Guidance on SARS-CoV-2 Surrogate Selection

1 | SCOPE

1.1 This document provides guidance on viruses that may be used in SARS-CoV-2 research focusing on environmental survival and decontamination strategies. SARS-CoV-2 virus is the causal agent of COVID-19.

1.2 The following criteria were used when selecting the recommended surrogates: enveloped viruses, availability, mammalian origin, categorized as BSL2. Respiratory viruses were preferred but not mandated.

1.3 The final decision as to the acceptability of a surrogate is the responsibility of the test user.

2 | SIGNIFICANCE AND USE

2.1 Surrogate selection is an attempt to accelerate the knowledge base through broader testing of coronaviruses. This research will be facilitated by testing across many labs to include labs that only test BSL2-level coronaviruses, as opposed to reliance at only a few BSL3 test facilities. The BSL3 labs are currently inundated with testing. Therefore, selection and documentation of BSL2 surrogates will empower numerous labs to contribute to coronavirus research, thereby mitigating the risk of anti-competition, a cornerstone of ASTM International.

2.2 Research is needed on strain-to-strain (agent-surrogate) comparisons, selection of debris to combine with different coronaviruses, test methods advancement, a need to increase virus titers to improve statistical confidence and optimizing test conditions for temperature and humidity. Agent-surrogate comparison testing is also needed in order to ascertain if SARS-CoV-2 has unnatural environmental or anti-microbial persistence compared to other coronaviruses.

3 | RECOMMENDATION

3.1 Knowledge on SARS-CoV-2 surrogates is a rapidly growing field. Therefore, ASTM encourages feedback on the surrogates listed in the table. Please contact ASTM Committee E35 at BMilewski@astm.org with additional information.

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Available</th>
<th>Genetic Material</th>
<th>Virus Type</th>
<th>Key Characteristics</th>
<th>Receptor</th>
<th>BSL/Cost</th>
<th>Number of Recommendations</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Coronavirus 229E</td>
<td>ATCC VR-740</td>
<td>ss RNA, positive sense, enveloped</td>
<td>coronavirus, alpha group</td>
<td>Human, Bat</td>
<td>Aminopeptidase N and ACE2</td>
<td>BSL2/ $593</td>
<td>12</td>
<td>Cell lines VRC-5 (ATCC #CCL-171); advantage: there are many 229E derived disinfection data available. Pitfall: 22E belongs to alpha group, not beta. Not certain whether 229 could well represent inactivation profile of COVID 19 (please refer to comments for OC43). A549 cells express ACE2 receptor (PMID 19685004). The titer from collection ~ 4.5log_{10}mL TCID50. Grow fast but do not produce high titer population. Strain 229E can also grow on WI-38 cells.</td>
</tr>
<tr>
<td>Human Coronavirus NL63</td>
<td>BEI #NR-470</td>
<td>ss RNA, positive sense, enveloped</td>
<td>coronavirus, alpha group</td>
<td>Bat, Swine, Human</td>
<td>ACE2</td>
<td>BSL2</td>
<td>3</td>
<td>Cell line Macaca mulatta kidney epithelial cells (LLC-MK2) (ATCC # CCL-7.1), testing using the OECD method and ASTM E1053 method. NL63 is available from BEI Resources. Will be appropriate surrogate when virus receptors are the object studies as it utilizes the same receptors as SARS.</td>
</tr>
<tr>
<td>Feline Infectious Peritonitis virus</td>
<td>ATCC VR-2009, ATCC VR-2128, ATCC VR-1812</td>
<td>ss RNA, positive sense, enveloped</td>
<td>coronavirus, alpha group</td>
<td>Feline</td>
<td>Aminopeptidase N</td>
<td>BSL2/ $593</td>
<td>3</td>
<td>Used in disinfectant efficacy testing, mainly to the EN and ISO methods as it is validated for EN methods.</td>
</tr>
<tr>
<td>Human Coronavirus OC43</td>
<td>ATCC VR-1558, ATCC VR-759 (no longer available)</td>
<td>ss RNA, positive sense, enveloped</td>
<td>coronavirus, beta group</td>
<td>Human, Bat</td>
<td>Aminopeptidase N</td>
<td>BSL2/ $593</td>
<td>4</td>
<td>Recommended strain OC43 (ATCC # CCL-1558). OC43 strain is easy to cultivate on HT-8 cells which are available from ATCC (ATCC # CCL-244). This strain is genetically similar to SARS-CoV-2 (beta group); Compared to 229 E, OC43 appeared to be less stable on surfaces (Wames et al. mBIO 6(6) e10697), but showed high resistance to benzalkonium chloride than other CoV surrogate viruses (MHV, and CCV) (Kampf et al., 2020, J Hospital Infection 104:246-251, and Wood et al. J Hospital Infection (1988) 38, 283-295). OC43 will be the closest among human</td>
</tr>
<tr>
<td>Surrogate Name</td>
<td>Available</td>
<td>Genetic Material</td>
<td>Virus Type</td>
<td>Vector</td>
<td>Receptor</td>
<td>BSL/Cost</td>
<td>Number of Recommendations</td>
<td>Comments</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>------------</td>
<td>--------</td>
<td>---------</td>
<td>---------</td>
<td>--------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>SARS-CoV-1 (Strain: Urbani)</td>
<td>BEI #NR-18925</td>
<td>ss RNA, positive sense, enveloped</td>
<td>coronavirus, beta group</td>
<td>Human, bat, civet cat</td>
<td>ACE2</td>
<td>BSL3</td>
<td>1</td>
<td>Limited order (1 via a year).</td>
</tr>
<tr>
<td>SARS-CoV-2 (Strain: USA-WA1/2020)</td>
<td>BEI #NR-52281</td>
<td>ss RNA, positive sense, enveloped</td>
<td>coronavirus, beta group</td>
<td>Human, bat</td>
<td>ACE2</td>
<td>BSL3</td>
<td>3</td>
<td>This virus is as cultivable as other coronaviruses, thereby diminishing the need for surrogate viruses.</td>
</tr>
</tbody>
</table>
Similar survivability on surfaces is seen with HCoV-NL63 (up to 7 days) and 229E (5–6 days).

HCoV-NL63 is available through Zeptrometrix, and growth of the virus is possible through readily available cell lines such as Huh-7 and 293T cells.

Testing on the SARS-CoV-2 should only be utilized in BSL-3 until the world population has immunity to it via vaccination.

See tab below for CDC response

I agree with reducing the list to 5, or less, surrogates and with restricting the list to Coronaviridae.

I think we could work with the list of options for surrogates that you sent around, and I don’t think we need other options for surrogates. I think it would be good to narrow down the list to 5 options. I would also use a coronavirus, and leave other viruses off the list as an option. One of my top choices for a potential surrogate would be Human Coronavirus strain 229E.

Since some contract and academic labs have the actual SARS-CoV-2 virus available for testing, I would suggest selecting two or three potential surrogates and comparing them to the SARS-CoV-2. Then we can see which potential surrogate would give the best indication of how SARS-CoV-2 would perform.

- Are there any further comments on any of the surrogates? I believe the list contains plenty of appropriate surrogates. I have nothing else to add. Regarding the SARS-CoV-2, it looks like the Vero CCL-81 & Vero E6 cells lines may be used for the isolation & passaging of the virus (https://wwwnc.cdc.gov/eid/article/26/6/20-0516_article).
- Should we cut the list down to top 5 or should more strains be added? I would suggest cutting the list down to five surrogates.
- Should we stick to coronavirus? Only 1 suggestion was not a coronavirus. I would recommend sticking to coronavirus.

Contributions on test methods: (ISO 21702:2019; ISO 20743:2013; ASTM E1052; EN 14476)

1. I would agree that since both SARS-CoV-2 and suitable cell lines (and other detection/quantification methods) are available, it seems the necessity of using a surrogate should be driven by perhaps factors other than efficacy evaluation itself? For example, does the motivation to use surrogate include the following:
   1) having more than one virus (i.e. SARS-CoV-2) available for manufacturer to conduct disinfectant efficacy testing, so the testing may not be limited by availability of a single source (virus and suitable cell lines)
   2) having something that is less “risky” than SARS-CoV-2 so it is safer for people who are performing efficacy testing?
   3) having a higher variety of viruses so more people / companies would be able to provide contracted efficacy testing for manufactures? Currently, growing SARS-CoV-2 require a BSL3, so having other <=BSL2 surrogates would enable higher accessibility for efficacy testing? This is related to #2, but focusing on the cell line detection part.
   4) having a surrogate that is easier and safer to work with and can be used for disinfectant validation (i.e. process validation) in addition to disinfectant efficacy testing?
   5) having a surrogate that is easier and quicker to measure in high throughput?

2. When this list is proposed, it would be good to provide, if not already planned, the following along with the list of surrogates:
   1) necessity/motivation of proposing such a list of surrogate
   2) selection criteria of the list of surrogate

3. Depending on answer to #1, I think common model bacteria phage might be good to include as surrogate, especially for disinfectant validation where the evaluation process might occur outside lab under well-controlled conditions. If so, I would recommend, in addition to animal coronaviruses, to include bacterial phage such as coliphage (non-enveloped virus, host E. coli) and phi6 (enveloped virus, host Pseudomonas syringae). They are safe to use, much easier and faster to measure, and also commonly used as surrogate in environmental fate and transport study for human viruses.

I believe testing different strains of Coronavirus is not adequate. You may include other more resistant organisms (two orders of magnitude (Enveloped < Non-Enveloped < Bacteria < Spores such as Mycobacterial strains in your testing.)