Work Objective Guidance

Responsible Official Webinar

October 13, 2021

Components of a Work Objective

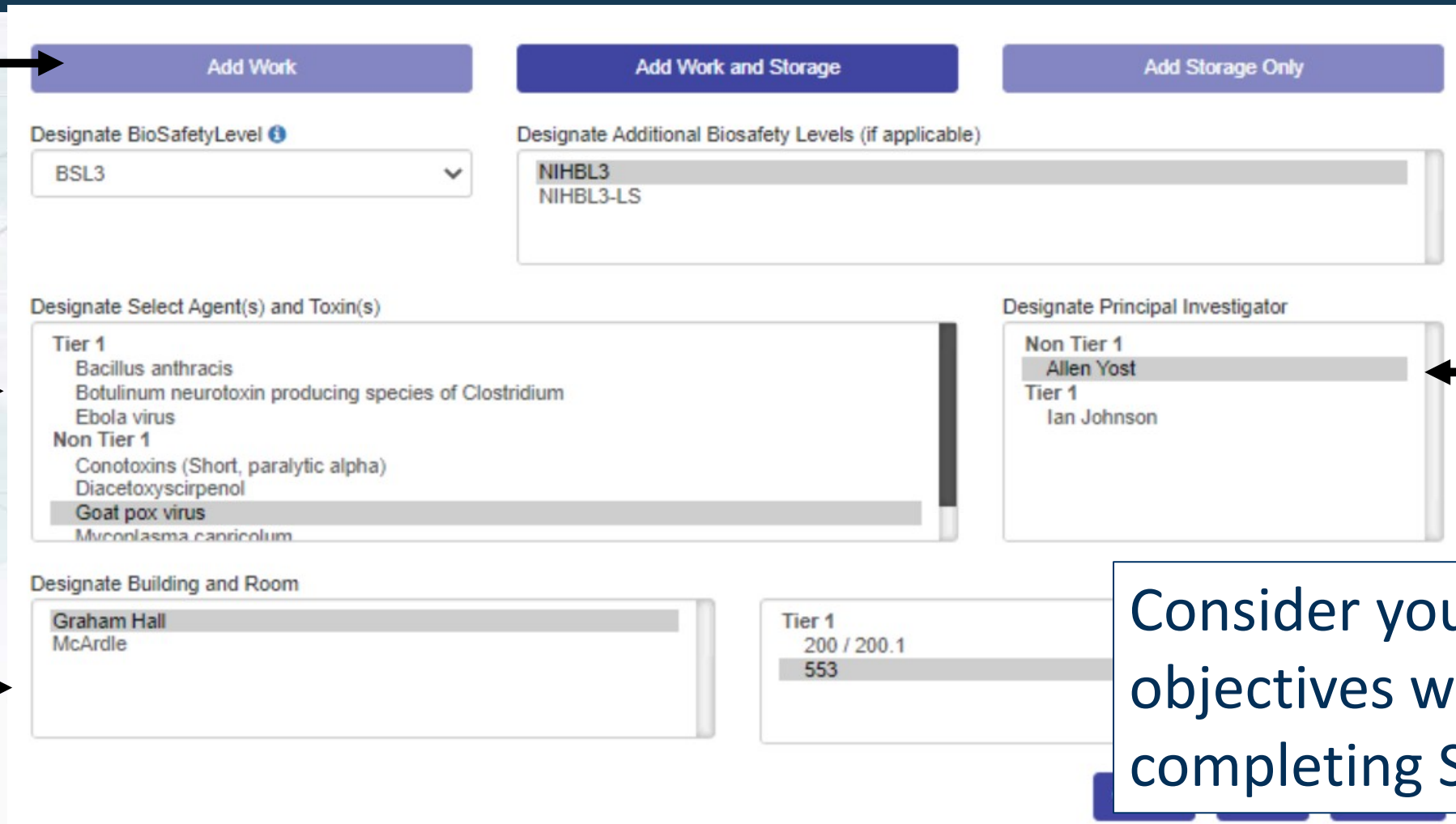
1. Type of work

2. Biosafety level

3. Select agent(s) and toxin(s)

4. Principal Investigator(s) (PIs)

5. Building and room(s)



Consider your work objectives when completing Section 6

Objective of Work

- Describe the work being performed. Include:
  - The assays used (e.g., Polymerase Chain Reaction (PCR), flow cytometry, cultures)
  - Animals used and method(s) of exposure
  - Any use or creation of recombinant material
  - If more than one select agent or toxin is listed, clearly indicate what work is associated with each agent or toxin, if not the same

Note: Only describe work specific to the room(s), agent(s)/toxin(s), and PI(s) selected

Example 1 for Objective of Work

- To determine the presence or absence of select agents and toxins in clinical and environmental samples. Work performed conforms to the methodologies established by the Centers for Disease Control Laboratory Response Network Program and includes extraction of nucleic acid, culture identification of select agents and toxins, real-time PCR, and protein detection by time resolved fluorescence. In addition, the laboratory performs studies to develop and validate real-time PCR assays, as well as assays using a mass spectrometer for diagnostic use using the select agents and toxins listed in our inventory.

Example 2 for Objective of Work

- We will determine the lethal dose, 50% (LD50) of *Burkholderia pseudomallei* and *Burkholderia mallei* strains (wild-type and mutants) in a mouse aerosol challenge model. The mutants to be tested will have mutations in virulence genes shown to contribute to the interaction of *B. pseudomallei* and *B. mallei* with host cells. We will also immunize mice with recombinant proteins corresponding to virulence genes shown to contribute to the interaction of *B. pseudomallei* and *B. mallei*, with host cells. Following this, we will test whether this immunization protects against *B. pseudomallei* and *B. mallei* infection in a mouse aerosol challenge model. The vaccine preparations will consist of purified recombinant *B. pseudomallei* and *B. mallei* proteins mixed with adjuvant.

Example 3 for Objective of Work

- Botulinum neurotoxin work involves development activities conducted in support of manufacturing process development, commercial manufacturing support and analytical method development for the biologics pipeline. The labs utilize equipment and instrumentation which include, but are not limited to: small incubators, centrifuges, filtration systems, High Performance Liquid Chromatography (HPLCs), gel electrophoresis systems, Ultraviolet-visible spectrophotometers, capillary electrophoresis, enzyme-linked immunoassay (ELISA), quantitative PCR instrumentation, standard PCR instrumentation, plate reader, shaker incubator, and plate washer, as well as support equipment such as biological safety cabinets, incubators, pipettes, balances, pH meters, refrigerators and freezers.

Example 4 for Objective of Work

- Research activities will focus on the development of live-attenuated vaccine platforms for the protection of multiple animal species including poultry, mice, and ferrets. Culture of highly pathogenic H5 and H7 influenza viruses: Highly pathogenic avian influenza viruses will be cultured in embryonated hen eggs or tissue culture cells. Reverse genetics regeneration of wild-type and vaccine strains: Influenza gene segments for wild-type highly pathogenic avian influenza viruses will be cloned into reverse genetics plasmids, and the plasmids will be transfected into 293T cells. The resulting virus will then be cultured in MDCK cells or embryonated hen eggs.

Guidance for Question 2

2. Provide an estimate of the maximum quantities (e.g., number of Petri dishes or total volume of liquid media) and concentration of each organism grown at a given time (e.g., 2 - 250 ml flasks of 105 cfu/ml). If select agent will not be propagated, indicate "no propagation of agent".

Agent	Maximum Quantity / Concentration
Bacillus anthracis	10 petri dishes; 15 x 11ml at 10e8 cfu/ml

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- Write '*Not Applicable*' for genomic material
- Do not list the maximum quantity the PI may *possess* for agents but rather the maximum quantity the PI may *work with* at a given time
- Include total volume, number of plates/slants and concentration
  - Example: four 75ml flasks at 10e9 pfu/ml

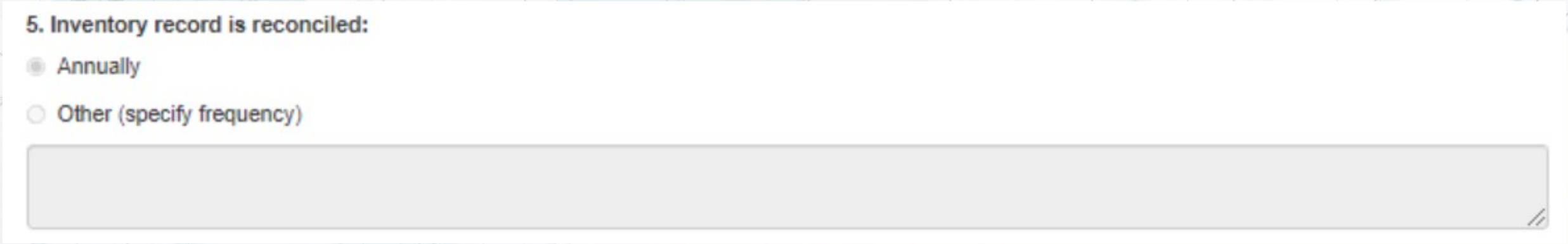
Guidance for Question 3

3. Provide an estimate of the maximum quantity of functional toxin held by the PI at any one time (e.g., 500 mg, 100 ml x 100 ug/ul).

Toxin	Maximum Quantity / Concentration
Conotoxins (Short, paralytic alpha)	500 mg

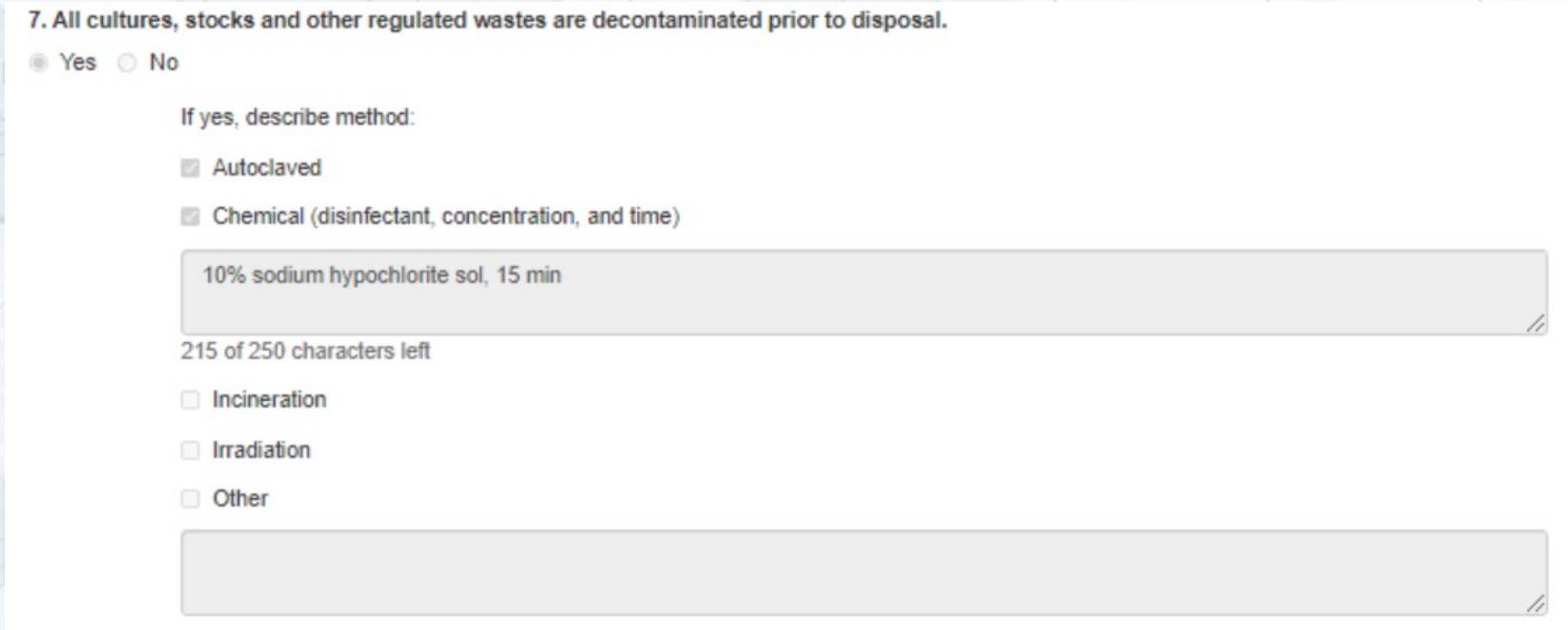
- List the maximum quantity of toxin the PI may possess at a given time, not the maximum quantity handled at a given time

Guidance for Question 5



- Use ‘Other’ if normal frequency is different from annually (e.g., quarterly)
- ‘Other’ is not intended for capturing uncommon circumstances, such as inventory audits following a change in PI

Guidance for Question 7



- Additional details are only needed for ‘Chemical’ or ‘Other’ options
- If a specific type of material is treated using more than one method, only list the primary method
  - For example, if PPE is autoclaved and then incinerated, only mark autoclave

Purpose of Question 9

9. Will work be performed with:

a. agents that will be propagated and produce regulated amounts of toxins or with registered toxins at or below the regulated amount?

○ Yes ◉ No

b. regulated nucleic acids, genetic modification of select agents or toxins, recombinant/synthetic nucleic acids or recombinant/synthetic organisms?

○ Yes ◉ No

c. animals?

○ Yes ◉ No

d. plants?

○ Yes ◉ No

e. arthropods?

○ Yes ◉ No

Purpose of Question 10

10. Will work be performed in:

a. BSL3Ag laboratory?

○ Yes ◉ No

b. BSL4/ABSL4 laboratory?

○ Yes ◉ No

General Reminders for Attachments

- Ensure the answers between the Attachments and/or Section 7A/C match
- If exposing animals to toxin, complete Attachment C
  - If a question doesn't apply, answer 'no' or write 'not applicable' in the text box
- If performing diagnostic testing on arthropods with no downstream work (e.g.; cultivation of select agent from field-collect arthropods), then complete Attachment E and answer yes to question 1

Attachment B: Work with regulated nucleic acids, genetic modification of select agents or toxins, recombinant/synthetic nucleic acids, or recombinant/synthetic organisms

Complete if you:

- Possess recombinant/synthetic nucleic acids that encode for functional forms of toxin and can be expressed in vivo or in vitro
- Possess nucleic acids that can produce infectious forms of select agent viruses (regulated genomic material)
- Possess genetically modified select agent viruses, bacteria, fungi or toxins
- Introduce or modify genetic elements
- Work with recombinant or synthetic nucleic acids or organisms
- Use reverse genetics systems to produce infectious forms of SA viruses or work with a complete set of reagents that could be used to rescue infectious virus

Attachment B: Work with regulated nucleic acids, genetic modification of select agents or toxins, recombinant/synthetic nucleic acids, or recombinant/synthetic organisms

Complete if you:

- Will perform a restricted experiment or possess the product of a restricted experiment
- Will perform experiments involving acquisition of increased/restored virulence (e.g., drug resistance, increased host range, enhanced transmissibility, infectivity, environmental stability)

For question 6, describe in detail what you will possess and/or create as it relates to the questions above

- If the work objective includes more than one select agent and/or toxin, indicate what information applies to each agent

Example 1 for Attachment B, Question 6

Kanamycin markers will be used for selecting transformants in *B. pseudomallei, B. mallei, F. tularensis* and *Y. pestis* that successfully retained vector with engineered resistance. These vectors/markers will be used for native gene knockouts, merodiploid construction, and gene dosage experiments. Zeocin markers will be used for selecting in *B. pseudomallei*. Use of fluorescent or bioluminescent-tagged select agents harboring plasmids with fluorescent/luminescent reporter genes or integrated fluorescent/luminescent genes, as well as engineering these strains in the case that we cannot obtain strains with these markers.

Example 2 for Attachment B, Question 6

Recombinant Rift Valley Fever viruses. These will be wild-type virus or viruses attenuated by alteration of protease cleavage sites, deletion of virulence genes, mutations that decrease virus replication, and insertion of reporters (e.g., GFP, luciferase, etc.).

Antiviral studies to look at the role of several drugs on the replication of virus in cell cultures or in animal models may include passive selection of viruses with reduced susceptibility to potential therapeutic compounds. Generation of recombinant infectious clones with reduced susceptibility may be performed to characterize such viruses for the purpose of identifying the mechanism of action of the therapeutic.

Questions?

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