

SARS-CoV/SARS-CoV-2 Chimeric Viruses Guidance Overview

June 29, 2023



Objectives

- Discuss the regulation of SARS-CoV/SARS-CoV-2 chimeric viruses and genomic material
- Provide resources to assist entities with compliance
- Provide examples of SARS-CoV/SARS-CoV-2 chimeric viruses
- Review the request processes for exclusions and restricted experiments



Regulations

42 C.F.R. Part 73.3 HHS select agents and toxins.

(b) 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.

42 C.F.R. Part 73.13 Restricted experiments.

(a)(3) Experiments 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.



Why is this Material Regulated?

- Combining a select agent virus (SARS-CoV) and a pandemic virus (SARS-CoV-2) has the potential to pose a severe threat to public health.
- Experiments to create SARS-CoV/SARS-CoV-2 chimeric viruses may result in the generation of a chimeric (hybrid) virus with the transmissibility of SARS-CoV-2 and the fatality rate of SARS-CoV.



Rulemaking Timeline



Nov 17, 2021

INTERIM
FINAL RULE
PUBLISHED



Dec 17, 2021

NOTIFICATION
OF POSSESSION
DEADLINE



Jan 18, 2022

PUBLIC
COMMENTS
DEADLINE



Feb 15, 2022

DSAT
REGISTRATION
DEADLINE



Mar 3, 2023

FINAL RULE
PUBLISHED &
GUIDANCE
DOCUMENT
POSTED



Final Rule – March 2023



FEDERAL REGISTER

The Daily Journal of the United States Government



® Rule

Possession, Use, and Transfer of Select Agents and Toxins- Addition 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 to the HHS List of Select Agents and Toxins

A Rule by the [Health and Human Services Department](#) on 03/03/2023



<https://www.federalregister.gov/d/2021-25204>



Guidance Document – March 2023



<https://www.selectagents.gov/compliance/guidance/chimeric/index.htm>



What is Regulated?

- SARS-CoV/SARS-CoV-2 chimeric viruses are select agents.
 - Result of adding nucleic acids coding for SARS-CoV virulence factors to SARS-CoV-2
- Genomic material for SARS-CoV/SARS-CoV-2 chimeric viruses is also subject to the regulations.
 - Positive strand RNA is an immediate precursor to virus production
- Complementary DNA or cDNA for SARS-CoV/SARS-CoV-2 chimeric viruses is **not** regulated.
 - RNA must no longer be present or capable of forming infectious virus



SARS-CoV/SARS-CoV-2 Restricted Experiments

- Restricted Experiments - 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” - the deliberate manipulation of SARS-CoV to incorporate nucleic acids coding for SARS-CoV-2 virulence factors would also be a restricted experiment.
 - “Nucleic acids” - entire genes, groups of genes, parts of a gene, or any modifications that may lead to phenotypic changes.

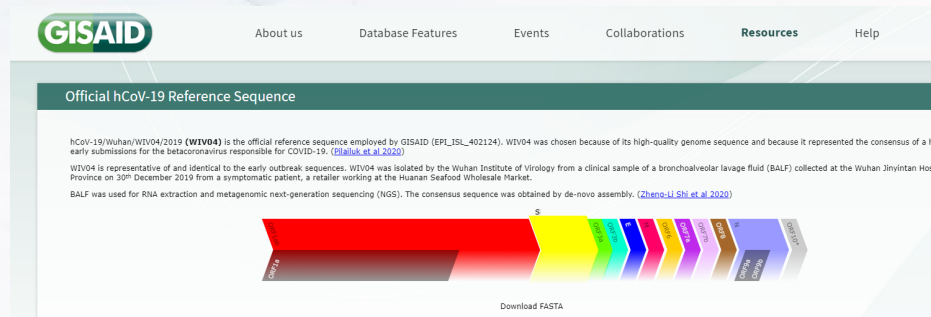


Resources to Assist Entities with Compliance

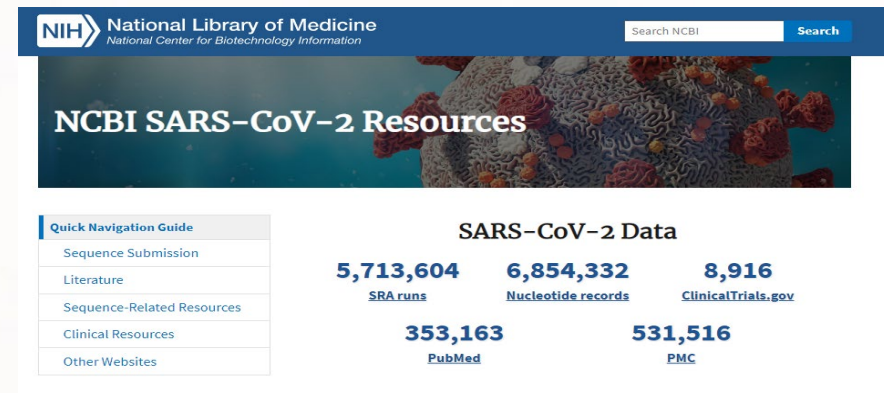


Public Sequence Repositories

- Determine whether proposed experiments involve the creation of SARS-CoV/SARS-CoV-2 chimeric viruses using:
 - National Library of Medicine (NLM)/National Center for Biotechnology Information (NCBI)
(<https://www.ncbi.nlm.nih.gov/sars-cov-2>)
 - Global Initiative on Sharing Avian Influenza Data (GISAID)
(<https://www.gisaid.org>)



The screenshot shows the GISAID website's 'Official hCoV-19 Reference Sequence' page. It features a navigation bar with 'About us', 'Database Features', 'Events', 'Collaborations', 'Resources', and 'Help'. Below the navigation bar, there is a section titled 'Official hCoV-19 Reference Sequence' with a green header. The main content area contains text explaining that the hCoV-19/Wuhan/WTIV04/2019 (WTIV04) is the official reference sequence. It mentions that WTIV04 was chosen due to its high-quality genome sequence and its representativeness. A diagram below the text shows a segmented bar representing the genome structure, with segments labeled 'ORF1a', 'ORF1b', 'S', 'E', 'G', 'C', and 'ORF3'. A 'Download FASTA' link is visible at the bottom of the diagram.



The screenshot shows the NCBI SARS-CoV-2 Resources page. It features a blue header with the NIH logo and the text 'National Library of Medicine National Center for Biotechnology Information'. A search bar is located in the top right corner. Below the header, there is a large banner with the text 'NCBI SARS-CoV-2 Resources' and a background image of a virus particle. To the left of the banner is a 'Quick Navigation Guide' with a list of links: 'Sequence Submission', 'Literature', 'Sequence-Related Resources', 'Clinical Resources', and 'Other Websites'. To the right of the banner is a 'SARS-CoV-2 Data' section with a table of statistics:

SARS-CoV-2 Data		
5,713,604	6,854,332	8,916
<small>SRA runs</small>	<small>Nucleotide records</small>	<small>ClinicalTrials.gov</small>
353,163	531,516	
<small>PubMed</small>	<small>PMC</small>	



Reference Sequences for SARS-CoV

- Tor2 and Urbani strains
 - <https://www.ncbi.nlm.nih.gov/nuccore/AY274119>
 - <https://www.ncbi.nlm.nih.gov/nuccore/AY278741>

An official website of the United States government [Here's how you know](#)

NIH National Library of Medicine
National Center for Biotechnology Information [Log in](#)

Nucleotide [Search](#)
Advanced

GenBank [Send to:](#) [Change region shown](#)
[Customize view](#)
[Analyze this sequence](#)

SARS coronavirus Tor2, complete genome
GenBank: AY274119.3
[FASTA](#) [Graphics](#)

An official website of the United States government [Here's how you know](#)

NIH National Library of Medicine
National Center for Biotechnology Information [Log in](#)

Nucleotide [Search](#)
Advanced [Help](#)

GenBank [Send to:](#) [Change region shown](#)
[Customize view](#)

SARS coronavirus Urbani, complete genome
GenBank: AY278741.1
[FASTA](#) [Graphics](#)



Virulence Factor Definition

Virulence factors:

- Are genes or gene modifications associated with virulence that lead to changes in structures, molecules, immunological regulatory systems, etc.
- 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.
- May impact vaccine sensitivity, resistance to medical countermeasures, pathogenicity, and disease severity.



Examples of SARS-CoV and SARS-CoV-2 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



Examples of Experiments – Restricted or Not Restricted



Experiment Example #1

Scenario

- A researcher studying SARS-CoV-2 gene function wants 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.

Would this be a restricted experiment?



Explanation - Experiment Example #1

Yes, this is a restricted experiment. The result would be a set of SARS-CoV-2/SARS-CoV chimeric viruses.

Explanation

- This experiment involves the creation of SARS-CoV-2 chimeric viruses.
- Specifically, nucleotides in regions associated with virulence will be exchanged for the SARS-CoV nucleotide sequence.
- Therefore, this would be considered a restricted experiment.



Experiment Example #2

Scenario

- 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.

Does this describe a restricted experiment?



Explanation - Experiment Example #2

Yes, this is a restricted experiment. The result would be a set of SARS-CoV2/SARS-CoV chimeric viruses.

Explanation

- 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.
- Therefore, this would be considered a restricted experiment.

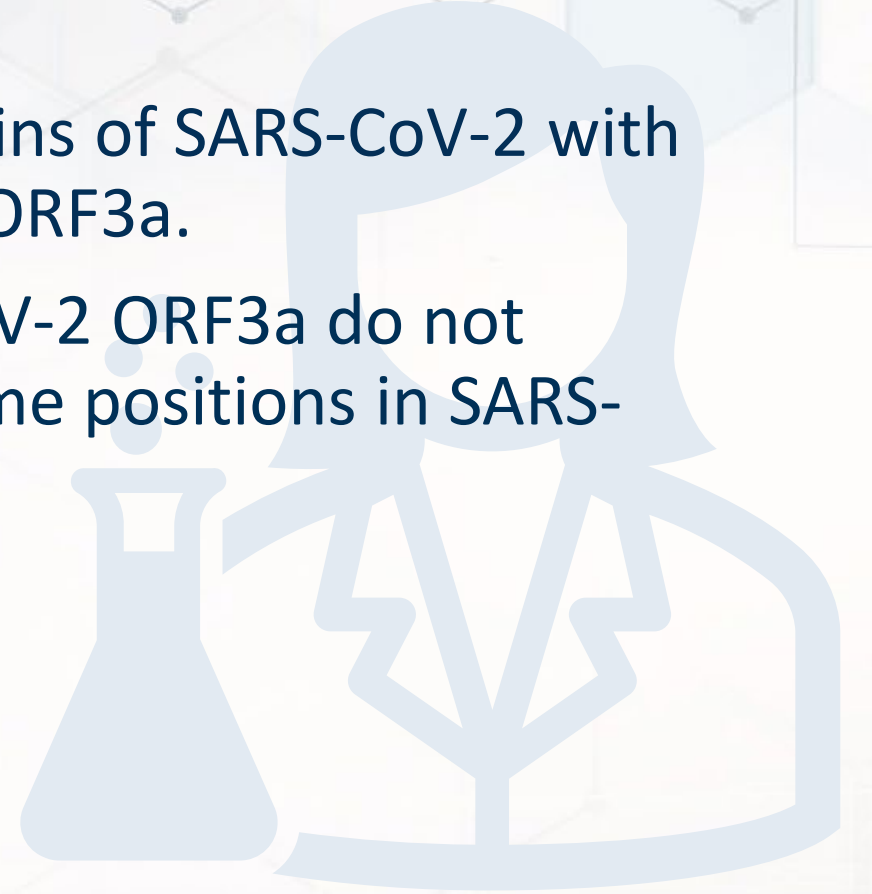


Experiment Example #3

Scenario

- An entity proposed four mutant derivative strains of SARS-CoV-2 with a series of alanine amino acid substitutions in ORF3a.
- The proposed alanine substitutions in SARS-CoV-2 ORF3a do not correspond to a homologous alanine at the same positions in SARS-CoV ORF3a.

Is this a restricted experiment?



Explanation - Experiment Example #3

No. The result would not be a restricted experiment.

Explanation

- SARS-CoV-2 is not a select agent.
- The proposed SARS-CoV-2 mutant strains are not chimeric viruses incorporating nucleic acids coding for SARS-CoV virulence factors into SARS-CoV-2. Therefore, the product 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 ORF3a.



DSAT Request Processes



Request to Conduct A Restricted Experiment

The following should be submitted via the **General Discussion** tab on the entity's 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 products



Method for creating the chimeric virus(es)



Identification and characteristics of the host organism used for molecular cloning (if applicable)



Synopsis of any planned animal experiments (if applicable) or other relevant animal work



Description of biosafety level including facility containment, equipment, and special practices to be utilized for the proposed experiment(s)



Scientific references or supporting documentation, particularly with respect to the biosafety aspects of the proposed experimental product



Expected timeframe of the proposed experiment(s)



Description of long-term and short-term storage plans for samples



Request to Possess Product of a Restricted Experiment

The potential receiver must submit the following documentation to DSAT for review prior to transfer:



Synopsis of 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 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)



Description of the transferor (e.g., entity, name of person initiating the transfer, point of contact information)



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.



Requesting Exclusions



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



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.



Information regarding results from tests that were conducted to differentiate animals exposed to the attenuated strain from those infected with the wild-type organism.



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.



Quantitative measures demonstrating a change in virulence in an appropriate animal model with appropriate controls.



Related published scientific papers supporting the methods and data provided for the exclusion.



Exclusion of Nucleic Acids from Positive-Stranded RNA Viruses

Nucleic acids:

- Must be incapable of forming infectious virus
- Require in-house validated inactivation and viability testing

Refer to the Guidance on the Inactivation or Removal of Select Agents and Toxins for Future Use for additional information.

<https://www.selectagents.gov/compliance/guidance/inactivation/index.htm>



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.
- Guidance on the Inactivation or Removal of Select Agents and Toxins for Future Use (<https://www.selectagents.gov/compliance/guidance/inactivation/index.htm>)
- Exclusion Guidance Document (<https://www.selectagents.gov/compliance/guidance/exclusions/index.htm>)
- Guidance on the Regulation of Select Agent and Toxin Nucleic Acids (<https://www.selectagents.gov/compliance/guidance/nucleic/index.htm>)
- Restricted Experiments Guidance (<https://www.selectagents.gov/compliance/guidance/restricted/index.htm>)
- Biosafety/Biocontainment Plan Guidance (<https://www.selectagents.gov/compliance/guidance/biosafety/index.htm>)
- Final Rule, Federal Register vol. 88, March 3, 2023 Possession, Use, and Transfer of Select Agents and Toxins-Addition 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 to the HHS List of Select Agents and Toxins
<https://www.federalregister.gov/documents/2023/03/03/2023-04323/possession-use-and-transfer-of-select-agents-and-toxins-addition-of-sars-covsars-cov-2-chimeric>



Summary

- SARS-CoV/SARS-CoV-2 chimeric viruses resulting from the deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors are select agents and regulated by 42 CFR Part 73. The genomic material is also regulated.
- Experiments involving the creation of SARS-CoV/SARS-CoV-2 chimeric viruses are potentially restricted experiments and require review by the DSAT prior to conducting work or possessing resulting products.
- Refer to the referenced resources for compliance assistance.



www.selectagents.gov

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Animal and Plant Health Inspection Service.

