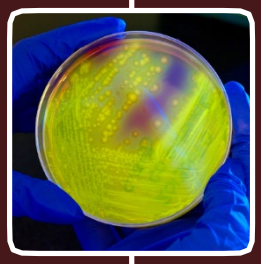

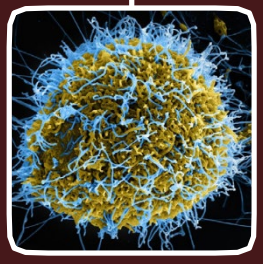

Regulation of SARS-CoV/SARS-CoV-2 Chimeric Viruses Guidance

March 2023

Centers for Disease Control and Prevention
Division of Select Agents and Toxins

Animal and Plant Health Inspection Service
Division of Agricultural Select Agents and Toxins

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Change/Highlight Section

Revisions: This is a living document subject to ongoing improvement. Feedback or suggestions for improvement from registered select agent entities or the public are welcome. Submit comments directly to the Federal Select Agent Program (FSAP) via CDC's Division of Select Agents and Toxins (DSAT) at LRSAT@cdc.gov.

Revision History:

Introduction

This document provides information to assist entities in meeting the requirements for conducting a SARS-CoV/SARS-CoV-2 chimeric virus restricted experiment or possessing the products resulting from such restricted experiments.

Regulation of SARS-CoV/SARS-CoV-2 Chimeric Viruses and Genomic Material

Since SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any intentional manipulation of SARS-CoV-2 to include nucleic acids coding for SARS-CoV virulence factors are select agents, entities are required to obtain prior approval from DSAT to possess, use, or transfer these agents. Additionally, experiments to create these chimeric viruses must be submitted to the FSAP for prior approval.

Nucleic acids encoding for genetically modified select agents that can produce infectious forms of any of the select agent viruses are also regulated as select agents. This is currently limited to positive-strand RNA virus genomic material. The positive strand RNA SARS-CoV/SARS-CoV-2 genome is regulated since it is an immediate precursor to virus production. Regulation is limited to positive strand RNA forms of the viral genome that can be translated into protein precursors for virus production. Complementary DNA (cDNA) copies of the SARS-CoV/SARS-CoV-2 chimeric virus genome are not regulated because the cDNA would first need to be transcribed into RNA and translated into protein. Therefore, the cDNA would not be an immediate precursor to virus.

SARS-CoV/SARS-CoV-2 Chimeric Virus Restricted Experiments

The Department of Health and Human Services (HHS) select agent regulations, 42 CFR Part 73, were also modified as part of this rulemaking to designate SARS-CoV/SARS-CoV-2 chimeric virus restricted experiments as those that involve the creation of SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors or vice versa. “Vice versa” means that the deliberate manipulation of SARS-CoV to incorporate nucleic acids coding for SARS-CoV-2 virulence factors would also be a restricted experiment and require prior approval from FSAP before generating the chimeric virus. The phrase “nucleic acids” includes entire genes, groups of genes, parts of a gene, or any modifications that may lead to phenotypic changes.

The restricted experiment provision applies to the deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors,or vice versa. If virulence is diminished instead of enhanced in the SARS-CoV/SARS-CoV-2 chimeric virus, the entity can submit an exclusion request for the resulting chimeric virus. If non-deliberate modifications occur that are consistent with the description above, entities should contact FSAP for further guidance.

Resources to Assist Entities Assessing Whether Their Work is Regulated or Considered a Restricted Experiment

There are several factors to consider when assessing whether proposed work may be regulated or a restricted experiment. Review of genetic sequence information and the incorporation of nucleic acids coding for virulence factors are key elements to consider in this assessment.

Entities should refer to public sequence repositories to determine whether the experiment they are considering may involve the creation of SARS-CoV/SARS-CoV-2 chimeric viruses. SARS- CoV/SARS-CoV-2 chimeric viruses result from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors or vice versa.

Public sequence repositories such as the National Center for Biotechnology Information (NCBI)/National Library of Medicine (NLM) (https://www.ncbi.nlm.nih.gov/sars-cov-2) and Global Initiative on Sharing Avian Influenza Data (GISAID) (https://www.gisaid.org) contain references for the SARS-CoV-2 genome, including variant information. For SARS-CoV, reference sequences from common laboratory strains to include Tor2 and Urbani can be found here: https://www.ncbi.nlm.nih.gov/nuccore/AY274119 and https://www.ncbi.nlm.nih.gov/nuccore/AY278741 for comparison to SARS-CoV-2.

Virulence factors are those genes or gene modifications that are associated with virulence, including those that lead to changes in structures, molecules, immunological regulatory systems, etc. Virulence factors determine a pathogen's ability to replicate, modify infectivity and host defenses, spread within the host and to other individuals (transmissibility), and produce products that are toxic to the host. These factors may impact vaccine sensitivity, resistance to medical countermeasures, pathogenicity, transmissibility, and disease severity.

Experiments with these virulence factor genes alone are not regulated. However, experiments to create SARS-CoV/SARS-CoV-2 chimeric viruses from the deliberate transfer of virulence factors from SARS-CoV to SARS-CoV-2 or vice versa are regulated. The following list of virulence factors is not all-inclusive but is provided to assist entities in determining if the work they are performing is subject to the select agent regulations.

Examples of Virulence Factors:

- Open Reading Frame 3a structural protein (ORF3a)
- Open Reading Frame 8b structural protein (ORF8b)
- Envelope protein (E), spike glycoprotein (S), transmembrane glycoprotein (M), and double membrane vesicle protein (DMV)
- Non-structural proteins (NSP) 1,3,4,6,15,16
- Suppressors of cytokine signaling (SOCS)

See Appendix for additional examples of SARS-CoV and SARS-CoV-2 virulence factors.

It is the entity's responsibility to submit proposed work to create SARS-CoV/SARS-CoV-2 chimeric viruses from the deliberate transfer of virulence factors from SARS-CoV to SARS-CoV-2 or vice versa to FSAP for review and approval prior to initiating work.

Examples of SARS-CoV/SARS-CoV-2 Chimeric Virus Restricted Experiments

The purpose of this section is to give examples of experiments that meet the definition of a restricted experiment and would therefore require prior approval before creating the chimeric virus. The experiments described here are not exclusive. If you are uncertain whether a potential chimeric virus is regulated or an experiment is restricted and requires prior approval, please contact FSAP for further guidance.

Example 1: A researcher wants to understand the gene functions in SARS-CoV-2 to identify potential targets for therapeutic drug development. The researcher wishes to replace SARS-CoV-2 genes thought to be associated with cell death or inflammation, such as ORF3a and NSP4, with SARS-CoV genes. The result would be a set of SARS-CoV-2/SARS-CoV chimeric viruses. Since this experiment involves the creation of SARS-CoV-2 chimeric viruses where nucleotides in regions associated with virulence would be exchanged for the SARS-CoV nucleotide sequence, it would be considered a restricted experiment.

Example 2: A researcher wants to create chimeric viruses by replacing nucleic acids within the receptor-binding domain of SARS-CoV-2 spike protein with the corresponding nucleic acids from the SARS-CoV spike protein to understand the importance of different amino acid sequences on viral binding. The binding properties of spike protein play an important role in transmissibility and virus pathogenicity. It is possible that making changes to this region could result in a more transmissible virus than the parent.
Since this experiment involves the creation of SARS-CoV-2 chimeric viruses where nucleotides in regions associated with virulence would be exchanged for the SARS-CoV nucleotide sequence, it would be considered a restricted experiment.

Example 3: A pharmaceutical company is interested in developing an mRNA vaccine that results in production of neutralizing antibodies against the N-terminal domain of the spike protein of SARS-CoV and SARS-CoV-2. To identify the best sequence to use in the mRNA vaccine, the company would like to create a series of SARS-CoV-2 viruses with different nucleotide sequences in the N-terminal domain, including the sequence from SARS-CoV. The viruses would be used to infect an animal model and analyze the neutralizing antibodies created. The result would be a set of SARS-CoV-2/SARS-CoV chimeric viruses. Since this experiment involves the creation of SARS-CoV-2 chimeric viruses where nucleotides in regions associated with virulence would be exchanged for the SARS-CoV nucleotide sequence, it would be considered a restricted experiment.

Example 4: A biotech company is studying the role of coronavirus proteins in viral infection. They plan to create a series of chimeric SARS-CoV viruses where SARS-CoV-2 Open Reading Frames (ORFs) for regulatory or accessory proteins thought to be associated with virulence will be incorporated. The effect on virus-host interactions and pathogenicity for many of these ORFs is not known. The result would be a set of SARS-CoV-2/SARS-CoV chimeric viruses. Since this experiment involves the creation of SARS-CoV chimeric viruses in which nucleotides that are expected to be associated with virulence would be exchanged for SARS-CoV-2 nucleotide sequences, it would be considered a restricted experiment.

Example of a SARS-CoV/SARS-CoV-2 Chimeric Virus Experiment that does not meet the Definition of a Restricted Experiment

The purpose of this section is to give an example of an experiment that does not meet the definition of a restricted experiment and would therefore not require prior approval before creating the chimeric virus. If you are uncertain whether a potential chimeric virus is regulated or an experiment is restricted and requires prior approval, please contact FSAP for further guidance.

Example: An entity proposed four mutant derivative strains of SARS-CoV-2 with a series of alanine amino acid substitutions in ORF3a. The four proposed SARS-CoV-2 mutant strains containing alanine amino acid substitutions in ORF3a are not select agents. These proposed SARS-CoV-2 mutant strains are not chimeric viruses incorporating nucleic acids coding for SARS-CoV virulence factors into SARS-CoV-2 and, therefore, would not be subject to the requirements of the select agent regulations. This determination is based solely on the observation that the proposed alanine substitutions in SARS-CoV-2 ORF3a do not correspond to a homologous alanine at the same positions in SARS-CoV open reading ORF3a.

Request Process to Conduct SARS-CoV/SARS-CoV-2 Chimeric Virus Restricted Experiments

The entity's Responsible Official should submit any request to conduct a restricted experiment via the General Discussion tab on the landing page in the eFSAP information system. The request should not be submitted as part of an amendment to an entity's registration. Doing so may delay approval of other changes proposed in the amendment while the restricted experiment request is under review. The entity may only amend their registration after the restricted experiment is approved. The registration is updated by submitting the appropriate Animal and Plant Health Inspection Service (APHIS)/CDC Form 1 section updates related to the experiment. See Responsible Official Guidance Document (selectagents.gov) for additional information on how to update an entity's registration.

When requesting to conduct a restricted experiment, the entity should submit the following relevant information via the General Discussion tab on the landing page in the eFSAP information system:

- Synopsis of the proposed experiment(s) and the intended objective(s).
- Description (including relevant sequence alignments) of the modified nucleic acid sequences (if applicable) and the predicted biological characteristics of the synthetic/recombinant product.
- Method for creating the chimeric virus(es).
- Identification and characteristics of the host organism used for molecular cloning (if applicable).
- Description of biosafety level including facility containment, equipment, and special practices to be utilized for the proposed experiment(s).
- Synopsis of any planned animal experiments (if applicable) or other relevant animal work.
- Expected timeframe of the proposed experiment(s).
- Description of long-term and short-term storage plans for samples.
- Scientific references or supporting documentation, particularly with respect to the biosafety aspects of the proposed experimental product.

If an entity is unsure if their proposed work is considered a restricted experiment, they should contact FSAP for assistance.

Request to Possess the Product Resulting from a SARS-CoV/SARS-CoV-2 Chimeric Virus Restricted Experiment

Section 13 of the select agent regulations provides that the possession of the product from a restricted experiment is regulated. Therefore, an entity must submit a request through the eFSAP information system for permission to possess the product. If the possession of the product from a restricted experiment is approved, the entity must submit a request to amend their registration to update the relevant information in accordance with Section 7 of the regulations. This includes updating the entity's work objectives and designating a Principal Investigator that would possess the product.

The requirements outlined above apply to products resulting from a restricted experiment conducted inside of the United States or products imported into the United States.

Possession of a product resulting from a restricted experiment requires pre-approval from FSAP before a transfer request is approved. The potential receiver must submit the following documentation to FSAP for review prior to transfer:

- Synopsis of the proposed experiments (if applicable) and the intended objectives.
- Description of biosafety level including facility containment, equipment, and special practices to be utilized for the proposed experiments.
- Synopsis of any planned animal experiments (if applicable) or other relevant animal work.
- Description of the transferor (e.g., entity, name of person initiating transfer, point of contact information).
- Description of occupational health and medical surveillance procedures (applicable to non-Tier 1 select agents as recommended in *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) (e.g., SARS–CoV).
- Scientific references or supporting documentation, particularly with respect to the biosafety aspects of the proposed experimental product. The supporting documentation should reflect the safety measures in place that would support the request to acquire the product of a restricted experiment from another entity.

If the request to possess the product from a restricted experiment is approved, the entity should then request to amend their registration by submitting the appropriate APHIS/CDC Form 1 section updates.

In addition, the APHIS/CDC Form 2, Request to Transfer Select Agents and Toxins, must be completed and submitted through the eFSAP information system prior to transferring any select agent(s). Please refer to the APHIS/CDC Form 2 instructions and guidance documents for more information.

Seeking Exclusions for Attenuated SARS-CoV/SARS-CoV-2 Chimeric Viruses

Section 3 of the select agent regulations provide criteria for the exclusion of select agents that have been modified to be attenuated such that they no longer have the potential to pose a severe threat to public health and safety. Some of the criteria used to determine if an attenuated SARS-CoV/SARS-CoV-2 chimeric virus should be excluded are given below.

The applicant's request should contain the following:

- Documented history of not causing disease in humans or relevant animal models.

- Defined genetic mutations or alterations known to attenuate virulence in humans or relevant animal models.
- Data showing the mutations have a low frequency of reversion to wild-type virulence.
- Level of difficulty in engineering the attenuated strain to restore wild-type virulence. For each pathogen, the sample size and type of animal models used to test virulence is important.
- Quantitative measures demonstrating a change in virulence in an appropriate animal model with appropriate controls.
- Information regarding results from tests that were conducted to differentiate animals exposed to the attenuated strain from those infected with the wild-type organism.
- Related published scientific papers supporting the methods and data provided for the exclusion.

FSAP will grant or deny the request and will state, in writing, the reasons for the decision. Exclusions are effective upon notification to the applicant.

If an excluded attenuated strain is subjected to any manipulation that restores or enhances its virulence, the resulting select agent will be subject to the select agent regulations [42 CFR 73.3(e)(2)].

For regulated nucleic acids from positive-stranded RNA viruses to be excluded from the regulations, they must be rendered incapable of forming an infectious virus using an in-house validated inactivation procedure that includes viability testing. For additional information about inactivation requirements, see Guidance on the Inactivation or Removal of Select Agents and Toxins for Future Use.

Useful Links and Resources

- Burrell, C. J., Howard, C. R., & Murphy, F. A. (2016). Fenner and White's medical virology. Academic Press.
- DHS Science and Technology (2022). Master Question List for COVID-19 (caused by SARS CoV-2) Monthly Report. US Department of Homeland Security. Retrieved January 18, 2022, from https://www.dhs.gov/sites/default/files/2022-01/22_0111_st_mql_sars_cov-2.pdf.

- Inactivation Guidance Document
- Exclusion Guidance Document
- Guidance on the Regulation of Select Agent and Toxin Nucleic Acids
- Restricted Experiments Guidance
- Select Agents and Toxins Biosafety/Biocontainment Plan Guidance
- Interim Final Rule, Federal Register vol. 86, No. 219. November 17, 2021 (Link: https://www.federalregister.gov/d/2021-25204)
- Federal Select Agent Program (selectagents.gov)
- Responsible Official Guidance Document

Appendix

Table 1. Examples of SARS-CoV and SARS-CoV-2 Virulence Factors[1]

Protein	Virus	Examples of Functions	References*
nsp1	SARS-CoV-2	Binds to human ribosome machinery & shuts down translation; blocking immune response Interference of JAK/STAT pathway	(5,3) (1)
nsp1	SARS-CoV	Dysregulated IFN production Inhibition of RIG-I like receptors (MDA5, PKR)- mediated IFN-β	(1,3)
nsp3	SARS-CoV	Dysregulated IFN production Inhibition of Rig-I like receptors (MDA5, PKR)-mediated IFN-β	(1,3)
nsp4	SARS-CoV	Dysregulated IFN production Inhibition of RIG-I like receptors (MDA5, PKR)- mediated IFN-β	(1,3)
nsp6	SARS-CoV	Dysregulated IFN production Inhibition of RIG-I like receptors (MDA5, PKR)- mediated IFN-β	(1,3)
nsp7	SARS-CoV	Antagonizes type-I IFN	(2,3)
nsp15	SARS-CoV	Antagonizes type-I IFN	(2,3)
DMV (double membrane vesicles)	SARS-CoV	Dysregulated IFN production Inhibition of Rig-I like receptors (MDA5, PKR)-mediated IFN-β	(1)
ORF8b/ab	SARS-CoV	Dysregulated IFN production Inhibition of Rig-I like receptors (MDA5, PKR)-mediated IFN-β	(1,3)
ORF6	SARS-CoV	Dysregulated IFN production Inhibition of RIG-I like receptors (MDA5, PKR)-mediated IFN-β	(1,3)
M protein	SARS-CoV	Dysregulated IFN production	

[1] This list is subject to change, including the addition or removal of factors.

Protein	Virus	Examples of Functions	References*
		Inhibition of RIG-I like receptors (MDA5, PKR)-mediated IFN-β	(1)
endonuclease	SARS-CoV	Dysregulated IFN production Inhibition of RIG-I like receptors (MDA5, PKR)-mediated IFN-β	(1,3)
Nsp16	SARS-CoV-2	Disguises viral mRNA as eukaryotic mRNA that is methylated at the 5' cap, thereby avoiding recognition by cytoplasmic PRR and MDA5, and downstream type I IFN responses	(3)
E protein	SARS-CoV / SARS-CoV-2	Alteration of Ca2+ concentrations	(1,3)
ORF3a	SARS-CoV/ SARS-CoV-2	Interaction with TRAF3 and ASC Interaction with NLRP3 leading to pyroptosis	(1) (3,4)
ORF8b	SARS-CoV	Interaction with NLRP3 leading to pyroptosis	(1,3)
SUDNsp3c	SARS-CoV	Induction c-Jun/c-Fos pathway	(1)
S protein	SARS-CoV/ SARS CoV-2	Exhaustion of NK cells	(1)
S protein	SARS-CoV	Induces the expression of IL6, IL8, CXCL10 and TNF through NF-kB activation in macrophages	(2)

Protein	Virus	Examples of Functions	References*
M protein	SARS-CoV	Blocks IFN-B production by impairing the formation of TRAF3-TANK-TBK1/IKK complex	(2,3)
N protein	SARS-CoV	Antagonizes type I IFN production by blocking IRF-3 phosphorylation	(2,3)
3a	SARS-CoV	Downregulates the expression of the type I IFN receptor (IFNAR) leading to blockade on IFN signaling	(2)
3b	SARS-CoV	Antagonizes type I IFN production by blocking IRF-3 phosphorylation. Inhibits IFN signaling	(2)
6	SARS-CoV	Antagonizes type I IFN production by blocking IRF-3 phosphorylation	(2)
7a	SARS-CoV	Activates NF-kB and upregulates the expression of the proinflammatory mediators IL8 and CCL5	(2)

*Appendix References

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3. Gorkhali, R., Koirala, P., Rijal, S., Mainali, A., Baral, A., & Bhattarai, H. K. (2021). Structure and function of major SARS-CoV-2 and SARS-CoV proteins. *Bioinformatics and Biology Insights*, 15, 11779322211025876.
4. Ren Y, Shu T, Wu D, Mu J, Wang C, Huang M, Han Y, Zhang XY, Zhou W, Qiu Y, Zhou X. The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. *Cell Mol Immunol.* 2020 Aug;17(8):881-883. doi: 10.1038/s41423-020-0485-9. Epub 2020 Jun 18. PMID: 32555321; PMCID: PMC7301057.
5. Vann KR, Tencer AH, Kutateladze TG. Inhibition of translation and immune responses by the virulence factor Nsp1 of SARS-CoV-2. *Signal Transduct Target Ther.* 2020 Oct 9;5(1):234. doi: 10.1038/s41392-020-00350-0. PMID: 33037187; PMCID: PMC7545794.