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Comparison of six commercial systems for the detection of non-O157 STEC in meat and vegetables

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) are important pathogens transmitted by food that may cause severe illness in human beings. Thus, systems for STEC detection in food should have increasingly higher sensitivity and specificity. Here we compared six commercial systems for non-O157 STEC detection in meat and vegetables and determined their sensitivity, specificity and repeatability. A total of 46 samples (meat n = 23; chard n = 23) were experimentally contaminated with strains O26:H11, O45:H-, O103:H2, O111:NM, O121:H19 and O145:NM isolated in Argentina. Strain detection was confirmed by isolation according to ISO 13136:2012. Detection of the *stx* and *eae* genes in meat samples was highly satisfactory with all commercial kits, but only five had 100% sensitivity and specificity in chard. Of four kits evaluated for serogroup detection, three had 100% sensitivity and specificity, and one had 93.7% sensitivity and 100% specificity. All kits were adequate to analyze meat but not vegetable samples, and were not therefore validated for the latter matrix. The challenge for microbiology laboratories is to identify the advantages and disadvantages of the available kits for STEC detection in food based on a clear knowledge of the particular needs of each laboratory.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and non-O157 STEC serogroups are important emerging foodborne pathogens recognized globally because they cause disease outbreaks and severe illness, including diarrhea, hemorrhagic colitis (HC) and potentially fatal haemolytic uremic syndrome (HUS) in humans (Karmali et al., 2010).

Escherichia coli serotype O157:H7 is mostly associated with outbreaks and sporadic cases of HC and HUS in many countries (Leotta et al., 2008). However, non-O157 STEC have been implicated in contamination incidents around the world, and the number of reported cases has steadily increased every year. Between 2000 and 2010, the Foodborne Diseases Active Surveillance Network (FoodNet) noted an increase in the incidence of non-O157 STEC from 0.12 to 0.95/100 000 (Tseng et al., 2016). Based on data from STEC serogroups associated with human disease, seven major serogroups are currently identified: O157, O26, O45, O103, O111, O121 and O145 (MLG 5C.00, 2019).

In the 1980s, STEC outbreaks were associated with the consumption of uncooked meat products. However, a large number of food products such as sprouts, vegetables and unpasteurized juices are also currently associated with serious outbreaks (Yang et al., 2017), particularly because they are often consumed without additional processing (Gao et al., 2018).

The notification of STEC serogroups varies among countries. For instance, some countries have zero tolerance for all STEC in chilled beef (RASFF). In the European Union, the absence of the six main STEC serogroups (O157, O26, O103, O111, O145 and O104:H4) is mandatory in sprouts (Commission, 2013). In the United States, the previously mentioned serogroups plus O45 and O121 are searched for in the matrices of ground beef and beef manufacturing trimmings (FSIS and Agriculture, 2014). In Argentina, the search for STEC serogroups

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O157:H7/NM, O26, O103, O111, O145 and O121 in ground beef, ready-to-eat foods, sausages and vegetables is mandatory in relation to the strains frequently associated with severe disease in human beings (Justice, 2017).

Currently, more than 30 commercial equipment and kits that meet the control requirements are available for STEC detection and isolation. STEC strains are considered highly pathogenic when they present genes stx_1 and/or stx_2 and *eae,* and belong to one of the above mentioned serotypes (Beutin and Fach, 2014). These genotypic virulence factors have been used to develop screening methods based on molecular detection strategies (EFSA, 2013), such as real-time polymerase chain reaction (RT-PCR) systems, which have gained importance in recent years for the examination of food samples and the environment. Currently, three reference methods include RT-PCR to detect the *stx* and *eae* genes in food (FDA, 2018; ISO, 2012; MLG 5C.00, 2019).

The aim of this study was to compare six commercial systems for the detection of non-O157 STEC in meat and vegetables.

2. Material and methods

2.1. Commercial kits

The participating companies provided the equipments, kits and consumables necessary to carry out the tests (Table 1). The following commercial kits were tested: Assurance GDS® (AgriNEA – MilliporeMerck, Darmstadt, Germany), GENE-UP® (bioMérieux, France), Pall GeneDisc® System (Pall Corporation, New York, US), Foodproof® (BIOTECON Diagnostics GmbH, Potsdam, Germany), PATHfinder (Generon S.p.A., San Prospero, Italy) and RapidFinder™ (Thermo Fisher Scientific Inc, Massachusetts, US). The enrichment broths were used as recommended by the manufacturers (Table 1). Detection of *stx* and *eae* genes was performed with all kits, while serogroup detection was carried out when the kits included such test. Results were confirmed by isolation according to ISO 13136:2012, which is considered the gold standard method (ISO, 2012).

2.2. Strains

Except for ATCC 25922, all non-STEC and non *E. coli* strains used for the sensitivity, specificity and repeatability tests belonged to the IGEVET collection and were isolated in Argentina. The STEC strains used in this study were O26 and O103 isolated from beef products, O111 isolated from patients with diarrhea, O121 isolated from the environment and O145 isolated from a patient with HUS. Non-*E. coli* and non-toxigenic *E. coli* strains were isolated from beef products and the environment. They were stored at −70 °C and grown in 4 mL brain heart infusion broth (Biokar, Zac de Ther, France) at 37 ± 1 °C for 18–24 h. Serial strain dilutions were performed in buffered peptone water (Biokar) and the inoculum level was confirmed by plating on plate count agar (Britania, BA, Argentina). Plates were incubated at 37 °C for 18–24 h. The samples were inoculated with 1 mL of a diluted culture of each strain to achieve a target inoculum for each test (sensitivity, exclusivity and repeatability).

2.3. Matrices

Samples of canned minced meat (n = 138, 25 g each) and canned chard (n = 138, 25 g each) were collected in Whirl-Pak filter bags (Nasco, Fort Atkinson, WI, US) under sterile conditions. Of these, 46 meat and chard samples (23 each) were used to test each kit.

2.4. Laboratories

The samples were experimentally contaminated at the Laboratorio de Seguridad Alimentaria of IGEVET (UNLP-CONICET LA PLATA) and sent to the two testing laboratories under refrigeration conditions (4 ± 1 °C) 12 h after inoculation.

The tests were carried out under controlled conditions in accordance with ISO 17043 in two laboratories with the same expertise in the detection of non-O157 STEC: 1) commercial systems Assurance GDS®, GENE-UP® and Pall GeneDisc® System were tested in Stamboulian Servicios de Salud, Laboratorio de Alimentos, División Higiene y Seguridad Alimentaria y Ambiental, and 2) Foodproof®, PATHfinder and RapidFinder™ were analyzed in Dirección General de Higiene y Seguridad Alimentaria, Gobierno de la Ciudad de Buenos Aires.

2.5. Sensitivity

The six non-O157 STEC serogroups with different stx_1, stx_2 and *eae* combinations were used for the sensitivity analysis, namely, O26:H11 (stx_1, *eae*), O103:H2 (stx_1, *eae*), O111:NM (stx_1, *eae*), O121:H19 (stx_2, *eae*) and O145:NM (stx_2, *eae*). Only one non-toxigenic (*eae*) strain was used (*E. coli* O45:H-) because there have yet to be toxigenic isolates of these serogroups in Argentina. Ten samples contaminated with at least one strain, or a combination of two, were used for each matrix. Two levels of inoculum (10 and 100 CFU/25 g) were used for each strain (Table 2).

2.6. Specificity

Nine non-*E. coli* and non-toxigenic *E. coli* strains at a single inoculum level (1×10^3 to 1×10^4 CFU/25 g) were used for the specificity trials (Table 3). Ten samples contaminated with at least one strain, or a combination of two strains, were used for each matrix.

2.7. Repeatability

Repeatability was tested in three samples, two contaminated with the strains used for the sensitivity assay, and one non-inoculated negative sample (Table 4). All samples were processed in triplicate by the same operator and under the same conditions.

2.8. Interpretation of results

In the first screening step, samples were considered *stx/eae*-negative when neither gene was detected or when they tested positive for only one of the two virulence factors. Samples were considered *stx/eae*-positive when both target genes were detected.

In serogroup screening, samples were considered negative when no signal or an unexpected signal for any serogroup was detected, and positive when a positive signal to the expected serogroup was detected. Results obtained with kits that, according to the manufacturers' indications, could identify cross-reacting strains were also considered positive when both serogroups were detected, even if only one of them had to be tested. The Assurance GDS® kit clearly states that certain STEC strains belonging to the O145 serogroup may also be *E. coli* O157:H7positive. Similarly, Pall GeneDisc® Top 7 does not detect virulence genes of serogroups O121 and O45 separately. RapidFinder™ does not discriminate between serogroups; therefore, a sample was considered positive when it gave a positive signal for the presence of any of the serogroups, even without identifying which.

2.9. Statistical analysis

Kappa coefficient (κ) was used to determine the rate of agreement of qualitative tests with the contamination pattern previously described and confirmation for isolation (ISO, 2012). Specificity, sensitivity and 95% confidence intervals of each kit were computed with isolation as the gold standard. All statistical analyses were performed using WinEpiScope software version 2.0 (Thrusfield et al., 2001).

Table 1
General characteristics and conditions of the commercial kits analyzed.

		Assurance GDS®	Foodproof®	GENE-UP®	Pall GeneDisc® System	PATHfinder	RapidFinder™
General	**Kit**	MPX Top 7 STEC MPX ID for TOP STEC	Foodproof® STEC screening lyoKit Foodproof® STEC Identification lyoKit	VIDAS® UP EC serogroups GENE-UP® STEC *stx* & *eae* GENE-UP® top 6 GENE-UP® O157:H7	GeneDisc® STEC – O157 GeneDisc® TOP 7	PATHfinder *E.coli* VTEC stx_1-stx_2 & *eae*-IPC duplex assay	RapidFinder™ STEC Screening Assay RapidFinder™ STEC Confirmation Assay
	PCR	GDS® Biocontrol Pick Pen®	ABI 7500 fast	mini VIDAS® + GENE-UP®	GeneDisc® Cycler	ABI 7500 fast	ABI 7500 fast
	Target	*stx* + *eae* + O group	stx_1 + stx_2 + *eae*	*stx* + *eae*	*stx* + *eae* + O157	stx_1 + stx_2 + *eae*	stx_1 + stx_2 + *eae* + O157
	Validation	AOAC Food products and environmental surfaces	AOAC Meat, seafood, milk, fruits, vegetables and environmental samples	AOAC Food products	AOAC Raw meat, dairy products and vegetable products	No Meat industry and fishery products	AOAC Ground beef and beef trim samples
	Number of samples that could be analyzed simultaneously	36	96 screening 48 serotyping	12 mini VIDAS® 96 PCR	12 STEC and 6 Top 7 for each thermal cycler	100	96
	Enrichment broth	Own kit	Tryptic soy broth Buffered peptone water		Buffered peptone water	Modified tryptic soy broth	Tryptic soy broth
DNA extraction	**Reagent storage temperature**	Fridge (2–8 °C)	Room (20–25 °C)	Fridge (2–8 °C)	Fridge (2–8 °C)	Fridge (2–8 °C)	Fridge (2–8 °C) and freezer (− 20 °C)
	Time for DNA extraction	0	40 min	0	10 min	15–17 min	40 min
PCR	**Reagent storage temperature**	Fridge (2–8 °C)	Fridge (2–8 °C)	Room (20–25 °C)	Fridge (2–8 °C)	Freezer (− 20 °C)	Fridge (2–8 °C)
	Time for the preparation of reagents prior to the incorporation of the DNA extract	5 min	15 min	5 min	15 min	Seconds	0
	Time to obtain the final results	85 min	65 min	58 min	65 min	45 min	50 min
Immunoconcentration	**Number of simultaneous samples**	8	N/A	12	N/A	N/A	N/A
	Time	30 min/29 samples	N/A	38 min	N/A	N/A	N/A
	Used selective agarized plates	Yes	N/A	Yes	N/A	N/A	N/A
	Time of conservation of the immunoconcentrate	It has no time of conservation, it is sown directly	N/A	3 days	N/A	N/A	N/A
Identification of serogroups	**Reagent storage temperature for DNA extraction**	Fridge (2–8 °C)	N/A	N/A	Fridge (2–8 °C)	N/A	Fridge (2–8 °C) and freezer (− 20 °C)
	Time for DNA extraction	0	N/A	0	0	N/A	40 min
	Reagent storage temperature for serogroup PCR	Fridge (2–8 °C)	N/A	Fridge (2–8 °C)	Fridge (2–8 °C)	N/A	Fridge (2–8 °C)
	Time for the preparation of reagents prior to the incorporation of the DNA extract	30 min/29 samples	N/A	0	15 min	N/A	N/A
	Compatibility of different serogroups in the same run	O26, O103, O157, O111, O145, O121, O45	N/A	O26, O103, O111, O145, O121, O45	O26, O103, O157, O111, O145, O121, O45	N/A	Big 6 without differentiation and O157
	Time to obtain the final results	85 min	N/A	65 min	55 min	N/A	40 min

N/A: not applicable.

Table 2
Non-O157 strains and bacterial inoculum used in meat and vegetable samples for the sensitivity assay.

SAMPLE	MEAT			
	Strain	CFU/25 g	Strain	CFU/25 g
M1			*E. coli* O26:H11	2.8×10^{2}
M2			*E. coli* O145:NM	2.2×10^{2}
M3	*E. coli* O111:NM	13		
M4	*E. coli* O103:H2	18		
M5	*E. coli* O26:H11	38		
M6	*E. coli* O103:H2	18		
			E. coli O26:H11	2.8×10^{2}
M8			*E. coli* O121:H19	1.2×10^{2}
M9	*E. coli* O145:NM	8	*E. coli* O103:H2	1.5×10^{2}
M10			*E. coli* O111:NM	0.9×10^{2}
SAMPLE	VEGETABLES			
	Strain	CFU/25 g	Strain	CFU/25 g
V1	*E. coli* O26:H11	38		
V2	*E. coli* O145:NM	8		
V3			*E. coli* O111:NM	0.9×10^{2}
V4			*E. coli* O103:H2	1.5×10^{2}
V5			*E. coli* O26:H11	2.8×10^{2}
V6			*E. coli* O103:H2	1.5×10^{2}
			E. coli O45:H-	1.0×10^{2}
V7	*E. coli* O26:H11	38	*E. coli* O121:H19	1.2×10^{2}
V8	*E. coli* O121:H19	22		
V9	*E. coli* O103:H2	18	*E. coli* O145:NM	2.2×10^{2}
V10	*E. coli* O111:NM	13		

Table 3
Non-*E. coli* and non-toxigenic *E. coli* strains and bacterial inoculum used in meat and vegetable samples for the specificity analysis.

Strain	CFU/25 g
Citrobacter freundii	2.3×10^{4}
E. coli ATCC 25922	2.1×10^{4}
E. coli O157:H-	1.8×10^{4}
E. coli O119:H25 (*eae*)	6.1×10^{3}
E. coli O130:H11 (stx_1, stx_2)	1.9×10^{4}
Escherichia hermanii	5.0×10^{3}
Proteus spp.	4.5×10^{4}
Pseudomonas aeruginosa	1.1×10^{4}
Salmonella Derby	3.4×10^{4}

Table 4
Non-O157 strains and bacterial inoculum used in meat and vegetable samples for the repeatability analysis.

Strain	CFU/25 g
E. coli O145:NM (stx_2, *eae*)	2.2×10^{2}
E. coli O26:H11 (stx_1, *eae*)	2.8×10^{2}
Negative	

3. Results

3.1. Screening for stx and eae genes

The results obtained with Assurance GDS® GENE-UP®, Pall GeneDisc® System, Foodproof® and RapidFinder™ had 100% sensitivity and 100% specificity for the *stx* and *eae* genes in both matrices. As expected, 100% repeatability was obtained in triplicate samples.

Both genes were detected correctly in all meat samples analyzed with PATHfinder. However, a false negative (93.8% sensitivity) was detected in vegetable samples (a matrix not validated with this kit), whereas specificity was 100%. Again, repeatability was 100%. Results of *stx* and *eae* screening are presented in Supplementary Table 1.

3.2. Detection of STEC serogroups

Four kits were evaluated for the detection of O26, O45, O103, O111, O121, O145 serogroups. The results obtained are presented in Supplementary Table 2.

Assurance GDS® uses an immunomagnetic separation (IMS) step with PickPen® prior to specific PCR performance. The analysis of meat and vegetable samples resulted in 100% sensitivity and specificity.

GENE-UP® is used in combination with other equipment. Thus, an immunoconcentration step of the culture broth in the VIDAS® equipment is followed by PCR for the individual detection of each serogroup. The analysis of meat and vegetable samples resulted in 100% sensitivity and 100% specificity.

Pall GeneDisc® System is based on PCR for the detection of serogroups, combining the detection of virulence factors of each serogroup with the presence of the *eae* variant gene for each. Both vegetable and meat samples had 93.7% sensitivity and 100% specificity with this method.

The results obtained with RapidFinder™ were not considered for the statistical analysis, because it reports the detection of one serogroup (O26, O45, O103, O111, O121 and O145), without specifying which. The samples contaminated with STEC were detected as positive without discriminating the serogroup. This kit presented 100% specificity.

All the results obtained were confirmed with ISO 13136:2012 (ISO, 2012). Results of the concordance (Kappa coefficient), sensitivity and specificity analyses of the tests carried out with each kit are shown in Tables 5 and 6.

4. Discussion

Shiga toxin-producing *Escherichia coli* are important pathogens transmitted by water and food (Torres et al., 2018). Thus, systems for STEC detection in food should have increasingly higher sensitivity and specificity.

In this study, we compared six commercial systems and evaluated their sensitivity, specificity and repeatability against two food matrices associated with risk of STEC-induced illness. All the kits could detect native STEC strains from Argentina, which were used to mimic real practice in food analysis laboratories of the region.

The detection of *stx* and *eae* genes in meat samples was highly satisfactory with kits having an international or inter-laboratory validation with this matrix. The screening results obtained with Assurance GDS®, Pall GeneDisc® System and RapidFinder™ coincided with previously published works (Beutin et al., 2009; Cloke et al., 2016; Feldsine et al., 2016; Fratamico and Bagi, 2012; Fratamico et al., 2017)

Table 5
Results of the statistical analysis in meat samples with the different methods tested.

	Kappa			Sensitivity (%)			Specificity (%)	
		LL[a]	UL[a]		LL	UL		LL UL
Screening								
Assurance GDS®	0.966	0.899	1	100			100	
Foodproof®	0.966	0.899	1	100			100	
GENE-UP®	0.966	0.899	1	100			100	
Pall GeneDisc® System	0.966	0.899	1	100			100	
PATHfinder	0.966	0.899	1	100			100	
RapidFinder™	0.966	0.899	1	100			100	
Serogroups								
Assurance GDS®	0.966	0.899	1	100			100	
GENE-UP®	0.966	0.899	1	100			100	
Pall GeneDisc® System	0.931	0.798	1	93.7	81.9	100	100	
RapidFinder™	0.966	0.899	1	100			100	

[a] LL: Lower limit. UP: Upper limit.

Table 6
Results of the statistical analysis in vegetable samples with the different methods tested.

	Kappa			Sensitivity (%)			Specificity (%)	
		LL[a]	UL[a]		LL	UL		LL UL
Screening								
Assurance GDS®	0.966	0.899	1	100			100	
Foodproof®	0.966	0.899	1	100			100	
GENE-UP®	0.966	0.899	1	100			100	
Pall GeneDisc® System	0.966	0.899	1	100			100	
PATHfinder	0.931	0.798	1	93.8	81.9	100	100	
RapidFinder™	0.966	0.899	1	100			100	
Serogroups								
Assurance GDS®	0.966	0.899	1	100			100	
GENE-UP®	0.966	0.899	1	100			100	
Pall GeneDisc® System	0.863	0.682	1	87.5	71.3	100	100	
RapidFinder™	0.966	0.899	1	100			100	

[a] LL: Lower limit. UP: Upper limit.

and the certified validation of each kit. The results obtained with GENE-UP® and Foodproof® were in accordance with their respective certified validation. PATHfinder was not validated in vegetables, obtaining deviations when determining sensitivity. The type of food and the processing applied affect the competence of the methods as well as the target microorganisms that are expected to be present; hence, validation according to the matrix to be considered becomes important (Jasson et al., 2010).

Considering that recent outbreaks have been associated with fresh products with a short shelf-life, results should be obtained as quickly as possible (Feng, 2014). All the kits tested in the present study met the minimum requirements proposed by FSIS for STEC detection, such as validated for testing relevant foods, fit for the intended purpose and application and performed per the conditions of the validated protocol by a laboratory that assures the quality of the analytical results (FSIS, 2019). However, screening results, as well as whether all the virulence genes identified are in the same viable microorganism should be confirmed by isolation (Fratamico and Bagi, 2012).

Some STEC isolation methods are currently available to identify contaminated food products (FDA, 2018; ISO, 2012; MLG 5C.00, 2019). Brusa et al. (2016) reported that there is no single method or combination of isolation methods capable of identifying all STEC serogroups. Accordingly, it would be necessary not only to combine multiple bacteriological tools but also to adapt the most appropriate set of techniques based on the regional prevalence of STEC. As many pathogens, STEC are usually present in a low number of cells in food samples, so that their isolation can be optimized using a previous step of serogroup-specific immunoconcentration (Rivas et al., 2015). One drawback is that the methods for detecting non-O157 serogroups have not yet been refined as compared with those used for O157 serogroup screening (Hallewell et al., 2017; Kraft et al., 2017). Some authors have described difficulties in isolating certain STEC serogroups (Feldsine et al., 2016; Fratamico et al., 2017; Hallewell et al., 2017). In the present study, all the serogroups could be isolated using the ISO 13136:2012 (ISO, 2012) methodology. However, it should be mentioned that we used controlled contaminations with non-stressed strains.

In this work, four different kits for serogroup identification were evaluated, obtaining satisfactory results according to the manufacturers' indications. The GENE-UP® system in combination with IMS using VIDAS® yielded the expected results, without delivering crossed reactions among serogroups. In the case of Assurance GDS® and Pall GeneDisc® System, they only produced crossed results between two O-groups. The RapidFinder® kit indicated the presence of any of the serogroups, without accurately determining which one. In these cases, it is necessary to carry out the IMS of serogroups to identify which is actually present in the sample (Cloke et al., 2016; Fratamico et al., 2017). The satisfactory results obtained with Assurance GDS® and RapidFinder® in the detection of STEC serogroups coincided with previously published works by various authors (Cloke et al., 2016; Feldsine et al., 2016; Fratamico et al., 2017). With respect to the deviations observed in serogroup detection using Pall GeneDisc® System, some authors described similar results, although in relation to serogroups different from those identified in the present work. Probably, their detection was prevented by the possibility of sharing the virulence genes chosen to carry out the PCR (Beutin et al., 2009) or the possibility of losing the specific *eae* genes (Fratamico and Bagi, 2012).

Zhao et al. (2014) suggested that pre-concentration (filtration, size fractionation, centrifugation or IMS) is the preferred option, since it can increase sensitivity several times by increasing the number of target organisms per unit volume. These benefits were reflected in the results obtained in this trial, since 100% sensitivity and specificity were obtained in equipments performing IMS before PCR.

The offer of rapid detection methods is broad and contributes to facing regulatory pressures. Some agencies such as FSIS (FSIS, 2019) mention the use of a particular commercial system, not being a requirement in itself, since they also offer second options that they themselves use and can better adapt to the particular conditions of each laboratory. In general, an ideal method must be sensitive, rapid and specific (Mangal et al., 2015). In addition, the following variables should be considered when choosing a new equipment or kit: cost, standardization process and validation (robustness, reproducibility, negative predictive value, positive predictive value, concordance), food matrix target, consumption and maintenance of reagents, labor cost, operating procedure, time to obtain the final results, number of samples analyzed simultaneously, biosecurity in the laboratory, among others (Chiueh et al., 2001; Jasson et al., 2010; Leotta, 2009). When deciding which kit to use, it is important to consider the speed and certainty of the results, for example, the release of a batch of product.

All six commercial methods evaluated in this study were adequate for meat analysis. In case of disadvantages when analyzing vegetable samples, the corresponding kit was not validated for this matrix. The challenge for microbiology laboratories is therefore to find advantages and disadvantages based on a clear knowledge of the particular needs of each laboratory adopting a rapid method for STEC detection in food. Although the results obtained with the different diagnostic kits indicated an excellent discriminatory potential, further studies should be carried out with larger sample sizes and using additional negative controls and samples with lower concentrations, as well as naturally contaminated samples.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.103273.

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