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This edition of the *BRL Bulletin* will discuss the new requirements for testing biologic materials that is outlined in the UIC ACC policy "Biologic Material Testing." This *Bulletin* will also discuss the authentication of key biological or chemical resources and methods of authentication. Biologic materials are, in the broadest sense, materials that are derived from living organisms. Biologic materials that are that are derived from or passaged through rodents, and then administered to rodents, are of greatest concern for colony biosecurity. Examples of biologic materials include cell lines, tumors, serum, tissues, antibody preparations, hybridomas, and basement membrane matrix. Contamination of biologic material with murine pathogens represents a significant biosecurity risk to the animal colony because of possible infection of rodents that have been inoculated with, or exposed to, contaminated biologic material with subsequent spread to other rodents.[2, 4] Some murine pathogens have been documented to interfere with research through contamination of biologic materials (e.g. altering tumor kinetics, interfering with the host immune system, increasing premature mortality of rodents, etc.).[1,2,3,4] Contamination of biologic materials with zoonotic murine pathogens can also put research staff at risk (e.g. lymphocytic choriomeningitis virus, known as LCMV).[3,4] Testing of biologic materials is necessary to protect both the animal colony and the research staff, and to ensure that high quality and reproducible research results are obtained from rodent subjects.

The NIH released Notice NOT-OD-16-011 which states that an "Authentication of Key Biologic and/or Chemical Resources" form is required for grant applications.[5] The NIH states that biologic and chemical resources "include, but are not limited to, cell lines, specialty chemicals, antibodies, and other biologics."[5] The authentication of biological and chemical resources is an important aspect of increasing rigor and reproducibility of scientific research. Working with misidentified biological and chemical resources can lead to inaccurate research conclusions, the propagation of inaccurate conclusions in published literature, and the added expense of having to repeat research aims. The authentication form should explain what type of testing will be performed and the frequency of testing. Aside from the NIH requiring authentication, many journals are also requiring that authentication procedures be described in submitted manuscripts. Methods of authentication and testing biologic material for pathogens will be described below to help investigators plan their testing for grant submission, and plan biological material administration to rodents housed at UIC.

Biologic Material Testing

As stated above, the biologic materials of concern are cell lines, tumors, serum, tissues, antibody preparations, hybridomas, and basement membrane preparations (e.g. Matrigel®) that are derived from, or passaged through rodents or rodent biologic material. From a biosecurity standpoint the biologic materials that are of greatest risk to the health of the UIC rodent colony are those that originated from outside of UIC. These include biologic materials that are obtained from investigators at other institutions or from commercial entities. Other institutions or

commercial entities may have rodents that are of a different health status than the rodent colony at UIC and biologic materials obtained from, or passaged through them, can become contaminated with pathogens excluded from UIC colonies. If these materials are introduced into rodents at UIC, there is the potential for the experimental animals to become infected and for the disease to spread within a room, or even within a facility. Investigators should ask for proof of testing for murine pathogens prior to obtaining biologic material from another institution or a commercial entity.

What needs to be tested?

If testing for rodent pathogens has not been performed, or if there is not recent proof of testing, then investigators should request extra biologic material so testing can be performed. Investigators must complete section 7.b in the ACC Form A "Protocol for Animal Use" and identify the biologic materials that will be used, identify if they are of murine origin, or passaged through rodents, and the source of the material. All test results are to be sent to OACIB and reviewed by a BRL veterinarian prior to initiating *in vivo* work. The type of biologic material and the origin will determine what testing is required, which is outlined below:

Tumors, cell lines, tissues, and hybridomas that were derived from rodents at academic or commercial entities (e.g. ATCC) outside of UIC, derived from rodents held in quarantine rooms at UIC, or derived from non-rodent sources that have been passaged through or exposed to rodents or rodent biologic material outside of UIC need to be tested for all of the required pathogens in the ACC Policy "Biologic Material Testing."

Serum, antibodies, and basement membrane matrix derived from rodents at other academic institutions, derived from rodents held in quarantine rooms at UIC, or derived from non -rodent sources that have been passaged through or exposed to rodents or rodent biologic material outside of UIC need to be tested for all of the pathogens in the UIC Biologic Materials Panel.

Serum, antibodies, and basement membrane matrix derived from rodents at a reputable commercial entity or from the general UIC rodent colony do not need to be tested. There are many commercial suppliers of biologic material, such as basement membrane preparations, antibody, and serum. When purchasing biologic material from commercial suppliers it is important to ask for documentation that material has been tested for rodent pathogens. Many suppliers are able to easily provide this information. If a supplier is not able to provide testing results, or does not perform testing, it is best to try to find another source for the product.

How is testing performed?

The current best practice for testing biologic materials for rodent pathogens is by PCR. Other methods that may be used are the mouse antibody production (MAP) and rat antibody production (RAP) tests. MAP and RAP testing are time consuming and depending on the pathogen, may not be the most sensitive method to identify contamination, which is why PCR testing is preferred for biologic material testing. Recommended commercial laboratories for biologic material pathogen testing are IDEXX BioResearch (UIC Biologic Materials Panel for mice and IMPACT IV Panel for rats) and Charles River Laboratories (Mouse Essential Panel and Rat Essential Panel). After initial testing, it is recommended that there be a plan in place for routine testing of biologic materials. Changes in cell kinetics or morphology, loss of cell lines, or unexpected

in vivo results are all possible indicators of contamination with murine pathogens. The veterinary staff should be contacted to discuss testing for rodent pathogens when any of the above alterations occur.

Contamination with Human Pathogens

Biologic materials that are of human origin may be contaminated with human pathogens. It is important to understand the risks associated with working with human biologic material and to consider testing for pathogens. The procedures described in OSHA's Bloodborne Pathogens standard (29 CFR 1910.1030)[6] should be followed when handling human origin biologic material. The minimum recommended pathogen testing is for blood borne viruses such as human immunodeficiency virus (HIV), hepatitis B, and hepatitis C. If the biologic material is positive for these or other pathogens, it is recommended to contact the UIC Environmental Health and Safety Office to discuss appropriate procedures for handling the biologic material. Human origin biologic material only needs to be tested for human pathogens once because it should not become contaminated with human pathogens when being handled by laboratory personnel.

Authentication of Key Biological or Chemical Resources

As part of the initiative to increase scientific reproducibility the NIH expects that key biologic and chemical resources be authenticated.[5] The NIH expects investigators to describe how authentication will be performed and the frequency of authentication in the grant submission. Even if an investigator has a non-NIH funding source, many scientific journals require that there be information regarding authentication in submitted manuscripts. Aside from the requirements of the NIH and journals, high quality data collection and the subsequent conclusions are dependent on confirmation that the material you think you are working with is actually what you are working with.

There are two different types of authentication, interspecies and intraspecies. Interspecies authentication analyzes the biologic material to determine the species of origin, usually by using PCR. For example, when working with a cell line that was derived from mice, there should only be mouse DNA in the tissue. If an investigator is working with a patient derived xenograft (PDX), the initial sample obtained from the human patient should only contain human DNA; however, after being passaged through mice there should be both human and mouse DNA. If the PDX is tested after several passages and there is only mouse DNA then that would mean that at some point in time a tumor of mouse origin was collected instead of the human origin PDX.

The other form of authentication is intraspecies testing which determines if the genetic profile of the biologic material is what is expected. In other words, intraspecies testing rules out misidentification or cross contamination. Intraspecies testing uses short tandem repeats at a minimum of 9 loci to determine the genetic profile of the biologic material. The genetic profile is compared to reference profiles (either published profiles or historical profiles that the laboratory has maintained) to determine if there are any changes from the expected, or baseline, genetic profile. The loss of alleles may happen over time as biologic material is worked with and passaged, but a >80% allele match with a reference baseline profile would still be expected. If a biologic material gains alleles over time, or has a completely different profile, contamination or misidentification is suspected. One of difficulties of biologic authentication is that it can be difficult to navigate different websites

to find known profiles. Currently the DSMZ online short tandem repeat database is the most user friendly (https://www.dsmz.de/services/services-human-and-animal-cell-lines/online-str-analysis.html).

There are commercial laboratories that perform authentication such as IDEXX BioResearch, Charles River Laboratories, ATCC, and many others (https://www.promega.com/products/cell-authentication-sample-identification/cell-line-authentication/cell-line-authentication-testing/?tabset0=3). UIC investigators are fortunate to have access to the UIC Research Resources Center (RRC) which provides authentication services for human cell lines. The RRC DNA Services Facility uses the GenePrint® 10 System to assess nine loci. They also provide interspecies testing on request and offer *Mycoplasma* sp. contamination testing.

There are no current standards for frequency of authentication, but if routine authentication is performed it is easier to determine when contamination or misidentification occurred. IDEXX BioResearch recommends that authentication be performed when a biologic material is acquired or created to establish baseline, when expanding and banking materials for later use, at the beginning and end of a research aim, and as a standard quality control for lines in continuous use. If biologic materials yield unexpected results they should be tested to confirm their identity.

Testing biologic materials for pathogens and authentication may at first seem like a burden; however, these steps are necessary to produce high quality and reproducible research, and keep the rodent colony and personnel safe.

References

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NIH Notice NOT-OD-16-011: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-011.html

OSHA's Bloodborne Pathogens standard (29 CFR 1910.1030): https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051