Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

are suitable for nucleic acid testing have been recognised by some manufacturers who have developed specific transport media to inactivate the viruses of interest and to minimise the degradation of nucleic acid.[6] Some of the major manufacturers of VTM solutions also offer products with additives to reduce nuclease activity but most of these also preclude opportunities to undertake virus culture. However, these limitations are often not clearly disclosed and may not be apparent to purchasing departments, especially during a pandemic, when any VTM may be mistakenly thought 'fit for purpose'.

In conclusion, the results of this study provide examples of how the composition of a VTM could have an impact on the outcome of nucleic acid based testing and, in particular, situations where either there is a need to detect RNA that is not packaged into a nucleocapsid or where RNA constructs may be diluted in a VTM for use as a positive control in an assay or perhaps for proficiency testing. Finally, and particularly in the face of a pandemic, users should be reminded that products fit for one purpose may not be suitable for an alternative use. A product that may be eminently suitable for virus culture purposes could result in misleading results if used for nucleic acid-based tests.

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P. D. Kirkland, M. J. Frost

Virology Laboratory, Elizabeth Macarthur Agriculture Institute, Menangle, NSW, Australia

Contact Dr P. D. Kirkland.
E-mail: peter.kirkland@dpi.nsw.gov.au

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Contamination of SARS-CoV-2 RT-PCR probes at the oligonucleotide manufacturer

Sir,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China, in December 2019 as the aetiological agent of Coronavirus disease 2019 (COVID-19).[1,2] Since then, the disease has spread rapidly worldwide and the World Health Organization (WHO) declared a pandemic on 11 March 2020.[3,4] At the beginning of the outbreak, rapid development and implementation of reliable detection methods became an immediate priority for clinical laboratories worldwide, and reverse transcription polymerase chain reaction (RT-PCR) methods, including those provided by the WHO,[5,6] have been implemented broadly. At the early stage of the outbreak, however, positive control material for RT-PCR assays (from positive patient samples, or viral culture) were not readily available. In such circumstances laboratories often turn to using synthetic controls (synDNA fragments or plasmids).[7,8] These synthetic controls have their advantages, particularly in that the controls can be acquired as readily as PCR primers and probes. Yet, depending on how they are designed, precautions must be taken when handling such controls as trace amounts of this material can potentially cause contamination in the same way as that caused by PCR products. Here we report contamination of a SARS-CoV-2 probe that our evidence suggests occurred at the oligonucleotide manufacturer, and was due to the manufacturer synthesising full length control oligonucleotides (spanning from the forward to reverse primers) in parallel with our probe orders.

Two commonly used assays, E-gene and RdRP, reported by Corman *et al.*,[5] were utilised by our laboratory in the early stages of the pandemic. To establish the assays, we were fortunate enough to have nucleotide stocks and positive control material provided to us from another local laboratory and the assays performed well in our hands. Needing new oligonucleotide stocks, we ordered primers and probes from supplier 'X' on 28 January 2020. The primers arrived on 31 January and were subjected to routine quality control checks. These included checking and recording oligonucleotide batch and reconstitution details, and master mix using new primers or probe was prepared and tested against previously checked reagents. The new primers passed quality control checks. The probes arrived on 11 February, however both were contaminated, providing positive results in the negative controls of both the E-gene and RdRP assays.

Suspecting that the probes had been contaminated by 'full length control oligonucleotides' (ordered by customers from elsewhere) we developed a series of alternative flanking primers for both assays (Fig. 1, Table 1). Each of these alternative flanking primers was designed to gradually 'step away' from the original target region. Full-length synthetic controls typically would only contain sequences from the original target region (i.e., not any additional sequences sitting outside of the original primer pair), and so this type of contamination can be identified by testing alternative primer sets targeting regions further away from the original target site. Therefore, for our reagents, if the contamination was from synthetic controls, the original primers would produce false positive results while the flanking primers would generate negative results when testing non-template controls (NTCs). The flanking primers as well as the original primers from supplier X were tested against the supplier X probes. The experiments were replicated using probes sourced from another supplier, supplier 'Y'. Two SARS-CoV-2 positive clinical samples and two NTCs were tested in each primer probe combination. This study was approved by the Children's Health Queensland Human Research Ethics Committee (HREC/LNR/19/QCHQ/49476).

All results are shown in Table 2. In brief, the known positive samples were positive by all oligonucleotide combinations. Notably, the NTCs were only positive in the supplier X probes using the original supplier X primers, and not in any other NTC, including the original supplier X primers with the supplier Y probes. Of concern were the cycle threshold (Ct) values for the supplier X probe NTCs for the RdRP assay which were very low at ~26 cycles (Table 2), indicating very high levels of contamination, whereas the NTC Ct values for the E assay were ~36 cycles. These results show the supplier X probes were contaminated with nucleic acid fragments consistent with the size, but not larger than, the expected PCR products for the E and RdRP assays. We have since contacted supplier X and they have now implemented new quality control measures to address this issue.

Overall, our study highlights the potential for contamination of oligonucleotide probes at the manufacturer and is due to customers ordering 'full length control oligonucleotides'. This is alarming in the context of reagent shortages

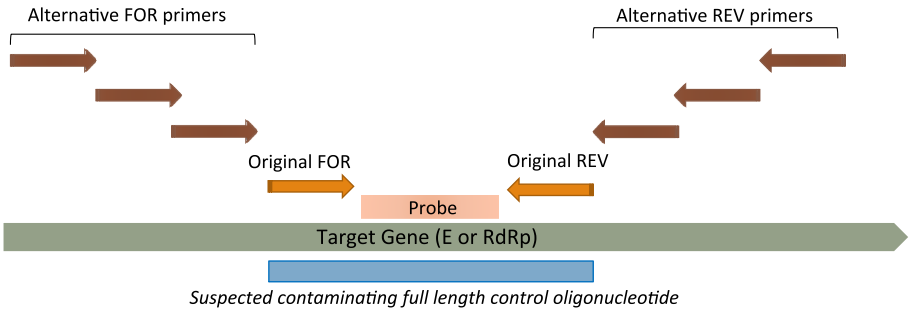

Fig. 1 Illustration of flanking primer designs for detection of contaminated probe. Note that six additional primers were designed for the E-gene but only four for the RdRP assay. FOR, forward primer; REV, reverse primer.

Table 1 List of oligonucleotides used in this study

Name	Oligonucleotides	Nucleotide position[a]	Notes
Sarbeco_E-F1	5′ACAGGTACGTTAATAGTTAATAGCGT	26237–26262	E-Gene Original primer[5]
Sarbeco_E-R2	5′ATATTGCAGCAGTACGCACACA	26328–26349	E-Gene Original primer[5]
Sarbeco_E-probe	5′ACACTAGCCATCCTTACTGCGCTTCG	26300–26325	E-Gene Original probe[5]
Sarbeco_E-altF2	5′CTTATGTACTCATTCGTTTCGGAAGA	26210–26235	E-Gene Flanking primer
Sarbeco_E-altF3	5′GTAAGCACAAGCTGATGAGTACGA	26185–26208	E-Gene Flanking primer
Sarbeco_E-altF4	5′ GACGACGACTACTAGCGTGCCTT	26161–26183	E-Gene Flanking primer
Sarbeco_E-altR2	5′ GAAGGTTTTACAAGACTCACGTTAACA	26350–26376	E-Gene Flanking primer
Sarbeco_E-altR3	5′ GAAGAATTCAGATTTTTAACACGAGAGTAAA	26385–26415	E-Gene Flanking primer
Sarbeco_E-altR4	5′ GTTCGTTTAGACCAGAAGATCAGGAA	26421–26446	E-Gene Flanking primer
RdRP_SARSr-F2	5′ GTGARATGGTCATGTGTGGCGG	15399–15420	RdRP Gene Original primer[5]
RdRP_SARSr-R1	5′ CARATGTTAAASACACTATTAGCATA	15473–15498	RdRP Gene Original primer[5]
RdRP_SARSr-P2	5′ CAGGTGGAACCTCATCAGGAGATGC	15438–15462	RdRP Gene Original probe[5]
RdRP_SARSr-altF3	5′ GTTTCTATAGATTAGCTAATGAGTGTGCTCAA	15360–15391	RdRP Gene Flanking primer
RdRP_SARSr-altF4	5′ CTTGTTCTTGCTCGCAAACATACAA	15314–15338	RdRP Gene Flanking primer
RdRP_SARSr-altR2	5′ GCATTAACATTGGCCGTGACA	15505–15525	RdRP Gene Flanking primer
RdRP_SARSr-altR3	5′ TCGGCAATTTTGTTACCATCAGTAGATA	15531–15558	RdRP Gene Flanking primer

[a] Nucleotide position used reference genome Genbank ID: MN938384.

Table 2 E-gene and RdRP RT-PCR results with different oligonucleotide combinations

Assay and oligonucleotides	Notes	SARS-CoV-2 positive clinical sample 1 (Ct)	SARS-CoV-2 positive clinical sample 2 (Ct)	NTC 1 (Ct)	NTC 2 (Ct)
E-gene					
F1/R2 + probe Y	Original primer set with probe from supplier Y	26.37	20.42	ND	ND
F1/R2 + probe X	Original primer set with probe from supplier X	29.04	22.59	35.44	37.05
F1/altR2 + probe X	Alternative primer combinations with probe from supplier X	29.51	23.15	ND	ND
F1/altR3 + probe X		29.3	23.25	ND	ND
F1/altR4 + probe X		30.14	23.71	ND	ND
altF2/R2 + Probe X		28.85	22.94	ND	ND
altF2/altR2 + Probe X		29.49	23.5	ND	ND
altF2/altR3 + Probe X		30.44	23.93	ND	ND
altF2/altR4 + Probe X		29.86	23.76	ND	ND
altF3/R2 + Probe X		29.32	23.2	ND	ND
altF3/altR2 + Probe X		29.87	23.84	ND	ND
altF3/altR3 + Probe X		30.51	24.24	ND	ND
altF3/altR4 + Probe X		30.22	24.24	ND	ND
altF4/R2 + Probe X		29.9	23.8	ND	ND
altF4/altR2 + Probe X		30.85	24.65	ND	ND
altF4/altR3 + Probe X		30.48	24.29	ND	ND
altF4/altR4 + Probe X		30.75	24.72	ND	ND
RdRP					
F2/R1 + probe Y	Original primer set with probe from supplier Y	29.48	23.97	ND	ND
F2/R1 + probe X	Original primer set with probe from supplier X	26	24.31	26.24	26.11
F2/altR2 + probe X	Alternative primer combinations with probe from supplier X	ND	28.74	ND	ND
F2/altR3 + probe X		29.27	23.63	ND	ND
altF3/R1 + probe X		ND	26.14	ND	ND
altF3/altR2 + probe X		28.49	22.97	ND	ND
altF3/altR3 + probe X		29.06	23.46	ND	ND
altF4/R1 + probe X		31.29	25.72	ND	ND
altF4/altR2 + probe X		28.64	23.05	ND	ND
altF4/altR3 + probe X		29.31	23.36	ND	ND

alt, alternative; Ct, cycle threshold; F, forward primer; ND, not detected; R, reverse primer.

and delays associated with the pandemic, and would have left our laboratory in a precarious position had we not also ordered probes from supplier Y. We affirm that synthetic controls can be useful as positive control material for rare or emergent diseases but should be manufactured and used carefully. Oligonucleotide suppliers should consider how to better handle such requests.

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Claire Y. T. Wang[1], Cameron Buckley[2], Cheryl Bletchly[3], Patrick Harris[2,3], David Whiley[2,3]

[1]Centre for Children's Health Research, Children's Health Queensland, Brisbane, Qld, Australia; [2]The University of Queensland Centre for Clinical Research, The University of Queensland, Brisbane, Qld, Australia; [3]Pathology Queensland Central Laboratory, Brisbane, Qld, Australia

Contact Claire Y. T. Wang.
E-mail: claire.wang@uq.edu.au

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Histopathology of cutaneous COVID-19 lesion: possible SARS-CoV-2 cytopathogenic effect

Sir,
On 1 April 2020 a 66-year-old woman was seen in the emergency room for dyspnoea. She also suffered from diffuse pain and severe fatigue for two days and headache, fever, dysgeusia and agenesis for four days. She had received a 1 g dose of azithromycin, with no improvement. Chest computed