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RESEARCH ARTICLE

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Proficiency testing for the detection of Middle East respiratory syndrome coronavirus demonstrates global capacity to detect Middle East respiratory syndrome coronavirus

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The first reported case of Middle East respiratory syndrome coronavirus (MERS-CoV) infection was identified in Saudi Arabia in September 2012, since which time there have been over 2000 laboratory-confirmed cases, including 750 deaths in 27 countries. Nucleic acid testing (NAT) is the preferred method for the detection of MERS-CoV. A single round of a Proficiency Testing Program (PTP) was used to assess the capability of laboratories globally to accurately detect the presence of MERS-CoV using NAT. A panel of eleven lyophilized specimens containing different viral loads of MERS-CoV, common coronaviruses, and in vitro RNA transcripts was distributed to laboratories in all six World Health Organization regions. A total of 96 laboratories from 79 countries participating in the PTP, with 76 of 96 (79.2%) reporting correct MERS-CoV results for all nine scored specimens. A further 10 laboratories (10.4%) scored correctly in eight of nine specimens of the PTP. The majority of laboratories demonstrated satisfactory performance in detecting the presence of MERS-CoV using NAT. However, some laboratories require improved assay sensitivity, reduced cross contamination of samples, and improved speciation of coronavirus subtypes for potentially complex clinical specimens. Further PTP and enhanced links with expert laboratories globally may improve the laboratory performance.

KEYWORDS
Middle East respiratory syndrome coronavirus, nucleic acid testing, quality assessment, quality assurance, World Health Organization

1 | INTRODUCTION

Middle East respiratory syndrome (MERS) is a viral respiratory disease first identified in Saudi Arabia in 2012 with cases continuing to be detected in the Middle East. Cases detected elsewhere have either been infected in the Middle East or been part of a chain of transmission originating in the Middle East. The disease is due to infection with the Middle East respiratory syndrome coronavirus (MERS-CoV), previously known as novel coronavirus 2012 or HCoV-EMC.[1,2] Human infection is thought to follow exposure to infected camels or human-to-human

Abbreviations: LRT, lower respiratory tract; MERS, Middle East respiratory syndrome; MERS-CoV, Middle East respiratory syndrome coronavirus; N, nucleocapsid protein gene; NAT, nucleic acid testing; ORF1a, open reading frame 1a; ORF1b, open reading frame 1b; PT, proficiency testing; PTP, Proficiency Testing Program; QA, quality assurance; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Programs; RdRp, RNA-dependent RNA polymerase; rRT-PCR, real-time reverse-transcription polymerase chain reaction; upE, upstream of the E protein gene; US CDC, United States Centers for Disease Control and Prevention; VIDRL, Victorian Infectious Diseases Reference Laboratory; WHO, World Health Organization.

TABLE 1 Invitation and participation of laboratories worldwide

	Invited		Responded		Agreed to participate		Received samples		Reported results	
WHO region	**Labs**	**Countries**	**Labs**	**Countries**	**Labs**	**Countries**	**Labs**	**Countries**	**Labs**	**Countries**
African region	13	13	7	7	7	7	7	7	6	6
Eastern Mediterranean region	23	17	19	16	18	15	18	15	17	14
European region	63	40	45	33	45	33	45	33	45	33
Region of the Americas	9	9	6	6	6	6	5	5	5	5
South-East Asia region	4	4	4	4	4	4	4	4	4	4
Western Pacific region	21	15	21	15	19	14	19	14	19	14
Total	**133**	98	**102**	81	**99**	79	**98**	78	**96**	76

Abbreviation: WHO, World Health Organization.

transmission primarily in healthcare settings. As of March 2018, the World Health Organization (WHO) had been notified of 2143 laboratory-confirmed cases of infection with MERS-CoV globally, including 750 deaths.[3]

Apart from epidemiologic clustering and virus-specific diagnostic testing, there is little clinically that distinguishes MERS-CoV infection from other severe viral respiratory infections such as severe acute respiratory syndrome or influenza. Nucleic acid testing (NAT) is the preferred method for detecting MERS-CoV. Suitable specimens for testing include lower respiratory tract samples (LRT), nasopharyngeal swabs, oropharyngeal swabs, nasal washes, and nasal aspirates. The LRT samples have been shown to contain the highest viral loads, possibly due to virus tropism for LRT cells.[4,5] Confirmation using real-time reverse-transcription polymerase chain reaction (rRT-PCR) assays require a positive result for at least two different specific targets on the MERS-CoV genome using a validated assay, or a positive rRT-PCR result for one specific target on the MERS-CoV genome plus MERS-CoV sequence confirmation from a separate viral genomic target.[6] Nucleic acid sequencing of an amplicon has also been recommended when there are discordant results on different assays,[6,7] confirming the specificity of the target.

It is important that laboratories develop technical capability to accurately and promptly identify MERS-CoV to implement appropriate infection control and isolation procedures to reduce the potential for transmission, and aid a rapid epidemiological investigation. External quality assessment programs are an essential tool for monitoring the diagnostic proficiency of laboratories and providing results that allow implementation of improved testing, thereby strengthening global capability in reducing spread. The WHO initiated a single round Proficiency Testing Program (PTP) for the detection of MERS-CoV by PCR, which was conducted in the first half of 2015 by the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP).

2 | METHODS

2.1 | Participation

The WHO identified 133 laboratories from 98 countries in all six WHO regions as potential participants. One hundred and two laboratories confirmed their interest and 99 subsequently confirmed their ability to receive the panel. A total of 96 laboratories located in 79 different countries returned results and participated in the PTP (Table 1). The additional verification step was introduced to ensure laboratories had all the documentation and permits in place that were required to import the material into their country.

2.2 | Panel description

The proficiency testing (PT) panel consisted of 11 specimens. This was made up of nine specimens (A to I) of inactivated (gamma irradiation; 50 kGy) MERS-CoV, human coronavirus OC43, human coronavirus 229E, and a negative control (specimen I), as well as two synthetic specimens (J and K) containing in vitro RNA transcripts. RCPAQAP have used similar transcripts in previous PT panels, and have shown that they are safe, stable, and reliable.[8] The design of these transcripts was based on the complete genome sequence of human betacoronavirus 2c EMC/2012 (GenBank accession number: JX869059.2). The following five regions of the MERS-CoV genome were included in the two synthetic specimens: a 385-nucleotide region upstream of the E protein gene (upE) covering nucleotides 27 312 to 27 696; 500 nucleotides of the open reading frame 1a (ORF1a) starting at nucleotide 10 923; a 502-nucleotide region of the open reading frame 1b (ORF1b) starting at nucleotide 18 054, as well as two regions; one from the RNA-dependent RNA polymerase (RdRp) starting at nucleotide 14 994 and another from the nucleocapsid (N) protein gene starting at nucleotide 29 523, with a length of 392 and 491 nucleotides, respectively. The five RNA transcripts were designed to yield positive results in RT-PCR assays that were originally published by Corman et al,[6,7] which formed the basis of the "*WHO interim guidance—laboratory testing for Middle East respiratory syndrome coronavirus.*"[8] Specimen J contained all five RNA transcripts, whilst specimen K contained a single transcript covering upE. This was designed to challenge participants with a sample (K) that would yield an equivocal result, as confirmatory testing to initial screening would return negative.

All specimens provided were lyophilized and tested for homogeneity and stability. Homogeneity was confirmed, and no significant sample degradation was detected after storage for 7 days at 37°C

and subsequent 21 days at −80°C. The MERS-CoV strain used was provided by Public Health England, and all coronaviruses included in the panel were prepared at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Melbourne, Victoria, Australia. Following gamma irradiation of the viruses, inactivation was confirmed and viral RNA was quantified using real-time PCR.[8] The relative measure of the concentration of virus-specific target was determined by generating standard curves using a set of MERS-CoV-specific and HCoV-specific primers to quantify the genome equivalents (GE) copies per millilitre of each specimen.[6,7] Three external referee laboratories confirmed sample characteristics.

2.3 | Assessment criteria

Participants were requested to test all specimens of the PT panel and

1. rule out or confirm the presence of MERS-CoV;
2. rule out or confirm the presence of a HCoV other than MERS-CoV;
3. identify the HCoV, if present.

Participating laboratories were assessed on their capacity to correctly analyze specimens A to I using their existing PCR detection protocols and reagents. This was performed qualitatively, with correct responses assigned on the basis of reporting the expected result eg MERS-CoV ruled out, other HCoV confirmed present and identified as human coronavirus 229E. Performance was assessed separately for the detection of MERS-CoV and the detection and identification of other HCoVs.

Results submitted for synthetic specimens J and K were not scored, as participants were likely to obtain varying results depending on the gene region and PCR marker used by the testing laboratory. In particular, participants performing MERS-CoV-specific testing according to the assay developed by the United States Centers for Disease Control and Prevention (US CDC) would expect a negative result for specimen J, as this assay targets a region of the N gene that is different to the region that was used to design the in vitro RNA transcript included in specimen J.[10] These specimens were included as they could provide interesting information in regard to how participating laboratories handle equivocal test results.

3 | RESULTS

3.1 | Capability to detect MERS-CoV and other HCoVs by PCR

Overall, 76 of 96 (79.2%) laboratories correctly reported the presence or absence of MERS-CoV in all nine scored specimens (Figure 1). An additional 10 (10.4%) laboratories correctly reported the presence or absence of MERS-CoV in 8 of 9 scored specimens, whilst 10 (10.4%) had at least two discordant results (Table 2). The absence of MERS-CoV in the negative control sample (specimen I) was correctly reported by 88 of 96 (91.7%) participants (Table 3). Five participants reported false-positive results, and three laboratories did not report a result for this specimen (I) for unknown reasons.

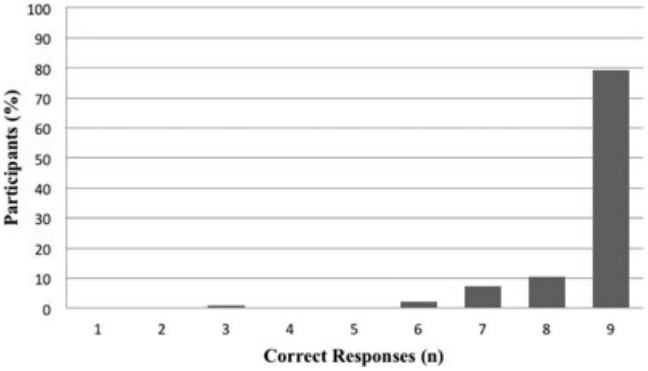

FIGURE 1 Participant performance in the nine scored specimens for Middle East respiratory syndrome coronavirus

A large proportion of participants reported that they have no or limited capability to test for human coronaviruses other than MERS-CoV. To account for this variability, performance was assessed separately for the detection of MERS-CoV and the detection and identification of other HCoVs. There were 69 of 96 (71.9%) laboratories that returned results regarding other HCoVs, with 27 (28.1%) correctly confirming or ruling out the presence of other HCoVs in all nine specimens (Table 2) and 29 (30.2%) correctly identifying the two other HCoVs included in specimen B (HCoV-OC43), specimen C (HCoV-229E), and specimen H (MERS-CoV & HCoV-229E). A summary of the performance of laboratories for all specimens is presented in Table 3.

3.2 | Limit of detection for MERS-CoV-specific PCR

The PT panel included a 10-fold dilution series (specimens D, E, F, and G), covering MERS-CoV RNA concentrations ranging from

TABLE 2 Participant performance in PT panel

	No. (%) of participants (n = 96) correctly reporting		
Number of correct results	MERS-CoV detection	Other HCoV detection	Other HCoV identification
9	76 (79.2)	27 (28.1)	N/A
8	10 (10.4)	15 (15.6)	N/A
7	7 (7.3)	2 (2.1)	N/A
6	2 (2.1)	2 (2.1)	N/A
5	0 (0.0)	1 (1.0)	N/A
4	0 (0.0)	4 (4.2)	N/A
3	1 (1.0)	8 (8.3)	29 (30.2)
2	0 (0.0)	6 (6.3)	26 (27.1)
1	0 (0.0)	4 (4.2)	4 (4.2)
0	0 (0.0)	27 (28.1)	37 (38.5)

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; PT, proficiency testing.

TABLE 3 Specimen characteristics and performance of participants

Specimen	Virus	Genome equivalents/μL[a]	No. (%) of participants (n = 96) with MERS-CoV correct	Other HCoV correct	Other HCoV not specified	Other HCoV ID correct	Other HCoV ID not specified
Differentiation							
A	MERS-CoV	4.3×10^2	94 (97.9)	47 (49.0)	48 (50.0)	N/A	N/A
B	HCoV-OC43	1.8×10^2	90 (93.8)	60 (62.5)	27 (28.1)	55 (57.3)	41 (42.7)
C	HCoV-229E	1.6×10^2	87 (90.6)	61 (66.7)	27 (28.1)	55 (57.3)	41 (42.7)
H	MERS-CoV and HCoV-229E	4.3×10^2 1.6×10^2	95 (99.0) –	37 (38.5) –	46 (47.9) –	33 (34.4) –	63 (65.6) –
I	Negative	–	88 (91.7)	63 (65.6)	33 (34.4)	N/A	N/A
Sensitivity							
E	MERS-CoV	4.3×10^3	94 (97.9)	45 (46.9)	49 (51.0)	N/A	N/A
D	MERS-CoV	4.3×10^2	94 (97.9)	44 (45.8)	51 (53.1)	N/A	N/A
F	MERS-CoV	4.3×10^1	94 (97.9)	46 (47.9)	50 (52.1)	N/A	N/A
G	MERS-CoV	4.3×10^0	92 (95.8)	47 (49.0)	49 (51.0)	N/A	N/A
Synthetic specimens							
J	upE ORF1a ORF1b RdRp N	4.3×10^3 4.6×10^2 1.6×10^2 2.5×10^3 ND	81 (84.4) – – – –	7 (7.3) – – – –	8 (8.3) – – – –	3 (3.1) – – – –	47 (49.0) – – – –
K	upE	4.3×10^3	49 (51.0)	28 (29.2)	19 (19.8)	3 (3.1)	54 (56.3)

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; N, nucleocapsid protein gene; ND, not determined; ORF1a, open reading frame 1; ORF1b, open reading frame 1; RdRp, RNA-dependent RNA polymeras; upE, upstream of the E protein gene.
[a]Genome equivalents after reconstitution of lyophilized specimens in 500 μL.

4.3×10^3 to 4.3×10^0 GE/μL. There were 94 of 96 participants (97.9%) reporting a correct positive result for specimens with RNA concentrations ranging from 4.3×10^3 to 4.3×10^1 GE/μL and 92 of 96 participants (95.8%) reporting a correct positive result for all four specimens, including the specimen with the lowest viral load (Table 3).

3.3 | Synthetic specimens

Eighty-one of ninety-six (84.4%) laboratories confirmed the presence of MERS-CoV in specimen J, which contained all five in-vitro RNA transcripts. For this specimen, seven (7.3%) laboratories ruled out the presence of MERS-CoV and eight (8.3%) participants did not specify. Specimen K contained one in-vitro RNA transcript, for which 49 of 96 (51.0%) laboratories reported the presence of MERS-CoV. Twenty-eight (29.2%) and 19 (19.8%) participants ruled out or did not specify the presence of MERS-CoV, respectively (Table 3).

3.4 | Methods used for the detection of MERS-CoV

All 96 participating laboratories tested the specimens of the PT panel for the presence or absence of MERS-CoV. The gene targets used by participants to confirm or rule out the presence of MERS-CoV in each of the 11 specimens were *upE*, *ORF1a*, *ORF1b*, *N*, and *RdRp* (Table 4).

Participants followed various protocols to confirm or rule out the presence of MERS-CoV. Examination of the set of gene targets that were used showed most participants either followed the *WHO interim guidance—laboratory testing for Middle East respiratory syndrome coronavirus* protocol,[8] or the assay developed by US CDC.[10] Both protocols recommend an initial screening assay against the gene target upE, with subsequent confirmation by one more PCR assays for the gene targets ORF1a/ORF1b/N (WHO) or N2/N3/RdRp (US CDC). In accordance with these two recommendations, 93.9% of participants performed at least two PCR assays per specimen to confirm or rule out the presence of MERS-CoV (Figure 2). TaqMan-based real-time PCR was the most commonly used PCR method with an average of 95.1% and 93.9% of participants using this method in the initial screening assay and the confirmatory second assay, respectively. Participants performing three or more PCR assays included conventional gel-based PCR assays, as well as nested and heminested assays in their testing regimen. The most commonly used PCR platforms include ABI real-time PCR systems (42 participants), Roche LightCycler (21 participants), Bio-Rad real-time PCR systems (11 participants), and the Qiagen rotor-gene (nine participants) and Stratagene systems (five participants). The majority of participating laboratories relied on manual RNA extraction using the Qiagen QIAamp Viral RNA Mini Kit (36 participants), followed by automated systems from BioMerieux (NUCLISENS EASYMAG) and Roche

TABLE 4 Target genes used by participants for MERS-CoV detection

	No. of participants (n = 96) testing for a MERS-CoV-specific target in each specimen[a]													
Target	A	B	C	D	E	F	G	H	I	J	K	Mean	SD	%
upE	94	94	92	93	93	93	93	93	92	94	95	93.3	0.90	97.2
ORF1a	49	45	46	49	49	49	49	49	46	51	53	48.6	2.29	50.7
ORF1b	32	31	32	32	32	32	32	32	32	32	36	32.3	1.27	33.6
N	21	20	21	21	21	21	21	21	19	22	32	21.8	3.46	22.7
NCV.N2	20	20	19	18	20	19	19	19	19	19	19	19.2	0.60	20.0
NCV.N3	19	13	12	22	19	19	19	19	12	19	18	17.4	3.38	18.1
RdRp	9	12	10	9	10	10	10	9	9	10	16	10.4	2.06	10.8

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; N, nucleocapsid protein gene; ORF1a, open reading frame 1; ORF1b, open reading frame 1; RdRp, RNA-dependent RNA polymeras; upE, upstream of the E protein gene.
[a]Assays include RT-PCR, conventional PCR and sequencing assays for N and RdRp.

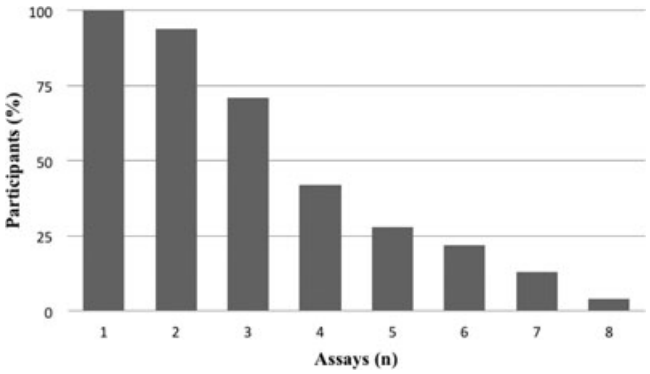

FIGURE 2 Average number of polymerase chain reaction assays performed per specimen, per laboratory

(Magna Pure System) used by 15 and five participating laboratories, respectively. A range of manual RNA extraction systems from Qiagen, Macherey-Nagel, and Roche were used by seven, five, and four participants, respectively.

4 | DISCUSSION

The PTP provides insight into the diagnostic methodology and performance of laboratories in detecting MERS-CoV worldwide. The high level of concordance between the 96 participating laboratories suggests a high global capacity to detect MERS-CoV. Despite this, a small number of laboratories performed poorly and the results of this PTP identify opportunities for future improvement. Incorrect results could be attributed to three main causes: (i) cross contamination, (ii) low sensitivity of the NAT (predominantly PCR), or (iii) transcription error. A number of laboratories reported a MERS-CoV false positive result for the human coronavirus samples (specimens B and C) and the negative sample (specimen I). These results were all reported with high C_t values, suggesting cross contamination of samples. There were four participants reporting false negative results for specimen G, which contained the lowest concentration of MERS-CoV. These participants consistently reported unusually high C_t values for specimens across the panel, indicating that these laboratories had relatively low sensitivity in their PCR assay and may not have been able to detect MERS-CoV at lower concentrations. This finding is similar to that of two previous external quality assessments, which reported some laboratories to have reduced assay sensitivity when assessed on a 10-fold MERS-CoV dilution panel.[11,12] These studies also demonstrated a high overall capacity for laboratories to detect MERS-CoV in China,[11] and worldwide.[12] Similarly, a study performed during the 2015 Korean outbreak reported a 100% MERS-CoV detection score amongst 47 participants.[13] However, the panel in this study only consisted of three specimens, limiting their assessment of assay sensitivity. Both the Korean and Chinese external quality assessments differed from this PTP in that they did not include other human coronaviruses for the assessment of assay specificity. Access to and participation in future quality assurance (QA) programs will provide the opportunity for improvement at a technical level. Offering an ongoing PTP for the detection of MERS-CoV and other human coronaviruses by PCR will ensure that this capacity will remain appropriate and provide continuing opportunities for improvement.

Results for the detection and identification of other human coronaviruses were less conclusive, with just 28.1% and 30.2% of laboratories scoring correctly in all samples, respectively. However these results were unsurprising, as a large proportion of participants reported they had no or limited capability to test for human coronaviruses other than MERS-CoV before the beginning of the PTP. The capacity to test for related organisms is important in distinguishing pathogens such as MERS-CoV from genetically related, but less virulent organisms such as other human coronaviruses.

The two synthetic specimens containing MERS-CoV complementary DNA were included in the PTP as they provided noninfectious, easily accessed, and scalable quantities of target analyte. Although these specimens were not scored, they were informative as for how participating laboratories handled equivocal test results. Specimen J contained five in vitro transcribed RNA transcripts, a design intended to give positive results for all NAT markers included in PCR protocols

recommended by the WHO. Specimen K contained a single transcript covering upE, a design that challenged participants with a sample that would yield an equivocal result. Participants would only get a positive result in an initial screening assay against upE, while confirmatory tests of this initial screen would be negative. The protocol recommended by the WHO advises further specimens to be collected in the absence of a confirmatory result. The US CDC protocol recommends reporting of an equivocal result and contacting the CDC for consultation. In the context of this PTP, the expected result for specimen K would have been "not specified" and a comment as for equivocal results and/or the necessity to collect and test a second sample. Interestingly, more than half of the participating laboratories reported the presence of MERS-CoV in specimen K despite the fact that confirmatory NAT assays against targets other than upE were negative. Additionally, a small number of laboratories were found to be only performing an average of one PCR assay per sample. These results highlight the need for laboratories to follow a NAT regimen with at least two independent assays. Laboratories need to have detailed guidelines for MERS-CoV PCR test interpretation as well as reporting instructions. By addressing these issues, it is anticipated that the global laboratory capacity for MERS-CoV detection will be enhanced.

Engaging international laboratories, especially those in countries with limited exposure to routine QA proved to be one of the major challenges in organizing and executing this PTP. Language barriers, limited resources, skepticism toward an unknown organization, as well as the need for laboratories to continue their routine testing alongside PTP sample testing most likely contributed to difficulties with engagement. Jurisdictional difficulties with specimen handling were also an administrative challenge. Despite the material not being infectious, several laboratories required a range of documentation such as import permits. Obtaining these documents was often time-consuming, and in more than one instance required 8 weeks or longer before specimens could be received. The responses to these challenges need to include early engagement with laboratories, as much local interaction as possible with regional WHO support, and effective communication with customs and courier officials to avoid delays at the dispatch stage of the PTP. The strong support from the WHO regional offices was key to successfully running the PTP in 96 laboratories from 79 countries. Contracting three different courier services ensured efficient distribution of the PTP globally, with 85% of laboratories receiving the panel within 5 days of dispatch.

In conclusion, participating laboratories had a high overall capacity to detect MERS-CoV. The PTP identified improvement opportunities at a technical level, including (i) the need for performance of confirmatory NAT assays, (ii) avoidance of cross contamination of samples, and (iii) care with clear identification of the particular coronavirus target and result. Laboratories need access to QA programs, and continued encouragement for engaging in these QA activities, which are in addition to, but important for their diagnostic functions. Facilitating involvement of laboratories in appropriate QA programs is essential to continuing accurate detection of pathogens. Accurate diagnosis is critical where a pathogen is infrequently seen, and detection may have significant community, public health, and social implications. Continuing involvement in well-regulated QA programs, attention to technical details, and staff development can avoid significant laboratory error and unnecessary public health concern.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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