Select Agent and Toxin Genetics: Regulatory Considerations

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DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

USDA

Genomic Material

Inactivation of Regulated Genomic Material

- For a sample to be excluded from the select agent regulations through the inactivation provisions, the virus and the genomic material must be rendered non-infectious and incapable of forming infectious virus using a validated inactivation procedure.
- Initial in-house validation of the inactivation procedure must be performed for both the virus and the regulated genomic material.

Policy on Work with Regulated Genomic Material

To work with Risk Group 3 (RG3), Risk Group 4 (RG4) and Veterinary Services (VS) agent (+) ssRNA genomic material in laboratories one containment level lower than the level required for the infectious virus, the following additional safety practices must be in place:

- The genomic material must be free of infectious virus before removing the genomic material from the laboratory designated to work with the live virus.
- For RG4 and Foot and Mouth Disease virus (FMDv) genomic work in BSL-3 laboratories, work must be performed inside a biosafety cabinet (BSC).
- For RG3 and VS agents (other than FMDv) genomic work in BSL-2 laboratories, work should be based on a risk assessment by the entity, although work in the BSC is preferred.
- No concurrent work with mammalian cell culture or *in vitro* translation experiments are conducted in the same laboratory.

Policy on Work with Regulated Genomic Material (Continued)

- No concurrent transfection work or *in vitro* translation reagents are used or stored in the same laboratory.
- Personal protective equipment must afford adequate mucosal membrane protection to avoid the risk of auto-inoculation and include the following:
  - Disposable or suite-dedicated lab coats.
  - Protective eyewear or face shield.
  - Gloves (latex, vinyl, nitrile, etc.) are chosen to resist those chemicals and/or solvents used in cloning procedures.
- Avoid glassware – plasticware is recommended.
- Avoid sharps, including needles and syringes.

Note: Regardless of the biosafety level used, the full-length genomes of any of the select agent viruses capable of producing infectious virus are regulated and must be handled in registered space.

Genetic Material Encoding BoNT

Recombinant/Synthetic Nucleic Acids Encoding for Toxins

42 C.F.R. 73.3(c) Genetic Elements, Recombinant and/or Synthetic Nucleic Acids, and Recombinant and/or Synthetic Organisms:

. . .

(2) Recombinant and/or Synthetic nucleic acids that encode for the toxic form(s) of any of the toxins listed in paragraph (b) of this section if the nucleic acids:

(i) Can be expressed *in vivo* or *in vitro*, or

(ii) Are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*.

https://selectagents.gov/regulations/interpretations/botulinum.htm

Regulatory Interpretation: Plasmids Containing Botulinum Neurotoxin Genes

Are plasmids containing full-length botulinum neurotoxin genes without a promoter for expression of the neurotoxin gene (DNA only, cannot be expressed even in a bacterial strain) subject to the select agent regulations?

- **No**. Plasmids containing genes encoding the full-length botulinum neurotoxin without a promoter for expression of the neurotoxin genes **are not regulated nucleic acids** because the plasmids cannot be expressed *in vitro* or *in vivo* as outlined in § 73.3(c)(2).

Are plasmids containing full-length botulinum neurotoxin genes with a promoter for expression of the neurotoxin gene (DNA only, can be expressed only in a bacterial expression strain) subject to the select agent regulations?

- **Yes**. Plasmids containing genes encoding the full-length botulinum neurotoxin with a promotor for expression of the neurotoxin genes **are regulated nucleic acids** because the plasmids can be expressed in the bacterial strain as outlined in § 73.3(c)(2).

Regulatory Interpretation: Plasmids Containing Botulinum Neurotoxin Genes (continued)

Are plasmids containing full-length botulinum neurotoxin genes with no promoter for expression of the neurotoxin gene in a bacterial strain subject to the select agent regulations?

- **No**. Plasmids containing genes encoding the full-length botulinum neurotoxin without a promoter for expression of the neurotoxin genes in a bacterial strain are not regulated nucleic acids because the plasmids cannot be expressed *in vitro* or *in vivo* as outlined in § 73.3(c)(2).

Are plasmids containing full-length botulinum neurotoxin genes with a promoter for expression of the neurotoxin gene in a bacterial strain subject to the select agent regulations?

- **Yes**. Plasmids containing full-length botulinum neurotoxin genes with a promotor for expression of the neurotoxin gene in a bacterial strain are regulated nucleic acids because the plasmids can be expressed in the bacterial strain as outlined in § 73.3(c)(2).

Regulatory Interpretation: mRNA Encoding Botulinum Neurotoxin

Question: Is mRNA from [botulinum neurotoxin producing species of *Clostridium*] [subject to the select agent and toxin regulations]?

It depends.

- In accordance with § 73.3(c)(2)(i), mRNA isolated from botulinum neurotoxin producing species of Clostridium are not regulated genetic elements because mRNA itself is incapable of being expressed in vitro or in vivo to produce botulinum neurotoxin.

However, if steps are taken to make mRNA isolated from botulinum neurotoxin producing species of *Clostridium* capable of being expressed *in vitro* or *in vivo* (e.g., packaging the mRNA for expression in a host, or preparing the mRNA for cell-free protein expression, or any other manipulation performed to render the mRNA capable of producing the toxin), those products would be regulated genetic elements subject to § 73.3(c)(2)(i).

- Additionally, if mRNA from a botulinum neurotoxin producing species of *Clostridium* is further manipulated for integration into a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*, those products would be regulated genetic elements subject to § 73.3(c)(2)(ii).

Regulatory Interpretation: mRNA Encoding Botulinum Neurotoxin (continued)

Question: Would the use of reverse transcription [of mRNA from botulinum neurotoxin producing species of *Clostridium*] to produce complementary DNA (cDNA) for fragmentation and additional sequencing, be [subject to the select agent and toxin regulations]?

It depends.

- In accordance with § 73.3(c)(2)(i), cDNA transcribed from mRNA isolated from a botulinum neurotoxin producing species of Clostridium are not regulated genetic elements because cDNA by itself cannot be expressed *in vitro* or *in vivo*. Use of this cDNA for fragmentation and additional sequencing would not render the cDNA subject to the select agent and toxin regulations.

However, if the cDNA encodes for the toxic form(s) of any of the toxins listed in § 73.3(b), and steps are taken to make that cDNA capable of being expressed i*n vitro* or *in vivo*; or if such cDNA is put in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*, those products would be regulated genetic elements or recombinant and/or synthetic nucleic acids subject to § 73.3(c)(2).

Genetically Modified Select Agents

Genetically Modified Select Agents or Toxins

42 C.F.R. Part 73.3(c) Genetic Elements, Recombinant and/or Synthetic Nucleic Acids, and Recombinant and/or Synthetic Organisms:

. . .

(3) HHS select agents and toxins listed in paragraph (b) of this section that have been genetically modified.

Exclusions

- Exclusions apply for select agents that meet various criteria provided in accordance with 42 C.F.R. §§ 73.3, 73.4; 9 C.F.R. §§ 121.3, 121.4; 7 C.F.R. § 331.3

- There are two categories of exclusion often related to genetically modified organisms:
  - Specific strains that meet regulatory exclusion criteria and the individual or entity can identify that the agent is within the exclusion category, such as low pathogenic strains of avian influenza virus which in some situations result from genetic modification of a highly pathogenic strain of avian influenza.
  - An attenuated strain of a select agent or a select toxin modified to be less potent or toxic, which is often attenuated through genomic modification.

Exclusion Considerations

Exclusions

- Regulatory exclusion criteria does exist for some organisms:
  - If specific agent regulatory criteria is listed, a genetically modified select agent that meets that criteria can be excluded as long as the entity can identify the agent is within the exclusion category.
  - In these situations, no formal exclusion review submission is required by FSAP, even if the excluded strain was obtained through genomic modification of a regulated strain.

Exclusions

- An attenuated strain of a select agent or a select toxin modified to be less potent or toxic, which is often attenuated through genomic modification:
  - Such agent attenuation requires a formal exclusion review by either or both DASAT and DRSC to determine if the resulting strain meets appropriate exclusion criteria.

Exclusions

- Key elements applying to genetically modified organism for exclusion include:
  - Defined genetic mutations or alterations known to attenuate virulence in humans or relevant animal or plant 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 or plant model used to test virulence is important.

Exclusions

- Key elements applying to genetically modified organism for exclusion include:
  - Quantitative measures demonstrating a change in virulence in an appropriate animal or plant model.
  - Information regarding tests that may be conducted to differentiate animals or plants exposed to the attenuated strain from those infected with the wild-type.

Exclusions

- Use of excluded attenuated strains requires individuals or entities to ensure attenuation remains after any manipulation that may restore or enhance the virulence or toxic activity:
  - Not only ensuring the genetic modification remains stable and intact, but also ensuring no other genetic modifications occur in other regions of the genome that can affect attenuation or toxic activity.
  - This may need to be confirmed through whole genome sequencing accompanying studies in the appropriate animal or plant model to characterize phenotypic expression after agent manipulation.


Modified
Excluded
Strains

Modified Excluded Strains

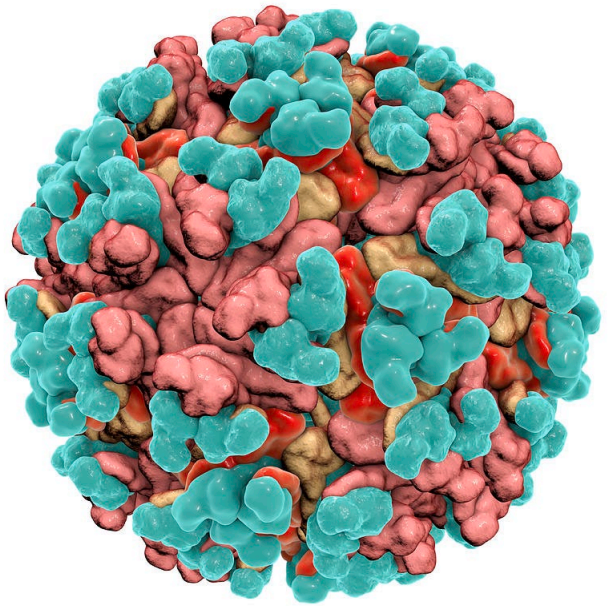
42 C.F.R. Part 73.3(e) An attenuated strain of a select agent or a select toxin modified to be less potent or toxic may be excluded from the requirements of this part based upon a determination by the HHS Secretary that the attenuated strain or modified toxin does not pose a severe threat to public health and safety.

. . .

(2) If an excluded attenuated strain or modified toxin is subjected to any manipulation that restores or enhances its virulence or toxic activity, the resulting select agent or toxin will be subject to the requirements of this part.

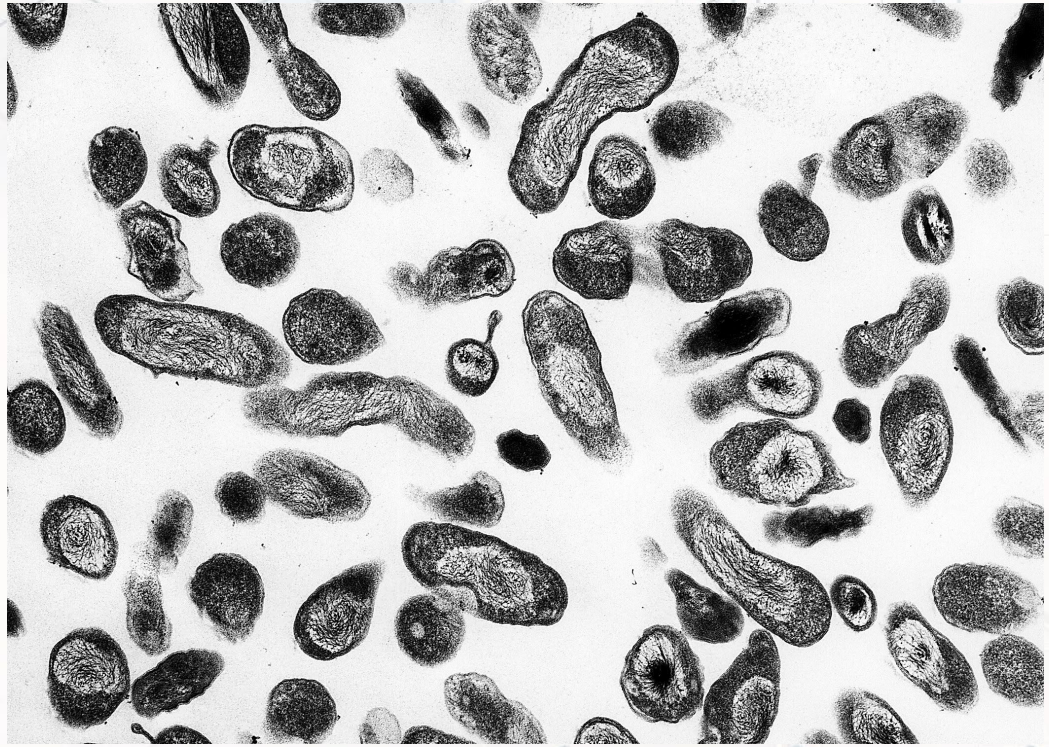
Venezuelan Equine Encephalitis Virus (VEEV) TC-83 (A3G)

- On September 1, 2022, FSAP published two Federal Register Notices (CDC and APHIS) regarding the excluded attenuated strain Venezuelan Equine Encephalitis Virus (VEEV) TC-83.
- New information has shown that a modification to the excluded attenuated strain Venezuelan Equine Encephalitis Virus (VEEV) TC-83 (A3G) has been shown to increase its virulence.
- Therefore, the modified VEEV strain TC-83 (A3G) is a select agent and subject to the select agent regulations.



Coxiella burnetii Phase II, Nine Mile Strain, Plaque Purified Clone 4 with Reversion to Wildtype *cbu0533*

- On August 10, 2023, CDC published a Federal Register Notice regarding *Coxiella burnetii* excluded strain with a reversion in virulence.
- DRSC determined that an excluded attenuated strain, *Coxiella burnetii* Phase II, Nine Mile Strain, plaque purified clone 4, has, in one instance, been shown to spontaneously mutate when passaged *in vivo.*
- The resulting mutant had enhanced pathogenicity and virulence.
- Therefore, *C. burnetii* Phase II, Nine Mile Strain, plaque purified clone 4 with reversion to wildtype *cbu0533* **IS NOT** an excluded strain but **IS** a select agent and therefore subject to the select agent regulations.



African Swine Fever Virus Exclusion Withdrawal, Strains ASFV-G-ΔMGF and ASFV-G-Δ9GL/ΔMGF

- On October 27, 2022, APHIS published a Federal Register Notice regarding African Swine Fever virus (ASFV) exclusion withdrawal for strains ASFV-G-ΔMGF and ASFV-G-Δ9GL/ΔMGF after identifying a reversion in virulence.
- DASAT determined that the multi-gene family (MGF) deletion, upon cell culture adaptation and passage in swine, did not maintain the attenuation characteristic of the original exclusion data.
- Information also became available of possible instability in the MGF regions of ASFV.
- Therefore, the phenotypic expression of virulence upon further passage in swine justified exclusion withdrawal and placing the two strains under regulatory oversight as select agents.

African Swine Fever Virus Exclusion Withdrawal, Strain ASFV-G-ΔI177L

- On May 6, 2024, APHIS published a Federal Register Notice regarding African Swine Fever virus (ASFV) exclusion withdrawal for strain ASFV-G-ΔI177L after identifying a reversion in virulence during swine backpassage studies.
- DASAT was informed of observation of clinical signs upon fifth backpassage of the cell culture adapted excluded strain, which included clinical signs of ASFV infection as well as gross pathology indicative of ASFV infection.
- Therefore, the phenotypic expression of virulence identified in swine backpassage studies justified exclusion withdrawal and placing the strain under regulatory oversight as select agents.
- Data is being compiled if the cell culture adaptation or the *in-vivo* selective pressure may be responsible for virulence reversion, but these situations demonstrate the critical need to characterize resulting virus populations after cell culture adaptation and reisolation from animal models.

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.