: oligonucleotides and PCR mastermix reagents were handled in one room under a PCR hood with specified laboratory coats. Sample preparation and RNA extraction took place in a second room. Sample RNA was added in a third room under a PCR hood. The synthetic E gene control was added last to the mastermix. All reagents were aliquoted and aliquots used once only. Contaminations from synthetic E gene present in primer batches upon delivery can be ruled out as well, although only one batch of E gene primers and probes was used with the QuantiTect and Superscript III setup, as only a certain proportion of samples showed the unspecific signals. Furthermore, the unspecific signals were significantly reduced in the Superscript III setup, which showed that its sensitivity was comparable to the QuantiTect setup. In addition, the initially used E gene primers and probe were separately used as templates with the RealStar kit and no amplification was observed, whereas the corresponding artificial E gene template delivered a clear S-shaped curve with this kit.

Commercial stocks of SARS-CoV-2 RNA may report low concentration values, leading to artificially increased apparent sensitivity of diagnostic assays

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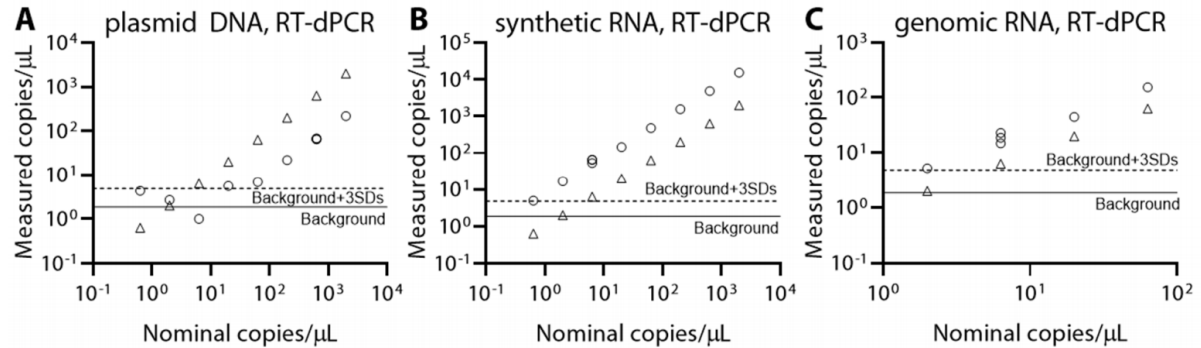


Fig. 2: Quantification of different SARS-CoV-2 nucleic acid targets using an RT-dPCR protocol. Plots show measured concentration (circles) for half-log dilutions of the label concentrations (triangles) based on supplier-reported values of (A) plasmid DNA (lot 508728 from IDT), (B) synthetic RNA (lot 70033953 from BEI), and (C) genomic RNA (lot 70033700 from BEI). Individual dilution series were repeated twice more for the 633 copies/ μL dilution with plasmid DNA and the 6.33 copies/ μL dilution for both RNA stocks. The background (solid line) was calculated by averaging no-template controls ($n=16$), and the assay detection limit (dashed line) was calculated as the background signal in no-template controls plus 3 standard deviations of the background signal (99.7% confidence).[9]

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HKU, Hong Kong SAR	ORF1b-nsp14, N
National Institute of Health, Thailand	N

1 **Pitfalls in SARS-CoV-2 PCR diagnostic**

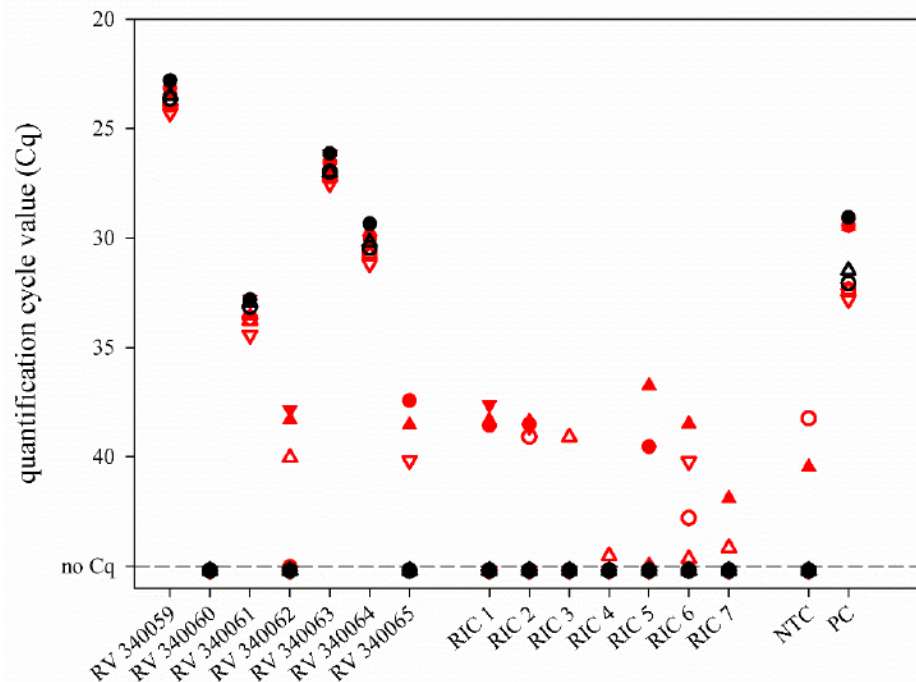
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3 Kerstin Wernike ^{a,1}, Markus Keller ^{a,1}, Fra

4 Groschup ^{a,*}, Martin Beer ^{a,*}

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- AgPath 1, duplex RT-qPCR, primers/probe set A-2
- ▲ AgPath 2, duplex RT-qPCR, primers/probe set A-2
- SSIII 1, duplex RT-qPCR, primers/probe set A-2
- △ SSIII 2, duplex RT-qPCR, primers/probe set A-2
- ▼ AgPath 2, singleplex RT-qPCR, primers/probe set A-2
- ▽ SSIII 2, singleplex RT-qPCR, primers/probe set A-2
- AgPath 1, duplex RT-qPCR, primers/probe set A-1
- ▲ AgPath 2, duplex RT-qPCR, primers/probe set A-1
- SSIII 1, duplex RT-qPCR, primers/probe set A-1
- △ SSIII 2, duplex RT-qPCR, primers/probe set A-1



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Volume 26, Number 8—August 2020

Research Letter

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

Ramona Mögling¹, Adam Meijer¹, Natasa Berginc, Sylvia Bruisten, Remi Charrel, Bruno Coutard, Isabella Eckerle, Vincent Enouf, Olav Hungnes, Gülay Korukluoglu, Thanos Kossyvakis, Andreas Mentis, Richard Molenkamp, Shaman Muradrasoli, Anna Papa, Fiona Pigny, Laurence Thirion, Sylvie van der Werf, and Chantal Reusken[✉]

On This Page

[Research Letter](#)

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

Dear Customer,

In the interest of one of our corporate values, transparency, please be aware that if you intend to use qPCR primers and/or probes for detection of SARS-CoV-2 (For Research Use Only. Not for Use In Diagnostic Procedures), the order may contain trace amounts of long oligo templates (commonly ordered for positive control assays).

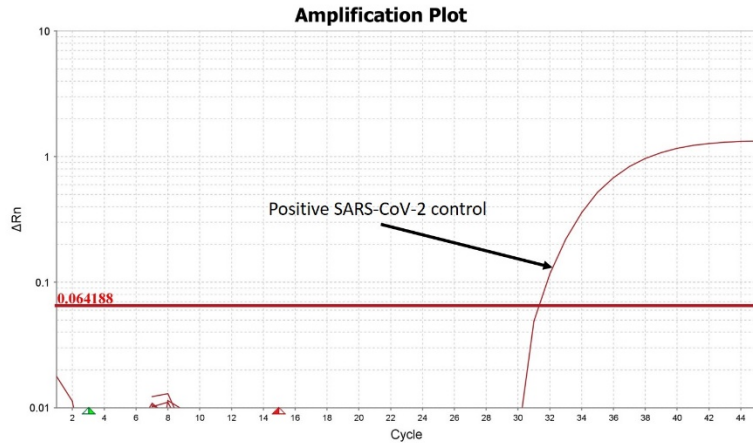
We are expending considerable resources to resolve this oligonucleotide industry problem that leads to signals in No Template Control assays and will provide another update as soon as a solution is in place.

We encourage you to run a No Template Control always, no matter the source of your oligonucleotides.

Thank you Kathryn Harris, Great Ormond Street NHS Foundation Trust

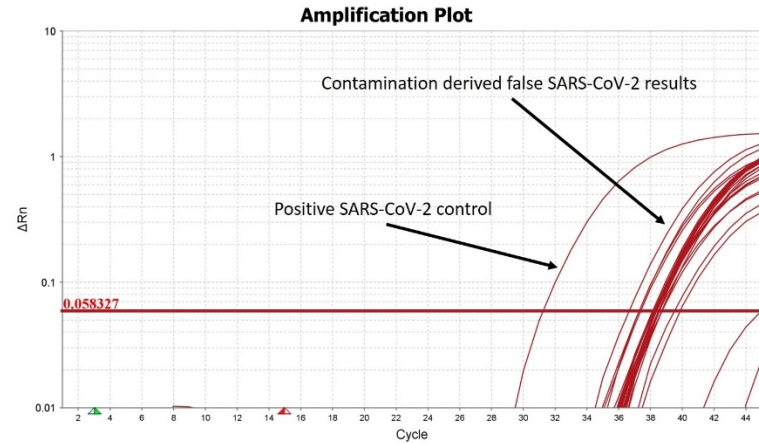


Duplex: N & Rnase P



Result: 60 negative patients

Triplex: N, Rnase P & PDVH



Result: 30 negative patients & 30 false positive patients

Contamination of reagents used for molecular detection of SARS-CoV-2 risks compromising diagnostic assays¶

PCR, the principal diagnostic method applied in the world-wide struggle against COVID-19, is capable of detecting a single molecule of a viral genome. Correctly designed PCR assays for SARS-CoV-2² should not cross-react with other (non-SARS) pathogen sequences and perform with very high analytical sensitivity². Such analytical performance is predicated on the ability of the method to detect the presence of the selected genetic target, without detection of a false-positive signal.¶

There is no “blank” signal in PCR diagnostics. False-positive results may occur during testing, but should not be considered as a background signal or factored into specificity calculations. There are situations where non-specific PCR signal may arise from sequence similarity, such as when measuring minority

A common practice for diagnostic PCR development is for the developer to commission the synthesis of a DNA target template as a positive control. The synthesis of these gene fragments is typically at nanomole scale and will produce in excess of a thousand trillion (10^{15}) copies of single stranded DNA. It is good practice to assure that this control template is made at different sites, usually from alternate vendors, from those making the other PCR reagents, to avoid this major potential source of contamination. However, as the number of laboratories creating assays and targets for the global SARS-CoV-2 pandemic is unprecedented, selecting different vendors may no longer prevent this source of contamination. There are already examples of such assay-derived contamination occurring⁸ and that it has hampered the diagnostic response to COVID-19⁹, with some vendors resigned to this

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Suggestions



Test for it

- **Assume reagents may contain contamination (Figure 1A). Quality control reagents prior to their use (primers, probes, PCR mastermix, water) using at least 10 negative control replicates (alongside a positive control).**
- **Aliquot reagents for single time use, especially nuclease-free water.**
- **Implement control procedures that include extraction blanks that contain carrier RNA; the latter (present in negative patient extracts) is important for measuring low level contamination. Consider using multiple extraction blanks to detect low level contamination.**
- **Further information on the source of contamination can be provided by including reverse transcription negative reactions; this will confirm DNA and not viral RNA as the source.**

Benchmarks

Transfer RNA Reduces the Formation of Primer Artifacts During Quantitative PCR

BioTechniques 27:50-52 (July 1999)

Recent advances have facilitated the

the field of fully quantitative PCR.

Current methodology allow identification of product different through the analysis of DNA m curves by plotting fluorescence function of temperature over the ciation temperature of amplified ucts (5). The outcome of the an can result in several possible ty fluorescent signal, of which, only

This work was supported, in part, by the British Natural Environmental Research Council (NERC) and by a Dutch Government funded Visiting Scientist Fellowship. In particular, I wish to thankfully acknowledge Dr. P. Kille and Mr. J.F. Huggett for continuous encouragement as well as Dr. J.E. Kam-menga and Ms. M. Arts for the provision of the nematodes used in this study. Address correspondence to Dr. Stephen R. Stürzenbaum, University of Wales, Cardiff, School of Biosciences, Biomedical Building, Cardiff, Wales, UK. Internet: sturzenbaumsr@cardiff.ac.uk

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Apply caution when results are close to assay the limit of detection

- **Beware of large numbers results with high Cq values.**
- **Consider the pattern of results. If low signal positives are not randomly distributed (e.g. if they occur adjacent to a high titre sample) this could point at cross-contamination. Consider repeating such low positive samples.**
- **Consider influences of pre-analysis and sample cross-contamination.**
- **If possible test for more than one SARS-CoV-2 target gene.**

Take preventive measures

- **Physically separate PCR setup and sample handling steps (and equipment) from those used for PCR analysis. Ideally use pre- and post-PCR rooms.**
- **Consider steps during preparation that may lead to contamination through aerosol production: pipetting (high throughput), centrifuges, etc. may lead to small amounts of aerosol that can result in cross-contamination.**

Suggestions



Get rid of it

- **Discard all reagents linked to contaminated reactions. While systematic evaluation may determine which reaction component is the culprit, it is often more resource efficient to start from scratch.**
- **Deep clean the laboratory using proven solutions that destroy nucleic acids (e.g. bleach and UV)**
- **If contamination persists, users may need to redesign assay to different part of the pathogen's genome**

Acknowledgements



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Thank you