UCD Centre for Food Safety,
UCD School of Public Health, Physiotherapy and Sports Science
University College Dublin,
Belfield, Dublin 4,Ireland.
Ph: (+353 1) 716 2868
cfs@ucd.ie

Standard Operating Procedure
for

Qualitative detection of SARS-CoV-2 in soft fruit, meat and packaging surface swabs

Version n.	Date	Comments
0	16-12-2020	SOP draft, based on the pilot study conducted at the UCD-CFS

1.0 Introduction

Coronavirus SARS-CoV-2 known to cause COVID-19 is an easily transmitted disease spreading from person-to-person via cough; sneeze; respiratory droplets and exhalate. Symptoms are well recognised, appearing 5-days after infection and include flu, sore throat, muscle aches and loss of taste or smell, conducible to respiratory illness responsible for the COVID-19 pandemic. The World Health Organization declared the outbreak a Public Health Emergency of International Concern on 30 January 2020, and a pandemic on 11 March 2020. SARS-CoV-2 has similar clinical presentation when compared with two other well-known acute respiratory infectious agents: severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS). SARS-CoV-2, SARS-CoV and MERS can circulate in animals and infect humans, thus representing a zoonoses.

SARS-CoV-2 is a Baltimore class IV virus and belongs to the broad family of viruses known as coronaviruses. It is a positive-sense single-stranded RNA (+ssRNA) virus, with a single linear RNA segment.

2.0 Scope

The "Qualitative detection of SARS-CoV-2 in soft fruit, meat and packaging surface swabs" is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in food samples and abiotic surfaces such as packaging of food.

Following liberation of viruses from the test sample, viral RNA is then extracted by magnetic separation or with the use of lysis and adsorption on silica. Target sequences within the viral RNA are amplified and detected by real-time RT-PCR. Results are for the identification of SARS-CoV-2 RNA and positive results are indicative of active contamination with SARS-CoV-2 of the matrices but do not rule out bacterial infection or co-infection with other viruses.

3.0 Terms and definitions

3.1 **Foodstuff.** Substance used or prepared for use as food.

3.2 **Food surface.** Surface of food, food preparation surface or food contact surface

3.3 **SARS-CoV-2.** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the strain of coronavirus that causes coronavirus disease 2019 (COVID-19).

3.4 **Detection of SARS-CoV-2.** Detection of SARS-CoV-2 RNA in a predetermined mass or volume of foodstuff, or area of food surface

3.5 **Process control virus.** Virus added to the sample portion at the earliest opportunity prior to virus extraction to control for extraction efficiency.

3.6 **Process control virus RNA.** RNA released from the process control virus in order to produce standard curve data for the estimation of extraction efficiency

3.7 **Negative RNA extraction control.** Control free of target RNA carried through all steps of the RNA extraction and detection procedure to monitor any cross-contamination events.

3.8 **Negative process control.** Target pathogen-free sample of the food matrix which is run through all stages of the analytical process.

3.9 **Hydrolysis probe.** Fluorescent probe coupled with two fluorescent molecules which are sterically separated by the 5′-3′-exonuclease activity of the enzyme during the amplification process.

3.10 **Negative RT-PCR control.** Aliquot of highly pure water used in a real-time RT-PCR reaction to control for contamination in the real- time RT-PCR reagents.

3.11 **External control RNA.** Reference RNA that can serve as target for the real-time PCR assay of relevance, e.g. RNA synthesized by *in-vitro* transcription from a plasmid

carrying a copy of the target gene, which is added to an aliquot of sample RNA in a defined amount to serve as a control for amplification in a separate reaction.

3.12 **Quantification cycle.** the PCR cycle at which the target is quantified in a given real-time PCR reaction.

3.13 **CT value.** This corresponds to the point at which reaction fluorescence rises above a threshold level.

3.14 **Theoretical limit of detection (tLOD).** Level that constitutes the smallest quantity of target that can in theory be detected. This corresponds to one genome copy per volume of RNA tested in the target assay, but varies according to the test matrix and the quantity of starting material.

3.15 **Practical limit of detection (pLOD).** Lowest concentration of target in a test sample that can be reproducibly detected (95 % confidence interval) under the experimental conditions specified in the method, as demonstrated by a collaborative trial or other validation. The pLOD is related to the test portion, the quality or quantity of the template RNA, and the tLOD of the method.

4.0 Principle

4.1 Virus extraction and lysis

The foodstuffs and food surfaces covered by this part of the SOP are often highly complex matrices and the target virus can be present at low concentrations. It is therefore necessary to carry out matrix- specific virus extraction and/or concentration in order to provide a substrate for subsequent common parts of the process. The choice of method depends upon the matrix, as described in details in the dedicated sections of this SOP.

4.2 RNA extraction

It is necessary to extract RNA using a method that yields clean RNA preparations to reduce the effect of PCR inhibitors. In this part of the SOP the chaotropic agent guanidine thiocyanate is used to disrupt the viral capsid. RNA is then adsorbed to silica to assist purification through several washing stages. Purified viral RNA is released from the silica into a buffer prior to real-time RT-PCR.

4.3 Real-time reverse transcription polymerase chain reaction (Real-time RT-PCR)

This SOP uses one-step real-time RT-PCR using hydrolysis probes. In one-step real- time RT-PCR, reverse transcription and PCR amplification are carried out consecutively in the same tube.

Real-time PCR using hydrolysis probes utilizes a short DNA probe with a fluorescent label and a fluorescence quencher attached at opposite ends. The assay chemistry ensures that as the quantity of amplified product increases, the probe is broken down and the fluorescent signal from the label increases proportionately.

Due to the low levels of virus template predicted, selection of fit-for-purpose one step real-time RT-PCR reagents and PCR primers and hydrolysis probes for the target viruses is important and for this SOP were chosen the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.