Detecting nCoV-2 in food samples using VETfinder solution

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1. Introduction

Food processing facilities have been deemed an essential service during the latest pandemic. However, it has been shown that social distancing in these environments is hard to maintain and possibly due to other factors, we regularly hear of reports of new outbreak clusters in these facilities.

Generon's VETfinder solution for the detection of nCoV-2 is based on Real-Time PCR. The flexibility of this technology allows the commercialization of a wide variety of tests for the food industry, including foodborne bacterial and viral pathogens, allergens and GMOs. VETfinder kits have already been demonstrated efficiently detect nCoV-2 in surface swabs enabling organisations to show employees and stakeholders of the commitment to protecting their health while restarting business operations, thus also protecting corporate brand reputation.

Generon solutions meet specific requirements of the food industry including: low cost and low limit of detection; the testing kits are not subject to shortages due to clinical nCoV-2 testing demand; can improve on-site testing capacity of food industries; provide resilience to new pandemics and enable increased microbial contamination monitoring. It is also highly unlikely that humans can contract COVID-19 from food or food packaging.

COVID-19 is a respiratory illness and the primary transmission route is through person-to- person contact and through direct contact with respiratory droplets generated when an infected person coughs or sneezes. Nevertheless, concerns have been raised regarding animals used as food being a transmitter of coronavirus disease (COVID-19) to humans.

It has been demonstrated that some animals can get infected by nCoV-2, but direct human infection is still unlikely (Sit T., 2020). The risk of indirect infection is still a real concern. Food animals and their products may, as is the case with other surfaces, potentially become contaminated with nCoV-2 when handled by people who are infected and actively shedding the virus (Fisher D., 2020).

Featured Products

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  Detection of nCoV-19 specific N gene in environmental and veterinary samples
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  Comma_XP Extraction kit
- **IL_SP54104 -** IndiSpin Pathogen Kit
- **IRMM nCoV-2 IVT reference synthetic ssRNA** EURM-019 342

Fig. VETFinder kit Generon

2. Experimental set-up

Detection of nCoV-2 in food can be challenging due to many factors. The matrix can indeed be very rich in nucleic acids which can compete with the target RNA for binding to the purification column. Porphirins and other PCR inhibitors might further reduce detection if present in the final extract. Hence, the Generon R&D team tested the capacity of the VETfinder solution to detect nCoV-2 in food products.

A homogenate of salmon fillet and of beef liver was prepared using a waring blender. Liver was selected because it is an organ with high DNA/RNA content and PCR inhibitors, thus it was possible to verify the effect of matrix interference in the detection of the target. An aliquot of 150 mg of each ground meat sample was spiked with 10^9 GU of target sequences using 10 µL of IRMM nCoV-2 IVT reference synthetic ssRNA (concentrated 10^8 GU/µL). 200 µL of PBS buffer 1X were added to facilitate sample homogenization by vigorous vortexing. For each ground meat, two other aliquots were used as blank samples. Intype IC RNA, a process control manufactured by Indical (Cat. # IC289970), was added to both spiked and blank samples. The sequence is chosen not to interfere or compete with IRMM nCoV-2 IVT RNA analysis in any of the process steps, it serves only as an internal control to monitor reverse transcription and amplification efficiency.

RNA of spiked and blank samples was then extracted using IndiSpin Pathogen Extraction Kit (Indical) following supplier protocol and eluted in 100 µL of AVE buffer. The same performances are guaranteed with GENERON COMMA_XP Kit.

In order to determine the limit of detection of the method, the RNA extracted from the blank samples was used to make serial 10-fold dilutions of the RNA extracted from the respective spiked samples (**Fig.1**)

RNA detection was performed using "VETfinder - Real-Time PCR kit for the detection of nCoV-19 specific N gene in environmental and veterinary samples" (PMB00C_N) using a Bio-Rad DW CFX-96 Deep Well Real-Time PCR system. Each reaction contained 20 µL of ready to use Mastermix and 5 µL of RNA sample (final volume 25 µL) (**Fig.2**).

Each reaction was run with initial conditions of 55°C for 10 min (one cycle), 95°C for 3 min (one cycle), followed by 45 cycles of 95°C for 15 s, and 58°C for 30 s, as instructed by the kit supplier.

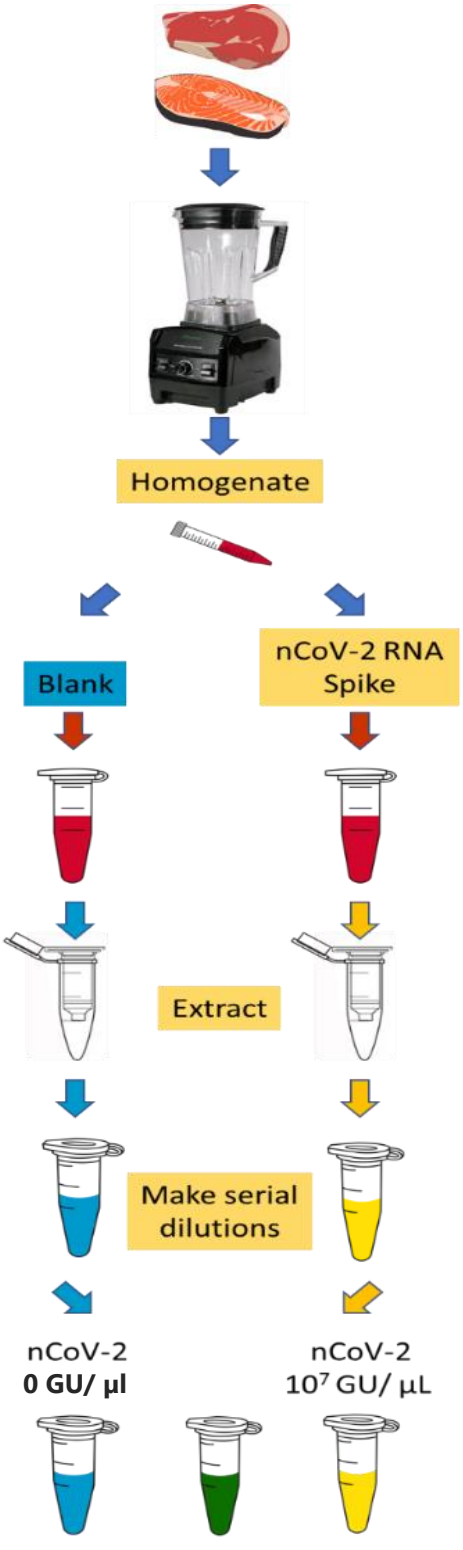

Fig.1 - Outline process chart for extraction of IRMM nCoV-2 IVT ssRNA from salmon fillet and beef liver.

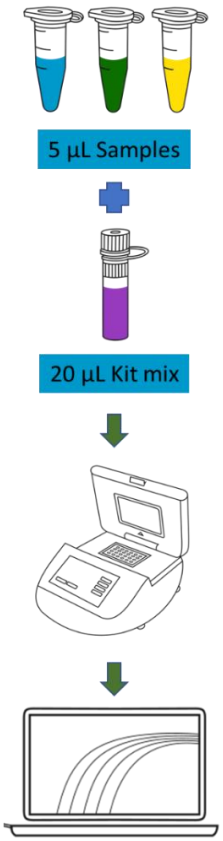

Fig. 2 - Outline process chart for setting up of PCR reaction mix.

3. Results

Spiking and extraction were performed on two different ground samples of both bovine liver (**Fig.3A**) and salmon fillet (**Fig.3B**). Detection was also run in duplicate on the 10-fold diluted samples. The graphs below show that for both salmon and veal liver it was possible to clearly detect the IRMM nCoV-2 IVT ssRNA down to 50 GU/reaction.

Graph (**Fig.3A**) shows fluorescence (RFU) against cycle number. The amplification curve indicated with number 1 represents amplification of nCoV-19 specific N gene in RNA extracted from bovine liver as it is ($5*10^7$ GU/ reaction). Curves marked with numbers from 2 to 7 represent N gene amplification in 10-fold diluted RNA. The 7^{th} dilution is the last detectable and that consists of 50 GU/reaction.

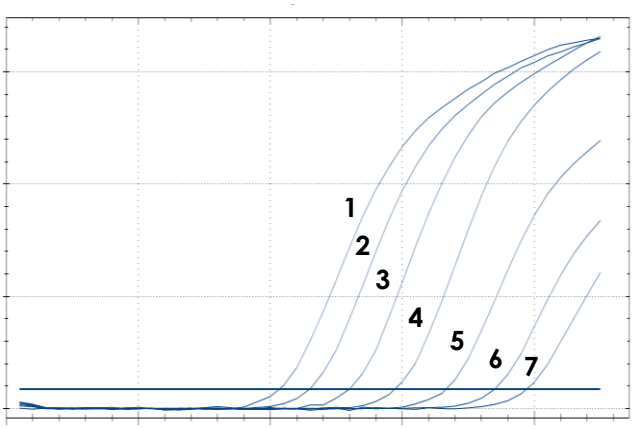

Fig. 3A - Real-time amplification profile of 10-fold serial dilutions of bovine liver spiked with IRMM nCoV-2 IVT RNA.

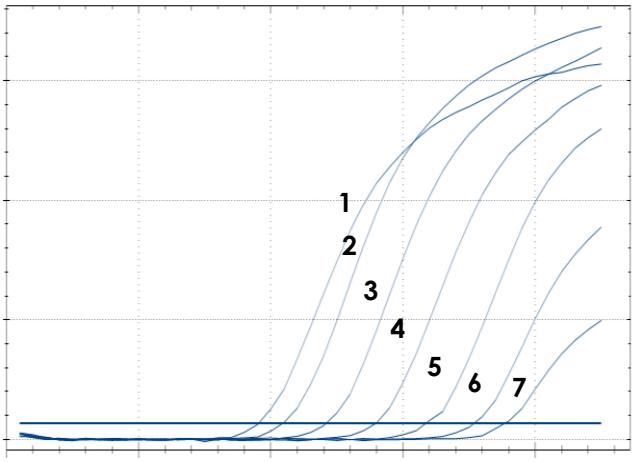

Fig. 3B - Real-time amplification profile of 10-fold serial dilutions of salmon fillet spiked with IRMM nCoV-2 IVT RNA

Graph (**Fig.3B**) shows fluorescence (RFU) against cycle number. The amplification curve indicated with number 1 represents amplification of nCoV-19 specific N gene in RNA extracted from salmon fillet as it is ($5*10^7$ GU/ reaction). Curves marked with numbers from 2 to 7 represent N gene amplification in 10-fold diluted RNA. The 7^{th} dilution is the last detectable and that consists of 50 GU/reaction.

4. Discussion

Although the probability of foodborne transmission of nCoV-2 may be low, this transmission route may cause higher rates of disease and morbidity and is of general concern for public health and food safety.

Hence, following recent episodes of food recalls and temporary production facility shut-downs due to suspect contamination with nCoV-2, we tested the possibility of detecting the presence of nCoV-2 in food products using VETfinder kits and RNA extraction kits used normally to test environmental samples (e.g. surface swabs and water).

We demonstrated that the presence of nCoV-2 RNA can be detected even in the presence of a large background of animal tissue RNA/DNA and other PCR interferents. The possibility to detect nCoV-2 down to minimal amounts gives the possibility to analyse large sampling units, at food production and processing sites where contamination "hot-spots" have been reported and where such risks have been identified in the food production and supply chain.

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