

# Multicenter Collaborative Trial Evaluation of a Method for Detection of Human Adenoviruses in Berry Fruit

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**Abstract** The qualitative performance characteristics of a qPCR-based method to detect human adenoviruses in raspberries were determined through a collaborative trial involving 11 European laboratories. The method incorporated a sample process control (murine norovirus) and an internal amplification control. Trial sensitivity or correct identification of 25-g raspberry samples artificially contaminated with between  $5 \times 10^2$  and  $5 \times 10^4$  PFU was 98.5%; the accordance and concordance were 97.0%. The positive predictive value was 94.2%. The trial specificity or percentage correct identification of non-artificially contam-

inated samples was 69.7%; the accordance was 80.0% and the concordance was 61.7%. The negative predictive value was 100%. Application of a method for the detection of human adenoviruses in food samples could be useful for routine monitoring for food safety management. It would help to determine if a route of contamination exists from human source to food supply chain which pathogenic viruses such as norovirus and hepatitis A virus could follow.

**Keywords** Human adenovirus · Multicenter ring trial · Detection

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## Introduction

There have been numerous outbreaks of disease caused by the consumption of berry fruits contaminated with enteric viral pathogens. The World Health Organisation (FAO/WHO 2008) identified norovirus and hepatitis A virus in fresh produce including berry fruits as a priority virus/commodity combination for which control measures should be considered. In the food industry, the major concepts such as HACCP have been directed at bacterial and fungal pathogens only. Equally as importantly, microbiological monitoring methods are used mainly at the end of the production chain. Also, analysing the impact of virus contamination of food has hitherto been based on gathering epidemiological information, which occurs only in response or as a reaction to disease outbreaks, and a coordinated and validated system or network does not yet exist to routinely and proactively monitor actual food samples. It is essential for thorough food safety management that systems are developed whereby viruses can be monitored at critical points throughout food supply chains.

But performing routine monitoring specifically for norovirus and hepatitis A virus may not actually be worthwhile. These viruses may be present as contaminants only very sporadically, or during outbreaks, and might be seldom detected even when food supply chains are vulnerable to contamination. It would be more effective to monitor for agents that would indicate that a route exists from source to points within the food supply chain which norovirus and hepatitis A viruses could follow to cause contamination. Adenoviruses infect both humans and a wide variety of animal species; they are shed in large numbers in the faeces of infected individuals (Granoff and Webster 1999) and are capable of robust survival (Rzeżutka and Cook 2004). Adenoviruses have been shown to be excreted by the populations of all geographical areas and to be the most abundant viruses detected in urban sewage without significant seasonal variation, and for these reasons have been proposed as indicators of human faecal contamination in water and food (Pina et al. 1998; Formiga-Cruz et al. 2002). Specific detection of adenoviruses from human or animal origin should be a useful tool for tracing the source of faecal viral contamination (Maluquer de Motes et al. 2004). Recent studies on the detection of human adenovirus in wastewater (Bofill-Mas et al. 2006), drinking water treatment plants (Albinana-Gimenez et al. 2009) and in recreational waters in Europe (Wyn-Jones et al. 2011) have shown their wide dissemination and support their applicability as indicators of faecal contamination. The European Framework 7 project “Integrated monitoring and control of foodborne viruses in European food supply chains (VI-TAL)” adopted the use of human adenoviruses as “index viruses” whose presence in a food supply chain such as that

for berry fruits will indicate, not specifically the presence of pathogenic virus types, but that a route of contamination exists from source to monitoring point which pathogenic viruses could follow. The study described here was conducted to test the robustness of a polymerase chain reaction (PCR) (qPCR<sup>1</sup>)-based method for detecting human adenoviruses in berry fruits, using raspberries as an example. The method incorporates a sample process control and an internal amplification control to verify its correct operation (D’Agostino et al. 2011).

## Materials and Methods

*Participating Institutes* The Food and Environment Research Agency (FERA), UK led the trial. Eleven laboratories from nine EU member states participated in the trials. They comprised the Veterinary Laboratories Agency (UK), Veterinary Research Institute (Czech Republic), University of Patras (Greece), University of Helsinki (Finland), Istituto Superiore di Sanita (ISS) (Italy), National Institute for Public Health and the Environment (the Netherlands), Wageningen University Research (the Netherlands), National Veterinary Research Institute (Poland), Scientific Veterinary Institute Novi-Sad (Serbia), Instituto Tecnológico Agrario de Castilla y León (ITACyL) (Spain) and University of Barcelona (Spain). Each participant was provided with a personalised standard operating procedure (SOP) for performance of this trial.

*Viruses* Human adenovirus (HAdV) serotype 2, used as the target virus in the trial, was kindly provided by Professor Rosina Girones of the University of Barcelona. It was propagated at ISS for six sequential passages in cultures of A549 cells (European Collection of Cell Culture, UK) and titrated by plaque assay, yielding stock titers of approximately  $4 \times 10^7$  plaque-forming units (PFU) ml<sup>-1</sup>. Murine norovirus (MNoV), used as the sample process control (SPCV) in the trial (Diez-Valcarce et al. 2011b), was obtained from Washington University Medical School of St. Louis. It was propagated for six sequential passages in cultures of RAW 267.4 cells (American Type Culture Collection). It was titrated by plaque assays, yielding stock titers of approximately  $10^8$  PFU ml<sup>-1</sup>. All virus stock suspensions were prepared by ISS.

*Trial Materials* Trial materials were prepared at the ISS by FERA staff, who coded each vial and alone knew the identity of the contents. There were nine coded vials, three of which contained  $100 \mu\text{l } 1 \times 10^6$  PFU ml<sup>-1</sup> HAdV (HIGH) suspension, three containing  $100 \mu\text{l } 1 \times 10^4$  PFU ml<sup>-1</sup>

<sup>1</sup> The term “qPCR” is used for qPCR throughout this article, in accordance with the recommendations of Bustin et al. (2009).

HAdV (LOW) suspension and three containing only cell culture medium (BLANK) were sent to each participant. Each participant was also sent one vial containing  $100 \mu\text{l}$  of  $5 \times 10^7$  PFU  $\text{ml}^{-1}$  MNoV (SPCV) suspension.

**Preparation of Trial Samples** Fresh raspberries were purchased separately by each participant from local sources. Nine 25-g raspberries portions were placed into plastic disposable weighing boats or similar receptacles. Three portions were artificially contaminated with  $5 \times 10^4$  PFU HAdV by pipetting  $5 \times 10 \mu\text{l}$  of the HIGH suspension onto the surface of the raspberries. Three portions were artificially contaminated with  $5 \times 10^2$  PFU HAdV by pipetting  $5 \times 10 \mu\text{l}$  of the LOW suspension onto the surface of the raspberries. Three portions were spiked with cell culture medium by pipetting  $5 \times 10 \mu\text{l}$  of the BLANK suspension onto the surface of the raspberries. All samples were left at room temperature for approximately 2 h until the suspending fluid was almost dry, and then processed following the method of Dubois et al. (2002). Immediately prior to commencing the process, all samples were spiked with  $1 \times 10^5$  PFU murine norovirus by pipetting  $10 \mu\text{l}$  of the SPCV suspension onto the surface of the raspberries.

**Extraction of Virus Nucleic Acids from Raspberries** The sample was processed using the method of Dubois et al. (2002). Approximately 25 g fruit was placed in a sterile beaker. Forty milliliters of Tris–glycine pH 9.5 buffer containing 1% beef extract and 6,500 U pectinase (e.g. Pectinex™ Ultra SPL solution, Sigma) was added to the sample, which was then agitated at room temperature for 20 min by rocking at 60 rpm. The pH was maintained at 9.0 throughout (if necessary adjusting using 4% w/v sodium hydroxide, extending the period of agitation by 10 min each time an adjustment was made. In strongly coloured berries, a change in colour of the eluate from blue/purple to red was considered indicative of acidification and was used to trigger pH adjustment). The liquid was decanted from the beaker through a strainer (e.g. a tea strainer) into one 50 ml or two smaller centrifuge tubes and centrifuged at  $10,000 \times g$  for 30 min at 4 °C. The supernatant was decanted into a single clean tube or bottle, and the pH was adjusted to 7.2. Volumes (0.25) of 50% (w/v) polyethylene glycol 8,000/1.5 M NaCl were then added and mixed by shaking for 1 min. The suspension was then incubated with gentle rocking at 4 °C for 60 min before centrifugation at  $10,000 \times g$  for 30 min at 4 °C. The supernatant was discarded, and the pellet was compacted by centrifugation at  $10,000 \times g$  for 5 min at 4 °C before resuspension in 500  $\mu\text{l}$  PBS. The suspension was then transferred to a chloroform-resistant tube, and 500  $\mu\text{l}$  1:1 chloroform:butanol (v:v) was added and mixed by vortexing. The sample was allowed to stand for 5 min and then centrifuged at  $10,000 \times g$  for

15 min at 4 °C. The aqueous phase was transferred to a clean tube and immediately used for nucleic acid extraction or stored at –20 °C. Nucleic acids were extracted using a NucliSENS® miniMAG® kit (bioMérieux) according to the manufacturer's instructions. The final elutions were performed with 100  $\mu\text{l}$  elution buffer, resulting in a 200- $\mu\text{l}$  nucleic acid extract. The nucleic acid extract was assayed immediately or stored at –70 °C. The extract was diluted to  $10^{-1}$  in nuclease-free water before assaying.

**Adenovirus qPCR** This assay was a duplex qPCR using the primers and conditions described by Hernroth et al. (2002), with the inclusion of an internal amplification control (IAC) (Diez-Valcarce et al. 2011a) and a carryover contamination prevention system utilising uracil N-glycosylase. The reaction contained 1  $\times$  TaqMan Universal PCR Master Mix (Applied Biosystems), 0.9  $\mu\text{M}$  each primer, 0.225  $\mu\text{M}$  adenovirus TaqMan probe (labelled with FAM), 50 nM IAC probe (labelled with VIC) and 100 copies of adenovirus IAC (Yorkshire Bioscience Ltd., UK). Ten microliters of the diluted nucleic acid extract was added to make a final reaction volume of 25  $\mu\text{l}$ . The thermocycling conditions were 2 min at 50 °C then 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C. Two PCR replicates were performed for each sample. In each PCR run, positive and negative amplification controls were included.

**Murine Norovirus Reverse Transcription qPCR (RTqPCR)** This assay was a one-step duplex reverse transcription qPCR using the primers and conditions described by Baert et al. (2008), with the inclusion of an IAC (Diez-Valcarce et al. 2011a, b). The reaction contained 1  $\times$  RNA Ultrasense reaction mix (Invitrogen), 0.2  $\mu\text{M}$  each primer, 0.2  $\mu\text{M}$  probe MGB-ORF1/ORF2 (labelled with FAM), 50 nM IAC probe (labelled with VIC), 1  $\times$  ROX reference dye (Invitrogen), 1  $\mu\text{l}$  RNA Ultrasense enzyme mix (Invitrogen) and 600 copies of murine norovirus IAC (Yorkshire Bioscience Ltd., UK). Ten-microliter sample of the diluted nucleic acid extract was added to make a final reaction volume of 20  $\mu\text{l}$ . The thermocycling conditions were 15 min at 50 °C, 2 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Two RTqPCR replicates were performed for each sample. In each run, positive and negative amplification controls were included.

**Definition of Analytical Method** In the frame of this collaborative trial, the analytical method is defined as the sample treatment (which includes virus extraction and concentration, and nucleic acid purification) coupled to the nucleic acid amplification assays for the target and the sample process control virus. Equally, a nucleic acid amplification assay is defined as a nucleic acid amplification reaction which contains an IAC.

**Reporting and Interpretation of Data** Raw data were reported by each participant to the trial leader, who translated the codes and analysed the data in collaboration with ITACyL. When an assay showed a quantification cycle (Cq, previously known as the threshold cycle) value  $\leq 40$  or 45 for murine norovirus or adenovirus respectively independently of the corresponding IAC Cq value, the result was interpreted as positive. When an assay showed a Cq value  $\geq 40$  or 45 for murine norovirus or adenovirus respectively with the corresponding IAC Cq value  $\leq 40$  or 45 for murine norovirus or adenovirus respectively, the result was interpreted as negative. When an assay showed both the target and its corresponding IAC Cq values  $\geq 40$  or 45, the reaction was considered to have failed. When a participant reported that at least one of the replicate HAdV assays was positive, they were considered to have identified the sample as being adenovirus contaminated. When a participant reported that both replicate HAdV assays were negative, but at least one replicate MNoV assay was positive, they were considered to have identified the sample as being adenovirus uncontaminated. When a participant reported that both replicate HAdV assays had failed, independently of the results of the MNoV assays, they were considered to have reported that the analysis of that sample had failed. When a participant reported that both replicate HAdV assays were negative and both replicate MNoV assays were negative, they were considered to have reported that the analysis of that sample had failed. Interpretation of the results followed the principles outlined by D'Agostino et al. (2011).

**Criteria for Inclusion of Results in the Statistical Analysis** The results from each participating laboratory were included unless they fell into one of the following two categories: (1) obvious performance deviation from the SOP and (2) presence of target amplicons in the negative amplification controls, indicating contamination of the reaction.

**Qualitative Statistical Analysis** The raw data sent by each laboratory were statistically analysed according to the recommendations of Scotter et al. (2001) and by the methods of Langton et al. (2002). The *diagnostic sensitivity* of the analytical method was defined as the percentage of positive samples giving a correct positive signal, i.e. using only the results of the analysis of the artificially contaminated samples. The *diagnostic specificity* of the analytical method was defined as the percentage of negative samples giving a correct negative signal, i.e. using only the results of the analysis of the non-artificially contaminated samples. *Accordance* (repeatability of qualitative data) was defined as the percentage chance of finding the same result, positive or negative, from two identical samples analysed in the

same laboratory under predefined repeatability conditions, and *concordance* (reproducibility of qualitative data) was defined as the percentage chance of finding the same result, positive or negative, from two identical samples analysed in different laboratories under predefined repeatability conditions. These calculations take into account different replication in different laboratories by weighting results appropriately. The *concordance odds ratio* (COR) was the degree of inter-laboratory variation in the results and expressed as the ratio between accordance and concordance percentages (Langton et al. 2002). The COR value may be interpreted as the likelihood of getting the same result from two identical samples, whether they are sent to the same laboratory or to two different laboratories. The closer the value is to 1.0, the higher the likelihood is of getting the same result. Confidence intervals for accordance, concordance and COR were calculated by the method of Davison and Hinckley (1997); each laboratory was considered representative of all laboratories in the “population” of laboratories, not just those participating in this analysis.

The positive predictive value of the analytical method is the proportion of the correctly identified contaminated samples. The negative predictive value of the analytical method is the proportion of the correctly identified uncontaminated samples, from all the samples reported as adenovirus uncontaminated. These values were calculated by the ISO 16140 method (Anonymous 2003).

## Results

**Participants' Results in the Collaborative Trial** Table 1 shows the participants' results from the analysis of raspberry samples artificially contaminated with  $5 \times 10^4$  PFU. All samples were correctly reported as contaminated, except in one case where the analysis of a sample had failed. Table 2 shows the participants' results from the analysis of raspberry samples artificially contaminated with  $5 \times 10^2$  PFU human adenovirus. Laboratory “4” did not perform analysis of the LOW artificially contaminated test samples. All samples were correctly reported as contaminated. Table 3 shows the participants' results from the analysis of the non-artificially contaminated raspberry samples. Here, four samples were reported as contaminated. Six sample analyses had failed.

**Qualitative Statistical Analysis** Table 4 gives the diagnostic specificity, diagnostic sensitivity, positive and negative predictive values, accordance and concordance values and the concordance odds ratio for the collaborative trial of the analytical method for the detection of human adenovirus on raspberries. The results of the analysis of the uncontami-

**Table 1** Participants’ results from the analysis of raspberry samples artificially contaminated with  $5 \times 10^4$  PFU human adenovirus (HIGH)

Laboratory	Sample A					Sample B					Sample C				
	HAdV		MNoV			HAdV		MNoV			HAdV		MNoV		
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.
1	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
2	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
3	+	+	+	+	C	F	+	-	+	C	F	F	-	-	AF
4	+	+	-	-	C	+	+	-	+	C	+	+	-	+	C
5	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
6	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
7	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
8	+	+	-	-	C	+	+	-	-	C	+	+	-	-	C
9	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
10	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
11	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C

*HAdV* human adenovirus, *MNoV* murine norovirus, *Rep.* replicate qPCR, *Int* interpretation, + target signal present, IAC signal present or absent, - target signal absent, IAC signal present, *F* target signal absent, IAC signal absent, *C* sample contaminated, *AF* analysis failed

nated samples by laboratory “8” were excluded because all their analyses failed.

**Discussion**

The method under trial proved capable of detecting adenoviruses in berry fruit at a level of at least  $10^2$  PFU per 25 g in artificially contaminated samples. Out of 66 samples analysed, only 1 had failed. This was due to the failure of the sample process as judged by the absence of a

signal from the SPCV in conjunction with the failure of the HAdV qPCR in both replicates. The statistical procedure used to analyse the trial results does not discriminate between negative results and failed analyses; it has been used several times to analyse the results of collaborative trials of PCR-based methods (Abdulmawjood et al. 2004; D’Agostino et al. 2004; Josefsen et al. 2004; Malorny et al. 2004; Wyn-Jones et al. 2011), but it would be advantageous to modify it for future similar studies. In some samples, other controls had failed, but overall, the samples could be legitimately reported as positive for HAdV. And the trial sensitivity was still very high, at 98.5%, which indicates

**Table 2** Participants’ results from the analysis of raspberry samples artificially contaminated with  $5 \times 10^2$  PFU human adenovirus (LOW)

Laboratory	Sample A					Sample B					Sample C				
	HAdV		MNoV			HAdV		MNoV			HAdV		MNoV		
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.
1	+	+	+	+	C	+	+	+	+	C	+	+	+	-	C
2	+	+	+	+	C	+	+	+	+	C	+	-	+	+	C
3	+	+	-	-	C	+	+	-	-	C	+	+	F	-	C
5	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
6	+	+	+	+	C	+	+	+	+	C	+	+	F	+	C
7	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
8	+	+	-	-	C	+	+	-	-	C	+	+	-	-	C
9	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
10	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C
11	+	+	+	+	C	+	+	+	+	C	+	+	+	+	C

*HAdV* human adenovirus, *MNoV* murine norovirus, *Rep.* replicate qPCR, *Int* interpretation, + target signal present, IAC signal present or absent, - target signal absent, IAC signal present, *F* target signal absent, IAC signal absent, *C* sample contaminated, *AF* analysis failed

**Table 3** Participants' results of the analysis of the non-artificially contaminated raspberry samples

Laboratory	Sample A					Sample B					Sample C				
	HAdV		MNoV			HAdV		MNoV			HAdV		MNoV		
	Rep.1	Rep. 2	Rep. 1	Rep. 2	Int.	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Int.
1	–	–	+	+	UC	–	–	+	+	UC	–	–	+	+	UC
2	–	–	+	+	UC	–	–	+	+	UC	–	–	+	+	UC
3	+	+	+	+	C	–	–	+	+	UC	F	F	–	–	AF
4	+	–	F	–	C	–	–	–	–	AF	–	–	–	–	AF
5	–	–	+	+	UC	–	–	+	+	UC	–	+	+	+	C
6	–	–	+	+	UC	–	–	+	+	UC	–	–	+	+	UC
7	–	–	+	+	UC	–	–	+	+	UC	–	–	+	+	UC
8	F	F	–	–	AF	F	F	–	–	AF	F	F	–	–	AF
9	–	–	+	+	UC	–	–	+	+	UC	+	+	+	+	C
10	–	F	+	+	UC	–	F	+	+	UC	F	–	+	+	UC
11	–	–	+	+	UC	–	–	+	+	UC	–	–	+	+	UC

*HAdV* human adenovirus, *MNoV* murine norovirus, *Rep.* replicate qPCR, *Int* interpretation, + target signal present, IAC signal present or absent, – target signal absent, IAC signal present, *F* target signal absent, IAC signal absent, *C* sample contaminated, *AF* analysis failed, *UC* uncontaminated

that the method can be used confidently to detect the presence of human adenovirus in berry fruits.

With the non-artificially contaminated samples, six analyses were reported to have failed. This highlights the value of an interlocking suite of controls when performing routine nucleic acid-based analysis for detection of viruses in foods, as they allow appropriate actions to be identified which should result in accurate reanalysis of failed tests (Bosch et al. 2011; D'Agostino et al. 2011; Rodríguez-Lázaro et al. 2007). It is unclear why the failed tests occurred in the trial, but they left 23 out of 33 samples being reported as uncontaminated, and this skewed the trial specificity to a lower value than that which has been observed in other trials (Abdulmawjood et al. 2004; D'Agostino et al. 2004; Josefsen et al. 2004; Malorny et al. 2004; Wyn-Jones et al. 2011). This proportion may not accurately reflect the actual number of false positives which might be expected in routine application of the current method, where analyses should not be expected to fail so often. The variability of results between laboratories here also affected the concordance and

concordance and the concordance odds ratios; however, the confidence intervals of each indicate that if the method was adopted by a wider selection of laboratories there would be a possibility of more uniform results. The negative predictive value of the method is excellent, as none of the artificially contaminated samples were reported as uncontaminated.

Four of the non-artificially contaminated samples were reported as contaminated with adenovirus. As a result, the trial specificity and the positive predictive value indicate that a proportion of false-positive results can be expected when using this method. However, a possible explanation is that the fruit used for these samples had in fact been contaminated with human adenovirus prior to purchase, and the positive results were not actually false. The method described in this study has been subsequently used to analyse berry fruit at point-of-sale in several European countries, and some of these samples have been positive for human adenovirus. It is recommended that any positive target amplicons are sequenced to confirm target identity when performing actual analysis of produce.

**Table 4** Statistical evaluation of the data obtained from the collaborative trial

Contamination level	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value	Accordance (%)	Concordance (%)	Concordance odds ratio (COR)
LOW + HIGH	98.5 (91.9, 99.7)	–	94.2 (86.0, 97.7)	–	97.0 (90.9, 100)	97.0 (91.2, 100)	1.0 (0.96, 1.0)
None	–	69.7 (52.7, 82.6)	–	100 (100, 100)	80.0 (60, 100)	61.7 (46.9, 93.3)	2.48 (0.85, 16.48)

Values in parentheses are the lower and upper 95% confidence intervals

The qPCR HAdV assay used in this study could be applied for quantitation of the target virus by estimating the number of HAdV genome copies based on an external standard. However, when the partners' results were converted into genome copies detected per sample (not shown), the level of between-laboratory variation was too great to be able to describe the performance characteristics of the method in quantitative terms. This is despite the fact that nucleic acid standard solutions were supplied along with the trial materials. The high between-laboratory variation may be caused by several factors, such as the condition in which the standard solutions have reached the partner institutes or operational differences between thermocyclers used in the various laboratories. These possibilities highlight a requirement for reliable reference materials and external quality control systems to be available, if routine monitoring of food supply chains for viruses is to be adopted efficiently.

Notwithstanding the above issues, the overall results of the collaborative trial were considered to show that the qPCR-based method for the detection of human adenoviruses in soft fruits was acceptably robust. The method was then employed within the VITAL project on gathering data on virus presence in various food supply chains. Forthcoming results (manuscripts in preparation) of this data gathering will reveal the usefulness of the index virus approach, and the information gained should assist consideration of measures which can be applied to block routes of virus contamination. The method described and tested in this study is a building block in the foundation of future systems for integrated monitoring and control of viruses in food supply chains.

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