SARS-CoV-2 Quality Control Frequently Asked Questions

1. **Why would I use an independent (third party) quality control?**
    a. There are typically two sources of control materials: Instrument, kit or method manufacturer (manufacturer-supplied controls) and manufacturers of control materials (independent or third-party controls).
        i. Manufacturer-supplied control is a quality control material manufactured under the same quality system as the instrument, kit or method it is intended to monitor and whose performance depends on design inputs from the instrument, kit or method manufacturer.
        ii. Independent (third party) Control Material is manufactured outside the quality system used to manufacture the instrument, kit or method it is intended to monitor and whose performance is independent of any design inputs from the instrument, kit or method manufacturer.
    b. Risk associated with using only manufacturer-supplied control materials is now globally recognized and discussed by national organizations and regulators. Here are some guidelines and recommendations from professional organizations:
        i. ISO15189 - Subclause 5.6.2.2
        Use of independent third-party control materials should be considered, either instead of, or in addition to, any control materials supplied by the reagent or instrument manufacturer"
        ii. Clinical and Laboratory Standards Institute (CLSI) ‣ CLSI C24-A3, Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition, 6.2.1 Relation to Calibrators Quality control materials should be different from the calibrator materials to ensure that the QC procedure provides an independent assessment of the measurement procedure's performance in its entirety, including the procedure for calibration of the measurement.
        iii. Department of Standards Malaysia ‣ Laboratory Accreditation Scheme of Malaysia. SC 2- Specific Criteria for Accreditation in the Field of Medical testing. 5.6.1 Quality Control
        The use of controls independent of those produced by the manufacturer of the test or analyzer is preferable
        iv. NATA (National Association of Testing Authorities), Australia ‣ Supplemental Requirements for accreditation in the field of Medical testing, AS 4633 (ISO 15189) Field Application Document, May 2007. 5.6.1 (ii) Internal quality control Controls independent of those produced by the manufacturer of the test or analyzer should be used

2. **Why would I use a full process control?**
    a. Nucleic acid methodologies employ many steps in order to achieve a reportable result. The purpose of using a full process control is to challenge each step in the assay procedure: extraction, amplification, and detection.

3. **What is the difference between synthetic RNA, encapsulated RNA and inactivated whole virus?**
    a. Synthetic RNA is synthetic nucleic acid with no human or animal infectious agents that is not protected, for example, by a protein coat. It is useful in diagnostic assays that do not incorporate an extraction method within their assay. You will hear the terminology of

"sample to result." Those methodologies incorporate nucleic acid extraction, amplification and detection in one combined assay format. In those instances, synthetic RNA QC is would not be suitable because the RNA is not protected during the extraction process.

b. Encapsulated RNA is synthetic RNA that is protected or presented in a format that mimics a virus particle, making it beneficial for use in the extraction, amplification and detection process.
c. Inactivated whole virus is a virus that has been rendered "inactive" through chemical and/or thermal means. Similar to encapsulated RNA, it is beneficial for use in the extraction, amplification and detection process.

4. How do I choose the right "synthetic" control for my assay?

a. Microbiologics offers several synthetic control options for SARS-CoV-2 that cover the most frequently targeted sequences by molecular assays. Different assay manufacturers have chosen different gene target for their assay. Therefore, it is important to choose a control that covers the same gene target. However, synthetic controls can be manufactured in many ways, i.e. complete genomic region or selected nucleotide sequences within the gene, etc. If the targets within the manufacturer's assay do not align to the synthetic control sequences, then the control will not amplify.

See table for target regions in our synthetic full process control HE0063S and HE0062S.

Genomic Region	Targets
Orf 8	Complete genomic region for gene: 14,250..14,450
Orf1ab/RdRP	5 targets including IP2 and IP4: 12,690 ..12,797 | 13,342 ..13,460 | 14,080 ..14,186 | 14,250..14,450 | 15,431 ..15,530
S (Spike)	Orf1b, Pancorona, and other targets: 18,778 ..18,909 | 24,354 ..24,900
M (Membrane protein or Matrix)	Complete genomic region for gene: 26,496 ..27,215
E (Envelope)	Complete genomic region for gene: 26,245 ..26,427 | 26,269 ..26,381
N (Nucleocapsid)	Complete genomic region for gene: 28,237 ..29,280

5. How is the Microbiologics SARS-CoV-2 whole virus inactivated?

a. Our SARS-CoV-2 whole virus control has been inactivated through chemical means.

6. When would I use a synthetic full process control vs an inactivated whole virus?

a. Both controls are used to challenge each step of the molecular assay procedure. While some would want to use a control that contains an actual virus synonymous to a patient sample, others prefer or are comfortable with using synthetically derived material. However, for synthetically derived full process controls, be mindful of the targets represented – refer to question #4.

7. Why are there A549 cells in some of your controls?

a. A549 cells are human lung basil epithelial cells. These have been added to our SARS-CoV-2 controls (HE0062S, HE0063S, HE0065N, HE0066NS) to ensure sample adequacy by different molecular assays.

8. **Why would I use a swab vs a pellet?**
    a. A swab best mimics the nasopharyngeal, oropharyngeal, and nasal samples collection process. A pellet mimics the nasopharyngeal wash/aspirate or nasal wash/aspirate collection process. However, much of the decision on whether to use a swab vs a pellet depends on preference and convenience.

9. **What are the benefits to using Microbiologics SARS-CoV-2 Quality Controls?**
    a. Comprehensive portfolio – Synthetic, Full Process and Inactivated Whole Virus
    b. Lyophilized controls improve stability and reduces shipping costs by eliminating need for dry ice.
    c. Suitable for conventional and automated testing platforms.
    d. Third party controls delivering an independent assessment of assay performance.