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## Review

## BEI Resources: Supporting antiviral research

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## ABSTRACT

The Biodefense and Emerging Infections Research Resources Repository (BEI Resources) provides unique, quality-assured reagents to the scientific community for use in basic research and product development involving biodefense and emerging infectious diseases. These include microorganisms (up to Biosafety Level-3) on the National Institute of Allergy and Infectious Diseases (NIAID) and Centers for Disease Control and Prevention (CDC) lists of Category A, B and C priority pathogens. In addition to live microorganisms, related products such as polyclonal antisera, monoclonal antibodies, isolated nucleic acid preparations, overlapping peptide arrays, purified proteins, and assay kits are also available. Many of these materials have direct or indirect applications in antiviral research. These reagents are available free of charge to all registered investigators, regardless of funding source or affiliation. Acquisition of new reagents for the repository is one of the critically necessary and challenging tasks for BEI Resources. Therefore, investigators are encouraged to deposit relevant items, so as to provide access to materials, relief from the burden of distribution, protection of intellectual property rights, and secure storage. In addition, BEI Resources has the capability of contracting for the preparation of specific reagents. If there is a resource needed to advance a specific research area, contact an NIAID program officer or use the “suggest a reagent” option on the BEI Resources homepage, [www.beiresources.org](http://www.beiresources.org).

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## 1. Introduction

The American Type Culture Collection (ATCC) was established in 1925 when a committee of scientists recognized a need for a central collection of microorganisms that would serve scientists all over the

world. The facility was originally located at the McCormick Institute in Chicago until the organization moved to Georgetown University in Washington, DC, in 1937. As research in the biosciences expanded, ATCC began to diversify its holdings, and as the collections grew ATCC occupied a series of sites, each providing more storage space. ATCC moved to its current state-of-the-art laboratory in Manassas, VA in 1998. The ATCC Virology and Rickettsiology collection was formed in 1958, and in 1986, formed a close relationship with the American Society of Virology in order to better

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serve the needs of the research community. The overall mission of the ATCC and the Virology collection is to acquire, authenticate, preserve, develop, and distribute biological materials, information, technology, and standards for the advancement and application of scientific knowledge. ATCC serves as a global nonprofit biore-source center and research organization that provides biological products and technical services to private industry, government, and academic organizations.

The National Institute of Allergy and Infectious Diseases (NIAID) awarded a contract to ATCC in September 2003 to provide unique and quality-assured reagents and resources to the scientific community for use in basic research and product development involving biodefense and emerging infections. This repository is called the Biodefense and Emerging Infections Research Resources Repository (“BEI Resources”). These reagents include microorganisms identified by the NIAID and the Centers for Disease Control and Prevention (CDC) as Category A, B and C priority pathogens up to Biosafety Level 3 (BSL-3). Other materials relevant to the field of antiviral research include standardized and well-characterized virus stocks, isolated viral nucleic acids, polyclonal and monoclonal antibodies, cloned genes, purified proteins, overlapping peptide arrays, and polymerase chain reaction (PCR)-based detection kits. BEI provides reagents that previously had limited availability or were unavailable, provides standardization and quality control of reagents, and provides a source for “one stop shopping” for these reagents.

While there is some overlap between the general ATCC Virology and the BEI collections, there are also distinct differences. The ATCC collection, for example, contains a diverse collection of human, animal, and plant viruses, while the BEI collection focuses on human pathogens and surrogates, including CDC and USDA select agents. Users are encouraged to examine both collections in order to find organisms and reagents relevant to their research.

## 2. Acquisition/deposits

Acquisition of new reagents for the repository is critical to its success. Some of the sources of biological materials are from the research community, existing domestic and international collections, field collections, clinical isolates, orphan collections (those in danger of loss or destruction due to lack of resources to maintain them, investigator turnover or retirement, etc.), and contract production of materials when necessary. Depositing into BEI Resources offers these advantages: (1) promotes access and use of the materials to the research community, (2) relieves researchers of the burden of distributing materials, (3) offers an alternative to institutions for secure storage of biological materials, (4) ensures regulatory compliance in shipping, and (5) protects the intellectual property of the depositor and allows for any distribution limitations to be set. Forms are available through the BEI Resources website to initiate the submission process. To assist with the deposit of reagents, the repository has a field acquisition specialist available who can assist researchers in filling out the deposit forms and packaging/shipment of reagents if requested.

The quality and usefulness of the BEI repository relies, in part, on the quality of the deposited reagents. However, it is often far more important that BEI obtain a critical reagent even if it may not have been fully characterized or be completely pure. In these cases, BEI can and does take on the responsibility of purifying and/or characterizing these reagents and making them available as rapidly as possible.

## 3. Production and preservation

Materials sent to BEI Resources are received by dedicated staff and checked by Quality Assurance personnel before being

**Table 1**

Examples of viruses available to authorized investigators through BEI Resources

Virus family	Viruses
Arenaviridae	Pirital, Junin (Candid-1)
Bunyaviridae	Hantaviruses: numerous Old and New World isolates California and La Crosse encephalitis, snowshoe hare
Coronaviridae	HuCoV NL63 Bovine coronavirus, transmissible gastroenteritis virus, infectious bronchitis virus, canine coronavirus, feline infectious peritonitis virus, porcine respiratory coronavirus
Enteroviridae	Enterovirus 71, various strains
Flaviviridae	Dengue types 1–4 Japanese encephalitis, numerous strains St. Louis encephalitis, yellow fever, West Nile
Orthomyxoviridae	Influenza A viruses, large number of named isolates
Paramyxoviridae	Newcastle disease, many isolates Human parainfluenza, several isolates
Poxviridae	Vaccinia, many strains Monkeypox Zaire 79 Monkeypox WRAR Monkeypox US 2003 Cowpox Brighton Red Ectromelia expressing murine IL-4
Togaviridae	Venezuelan equine encephalitis (various strains)

stored under appropriate conditions. Materials can be received and stored at any temperature and in a wide array of vialing formats. BEI Resources recognizes the importance and value of these reagents and takes special care when handling and storing them. BEI Resources is ISO 9001 accredited and uses documented and tracked procedures for receipt and storage.

Materials that are received in a ready-to-use format, such as those produced under contract, are made immediately available on our web site. Seed vials of live organisms that require expansion are placed into our production queue, and are given a priority relative to other items needing to be produced. While BEI Resources recognizes that all of these reagents are important, some prioritization must occur based on the immediate demand from the user community and with regards to NIAID research goals and priorities. Prioritization is set by NIAID based on the needs of NIAID-funded researchers, input from the BEI Science Advisory Committee, and continuing gap analysis by BEI Collection Scientists.

When expanding materials for distribution, BEI Resources maintains minimal passage number, preserves the materials to ensure that they remain unchanged, and stores the materials in a secure facility. Viruses (Table 1) are grown at the appropriate biosafety level (from BSL-2 up to and including BSL-3+). We are able to grow viruses and rickettsiae under any conditions necessary, including in adherent or suspension cell culture, using any necessary cell line from the BEI or ATCC collection, or in embryonated eggs (for viruses). These reagents may also be propagated at nearly any scale. Hybridoma cell lines are expanded either in vitro or as mouse ascites fluid and the antibodies purified from the culture supernatant or ascitic fluid (Table 2). Traceable production records are kept on all lots of materials.

**Table 2**

Examples of monoclonal antibodies available to authorized investigators through BEI Resources

Virus family	Targets of monoclonal antibodies
Arenaviridae	Junin virus
Coronaviridae	SARS Co-V E, M, S, N proteins
Flaviviridae	Dengue virus types 1–4
Filoviridae	Ebola virus proteins
Orthomyxoviridae	Influenza A H5N1 virus HA protein, numerous mabs
Poxviridae	Vaccinia virus A27L, B5R, L1R (ectodomain-specific)
Togaviridae	Venezuelan equine encephalitis virus

**Table 3**

Examples of purified viral DNA or RNA available to authorized investigators through BEI Resources

Virus family	Purified viral DNA/RNA
Coronaviridae	SARS-CoV gene clones Bovine coronavirus, transmissible gastroenteritis virus, infectious bronchitis virus, canine coronavirus, feline infectious peritonitis virus, porcine respiratory coronavirus
Enteroviridae	Enterovirus 71
Flaviviridae	Dengue virus types 1–4 St. Louis Encephalitis virus West Nile virus
Orthomyxoviridae	Influenza A H1N1, H2N2, H3N2, H5N2 genomic RNA
Poxviridae	Vaccinia virus genomic DNA, various isolates Monkeypox virus, various isolates Cowpox virus, Brighton Red Rabbitpox virus Raccoonpox virus
Togaviridae	Venezuelan equine encephalitis virus Eastern equine encephalitis virus Western equine encephalitis virus Pixuna virus

**Table 4**

Examples of recombinant viral proteins available to authorized investigators through BEI Resources

Virus family	Recombinant proteins
Coronaviridae	SARS Co-V S, N, 3CL proteins (S histidine-tagged)
Orthomyxoviridae	Influenza A HA proteins (H5, H6, H9, N2, N4)
Poxviridae	Histidine-tagged vaccinia virus A33R, B5R and other proteins

BEI Resources can produce materials in two different ways. Some reagents are produced internally. These include most live organisms, purified viral RNA/DNA (Table 3), and most monoclonal antibodies. For others, external production via commercial vendors or investigators at academic institutions may be required. In some cases, the reagents can be produced commercially and the characterization may be contracted back to a specific investigator or institution. Some are produced according to current Good Manufacturing Practices (cGMP), when needed. Examples of materials that have been produced externally are recombinant proteins (cGMP or non-cGMP) (Table 4), standardized assay reagents, serum standards (single or in panels), fill/finish (cGMP or non-cGMP), master seeds and working banks for pre-clinical trials, cGMP cell banks, monoclonal (Table 2) and polyclonal (Table 5) antibodies, peptide arrays (Table 6), and *in vivo* production of pathogens. There are two ways to request new reagents through BEI Resources: (1) visit the website and make a suggestion for a specific reagent, (2) contact a Program Officer at NIAID, who will work with the BEI Resources Project Officer to make the reagent available if they verify that the reagent is indeed necessary and that it will meet the needs of several investigators in the community.

**Table 5**

Examples of polyvalent antisera available to authorized investigators through BEI Resources

Virus family	Targets of polyvalent antisera
Coronaviridae	Bovine coronavirus, transmissible gastroenteritis virus, infectious bronchitis virus, canine coronavirus, feline infectious peritonitis virus, porcine respiratory coronavirus
Orthomyxoviridae	Influenza A virus HA and N proteins, numerous subtypes Influenza A viruses, numerous subtypes
Paramyxoviridae	Human parainfluenza, types 2, 3, and 4B Bovine parainfluenza type 3
Poxviridae	Vaccinia virus A27L, B5R, L1R and other proteins
Togaviridae	Venezuelan equine encephalitis virus

**Table 6**

Examples of peptide arrays available to authorized investigators through BEI Resources

Virus family	Peptide arrays
Bunyaviridae	Sin Nombre and Hantaan virus N, GPC, L proteins
Coronaviridae	SARS S, N, M, E proteins
Flaviviridae	West Nile virus structural and nonstructural proteins Dengue types 1–4 structural and nonstructural proteins
Orthomyxoviridae	Influenza A (H1N1, H3N2, H5N1) HA and other proteins Influenza B HA
Poxviridae	Vaccinia virus protein epitopes

#### 4. Authentication and quality control

BEI Resources conducts quality control and characterization of all reagents produced before they are distributed to the research community. While this process can be lengthy, it assures researchers that the reagents they are receiving are of the highest possible quality. Viruses and other materials produced at BEI Resources, or via external contracts, are subjected to a polyphasic authentication scheme. Authentication assays are broken into three categories: phenotypic, genotypic, and purity. Not all quality control tests are appropriate for all types of reagents. All reagents come with Product Sheets and Certificates of Analysis. In order to view these documents it is necessary to log into the website.

##### 4.1. Phenotypic assays

All viruses produced at BEI Resources are tested for viability by growth on an appropriate cell line and for the generation of the expected cytopathic effect. Further, the concentration of each virus is determined by plaque assay, tissue culture infectious dose (TCID<sub>50</sub>) titer, or by egg infectious dose (EID<sub>50</sub>) titer. When appropriate antibodies are available, viruses are tested for identity and reactivity using immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISAs) or Western blots. Drawing on more than 50 years of virology experience at ATCC, BEI Resources is able to compare each lot to an extensive base of historical data for many viruses in the collection.

##### 4.2. Genotypic assays

In order to confirm identity, virus cultures are subjected to PCR amplification using genus, species and/or strain-specific primers. While occasionally these assays are obtained from the published literature, more commonly they are developed and validated in-house by BEI Resources scientific staff. A number of these unique assays and controls are being made available to researchers. In several cases, quantitative real-time PCR assays have been developed and will be made available to registrants along with quantitation standards. In order to obtain sufficient resolution and discrimination, specific regions or genes within the viral genome are sequenced for identification purposes.

##### 4.3. Purity assays

Maintaining the integrity of reagents is critical for their successful downstream uses. All virology, cell biology, and immunology reagents are subjected to rigorous sterility testing. While it is impossible to test all samples for the presence of all possible contaminating organisms, every effort is made to ensure purity. This includes confirmation of the lack of cross-reactivity with antibodies or PCR primers to related organisms. Further, all reagents are tested and confirmed to be free of contaminating bacteria and fungi by culturing them in six different media, under both aerobic and

anaerobic conditions, at two different temperatures, for 21 days. The absence of *Mycoplasma* contamination is confirmed both by PCR-based testing (which is able to detect approximately 30 different species), and by direct culture for 21 days on both solid and broth medias. These tests are significantly more rigorous than required by United States Pharmacopeia (USP) guidelines. Tests for pH and to confirm the absence of endotoxin are done on materials designed to be used in animal challenge studies.

It is appropriate to make specific mention of viral nucleic acids (Table 3). BEI Resources has done extensive research into the production of high-quality isolated nucleic acids (either RNA or DNA) from viruses within the collection. These nucleic acids are made available to registrants, and are assayed for their suitability in PCR-based applications. Further, each lot of isolated nucleic acid is tested extensively for the presence of any residual viable source organism. This testing is critical for the safe distribution and use of isolated nucleic acids from pathogens in non-containment facilities in molecular assays. These nucleic acids are made available for diagnostics or other research work that does not require the live pathogen, or to researchers who lack access to containment facilities. U.S. Government regulations state that “Nucleic acids that can produce infectious forms of any of the select agent viruses listed in paragraph (b) of this section” are themselves considered select agents (42 CFR 73.3 and 73.4). This regulation applies largely to positive-sense RNA viruses, but also applies to other infectious clones of select agent viruses. However, except for those cases stated above, other isolated nucleic acids need not be treated as a select agent, further reducing the burden on researchers and enhancing the usefulness of the material.

## 5. Storage/distribution

After materials have been produced and preserved, they are ready for storage and distribution. BEI Resources has over 1300 square feet of  $-20^{\circ}\text{C}$  freezer space and 700 square feet of  $4^{\circ}\text{C}$  walk-in refrigerator space, several  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  mechanical freezers, and numerous liquid nitrogen freezers. Biological materials are distributed to registered researchers at no cost except for shipping and handling. The BEI Resources regulatory and compliance group ensures that the possession, use and transfer of the reagents are compliant with the various federal regulations and agencies. The group is available to assist investigators applying for permits and to answer questions that may arise.

## 6. Registration/ordering

Any qualified scientist or researcher, whether located in the U.S. or overseas, can register and receive reagents from BEI Resources. Access is not limited to NIH contractors or grantees. To register with BEI Resources, simply visit the website and download the appropriate forms. There are three different levels of registration based on the type of reagent requested by the investigator. Level 1 registration is for nonpathogenic BSL-1 level materials, Level 2 for non-select BSL-2 and BSL-3 reagents, and Level 3 for select agents. One of the major forms required for registration is the Material Transfer Agreement (MTA). When registering, one way to simplify matters is to have multiple investigators at the same institution sign a blanket MTA. In this way, one MTA goes through the process for all of the investigators listed. Typically, registration takes only 1–2 weeks for level 1 reagents, once all information is received by BEI staff. Higher level registrations may take some additional time due to the increased level of documentation required and additional verification needed. The most common cause of registration delays is due to incomplete forms or supporting documents, when needed.

Once you have registered with BEI Resources, a password will be mailed to you. Upon receipt of the password, orders can be placed directly through the website. Many of the items have a maximum amount that can be ordered without any additional approvals. If an amount is requested that is above the maximum, then a justification will be requested and the order will be forwarded to the Project Officer at NIAID for approval. All orders will be shipped within 7 working days upon receipt of Project Officer approval and any necessary permits.

## 7. Partnerships

NIAID has an Interagency Agreement with the Department of Defense (DoD) Critical Reagents Program (CRP). The CRP ensures the quality and availability of reagents that are critical to the successful development, testing and operation of biowarfare detection systems and medical biological products. The Chemical Biological Medical Systems-Joint Project Management Office (CBMS-JPMO) currently has critical reagent products that it has procured from various contractors or produced at specialized government repositories working with the CRP program. The DoD, through the CBMS-JPMO, is willing to make these products available to NIAID for basic research and product development activities, subject to availability and emerging DoD requirements. Therefore, BEI Resources is able to provide reagents from the CRP to the research community. In this case, the items are listed on the BEI Resources website, but the reagents are maintained and shipped from the various CRP sites. There are some limitations on the quantity of material that can be ordered through the CRP due to the cost required for NIAID to acquire it from DoD.

## 8. Outreach

The success of BEI Resources is measured by how well it serves the research community. In December 2006, NIAID convened a Scientific Review Committee (SRC) to review BEI Resources and make recommendations for what was still needed to benefit the research community. This committee was made up of 15 members with biodefense research and development backgrounds from private and public organizations as well as one representative from the DoD. This SRC will meet bi-annually to review the contents of the BEI repository from a curative perspective, providing guidance on what should be prioritized and added to the collection. At the December, 2006 meeting, the SRC recommended that Focus Groups be formed to address individual organisms and assist in acquiring those items significant to current research yet missing from the collection. BEI Resources worked to identify focus group leaders who then convened panels to review the repository reagents for their specific research focus. Sixteen different groups on the following topics were formed and convened earlier this year: (1) Alphaviruses, (2) Arenaviruses, (3) *Bacillus anthracis*, (4) *Brucella* species, (5) *Burkholderia* species, (6) *Clostridium botulinum*, (7) Coronaviruses, (8) *Coxiella* species, (9) Epsilon Toxin, (10) Filoviruses, (11) *Francisella tularensis* and *Yersinia pestis*, (12) Bunya- and Flaviviruses, (13) Orthomyxoviruses, (14) Poxviruses, (15) Ricin, and (16) Staphylococcal enterotoxin B. The focus groups made recommendations for the prioritization of individual organisms and have been working with BEI Resources to acquire these items.

## 9. Summary

BEI Resources is a valuable resource for the research community. It provides high-quality reagents free of charge except for

shipping and handling costs. Submissions of new reagents are strongly encouraged to increase the value of the repository, and feedback from the research community suggesting new reagents is also of great importance to ensure that BEI Resources continues to meet the needs of investigators. Along with suggestions, identi-

fication of specific commercial resources or academic contacts for acquiring the reagents is also beneficial and assists BEI Resources with obtaining the materials in a timely fashion. Please visit the website, [www.beiresources.org](http://www.beiresources.org), to see the reagents available to assist in your research.