High Consequence Pathogens: Attenuated Strain and Inactivation Verification

Scope/Summary: The goal of this policy is to provide a description of the steps that the CAB/ESCRO and Biosafety Program requires of those Principal Investigators (PIs) that wish to obtain and use attenuated variants or inactivated samples of high consequence human or animal pathogens (HCP), including but not limited to select agents. This policy applies to all samples known or suspected to contain SARS-CoV-2.

I. General Policies and Procedures:

- In general attenuated variants or inactivated samples may be handled at lower containment levels than the fully virulent or resistant wild type parent strain. In these instances, before permission is given to begin to work with the variant or inactivated sample at a lower biosafety containment level, the PI must verify that the strain that they received in their laboratory:
    - (i) has the expected genotype (e.g. deletion of certain genes; the presence of specific mutations in specific genes; the absence of specific plasmids containing virulence or toxin genes)
    - (ii) has the expected phenotype (e.g. if the attenuation is expressed as a limited host range; inability to form a plaque upon infection of a particular host cell; loss of ability to grow on specific media),
    - (iii) was inactivated using a validated procedure
- Confirmatory tests must be done at the containment level used for work with the wild type parental strain. Lower containment levels will only be considered by the committee once the attenuation is documented. The confirmatory experiments may be done by the sending laboratory or in some cases by the MIT investigator with CAB/ESCRO approval. The CAB/ESCRO will review the experimental data, inactivation procedure, or documentation before making a decision. The committee will consider the stability of the attenuated genotype: the kind of attenuating mutation is important in that deletions are less likely to revert. The committee will also review whether the inactivation procedure is appropriate for the types of samples to be used (e.g. based on published literature or validated by the supplier). Cases where the specific attenuation or inactivation methods and details can't be obtained will not be approved.

II. Prior to the Request to Obtain an Attenuated or Inactivated Strain:

- Prior to a request to obtain an attenuated or inactivated strain of a high consequence pathogen, investigator(s) must contact the EHS Biosafety Program. The PI and Biosafety Officer will work together to identify an appropriate and feasible method or test[1] that may be used to verify the presence of the expected mutations that result in the

[1] Examples of accepted methods for verification of appropriate genotype: DNA sequencing: PCR (using specified primers): RFLP

attenuation[2] or confirm that the inactivation procedure is effective. Once the method used to verify attenuation has been agreed upon, the investigator must amend their Biological Research Registration (BRR) to cover this agent and include a description of the proposed test and experiments or detailing the inactivation procedure used; attenuated or inactivated agents can't be sent to MIT before the BRR amendment has been approved.

III. Upon Receipt of the Attenuated Strain:

- Upon receipt of the strain, the MIT PI will verify receipt of the expected genotype using the agreed test. This will be done at the containment level appropriate for a fully virulent wild type strain. If the sender includes sufficient attenuation test results for that lot of attenuated agent no further testing may be required as decided by the CAB/ESCRO and EHS Biosafety Program. If this is possible it must be worked out before the strain is shipped. Upon arrival of the shipment, the PI and the Biosafety Officer will review the attenuation test results. These test results will be shared with the CAB/ESCRO. If in the opinion of the Biosafety Officer or CAB/ESCRO members, the results are not clear or do not support the assertion that the strain is attenuated, the investigator may not work with the strain. The Biosafety Officer will contact the source laboratory or entity and inform them of the issue. The investigator may not work with the unverified "attenuated" strain. All work with that "new" strain must be at the higher containment level appropriate for the fully virulent parent strain until the issues surrounding documentation of the attenuated genotype are resolved to the satisfaction of the BSO and CAB/ESCRO. If this is not feasible for any reason, all samples of the agent will be inactivated and disposed of (e.g. autoclaved or chemically inactivated) as determined by the EHS Biosafety Program. Verification of inactivation and disposal will be sent to the Biosafety Officer and communicated to the CAB/ESCRO. The PI may try to obtain an attenuated strain from another source. If that is the case the same process as outlined above will be followed. Depending on the mechanism of attenuation the CAB/ESCRO may require that the PI retest isolates periodically.

Revision History:

Approved 01/2002
Reviewed and approved 03/2004
Revised and approved 01/2008
Revised and approved 12/2021

[2] If appropriate laboratory space is not available and the verification test cannot be conducted at MIT, the MIT investigator should request that the sender or source of the strain provide data for that isolate that clearly documents the attenuation. The verifying test must include both positive and negative controls and be sensitive enough to differentiate the strain or isolate from the archetypical wild type or parental strain. If data cannot be obtained from the sender then the MIT investigator may not obtain the strain for use in their laboratory.