# Extraction and Detection of Viruses from Food and Environmental Samples

LIFE TECHNOLOGIES™
FOOD SAFETY

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## ABSTRACT

Food borne disease caused by norovirus and hepatitis A virus are increasing every year. Therefore food industries have to take in account virus food safety issue. This underlines the need for methods to identify viral contamination in order to offer analytical solutions to food industries and laboratories. Theses methods have to be in compliance wit the ISO/TS 15216. The objective of this study was to develop a complete workflow to offer testing laboratories simple, sensitive, fast, standardized, reliable and cheap analytical methods.

Methods for elution and concentration of virus were based on those described in the standard method for virus detection in food (ISO 15216-1 and 2). Nucleic acid were extracted using magnetic beads in association with specific extraction reagents. The extraction was performed on the BeadRetriever™ system. RNA were detected using for the targeted viruses (Mengo virus, norovirus GI and GII, hepatitis A virus). The method was validated on artificially contaminated samples (soft fruits, salads, surface, shellfish, bottled water, herbs, complex foods) at 2 different levels for NoV GI, GII and HAV (500 or 1000 genome copies). All experiments and amplification reactions were performed in triplicate. The complete workflow was also validated on 15 naturally contaminated shellfish previously detected positive in Ceeram's laboratory.

Using the complete workflow on artificially contaminated samples, extraction recoveries were systematically higher than 1% whatever the matrix as recommended in the ISO/TS 15216. For all of the sample types, a limit of detection of 500 genome copies was obtained with a good reproducibility. All of the viruses on the naturally contaminated shellfish have been detected using the developed workflow, even those with a level of contamination below 500 copies/ g of digestive tissue.

## INTRODUCTION

According to the Robert-Koch-Institute (RKI) Human Norovirus is the food-borne disease with the most reported incidents in Germany, peaking with up to 5.696 reported outbreaks in the winter season 2009-2010 (Nov – Apr). Globally, tens of millions of individuals contract Hepatitis A and Norovirus each year. In partnership with the European Centre for Expertise and Research on Microbial Agents (CEERAM), Life Technologies provides a suite of RT-qPCR solutions that detect Norovirus GI, Norovirus GII, Hepatitis A and Hepatitis E in a variety of sample matrices such as shellfish, soft fruits and water, with exceptional sensitivity and specificity. Helping food producers, processors and retailers to preserve the quality of their products and protect the health and well being of their customers.

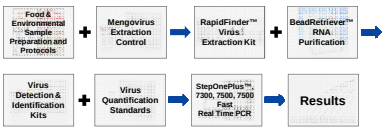
## MATERIALS AND METHODS

The aim was to optimize the protocol for virus elution and concentration from shellfish and soft fruits to have protocols close to the ones described in the ISO/TS 15216 for these 2 types of matrices. These 2 matrices were selected as they constitute the most difficult samples to analyze.

For oysters, after proteinase K treatment following ISO/TS 15216 recommendations, the supernatant was not very clear and it was difficult to resuspend the beads in solution and bind them to the magnet. For fruit samples, a clarification step with chloroform-butanol is used to eliminate some inhibitors, colors and particles. Therefore, such a clarification step was tested on shellfish samples.

For soft fruit tests with NoV were performed. The experiments were conducted on artificially contaminated oysters and raspberries with 1000 genome copies of NoV. A dilution was made to have the desired concentration of 10,000 genome copies/ mL. The artificial contamination was made with 100 µL to have an input of 1000 copies. For virus elution and concentration, protocols were used exactly as described in ISO/TS 15216. RNA extracts were tested pure and diluted. The criteria for selection were simplicity of the method, accordance with ISO/TS, Mengo virus recovery higher than 1% and the detection of target viruses.

**Figure 1. Virus Extraction and Detection from Food and Environmental Samples:**




The RapidFinder™ Virus Extraction Kit, when used as a part of the Life Technologies™ workflow for detecting norovirus, hepatitis A virus, and hepatitis E virus, provides food and environmental testing customers with:

•Speed—faster processing time than competitive viral extraction solutions
•Flexibility—as few as 1 and as many as 15 samples run during a single cycle
•Confidence—fewer hands-on steps means reduced risk of cross-contamination

The RapidFinder™ Virus Extraction Kit uses the proven technology of magnetic silica beads in order to ensure higher sensitivity and improved capture performance. The viral extraction reagents are optimized to produce fewer false-negatives due to effective PCR inhibitor removal. The kit is designed for use with the BeadRetriever™ System, which enables rapid purification and concentration of selected virus RNA targets from a broad range of samples. This easily-operated, automated sample preparation system generates highly-reproducible results, even for the most challenging sample types.

**Figure 2. BeadRetriever™ System:**

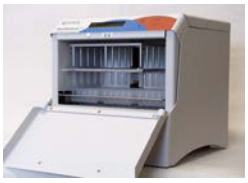
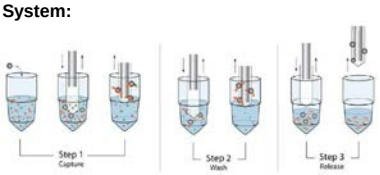


**Figure 3. Principle Behind BeadRetriever™ System:**




The principle of the BeadRetriever™ system is based on the use of magnetic rods covered with disposable, specially designed tip combs and tube strips. The instrument functions without any dispensing or aspiration parts or devices. Samples and reagents including magnetic beads are dispensed into the tube strips according to the corresponding kit instructions. The steps of the protocol that are selected by the user via the keypad and display have already been preloaded in the on-board software.

## RESULTS

**Table 1. Detection of Norovirus GI from Soft Fruit:**

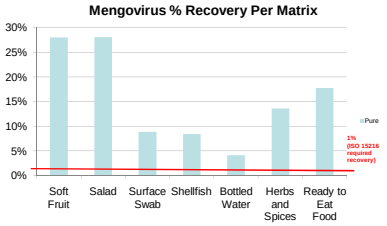
| Results for Norovirus GI Detection in Soft Fruits | | Mean Ct values (n=3) (Extraction Recovery % based on Mengovirus) | |
|---|---|---|---|
| | | 1000 copies | 500 copies |
| A | pure | 36.03 (32%) | 38.33 (35%) |
| | 1/10 | 36.25 (32%) | 39.09 (35%) |
| B | pure | 36.83 (48%) | 39.28 (30%) |
| | 1/10 | 38.62 (48%) | 39.17 (30%) |
| C | pure | 36.82 (35%) | 37.77 (28%) |
| | 1/10 | 38.21 (35%) | 38.76 (28%) |

**Table 2. Detection of Norovirus GII from Soft Fruit:**

| Results for Norovirus GII Detection in Soft Fruits | | Mean Ct values (n=3) (Extraction Recovery % based on Mengovirus) | |
|---|---|---|---|
| | | 1000 copies | 500 copies |
| A | pure | 37.31 (32.1%) | 37.32 (35.5%) |
| | 1/10 | 36.08 (32.1%) | 36.33 (35.5%) |
| B | pure | 37.07 (48.0%) | 36.54 (30.1%) |
| | 1/10 | 37.48 (48.0%) | 35.88 (30.1%) |
| C | pure | 35.54 (35.0%) | 37.47 (28.5%) |
| | 1/10 | 35.83 (48.0%) | 36.39 (28.5%) |

This experiment was performed on raspberries. No contamination was detected in negative samples. A, B and C represent 3 different iterations of the sample prep protocol. A represents addition of Isopropanol before lysis buffer addition, B represents addition of isopropanol after lysis buffer addition and C represents Isopropanol mixed into the lysis buffer. Protocol C was chosen as the best protocol for soft fruits.The results presented in the above tables are the mean Ct values obtained for 1000 and 500 genome copies. Samples were tested as pure elutions and as 1/10 dilutions to determine the level of inhibition. The ceeramTools® Norovirus GII assay was used for detection.

**Figure 4. Mengovirus % Recovery From Multiple Matrices**




This graphic shows the recovery percentages of Mengovirus in different matrices including raspberries (soft fruit), lettuce (salad), green pepper swabs (surface swabs), oysters (shellfish), bottled water, dill (herbs and spices) and mixed salad (ready to eat food). In all instances, the kit is able to recover greater than 1% of the control virus and detect down to 500 copies/reaction for NoV GI + II as well as HAV. This exceeds the requirements stated in 15216:213 / 1 & 2 for the applied extraction control. All extraction were performed independently in triplicates per food matrix.

## CONCLUSIONS

By combining ceeramTools® detection kits and Life Technologies reagents and BeadRetriever™ equipment, a complete workflow starting from the samples up to amplification results has been developed. This diagram of procedure is in accordance with the ISO/TS 15216. Whatever the targeted matrices, a limit of detection for the method of 500 genome copies has been obtained. The developed method has also been validated on naturally contaminated samples.

As a part of Life Technologies virus detection workflow, the RapidFinder™ Virus Extraction kit provides:

- All-in-one kit, optimized specifically for food and environmental samples
- Automated RNA purification using a proven immunomagnetic separation platform
- A solution with flexible throughput for as many as 15 samples
- Highly efficient extraction, on even the most challenging food matrices
- A complete workflow consistent with ISO Standards 15216:2013 / 1 & 2

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