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RESEARCH

Navigating the UKAS Accreditation Process for Detecting Norovirus and Hepatitis A Virus in Fresh and Frozen Produce: a Case Study

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Abstract

Given the urgent requirement for more laboratories to develop proficiency in detecting foodborne viruses, this case study charts the path to accreditation, demystifying the process of validating a method for detecting norovirus and hepatitis A virus in fresh produce. Securing accreditation is crucial to ensuring dependable and precise food analysis, particularly relevant for products frequently consumed raw, which are at risk of contamination by foodborne viruses. The study provides an in-depth look at the stringent procedures integral to achieving precision and dependability in results, underscoring the pivotal role of competency checks involving artificial contamination of samples. The case study also navigates the integral role of both external and internal quality assurance processes in affirming the consistency and accuracy of laboratory testing methods. The findings of this case study are transformative, amplifying confidence in laboratory results and potentially catalysing improvements in public health by ensuring accurate virus detection and identification in food samples. Furthermore, the accreditation process, as detailed in this case study, could pioneer a path for other laboratories, fostering best practices in virus detection and identification.

Keywords Norovirus · Hepatitis A virus · Accreditation · Food safety · Molecular detection

Introduction

The United Kingdom Accreditation Service (UKAS) is the national accreditation body in the UK appointed by the government to assess and accredit organizations that provide services including certification, testing, inspection, and calibration. UKAS accreditation covers biological, microbiological, and biochemical testing and measurement, including the examination of plant material, animal health, food, drugs, and pharmaceuticals. This case study explores the journey towards achieving UKAS accreditation for a method for the detection of norovirus and hepatitis A virus in fresh and frozen produce, based on ISO/TS 15216-2:2013 "Microbiology of the food chain—Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR—Part 2: Method for detection" (Anonymous 2013). The TS, or technical specification, designation indicates that the document was highly specialised with a limited application scope, but it provided an important foundation for the development of the later ISO15216-2:2019 standard which is now fully published (Anonymous 2019). The 2019 version of the ISO15216-2 standard introduced several changes to the methods and terminology used in the 2013 version. These changes included:

(i) A requirement to use a suitable buffer for the dilution of control materials was added.
(ii) The method for generating process control virus RNA for the standard curve was changed.
(iii) Breakpoints with defined temperature and time parameters in the extraction methods were added.
(iv) The terminology was changed from amplification efficiency to RT-PCR inhibition.
(v) Extra real-time RT-PCR reactions for sample RNA and negative controls were added.
(vi) Method characteristics and the results of method validation studies were included.

These changes resulted in a standard that was not only more precise but also provided more comprehensive

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information; however, in technical terms of how the method was to be performed, there was little change between this new version and the 2013 version.

The insights gleaned from this case study could be beneficial to laboratories worldwide seeking to implement similar methodologies and meet their local accreditation requirements.

Background

In recent years, there has been a decline in the number of laboratories capable of conducting virological analyses of foods, both in the UK and globally. This, coupled with the fact that existing laboratories often specialise in specific food products such as shellfish, highlights the need for expanding capabilities in diverse food categories. This case study is based on the method established at Campden BRI (Chipping Campden, GL55 6LD, UK), which, in 2018, became the first laboratory in the UK to receive UKAS accreditation for detecting norovirus and hepatitis A virus in fresh and frozen produce analysis. Although this laboratory has since rescinded its UKAS accreditation status for virus analysis in foods, the lessons from the experience remain crucial. Over a 6-year period, extensive use of the method was conducted on a wide variety of foodstuffs. The focus was predominantly on the detection of norovirus GI/GII and hepatitis A virus, as the extraction process for these viruses is identical regardless of the target virus to be detected. The products tested ranged from fresh or frozen berry fruits, such as raspberries, blueberries, and strawberries, to leafy green salad vegetables, including open-headed lettuce types (including butterhead and romaine) and spinach. These food items are frequently enjoyed uncooked in salads, as healthy snacks, or used in the preparation of desserts, garnishes, or blended drinks. Given that they do not undergo any sterilisation or "kill" process, their importance in relation to food safety is heightened.

The independent accreditation of a method, especially in cases where it has not been previously established, is integral. This allows commercial entities to deliver precise and trustworthy food analyses, which is a fundamental requirement for helping clients ensure the standards of safety and quality of the food products that are consumed are met. The description of the accredited method, including the specific deviations made from the ISO/TS 15216-2:2013 standard method and the justification for these deviations, provides valuable information for other laboratories looking to implement this methodology and achieve UKAS accreditation or its equivalent for the detection of norovirus and hepatitis A virus in fresh and frozen produce. Additionally, it demonstrates the importance of continuously optimising and refining detection methods to ensure the highest level of accuracy and reliability in the food safety analysis.

The Accreditation Standard ISO/IEC 17025: the Foundations

Securing UKAS accreditation for a novel method, which has not been previously accredited in any laboratory, fundamentally necessitates adherence to the ISO/IEC 17025 standard, formally titled "General Requirements for the Competence of Testing and Calibration Laboratories". This standard (available at https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:ed-3:v1:en), provides a robust framework for affirming the technical competence and integrity of the testing methods employed by a laboratory. Compliance with ISO/IEC 17025 is not just beneficial but a critical prerequisite in the pursuit of UKAS accreditation. By adhering to this standard, laboratories demonstrate their capability to produce precise, accurate, and reliable results, thereby meeting the stringent requirements for UKAS accreditation.

Compliance with the management requirements of ISO/IEC 17025 ensures that the laboratory has a well-established and functional quality management system (QMS). The QMS oversees all aspects of the operation, from document control to preventive and corrective actions, essential for maintaining consistency and strict adherence to the operational parameters of the testing procedure. The technical requirements, on the other hand, ensure that all the elements directly affecting the reliability of the test results are up to the mark. These elements include personnel competence, the validity and appropriateness of the methods used, verification that measurements and calibrations conform to national standards, ensuring there is a suitable testing environment, the correct handling of test items, and quality assurance of results.

Achieving UKAS accreditation means successfully meeting all these requirements and demonstrating consistent proficiency and accuracy of the method on an ongoing basis. It is an exhaustive process, requiring the laboratory to produce evidence for its proficiency, competency, and adherence to quality systems. Moreover, accreditation emphasizes the continual improvement approach which is intrinsic to ISO/IEC 17025, encouraging the laboratory to consistently identify and implement enhancements to its system and operations. In essence, aligning with the ISO/IEC 17025 standard allows a laboratory to demonstrate technical competency, robust operational structures, and the ability to generate reliable and accurate results for a testing method.

Gathering the necessary evidence is a crucial step in the accreditation process. It involves providing specific data that attests to conformity with the minimum requirements as set out in the standard method. Even though Campden BRI's approach was based on the ISO15216-2:2013

method, it did not strictly adhere to it (see below). Therefore, the minimum evidence required was definitive proof that the target viruses, namely, norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII), and hepatitis A virus (HAV), could be detected in relevant matrix types using the modified method.

Compliance with a standard does not necessarily mean strict, unvarying adherence. Based on ISO/TS 15216-2:2013, our method incorporated certain deviations aimed at enhancing the efficiency and sensitivity of the detection process. These deviations were not arbitrary but were necessitated by practical experience and the nature of the foodstuffs tested. In the next section, these methodological alterations are detailed, highlighting the rationale behind them and their impact on the overall performance of the method.

Methodological Adaptations: Tailoring the ISO15216-2 Standard for Enhanced Virus Detection

The scope and application of the ISO15216-2 standard in the context of its application to the fresh produce category offer a method for analysing a variety of fresh and frozen berries, as well as certain salad vegetables, for the presence of norovirus and hepatitis A virus. The 2013 version of the ISO15216-2 standard, also known as ISO/TS 15216-2:2013 (Anonymous 2013), provided a method for the qualitative detection of hepatitis A virus (HAV) and norovirus genogroups I (GI) and II (GII) from food samples or surfaces. The TS was followed to the nucleic acid extraction stage, and then instead of performing RT-PCR using in-house prepared reagents as described in the standard method, we used CEERAM commercial kits for RT-PCR detection (ceeramTOOLS® real-time RT-PCR kits, bioMérieux). The primers and probes used in those kits are sold "in conformity" with the ISO/TS 15216-2 standard.

The method describes a process of liberation and concentration of NoV genogroups 1 (GI) and II (GII) and HAV from soft fruit and salad vegetables such as strawberries and raspberries (fresh or frozen) and lettuce; however, the method is not limited to just these commodities—it has been used successfully on other fresh produce types.

The sample treatment comprises five basic steps: (1) sample receipt, (2) removal of viruses from the food surfaces, (3) removal of food solids, (4) concentration of suspended viruses, and (5) extraction/detection of nucleic acids.

Viruses are removed from food surfaces by washing in an alkaline (pH 9.5) buffer containing 1% beef extract, and food solids are removed by centrifugation at 10,000 × *g*. The concentration of suspended viruses is performed using PEG precipitation. The procedure for nucleic acid extraction is left to the discretion of the user. The detection assay is RT-qPCR; in ISO/TS 15216-2, it is employed to give qualitative (presence/absence) results.

Given the immense diversity of berry fruits and salad vegetables available on the market, it is impractical to confirm the effectiveness of the method for every single food type. Therefore, the performance of this method was verified based on representative samples, including fresh strawberries, frozen raspberries, and open-leaf lettuce. The selection of these particular items was made considering the high frequency of outbreaks associated with them (Chatziprodromidou et al., 2018). Moreover, these samples present a challenge for the analyst, making them ideal representatives for both fresh and frozen produce categories. The protocol has been applied to other food types outside of these categories, such as dates, pineapple slices, and fresh seaweed (unpublished). However, these items are not considered within the standard's scope and thus cannot be reported under UKAS conditions.

The working group that developed the ISO15216 standard did not extend its validation to other food types simply due to the enormity (and cost) of the task: validating every potentially at-risk product was impractical. Instead, the group focused on food types that are most likely to be contaminated with foodborne viruses. Therefore, the ISO method primarily covers and has been validated for these high-risk food categories. The validation studies are described in detail in Annex 1 of ISO15216-2:2019.

Deviations from the Standard

The following provides a description of variations to the ISO/TS 15216-2 that we employed within our method, with a justification for each. These deviations arise from the method's sustained implementation over several years, having been applied to a diverse range of fresh and frozen produce samples artificially contaminated with norovirus and hepatitis A virus. The individual circumstances underpinning the decisions to introduce these variations are too extensive to list in detail. The method consistently met or exceeded acceptable result criteria across these varied applications.

In section 8.2.4 of the standard, instructions are provided for the analysis of soft fruit and salad vegetables. Firstly, the standard stipulates a sample size of 25 ± 0.3 g for soft fruits or salad vegetables, which should be chopped to an approximate size of 2.5 × 2.5 × 2.5 cm unless individual fruits are smaller than this. However, instead of chopping the berry fruits, whatever size they were, we analysed them whole. This was done to avoid the release of acidic juices into the buffer used to release viruses from the fruit. The buffer is at a pH of 9.5, and the addition of acidic juices could weaken its action, leading to a lower efficiency of virus displacement

from the fruit surfaces. Furthermore, there is very limited evidence to suggest that viruses can be internalized within fruits or vegetables. Thus, chopping the fruits or vegetables to expose their interiors can inadvertently release inhibitory substances that can affect downstream detection without increasing the detachment of viruses from the surface. Therefore, our sample weight was defined as "25–30 g", excluding larger strawberries and vegetables such as vine tomatoes. This is because some strawberries and tomatoes are in excess of this weight, in which case one whole large fruit was analysed. Where smaller fruits are analysed, it is not possible to get exactly 25 g, so the range between 25 and 30 g allows for smaller whole fruits to be analysed.

Secondly, we found that the ISO15216:2 recommendation of transferring the fruits/vegetables to a 400-ml stomacher mesh filter bag and using a mechanical rocker was ineffective for immersing larger fruits or vegetables in 40-ml buffer, and the composition of the mesh filter led to considerable buffer retention, leading to potential losses of the virus. To overcome this challenge, we employed the use of 250-ml wide-necked pots, which we rolled at a rate of 60 rpm, ensuring effective immersion of the fruits/vegetables in the buffer. We also opted for BagFilter® bags (Fisherbrand™ BagFilter™ S 400 Lateral Pouring Filter Bag) instead of mesh filter stomacher bags to minimize buffer loss. The standard mesh filter stomacher bags have a very large surface area which is absorbent. In normal bacterial analysis methodologies, these mesh filter bags are designed to be squeezed so that the remnants of the fluids are extracted from the mesh filter and removed while keeping the food debris within the mesh bag; however, we do not want to disrupt or damage the sample due to the potential release of inhibitory substances which may be released into the buffer. The BagFilter® bags help us overcome this problem as they have a fabric filter with a porosity of less than 250 microns which is welded to the inside bag along its length, thereby allowing the buffer to pass through to a chamber where it can be decanted without the same level of absorbance as the mesh filter and thus less loss of virus.

The amount of PEG/NaCl solution that should be added to the supernatant after the first clarification/centrifugation is stated as follows: "Add 0.25 volumes of 5 × PEG/NaCl solution (5.3.1) (to produce a final concentration of 100 g/l PEG 0.3 mol/l NaCl)". Irrespective of the volume of supernatant retrieved, we added a standard 10 ml of PEG/NaCl to the sample, a volume that we had previously tested and found to be suitable without causing adverse effects (unpublished) since the amount of supernatant recovered was generally close to 40 ml each time the method was performed.

Lastly, our method incorporated an internal amplification control (IAC) instead of the recommended external amplification control (EAC). While an EAC is recommended to monitor the effects of inhibitors, our experience has shown that an EAC can be unreliable due to the absence of a confirmation step (see below for an explanation). Thus, the use of an IAC provided a more dependable and robust alternative.

Navigating Control Limitations: the Case for Use of an Internal Versus an External Amplification Control

The ISO/TS 15216 standard suggests using an external amplification control (EAC) to identify any potential inhibitors that might affect the outcomes of PCR reactions. These EACs are extensively explained in the CEFAS Generic Protocol and were initially developed by Le Guyader et al. (2009). However, after rigorous examination and practical application of the methodology, we identified certain limitations not explicitly discussed in these references. Accordingly, we decided to shift towards an alternative: employing an internal amplification control (IAC) which comes as standard in many commercially available molecular detection kits. In this section, we will examine the thought process that fuelled this shift, its impact on enhancing the accuracy of norovirus detection, and how it bolsters our trust in the results. This is particularly important given that most samples tested—especially when using this protocol as a screening method—will return a negative result for the target virus. This change is therefore essential to providing us with greater confidence in the veracity of these negative results.

EACs as recommended for use in the ISO method are derived from the sequences that specify the capsids of the NoV GI.1 "Norwalk" strain and the NoV GII.4 "Lordsdale" strain. However, the use of these EACs presents significant challenges. A primary concern is that the same primers and probes are utilised for both the EAC and any NoV GI or GII strains extracted from the sample. Consequently, EAC amplicons can only be distinguished from NoV GI or GII amplicons through sequencing or digestion with a restriction enzyme. The EAC contains an additional 6 nucleotides that are the recognition site for the Bam H1 restriction enzyme. This was presumably included to be able to confirm amplicons post-PCR through an additional step (restriction or sequencing). Yet, neither the ISO15216 method nor the CEFAS Generic Protocol method proposes such a step in the event of a positive signal being obtained from a sample. It appears that the Bam H1 sequences were included due to the recognition that the EAC had the potential to contaminate test wells on a plate for the reasons given above. Consequently, it was considered necessary to be able to verify potential false positives through post-PCR manipulation. Without this differentiation, any "positive" result cannot be verified as a true positive result and is not simply a result of contamination by the EAC. Given the nature of the EAC, it would be crucial to confirm the identity of positive

results since in essence there are an equal number of controls which are indistinguishable from the target which increases the likelihood of cross-contamination across the PCR plate and consequently raises the risk of false positive results. However, this confirmatory step is omitted in the ISO15216 standard, thus substantiating our preference for an IAC as a more appropriate amplification control (D'Agostino and Cook 2018).

Moreover, if a test sample reaction well returns a negative result but the EAC reaction well produces a signal, it still leaves no means of knowing unambiguously if the amplification reaction worked correctly in the test well. A further drawback is the requirement to use double the volume of reagents since the EAC is in a separate reaction well. This increases the space used on the PCR plate, effectively doubles the number of reactions, and thus substantially increases the cost of the analysis.

In contrast, the ceeramTOOLS® range of test kits we used, which include norovirus GI/GII and HAV, incorporate internal amplification controls. The ISO15216 method does allow for alternative methods of assessing inhibition and states that "alternative approaches for RT-PCR inhibition control that can be demonstrated to provide equivalent performance to the use of EC RNA are permitted". Therefore, we decided to employ the ceeramTOOLS® range of test kits with an internal amplification control for commercial sample analysis. NOTE: We anticipated that this approach would offer at least an equivalent, if not, superior performance compared to the use of EACs. While we utilised ceeramTOOLS®, there are several other available detection kits on the market that also incorporate internal amplification controls and could serve as feasible alternatives. For those considering custom assay design, guidelines on the practical aspects of creating an IAC are available (Hoorfar et al., 2004; Cook et al., 2013).

Ensuring Analytical Precision: the Role of Competency Checks in Laboratory Accreditation

Laboratory accreditation is a process that is as rigorous as it is critical. One of the fundamental aspects of this process, which often goes unnoticed, is the role of competency checks. These checks are more than just a procedural requirement; they form the backbone of the laboratory's commitment to accuracy, reliability, and excellence. The competency checks focused on the ability of laboratory staff to correctly analyse and identify artificially contaminated produce. This is a rigorous exercise designed to evaluate and validate the analytical skills of analysts. Artificial contamination of samples with norovirus and hepatitis A virus was carried out at four distinct levels of virus in the samples: zero (a control group), 10^1–10^2, 10^2–10^3, and 10^3–10^5 genome copies per sample. Each of these levels was tested in singlicate on a minimum of two separate occasions, leading to a total of at least eight samples for the complete procedure.

Artificial contamination was performed using commercially supplied, quantified stocks of virus (PHE LENTICULE® Discs—*LENTICULE* is a trademark of the UK Health Security Agency). These lenticules are provided in plastic vials and comprise control-dried faecal material containing norovirus (either GI or GII) in tablet format, encapsulated within a silica gel desiccant. Importantly, the number of virus genome copies per disc is quantified for each batch, allowing for a rigorous and quantifiable approach to seeding. As such, the seeding levels in this study are defined in terms of genome copies. Each sample was labelled with a unique code by the competency assessor(s). This crucial step ensures that the analyst is blind to the identity of the samples until the completion of the assessment, thereby eliminating any potential bias and maintaining the integrity of the procedure. Once the analysis is complete, the analyst reports the results to the assessor(s), who then evaluate the data against the set criteria.

The competency evaluation process is stringent, with clearly defined success criteria:

(i) The accreditation process requires the detection of artificially introduced viruses at various levels in the test samples. At the highest contamination levels, between 10^3 and 10^5 genome copies of each virus, the expectation is for complete identification of all samples. There is also an expectation that all uncontaminated samples will be correctly identified. For lower contamination levels, specifically 10^1–10^2 or 10^2–10^3 virus genome copies, perfect identification is not a strict requirement. Partial positive results are acceptable and are expected at the lower levels. Acceptable criteria for the verification of the method is an important aspect to be discussed with the local accreditation body assessors where this may not be specifically covered in international standards, e.g., if these standards only cover verification of methods to detect bacteria. Future ISO standards focusing on viruses may well cover this aspect of verification, so it would be prudent for those wishing to validate alternative methods or verify the performance of reference methods within their laboratory to keep up to date with the ISO 16140 series (microbiology of the food chain).

(ii) For every uncontaminated sample, the sample process control virus (SPCV) must be detected and demonstrate a minimum extraction efficiency of 1%. This means that if we assume the lowest allowable recovery rate of 1% and if you have for example 10^3 (or 1000) copies of the target virus in a sample, you expect to detect at least 10

copies after the extraction process. This scenario gives us the minimum numbers likely to be present in the sample based on this lowest permissible recovery rate. As previously mentioned, there may be times when the recovery of SPCV is less than 1% when the target virus is present.

(iii) All norovirus and hepatitis A virus internal amplification controls must produce a signal in the absence of a target signal. This is to validate the effectiveness of the amplification process. Again, if a positive target virus signal is obtained, the IAC may or may not be present. In this case, it is redundant since the presence of a target virus signal shows the amplification process has worked correctly.

(iv) Uncontaminated samples should not yield any signal for norovirus and/or hepatitis A virus. This validates the specificity of the detection process.

(v) The negative RT-PCR control must not produce any signal for norovirus and/or hepatitis A virus, demonstrating the absence of contamination in the testing process and in the setup of the PCR plate.

Proficiency Testing—External and Internal

Both external and internal quality assurance processes play a pivotal role in verifying the consistency and accuracy of laboratory testing methods. They function as safeguards that ensure the results delivered by a laboratory are reliable and accurate, contributing to the validation of the methods used and providing the evidence required for UKAS accreditation.

The external quality assurance (EQA) process typically involves participation in proficiency testing schemes organised by an external reputable body, such as the UK Health Security Agency (UK HSA). In these schemes, laboratories are given test samples containing known quantities of specific viruses at varying levels. By analysing these samples blindly and comparing their results with those from other laboratories, they can benchmark their own performance, identifying any discrepancies and areas for improvement. Over time, continued participation in EQA provides an array of data points across different viral load levels. This, in turn, allows laboratories to determine their own method's sensitivity and limit of detection. Even if the method under review is designed for a presence-absence outcome, having this range of detection data can provide additional insight into its performance. For example, in our laboratory between October 2017 and October 2019, six external proficiency tests were carried out. On some occasions, all 3 target viruses were present at varying levels (NoV GI, NoV GII, and HAV). On other occasions, some samples contained no target viruses. Successful analysis of the samples provides UKAS assessors with data that can be compared with other laboratory data, thus serving as an independent check on performance within the laboratory.

Internal quality assurance (IQA) reinforces EQA by consistently monitoring and assessing the efficacy of the laboratory's methods. A pivotal part of this process is the usage of IACs and the sample process control viruses (SPCV) during each test run. The IACs are incorporated into the assay to detect any potential inhibition in the PCR process, providing real-time feedback on the test's performance. If the IAC does not yield the expected result, it might indicate a problem in the PCR amplification, whether due to reagent issues or sample-related inhibitors. This immediate feedback allows any technical issues to be addressed promptly. Simultaneously, the use of SPCVs provides valuable information on the overall efficiency of the virus extraction and detection processes. The SPCV mimics the behaviour of the target viruses (hepatitis A and norovirus) within the test system. The consistent performance of the SPCV is indicative of a well-controlled testing process.

By routinely observing the performance of the IACs and SPCVs, a substantial body of data is accumulated, illustrating the method's stability and reliability over time. This wealth of performance data plays a crucial role in the UKAS accreditation process, providing clear evidence of the method's sustained precision and accuracy. This IQA approach, therefore, not only ensures the validity of each test run but also contributes to the continuous improvement and refinement of the overall laboratory operation.

Examples of the performance of IACs on two different matrices are shown in Figures 1 and 2. The tables show the Ct values of the IACs from several artificially contaminated samples as well as negative controls which demonstrates that the method is able to effectively extract nucleic acid from virus suspensions obtained from food samples without any obvious signs of inhibition present, with less than 1 Ct value difference between all IACs. Building up this data over time gives proof of the reliability and robustness of the method over the range of samples tested, and this is valuable evidence for UKAS assessors.

Another set of data accumulated over time which is useful to show UKAS assessors is a range of recovery efficiencies of the SPCV after analysis of different matrices. Table 1 shows the results of the % recovery of the sample process control virus (MgV) obtained from a subset of commercial sample analysis (anonymised). The minimum criteria of acceptance of a recovery efficiency of ≥ 1% have been achieved in all samples, showing that the method can successfully recover viruses through all stages of the method.

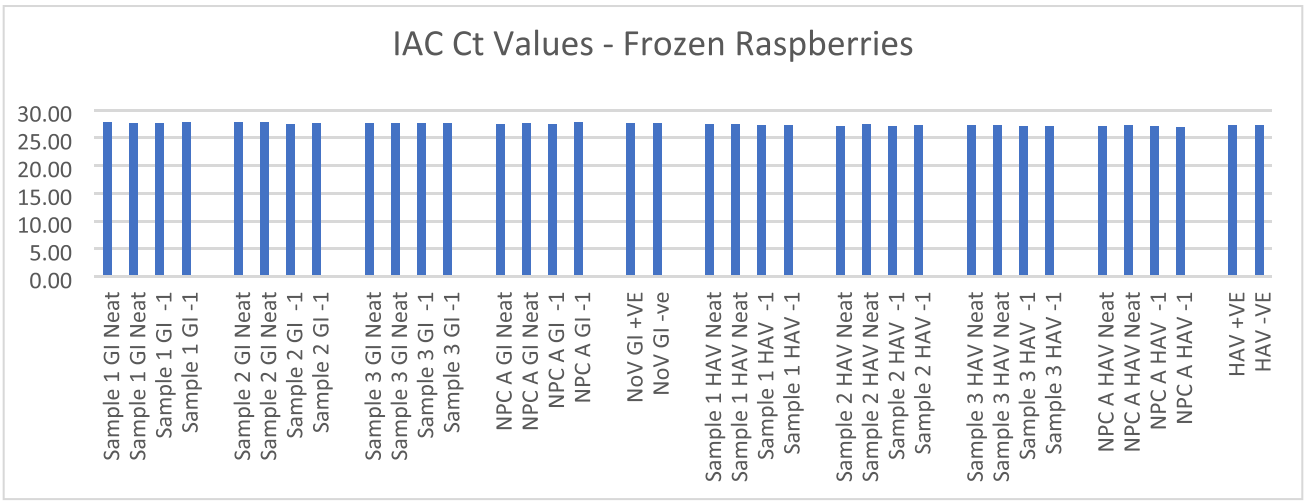

Fig. 1 IAC results for frozen raspberries

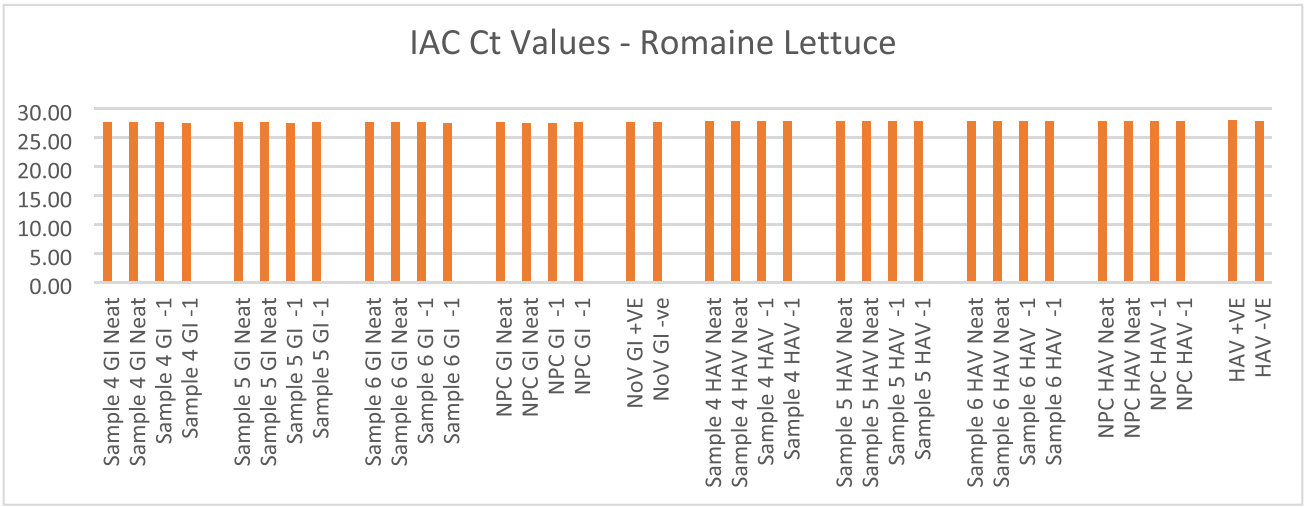

Fig. 2 IAC results for romaine lettuce

System Suitability Checks and Next Steps

The procedure for attaining accreditation for the method necessitates a variety of analytical apparatus. These pieces of equipment play a vital role in the detection of viruses. Fundamental to this methodology is a real-time PCR system used for the amplification and detection of viral nucleic acids (in this case, an Applied Biosystems 7500 PCR instrument). A thermo-mixer, which has a key function during the nucleic acid extraction stages, is also essential, as is an automated or semi-automated system for nucleic acid extraction. A precision balance is also a crucial part of this analytical setup, ensuring accurate measurements during the course of the analysis. These instruments undergo regular accuracy checks in accordance with strict UKAS standards. Complementing these tools is a refrigerated centrifuge that is critical for the sedimentation of particles at high speeds which is also serviced by UKAS-certified service engineers.

These primary pieces of equipment, particularly those that must maintain specific speeds, store reagents at particular temperatures, record operating times, or log weight measurements, must meet UKAS standards in terms of calibration and servicing. Each piece of equipment should visibly display a sticker indicating the dates of calibration and service, verifying its compliance with the rigorous standards set by UKAS. UKAS verification checks also require the use of calibrated weights. Besides these, a mini-centrifuge

Table 1 Efficiency of recovery of the SPCV from a variety of fresh produce items

Sample type	Target virus analysed	Mean % recovery SPCV*
Baby leaf spinach	NoV GI/GII/HAV	3.0
British curly leaf lettuce	NoV GI/GII/HAV	26.4
Wild rocket	NoV GI/GII/HAV	5.8
Little gem lettuce	NoV GI/GII/HAV	13.1
Plum vine tomatoes	NoV GI/GII/HAV	4.7
Cherry tomatoes	NoV GI/GII/HAV	1.4
Fresh raspberries	NoV GI/GII/HAV	3.1
Cranberries	NoV GI/GII/HAV	3.5
Fresh blackberries	NoV GI/GII/HAV	2.5
Frozen raspberries	NoV GI/GII/HAV	1.8
Frozen summer fruits	NoV GI/GII/HAV	3.3
Frozen berry mix	NoV GI/GII/HAV	1.1
Frozen black forest fruits	NoV GI/GII/HAV	1.4
Redcurrants	NoV GI/GII/HAV	1.2
Bistro salad	NoV GI/GII/HAV	4.8
Iceberg lettuce	NoV GI/GII/HAV	16.9
Little gem lettuce	NoV GI/GII/HAV	15.8
Wild rocket	NoV GI/GII/HAV	17.5
Baby spinach	NoV GI/GII/HAV	16.4
Blackberries	NoV GI/GII/HAV	7.8
Raspberries	NoV GI/GII/HAV	13.7
Strawberries	NoV GI/GII/HAV	17.5
Strawberries	NoV GI/GII/HAV	19.6
Cherry vine tomatoes	NoV GI/GII/HAV	9.0
Cherry tomatoes	NoV GI/GII/HAV	5.1
Sundried tomatoes	NoV GI/GII/HAV	12.4
Frozen chopped spinach	NoV GI/GII/HAV	13.7
Frozen blueberries	NoV GI/GII/HAV	2.3
Frozen blueberries and strawberries	NoV GI/GII/HAV	6.0
Fresh blueberries	NoV GI/GII/HAV	10.9
Fresh strawberries	HAV	2.4
Fresh strawberries	HAV	4.6

*Mean % recovery values shown are the result of duplicate RT-PCR reactions performed undiluted and at a 1:10 dilution

and an electronic roller are necessary for sample processing, although they do not require regular servicing or maintenance.

The analysis protocol necessitates the precise manipulation and measurement of various liquid volumes. To achieve this, a range of pipettes with different volume capacities is required. The precision of these pipettes is not taken for granted; instead, each one is subject to accuracy checks before the commencement of the analysis of a new batch of samples. This vital in-house procedure guarantees the integrity of the analyses, ensuring that the volumes used in all steps of the process are as exact as they can possibly be.

The process also includes temperature-regulated storage facilities such as a refrigerator for media storage and a freezer for reagent storage. The temperatures of both units are monitored daily to ensure optimal conditions for the preservation of reagents and samples and are also subject to UKAS verification checks.

Finally, system suitability checks entail the use of specific buffers and reagents, prepared either in-house or sourced from approved suppliers. These include phosphate-buffered saline (PBS), Tris/glycine/beef extract (TGBE) buffer, and polyethylene glycol (PEG)/sodium chloride (NaCl) solution. Reagents necessary for the RT-PCR stage are also sourced from approved suppliers, with all relevant certifications and technical notes readily available. Each piece of equipment and reagent used is meticulously recorded and maintained as per institutional management standards, in line with UKAS requirements.

Once the competency checks and system suitability checks are complete and the data has been compiled, the UKAS assessors are provided with a comprehensive package of all related information. This package includes certificates of calibration for all equipment, servicing reports, SOPs pertinent to the method, a list of all approved suppliers, details on data reporting and storage procedures, copies of all logbooks used, and examples of customer reports. A date is then agreed upon to carry out the assessment. By following this thorough procedure, it ensures that the laboratory maintains its high standard of analytical precision, thereby upholding the integrity of the accreditation and the commitment to its clients. It also provides the technicians performing the analysis with confidence that they are performing the method correctly.

Setting Out the Criteria and Performing the Verification

The method was assessed using the following criteria:

(i) Successful detection* of norovirus GI in lettuce
(ii) Successful detection of norovirus GII in lettuce
(iii) Successful detection of hepatitis A virus in lettuce
(iv) Successful detection of norovirus GI in fresh strawberries
(v) Successful detection of norovirus GII in fresh strawberries
(vi) Successful detection of hepatitis A virus in fresh strawberries
(vii) Successful detection of norovirus GI in frozen raspberries

(viii) Successful detection of norovirus GII in frozen raspberries
(ix) Successful detection of hepatitis A virus in frozen raspberries
(x) Successful recovery efficiency obtained using the sample process control virus** (a recovery efficiency of ≥1%)

*"Successful detection" is defined as the target viruses being detected in their respective reaction wells at a value of ≤ Ct 40 as per the ceeramTOOLS® kit instructions and that the controls have operated successfully.

**In addition to the target reference materials, a sample process control virus (SPCV) is added to the sample before analysis.

Operating at full efficiency, this method theoretically mediates the detection of between 20 and 200 virus genome copies per sample. However, no method is 100% efficient, and the efficiency of this method can be influenced by the nature of the sample matrix and at various stages within the methodology itself. Using the SPCV, the efficiency of recovery can be estimated by comparing the number of genome copies detected with those spiked into the sample. This allows the calculation of the efficiency of recovery for each sample.

Certified Reference Materials

LENTICULE® discs. Each batch of discs contains an expected range of genome copy numbers per disc as follows (subject to change depending on the batch used):

(1) Norovirus GI - PHE Catalogue No. RMNOROG1. Geometric mean value: 1.1×10^4 genome copies per LENTICULE® disc range $8.9 \times 10^3 - 1.4 \times 10^4$
(2) Norovirus GII - PHE Catalogue No. RMNOROG2. Geometric mean value: 2.5×10^3 genome copies per LENTICULE® disc
(3) Hepatitis A virus - PHE Catalogue No. RM000HAV. Geometric mean value: 1.8×10^3 genome copies per LENTICULE® disc

Overview of Controls Used

In order to confirm the correct performance of the method, a range of controls are included at various stages. The controls included are as follows:

(i) Sample process control virus. This is a non-target virus (mengovirus in this case) which is added in a known quantity to each sample prior to analysis. It is supplied as part of the ceeramTOOLS® mengovirus detection kit. When analysis is complete, it is used to confirm the successful performance of the method in the absence of target signals and to calculate the recovery efficiency. A recovery efficiency of ≥ 1% is considered a successful analysis. SPCVs are added to every sample, including the negative process control and the blank process control samples when used. It should be noted that sometimes a target virus signal may be detected even if the efficiency of recovery is less than 1%. The presence of the target virus signal supersedes the recovery efficiency value as the SPC is only useful where no target signal is obtained.
(ii) Negative process control. This is a target pathogen-free sample of the food matrix which is run through all stages of the analytical process. The standard method states "A negative process control sample shall be run in parallel to test samples at a frequency determined as part of the laboratory quality assurance programme". This control was done once during the testing. Note that it is not guaranteed that the matrix will be target-free. It cannot be guaranteed unless it has been grown specifically for the purpose.
(iii) Negative RT-PCR control. This is an aliquot of highly pure water used in a real-time RT-PCR reaction to control for contamination in the real-time RT-PCR reagents. Each of the target virus ceeramTOOLS® kits includes a negative control for real-time PCR reagents. The mengovirus kit does not include this, so an aliquot of sterile water is used for this purpose. These are included on each plate analysed.
(iv) Positive RT-PCR control. These are included in each of the ceeramTOOLS® target virus kits. They are added to each plate analysed.
(v) Blank sample process control. This is a matrix-free sample which consists of 40 ml of TGBE buffer plus the mengovirus SPCV. This is then taken through a complete analysis to ensure the matrix is not having a detrimental effect on the media and reagents. It is not essential to run this control on every plate. This control was done on 3 occasions during the testing.

When testing samples to monitor for inhibition, both undiluted and a 1:10 dilution are analysed in duplicate for each sample—this is in line with the recommendation within ISO15216 parts 1 and 2 due to the frequent possibility of inhibition outside the acceptable parameters.

Interpretation of Data

The correct interpretation of data obtained from the analysis of NoV GI/GII, HAV, and MgV (SPCV) targets is crucial for accurately reporting the presence or absence of these targets. In Table 2, the actions to be taken based on the specific

Table 2 List of potential results and their interpretation (assuming other included controls are acceptable—see "Considerations for PCR Controls" below)

Result example	Target virus (NoV GI/GII/HAV)	IAC	MgV (SPCV)	Acceptable (Y/N)	Action
1	Present	±	+ ≥1%	Y	Report as target present.
2	Present	±	+ <1%	Y	Report as target present.
3	Present	±	-	Y	Report as target-present & change stock of MgV. Report should indicate that sample recovery efficiency was unable to be calculated.
4	Absent	+	+ ≥1%	Y	Report as target absent.
5	Absent	±	+ <1%	N	Potential inhibition has occurred. Repeat the sample analysis.
6	Absent	-	+ ≥1%	N	IAC may have degraded. Do not report as target absent but change to a new detection kit if the PCR controls are all OK and re-run PCR.
7	Absent	±	-	N	Sample treatment failure. Change the stock of MgV (SPCV) and repeat the sample analysis.

Key: "+", present; "-", absent; "±", either present or absent. Note that at least one target signal appearing in either the undiluted or 1:10 dilution constitutes a successful test

results obtained are listed. Here, there are several possible result scenarios after the analysis of a sample.

Considerations for PCR Controls

The interpretation of results should also take into account the performance of PCR controls included in each PCR run. The following scenarios provide guidance for interpreting results based on the PCR controls:

(i) If any of the positive PCR control signals do not appear on a PCR plate without any target signals of NoV GI/GII or HAV in a test sample after the PCR run, a negative result cannot be reported. In such cases, the PCR detection kit should be changed to a new batch/lot number, and the PCR should be performed again.

(ii) If a target signal is detected in a test sample but not in the corresponding positive PCR control well, this indicates that the PCR has worked correctly, but there is a problem with the positive control material. In such situations, the kit should be changed to a new batch/lot number for the next set of samples, and the test sample should be reported as "present".

(iii) If any of the negative PCR control signals contain a positive signal, it suggests contamination has occurred. In such cases, any positive test result should not be reported as present in the sample. Thorough cleaning and decontamination of the working areas should be conducted, and the analysis should be re-run if a target is present in any of the test samples, as contamination may be the cause.

Table 3 Verification data for fresh strawberry samples

Sample type	Virus type	Average Cq value	Efficiency of recovery
Fresh strawberry	NoV GI neat	35.7	0.37
	NoV GI neat		
	NoV GI 1:10	39.9	0.3
	NoV GI 1:10		
	NoV GII neat	34.7	0.37
	NoV GII neat		
	NoV GII 1:10	39.0	0.3
	NoV GII 1:10		
	HAV neat	35.0	0.37
	HAV neat		
	HAV 1:10	38.2	0.3
	HAV 1:10		

Method Verification Results

Tables 3, 4, and 5 provide a summary of the verification data resulting from artificial contamination using 5 lenticule discs spiked onto the surface of fresh strawberries, frozen raspberries, and lettuce samples. This was used to provide evidence of the successful and correct identification of the target viruses from representative samples. Each PCR was performed in duplicate, and the resulting average Cq value is provided.

As can be seen from the results, in some cases the recovery efficiency was below 1%, but as stated above, sometimes a target virus signal may be detected even if the efficiency of recovery is less than 1%. The presence of the target virus signal in each case supersedes the

Table 4 Verification data for frozen raspberry samples

Sample type	Virus type	Average Cq value	Efficiency of recovery
Frozen raspberry	NoV GI neat	36.9	0.13
	NoV GI neat		
	NoV GI 1:10	39.2	0.2
	NoV GI 1:10		
	NoV GII neat	36.1	0.13
	NoV GII neat		
	NoV GII 1:10	38	0.2
	NoV GII 1:10		
	HAV neat	37	0.13
	HAV neat		
	HAV 1:10	39	0.2
	HAV 1:10		

Table 5 Verification data for lettuce samples

Sample type	Virus type	Average Cq value	Efficiency of recovery
Lettuce	NoV GI neat	36.2	5.0
	NoV GI neat		
	NoV GI 1:10	38.6	5.0
	NoV GI 1:10		
	NoV GII neat	35.3	5.0
	NoV GII neat		
	NoV GII 1:10	41.0	5.0
	NoV GII 1:10		
	HAV neat	35.3	5.0
	HAV neat		
	HAV 1:10	38.2	5.0
	HAV 1:10		

recovery efficiency value, as this is only useful where no target signal is obtained. In a commercial setting, when there is no target virus detected, a recovery efficiency below 1% would necessitate a complete retest of the sample to ensure accurate and reliable results have been obtained. Should the sample quantity remaining be insufficient for a retest, an alternative would be to retest the RNA eluate. Should this also yield an unsuccessful result, the test would then be categorised as "failed". This highlights the importance of recovery efficiency as a critical parameter in the detection of viruses in food samples.

Table 6 Uncontaminated samples of each type and a SPCV analysed to ensure no natural contamination of samples used during verification

Sample type	Target assay	RT-PCR result (Ct value)
Raspberry (frozen)	NoV GI	No signal (absent)
	NoV GII	No signal (absent)
	HAV	No signal (absent)
	SPCV	28.3
Strawberry (fresh)	NoV GI	No signal (absent)
	NoV GII	No signal (absent)
	HAV	No signal (absent)
	SPCV	34
Lettuce	NoV GI	No signal (absent)
	NoV GII	No signal (absent)
	HAV	No signal (absent)
	SPCV	32.2

It is also important to include samples that have not been artificially contaminated with the target viruses to confirm that the samples did not contain any background target viruses, as demonstrated in Table 6.

Technical Reviews, Quality Management Systems, Fitness for Purpose, and the Path to Accreditation

A crucial part of the UKAS assessment is the technical review of the laboratory's results which is an exhaustive analysis of the laboratory's proficiency testing and competency assessment results. It is a meticulous process that ensures that the laboratory's methods align thoroughly with the type of testing being performed. Every test result produced by the laboratory is scrutinized and evaluated, and every part of the methodology is reviewed. The purpose is to establish that the laboratory is not just producing results but accurate and reliable results; however, this technical review is not limited to just the results. It extends into the core of the laboratory's operations, examining how these results are achieved. It investigates the laboratory's methods, ensuring they are appropriate for the type of testing being performed. It is also an in-depth look into the procedural backbone of the laboratory, ensuring that the foundation of the laboratory's operations aligns with the stringent standards required for UKAS accreditation.

The UKAS assessors also conduct an in-depth review of the laboratory's quality management system (QMS). The QMS is the laboratory's operational blueprint—a comprehensive structure encompassing the laboratory's policies, procedures, and processes for managing quality.

The QMS outlines how the laboratory monitors and manages the quality of its testing, how it trains and manages its staff, and how it handles customer complaints and nonconformances. This QMS review is an intricate process that examines every aspect of the laboratory's quality management. It is designed to ensure that the QMS is not merely a collection of policies and procedures but is an effective and operational tool that consistently drives the quality of the laboratory's work. The QMS review also ensures that the laboratory's QMS aligns with the requirements of the relevant accreditation standard.

After completing both the technical and quality management system (QMS) reviews, UKAS assessors will evaluate whether the laboratory fulfils all accreditation standard criteria. Should the laboratory meet these requirements, it will gain accreditation for the specific method under examination. This newly accredited method is subsequently included in the public-facing UKAS schedule for the laboratory, providing transparency for clients seeking information on accredited methods. Generally, such accreditation holds validity for 2 to 4 years. During this tenure, the laboratory is subjected to regular assessments by UKAS to ensure continued adherence to the accreditation standard. As the virus detection method was a recent addition to the UKAS accreditation schedule, annual reassessments were mandated. Additionally, internal method auditing is carried out annually by a laboratory assessor.

Conclusion: Enhancing Trust Through Accreditation in Food Virology Testing

Ensuring that food products are safe for consumption is of utmost importance to both consumers and food business operators. While bacterial pathogen testing is often the focus of food safety measures, it is essential to recognize the equally significant role of testing for viral pathogens in ensuring food safety. Recent outbreaks of both norovirus and hepatitis A caused by the consumption of contaminated food products have highlighted the severe threat posed by viral pathogens to public health, making it even more crucial to acknowledge the significance of rigorous, precise, and reliable virology testing.

It is essential that virological testing should be held to the same level of strictness as bacteriological testing. This will help stakeholders understand its necessity and ensure the accuracy of results for customers. Accreditation, such as ISO/IEC 17025, plays a critical role in establishing the credibility and dependability of testing laboratories. This globally recognized stamp of approval indicates that a laboratory has met internationally accepted standards, providing assurance to clients and customers that the laboratory can consistently deliver valid results.

The key points outlined in this case study are not an exhaustive list of everything required for UKAS accreditation—the process can be lengthy and there are many other pieces of evidence that have to be provided for the UKAS assessors; however, it is hoped that since this has now been achieved for the detection of viruses in fresh produce, this will serve as a model for laboratories wishing to gain accreditation in this area in the future.

In conclusion, virological testing should be a pivotal component in the preservation of food safety, serving as both an informative and potentially preventive measure against outbreaks and a provider of precise prevalence data. By committing to rigorous, precise, and reliable virology testing procedures, and securing accreditation, we can substantially augment the safety of high-risk food items.

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Declarations

Competing Interests The author declares no competing interests.

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