Guidance on SARS-CoV-2 Surrogate Selection

1 | SCOPE

PREVIOUS NEXT | TOP BOTTOM

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

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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

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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 1 POTENTIALS FOR SARS-CoV-2 SURROGATE 16 APRIL 2020

Strain Name	Available	Key Characteristics					Number of Recommendations	Comments
		Genetic Material	*Virus Type*	*Vector*	*Receptor*	*BSL/Cost*		Add comments pertaining to the use of the surrogate in the laboratory
Human Coronoavirus 229E	ATCC VR#-740	ss RNA, positive sense, enveloped	coronavirus, alpha group	Human, Bat	Aminopeptidase N and ACE2	BSL2/ $593	12	Cell lines MRC-5 (ATCC #CCL-171); advantage: there are many 229E derived disinfection data available. Pitfall: 22E belongs to alpha group, not beta. Not certain whether 229 could well represent inactivation profile of COVID 19 (please refer to comments for OC43). A549 cells express ACE2 receptor (PMID 19685004). The titer from collection ~ 4.5$\log_{10}$/mL TCID50. Grow fast but do not produce high titer population. Strain 229E can also grow on WI-38 cells.
Human Coronoavirus NL63	BEI #NR-470	ss RNA, positive sense, enveloped	coronavirus, alpha group	Bat, Swine, Human	ACE2	BSL2	3	Cell line Macaca mulatta kidney epithelial cells (LLC-MCK2) (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.
Feline infectious peritonitis virus	ATCC VR-2009, ATCC VR-2126, ATCC VR-1812	ss RNA, positive sense, enveloped	coronavirus, alpha group	Feline	Aminopeptidase N	BSL2/ $593	3	Used in disinfectant efficacy testing, mainly to the EN and ISO methods as it is validated for EN methods.
Human Coronavirus OC43	ATCC VR-1558, ATCC VR-759 (no longer available)	ss RNA, positive sense, enveloped	coronavirus, beta group	Human, Bat	Aminopeptidase N	BSL2/ $593	4	Recommended strain OC43 (ATCC # CCL-1558), OC43 strain is easy to cultivate on HTC-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 (Warnes 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

								viruses. The titer from collection ~ 6.5 log_{10}/mL TCID50. Takes ~ a week to grow.
Murine hepatitis virus (MHV) / Murine Coronavirus	ATCC VR#-764, ATCC VR#-766, ATCC VR#-261	ss RNA, positive sense, enveloped	coronavirus, beta group	Mice	CEACAM1		1	NCTC 1469 cell line (ATCC® CCL-9.1): ATCC VR 764; 1-2 days of incubation, VR 766; 1-2 day(s) of incubation; VR 261, 4-7 days of incubation, also note that this virus is genetically similar to COVID 19 (beta group).
Transmissible gastroenteritis virus (TGEV)	ATCC VR#-763	ss RNA, positive sense, enveloped	coronavirus, alpha group	Swine	Aminopeptidase N	BSL2/ $593	1	
Canine coronavirus (CCV)	ATCC VR#-2068		coronavirus	Canine	Aminopeptidase N	$593	1	$593, ST cell line (ATCC CRL-1746) or CRFK cell (ATCC CCL): Naylor et. al. JCM 2001
Porcine respiratory coronavirus	ATCC VR#-2384	ss RNA, positive sense, enveloped	coronavirus, alpha group	Swine		BSL2/ $593	1	ST cell line (ATCC CRL-1746): 2-3 days of incubation.
Influenza A Virus (IFV), Strain H1N1 *NOTE: non-coronavirus surrogate*	ATCC VR#-1469	RNA	influenza		Sialic Acid		1	Cell line Madin-Darby Canine Kidney (MDCK) (ATCC # CCL 34); influenza virus for testing with the OECD method (a method similar to ASTM E2197), work with human coronavirus NL-63 with the OECD method and ASTM E1053 method.

SARS-CoV-1 and SARS-CoV-2 - NO SURROGATE 16 APRIL 2020

Surrogate Name	**Available**	**Key Characteristics**					**Number of Recommendations**	**Comments**
		Genetic Material	***Virus Type***	***Vector***	***Receptor***	***BSL/Cost***		**Add comments pertaining to the use of the surrogate in the laboratory**
SARS-CoV-1 (Strain: Urbani)	BEI #NR-18925	ss RNA, positive sense, enveloped	coronavirus, beta group	Human, bat, civet cat	ACE2	BSL3	1	Limited order (1 vial a year).
SARS-CoV-2 (Strain: USA-WA1/202)	BEI #NR-52281	ss RNA, positive sense, enveloped	coronavirus, beta group	Human, bat	ACE2	BSL3	3	This virus is as cultivable as other coronaviruses thereby diminishing the need for surrogate viruses. Availability of the actual causative agent of COVID-19 for testing reduces the need for surrogate viruses that may cause diseases that are less morbid and severe. This is BSL3. The severity of the disease is only relevant to products that intend to cure the disease.

TABLE 2 Comments and Questions

Can we assume that all of these strains can be handled in a BSL 2 laboratory? Did they mention this when submitting? This was a requirement but not sure if it was followed as there are many non-human viruses listed as well, which was also a requirement (desire).

Should we cut the list down to top 5 or should more strains be added? Yes, even down to 4 (OC43, Murine hepatitis virus as Beta Coronaviruses; 229E and NL63 as Alphacoronaviruses)

Should we stick to coronavirus? Only 1 suggestion was not a coronavirus. Yes, it is hardly justifiable not to use coronavirus as a surrogate for coronavirus.

I believe that the list of proposed surrogates is already too long. Virtually every coronavirus is listed there with a strong potential for confusion. I suggest that the influenzavirus should be deleted from the list. SARS-2 is also mentioned, but it requires BSL-3 facilities to handle it with the attendant risk to the health of lab workers.
Human respiratory coronavirus 229E has been in use as a surrogate for the more pathogenic coronaviruses over many years now with no issues. It can be cultured and its infectivity assayed relatively easily while requiring BSL-2 containment. 229E is already a part of certain ASTM standards (E35.15). Its use obviates the need for using animal pathogenic coronaviruses unless required for veterinary settings.
Please let me know if you need more information in the matter.

- Are there any further comments on any of the surrogates?
  - o The SARS-CoV-2 Virus which causes the COVID-19 disease is a BSL3 virus and would limit availability.
  - o Therefore, we must work a BSL2 surrogate that is accessible and safe to use in most labs.
- Should we cut the list down to top 5 or should more strains be added?
  - o I think the top 5 viruses would be sufficient and could even be reduced to three:
    - (1) Human Coronoavirus 229E,
    - (2) Human Coronoavirus NL63, and
    - (3) Human coronavirus OC43 o These are all of human origins, impact the respiratory tract, and are BSL 2 organisms. Correction: The 3 viruses are not necessarily of human origin. They are known to be able to infect humans.
- Should we stick to coronavirus? Only 1 suggestion was not a coronavirus.
  - o Yes, let's stick to the coronaviruses. Also, it would be best that the surrogates are involved in respiratory infections.
  - o The Feline infectious peritonitis virus (also known as Feline Coronavirus) primarily affects the feline GI tract.
- Test methods:
  - o ISO 18184:2019 (Textiles — Determination of antiviral activity of textile products)
  - o ISO 21702:2019 (Measurement of antiviral activity on plastics and other non-porous surfaces)
  - o Both use Influenza A (H3N2) or (H1N1) as surrogate for enveloped viruses and Feline Calicivirus as surrogate for nonenveloped viruses.

One note: human Coronavirus strain 229E can also grow on WI-38 cells. Human lunch fibroblast cell, ATCC CCL-75 $476

I believe adding a column for special requirements (e.g., BSL 2, BSL 3, availability) would be useful, and yes, use of a coronavirus is preferred, so I'm inclined to suggest that we delete other viruses at this time. Thanks for your important work.

I recommend the Human coronavirus NL63 (HCoV-NL63) when taking into account safety, ease of obtaining and culturing of the virus and known survival of the virus within the environment. I believe the group should only consider the 7 coronaviruses which are known to infect humans.

I would prefer a betacoronavirus to test against, but alphacoronaviruses cause a much milder disease in humans and from and safety standpoint would be more preferable to work with under BSL-2. HCoV-229E and HCoV-NL63 are both alphacoronaviruses and have a bat origin.

HCoV-NL63, like SARS-CoV-2 (also originated in bats), uses the same ACE-2 receptor for infection. NL063 was discovered in 2004, making it a more recent coronavirus than HCoV-229E.

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 additional 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.