MOLECULAR DETECTION OF NON-VIABLE SARS-COV-2 IN FROZEN MEAT PACKAGING

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I. INTRODUCTION

Faced with the rapid spread of SARS-CoV-2, in March 2020 the World Health Organization (WHO) issued a pandemic alert. According to Official Letter No. 09199.000448/2020-80, health control measures became mandatory to minimize the risk of transmission of the SARS-CoV-2 virus, since the Chinese health authorities adopted the testing of packaging for detection of genetic material from this virus. This detection on packaging can generate the cancellation of export authorization (1). The sanitization methods used to inactivate the virus, should also cause some degree of denaturation and/or structural disintegration of the viral RNA to prevent detection during sampling performed by Chinese sanitary authorities. However, commercially available sanitizers seemed not to have demonstrated effectiveness in inhibiting the amplification of the viral genome by RT-qPCR, resulting in several frozen-meat containers being rejected and returned to the exporter. Apparently, the use of these products still results in the detection of viral genomic copies, despite the virus no longer being viable and, therefore, no longer contagious/infectious.

The objective of this project was to evaluate the influence of potentially interfering substances (sodium hypochlorite 2%, 3% and RNAse) on the amplification of gene fragments present on the surface of secondary packaging (cardboard) used for frozen meat cuts.

II. MATERIALS AND METHODS

The secondary packaging (cardboard), from two different commercial brands used for shipping frozen meat were cut into 2cm x 2cm squares and then sprinkled with 100 µL of a nasopharyngeal swab sample containing inactivated SARS-CoV-2 with a viral load of E+06/µL and stained with Trypan Blue. Additionally, the same procedure was performed using the commercial SARS-CoV-2 genome 2019-nCov_N_Positive Control (IDT) with a viral load of E+05 genomic copies/uL stained with Trypan Blue as an internal process control. The cardboard squares (5 replicas of each type - A and B) were sprayed with 100µL of sanitizing solutions of 2% and 3% sodium hypochlorite, RNAse (Nuclease P1) and PBS (control), placed in a petri dish (1 dish for each cardboard square), vacuum packed and stored at -20ºC for 24h and 30 days, respectively. Each treatment was carried out with five repetitions on aliquots of cardboards A and B sprinkled with a sample with inactivated SARS-CoV-2, in addition to the negative control (PBS) and the internal process control (commercial SARS-CoV-2 commercial genome - CIP). For viral genomic detection, the samples were left for 1 h at room temperature in a laminar flow chamber. Next, the samples were sonicated, the viral genetic material extracted using Purelink Viral RNA/DNA Mini Kit (Invitrogen) and RT-qPCR performed.

III. RESULTS AND DISCUSSION

Analysis of the RT-qPCR results demonstrated that the viral genome was detected in all cardboard aliquots (both A and B), before and after the different treatments carried out. Analysis of external

cardboard samples sprayed with inactivated virus (E+06 genomic copies/mL) and subjected to treatments with 2%, 3% sodium hypochlorite and RNAse, after 24h, demonstrated an average viral load of 1.44E+03, 5.88E+03 and 2.39E+03, respectively. The mean values of the negative control (PBS treatment) and CIP were 9.13E+04 and 1.69E+04, respectively. The means found for treatment analyzes after 30 days of freezing the material were similar: 8.55E+04, 1.34E+05 and 1.31E+04, for treatments with sodium hypochlorite 2%, 3% and RNAse, respectively. The means for the negative and positive controls were 9.13E+04 and 1.31E+04, respectively. Regarding the analysis of internal cardboard samples after 24h, the average viral genomic load after treatment with 2%, 3% sodium hypochlorite and RNAse were 5.73E+04, 5.07E+03 and 9.56E+04, respectively. The mean values of the control and CIP were 1.18E+05 and 1.67E+04, respectively. As observed in the analysis of the external packaging, the mean viral load values for treatments after 30 days showed similar values. Treatments with 2%, 3% sodium hypochlorite and RNAse showed mean viral load values of 1.61E+05, 9.91E+03 and 3.33E+04, respectively. The mean values of the control and CIP were 2.27E+05 and 1.48E+04, respectively. The results demonstrate a loss of approximately 1 log of inactivated virus and commercial viral genome during material processing. An analysis of variance was carried out for statistical evaluation and comparison of the effect of treatments in the two packages, for the two incubation periods (24h and 30 days). After incubation at -20ºC for 24 hours, package A showed a reduction in viral load with the 3 treatments and better efficiency was observed with 2% Hypochlorite or RNAse, while package B showed a reduction in viral load only with 3% Hypochlorite treatment. Considering incubation for 30 days, packages A and B showed a reduction with RNAse treatment, however package B showed better efficiency after treatment with 3% Hypochlorite. Sodium hypochlorite in used concentrations is known to be capable of inactivating SARS-CoV-2 (2). However, genomic detection continues as nucleic acid fragments remain available originating false positive results.

IV. CONCLUSION

None of the potentially interfering substances tested were able to prevent viral-RNA detection in any of the project's treatment conditions. Although unable to cause infection due to the viral inactivation process, fragments of viral genetic material persist. This allows to detect viral-RNA using different molecular techniques. It means that molecular methods performed by sanitary authorities to prevent transmission of SARS-Cov-2 were not suitable, as they may have been identifying just RNA viral fragments of a non-viable and non-infectious pathogen, which has no risk for public health. Thus, molecular methods should be followed by viral culture of sampled material to confirm the presence of viable viruses and the real risk of infectious transmission. These findings could support the criteria for future international trade agreements.

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