

ATCC[®] connection[™]

ELF[®] Phosphatase Detection Kit

The ATCC[®] Stem Cell Center recently launched an easy detection assay for monitoring the state of embryonic stem cells (ESCs). The ELF[®] Phosphatase Detection Kit for Embryonic Stem Cells offers a 30-minute assay to determine whether embryonic stem cells (ESCs) have maintained their undifferentiated state or if they have begun to differentiate.

Endogenous alkaline phosphatase has been established as a marker for undifferentiated embryonic cells. ⁽¹⁻³⁾ The ATCC kit offers a simple and robust method using this marker to fluorescently detect and visualize alkaline phosphatase activity in ESCs. ⁽⁴⁻⁵⁾

Reliable, sensitive and stable, the assay enables researchers to confidently proceed with experiments knowing that their stem cell populations

have been maintained in the undifferentiated state (figures 1,2,3). The procedure is simple and requires no special skills. The protocol can be completed with visualization of fluorescent signal within 30 minutes. This novel fluorescence-based system can be used alone as an indication of the state of differentiation, or in conjunction with the detection of other stem cell markers to provide assessment of overall in vitro stem cell pluripotency.

Earlier methods to detect endogenous phosphatase activity provide adequate fluorescence but are time-consuming. The ATCC kit's soluble phosphatase substrate fluoresces weakly in the blue range. Once the phosphate is enzymatically removed, the resulting product forms an intensely

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Cryogenic Preservation of Bacteria

Cryogenic preservation is the act of freezing and storing cells at very low temperatures. Even after decades of research, the effects of the freezing and thawing process on living cells are not fully understood. It is known that cellular metabolism ceases when water – the essential foundation for biological processes – changes from liquid to solid state. As cells are warmed and the water returns to liquid, cellular function resumes.

Cryopreservation of living bacteria offers a way to stabilize cultures for long-term storage. Proper cryopreservation allows for the generation and maintenance of bacterial stocks and decreases the need for repeated subculturing, which can lead to contamination, genetic drift or mutation as continuously smaller portions of a population are selected. Low-temperature storage greatly reduces phenotypic and genotypic drift and enables cultures to be used as standards, helping to ensure reproducible results in a series of tests or experiments.

Several factors are critical to the stability and viability of a bacterial culture undergoing cryopreservation. Cell type, age, growth conditions, population size, cryoprotectant used, storage conditions and recovery methods all affect viability and stability. Before preparing bacterial cells for preservation, the culture's purity should be checked and its identity confirmed at the genus and species level. In addition, the culture's passage number should be checked to ensure it is in line with guidelines, such as those for USP or CLSI. The purity and identity of bacterial cultures should be examined again when they are removed from the frozen state. A good cataloging and data record-keeping system is also important to prevent misidentification and duplication of material during the preservation process.

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What's new

ATCC Awarded Contract For NCI Biospecimen Network Program

On November 7, 2005 ATCC® announced its award of a contract from the National Cancer Institute to design and implement a system to collect and store biological specimens and associated clinical data for use in cancer research. The NCI project's ultimate goal is to enhance the quality and availability of clinical biospecimens and associated data to the broader scientific community.

The contract award allows ATCC to apply its core competencies in the acquisition, authentication, preservation, storage and distribution of biological reference materials to managing clinical biospecimens for cancer researchers.

The contract represents a significant part of NCI's biospecimen network pilot program, aimed at speeding the discovery and validation of biomarkers for prostate cancer.

The need for validated standards to make research results reproducible and comparable was a major factor in NCI's selection of ATCC to design the biospecimen coordination system. ATCC is the global leader in the definition and establishment of standard biological materials necessary for consistent experimental methodology and the valid comparison of research data.

"ATCC is delighted to take this role in the biospecimen network project and to team

with NCI in delivering clinical biospecimen management services to cancer researchers around the world," ATCC President and CEO Raymond Cypess, DVM/PhD, said.

The Biospecimen Coordination System, which ATCC is charged with designing and implementing, will support the multi-site enrollment of patient volunteers as well as the collection of their biospecimens and associated clinical information. Harmonized approaches for ethical oversight, informed consent will be used. Standard operating procedures for clinical data collection and biospecimen preservation, processing, storage and dissemination will be developed based on ATCC's long history of expertise in this area.

"With ATCC's involvement in the National Biospecimen Network, NCI-funded investigators can take advantage of our organization's technical know-how and experience in biorepository management services," Cypess added.

Partnering with ATCC on the NCI contract will be global consulting firm BoozAllenHamilton and the Johns Hopkins University. BAH will be designing the bioinformatics infrastructure to enable the management of the data associated with the clinical specimens. JHU will provide pathology support.

Genomic DNA Packaging

ATCC announced a change in the package size of genomic DNA prepared from well-characterized microbial strains. Genomic DNA from bacteria, yeast and fungi will soon be available in a 5- μ g size. The change comes in response to requests from ATCC customers.

The existing 10- μ g size will be discontinued when the 5- μ g size becomes available. The ATCC catalog number will change to add '-5' as a suffix. For example: ATCC catalog number 11696D will change to 11696D-5 for the new 5- μ g size.

ATCC offers purified genomic DNA from several hundred well-characterized and authenticated microbial strains. This high-quality DNA has been isolated under aseptic conditions to prevent cross-contamination. Each lot of ATCC genomic DNA is evaluated for integrity, purity, and quality. Testing has been expanded for the 5- μ g size to include agarose gel electrophoresis, restriction digestion, spectrophotometry, 16S sequencing and suitability for amplification by PCR.

For more product information please visit the ATCC Web site at www.atcc.org or contact one of our technical service representatives at 800-638-6597 or tech@atcc.org.

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ATCC Acquisitions Strategy Targets Respiratory Pathogens, Disease Models and Reference Strains

Pathogens of the respiratory system, cell lines that serve as disease models and microbial reference strains are among the major targets for ATCC's recent strategy for accessioning new biological materials.

ATCC's acquisitions strategy for the upcoming year is defined by targeted, discerning accessioning of biomaterials that are of high value to the scientific community, promote the use of standards in life science research and foster the movement of medical applications from 'bench to bedside.' The acquisition targets are largely a response to the current research and development landscape, characterized by high concern over the threat of pandemic influenza, high levels of federal spending for acute respiratory distress syndrome and continued attention to seasonal influenza.

Seeking to serve the scientific enterprise, ATCC scientists have identified a list of acquisition target categories that have the highest utility for the widest range of research fields. In addition to respiratory pathogens and disease models, ATCC seeks hybridomas, microorganisms with fully sequenced genomes and immune effector cells. Also targeted are pathogens such as toxigenic strains, clinical isolates and food- and water-borne microbes. Rounding out the list of acquisition targets are host cells, relevant DNA clones and surrogates for pathogens.

Through participation in the National Science Foundation's Living Stock Collection (LSC), ATCC helps further the LSC mission to represent microbial diversity by seeking sequenced organisms, new and underrepresented genera and species, as well as extremophiles with relevance to bioremediation.

Targeted Acquisitions Strategy

In recent years, ATCC has moved far beyond its role as a repository for biological materials, transforming itself into a vibrant and active player in the life science R&D enterprise. ATCC's position as the world's leading biological resource center (BRC) represents a source of pride for the organization and its employees. BRCs serve a critical function for the scientific community by authenticating and



distributing standard biological materials that enhance the effectiveness and productivity of the life science research and development enterprise. As such, ATCC maintains a robust R&D program, engaging in a range of projects to support translational research and product development in addition to the organization's traditional function of accessioning new cultures from depositors who wish to advance science by sharing their discoveries with colleagues.

A number of factors have played a part in prompting the organization to endorse a philosophy of acquisitions that differs from its past stance, which tended to accept for deposit every sample sent to the organization. Among these is ATCC's increased scope of R&D and support activities in the last decade. Another factor is ATCC's mission to respond to the research needs of the wider scientific community and the science and technology marketplace. Others include a declining level of grant & contract funding for accessioning and maintaining biological materials coupled with an increased rate of discovery of new microbes and cell lines. As a result, ATCC, as a nonprofit organization, must rely more heavily upon culture distribution fees to cover the costs of accession including authentication and long-term preservation.

ATCC remains committed to acquiring those materials with the highest degree of utility and relevance to the research community in both the public and private sectors. However, it must balance the need to accession new materials for the scientific community with its other functions and services to the community as a biological resource center. Currently, a key factor in our acquisition protocol is a review and prioritization of the expected demand or

need for all potential deposits with input from the scientific community.

ATCC has heard a number of common complaints from members of the research community. Many involve the use of the ATCC Material Transfer Agreement (MTA). All parties in a transaction of research materials are afforded some benefit with the MTA. The document helps protect depositors' intellectual property rights. The MTA defines the duties of the recipients and their institutions, including the responsibility to properly handle, use, and dispose of the materials according to applicable laws and regulations. Third, MTAs protect the rights of the recipients by offering the terms of a warranty, which guarantees that if the received materials are not viable, the recipient is entitled to a refund or replacement. A fourth function of MTAs is to protect the rights of the culture collection, by indemnifying the distributor of the materials from claims relating to the use, handling, storage and disposal of the materials.

There remains a persistent misconception among members of the scientific community that ATCC receives government subsidies for some of its activities. In fact, ATCC does not receive dollars from federal, state or local governments, except those that are won through nationally competed grants and contracts. Further, ATCC does not receive any revenue from depositors – there is no cost for making a deposit to the general collection.

Growth and evolution of the organization's strategic directions, along with the targeted acquisitions approach may affect the turnaround time for accessioning of certain biological materials. In the event there is a delay in processing deposited biological materials for accession, please bear with us and understand that ATCC views these materials as extremely important. ATCC continues to support customer service and technical inquiries, so that depositors may receive information during the accession process.

The organization thanks you for your passion for life science research and your support of ATCC and its mission.

Bacteria

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Growth and preparation for cryopreservation

Bacteria being cryopreserved should be grown under optimal conditions on the recommended medium for each strain. The recommended media for ATCC Genuine Cultures™ can be found on the product sheet for each culture or at the ATCC website (<http://www.atcc.org>).

Among the factors influencing how well bacteria handle the cryopreservation process are whether or not the cells are grown in aerated conditions and at which point the cells are harvested. Microbial cells, particularly bacteria, grown under aerated conditions tolerate the stress of freezing better than statically grown or non-aerated cultures. T. Nei et al. (1) have studied the performance of aerated and non-aerated cultures of *E. coli* after the freezing process. Microbial cells harvested in late log or early stationary phase are generally more resistant to the stresses of freezing than younger or older harvested cells (2).

Cells to be cryopreserved can be harvested from broth culture, agar plates or slants. In addition, bacteria can be harvested from centrifugation by removing the supernatant and resuspending the pellet in the suspending medium. Cells to be frozen are generally suspended in fresh growth medium containing a cryoprotectant. For most bacteria, a concentration of 10^7 cells per ml is required for good recovery (3).

Cryoprotectant

Bacterial cultures can be protected during the freezing process by the addition of chemical agents called cryoprotectants. Cryoprotectants such as glycerol penetrate the cells to delay intracellular freezing and protect against solute concentration effects (4). The choice of a cryoprotectant depends upon the type of cell being preserved. For freezing non-fastidious bacterial cultures, ATCC recommends a final concentration of 10% glycerol. Such a concentration can be obtained by adding 20% sterile glycerol to an equal volume of culture medium containing cells. ATCC offers ready-to-use freeze media (catalog no. ATCC®20-2200) TSB with 10% glycerol for non-fastidious bacterial cultures.

The time between the addition of the glycerol to the cells and the cooling process is called the equilibration period. To ensure that the cryoprotectant has enough time to penetrate the cells, the cells should be allowed to equilibrate at room temperature for a minimum of 15 minutes, but no longer than 45-60 minutes (5). The cryoprotectant (glycerol) may be toxic to cells if the equilibration time is longer than 60 minutes.

This time is usually spent dispensing the cells and cryoprotectant into the vials. The most commonly used vials are plastic cryovials with volumes between 1.2 ml and 2.0 ml.

Cooling and Storage Conditions

Following application of the cryoprotectant, the cultures are ready for cooling. As cells start to freeze, ice forms first outside, then inside the cell. As the environment outside the cell starts to freeze, there is an increase in the concentration of solutes outside the cell. Because of the concentration difference across the bacterial cell membrane, water begins to leave the cell and continues to do so until a balance in solute concentration is obtained.

During this period, cooling rate is critical – cells can become too dehydrated if this situation is prolonged by cooling rates that are too slow. If the cells are allowed to cool too rapidly, greater amounts of intracellular ice formation can occur. The study by Nei et al. on the behavior of aerated vs. non-aerated cultures concluded that the aerated cells dehydrated faster during cooling than non-aerated cells. However, some bacterial strains cannot be grown in shake culture and must be grown on agar before cryopreservation.

Mazur et al. have suggested the ideal cooling rate for bacteria is approximately -1°C per minute (6). However, most non-fastidious bacteria will withstand less-than-ideal cooling rates and can be frozen by placing the vials on the bottom of a mechanical freezer at -60°C to -80°C for one hour. For more fastidious strains, more uniform cooling rates are required. A programmable-rate freezing apparatus can be used to obtain rates of $-1^{\circ}\text{C}/\text{minute}$.

After cooling, the vials are stored in the vapor phase of a liquid nitrogen freezer at -170°C . Storage temperatures below -130°C are

critical to the long term stability of bacterial cultures, because that is the point at which ice crystal formation stops. At temperatures above -130°C , ice crystals continue to grow and reform (7,8). Warming and recooling, even without complete thawing, can be detrimental to bacterial cultures. It is imperative that storage temperatures be maintained continuously and that the location, date and details of the freeze be recorded when the freezing process is completed. For most non-fastidious bacteria, -80°C is sufficient for short-term storage up to 5 years.

Recovery

To revive frozen cultures, thaw in a 37°C water bath and transfer all vial contents to the optimum growth medium for incubation at the proper temperature. More fastidious cells may require more time to recover from freezing. Cultures should be re-checked for viability and purity after the process to ensure that the material recovered from the preservation process remains unchanged.

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Choose ATCC cell cultures with confidence

It is well known that phenotypic and genotypic characteristics of cells can change when they are cultivated for extended periods. It is also recognized that stocks of commonly used cell lines maintained in many laboratories have experienced hundreds of passages. Furthermore, cross-contamination of animal cell lines with other cell lines has been acknowledged for 20 years. Mycoplasma infection of cell lines can lead to detrimental host effects including chromosomal aberrations, growth changes, alterations in gene expression and changes in virus and antibody production.¹⁻⁹

Obtaining cells from a reputable source such as ATCC is a critical first step in ensuring the quality and reproducibility of research data. Continuous subculturing or splitting increases the possibility of contamination and may lead to genetic drift. Well-characterized cell lines are the best starting materials for any research project.

Authentication & Quality Control

When a culture arrives at ATCC for deposit, a preliminary freeze is done while the original culture is checked for contamination and the species is verified. Cells are further characterized with a selection of tests when appropriate.

- Assays for microbes, including mycoplasma, determine whether the culture is free from contamination.
- Isoenzyme analysis, karyotyping, and immunological testing verify the species of origin.
- Morphologies of growing cells are recorded at low and high densities and are routinely made available to researchers on the ATCC website.
- Short tandem repeat (STR) profiling is used to confirm the genotype of all human cell lines
- The viability of the cell line prior to freezing and post-freezing is checked.
- Growth characteristics such as growth rate and cell density are recorded.
- Various specialized testing, such as determining the immunoglobulin subclass secreted by hybridomas or confirming expression of cellular markers by flow cytometry, may be employed.

Process and Quality Control

A large number of frozen vials are prepared in the first production run and a portion of these vials are set aside as seed stock, which remain available for preparing new vials for distribution. Quality testing is applied to the seed stock and is repeated for each new distribution lot. This system avoids prolonged

serial subculturing and minimizes the number of passages that the cells undergo before being sent to a customer.

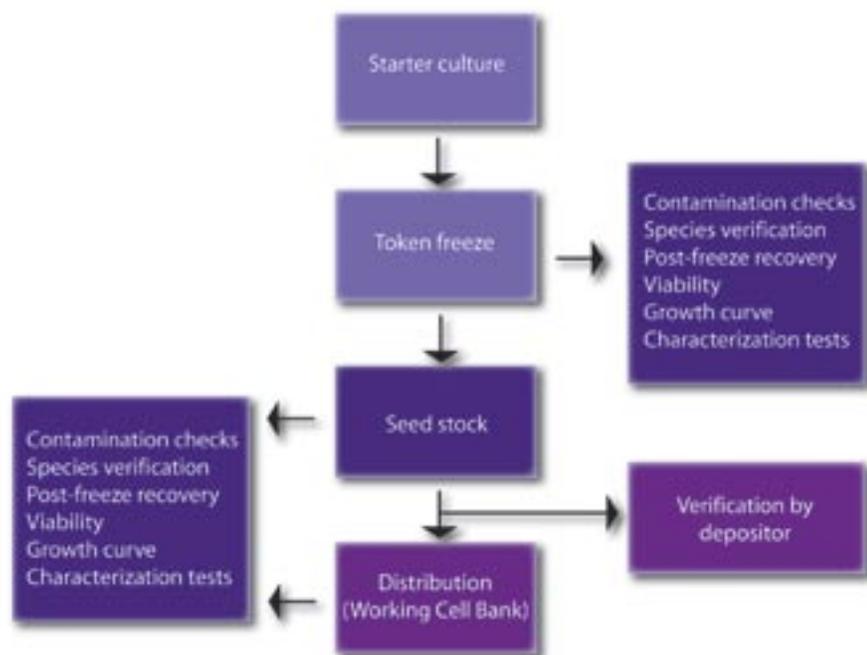
All cryopreserved material at ATCC is meticulously stored in liquid nitrogen vapor. Both the temperature and liquid nitrogen levels in the freezers are monitored continuously.

ATCC processes for accessioning, producing, storing and distributing cell lines ensure that researchers receive the highest quality reagents to conduct research. In addition, all cell lines are backed with dependable technical support.

Insist on the best materials for your research projects. Choose ATCC cell cultures with confidence.

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ELF

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fluorescent yellow-green precipitate at the site of enzymatic activity. It can be visualized with a Hoechst/DAPI longpass filter set.

The phosphatase precipitate has an exceptionally large Stokes shift of greater than 100nm (it exhibits a fluorescence emission that is separated from its excitation wavelength by greater than 100nm). Therefore, it is ideal for use with appropriate dual or multicolor applications and signals can be easily distinguished. This precipitate also provides a fluorescent signal that is extremely photostable. The excitation/emission of the reaction product is 345/530 nm.

The detection method has been optimized on several mouse and human ESC lines and is used routinely by the ATCC Stem Cell Center (SCC) on in-house stem cell research projects. SCC scientists depend on the assay regularly to ensure the cell lines they work with exist in an undifferentiated state. The phosphatase detection protocol is ideal for routine verification of pluripotency in ES cell lines.

Stem cell lines often are not as straightforward to culture as commonly used cell lines and exhibit a tendency to differentiate. Therefore, SCC scientists recommend running the assay after every two to three passages and always before running an experiment that requires undifferentiated stem cells.

The ELF® Phosphatase Detection Kit for Embryonic Stem Cells (catalog no. ATCC® SCRR-3010)

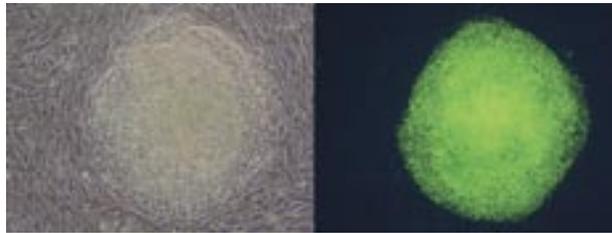


Figure 1 – Human ES cells, BG01V (ATCC® SCRC-2002™) surrounded by feeder cells are **fully undifferentiated**, as evidenced by the presence of continuous green fluorescence throughout the cell mass.



Figure 2 – Most of this BG01V colony is undifferentiated. A locus of cells has **differentiated**, as shown by the lack of fluorescence in one region (arrows).

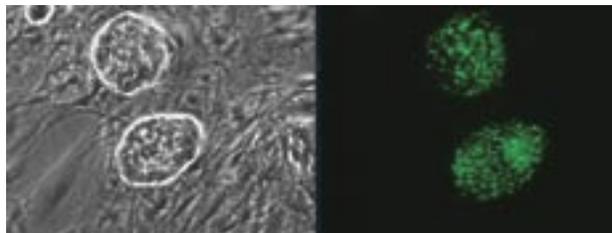


Figure 3 – The uneven fluorescence in these mouse ES cells, R1/E (ATCC® SCRC-1036™) indicates **nonuniform** maintenance of the undifferentiated state.

can be ordered from the ATCC online catalog or by calling 800-638-6597 in the United States, Canada, and Puerto Rico or 703-365-2700 elsewhere. The ATCC Stem Cell Center conducts ongoing research and stem cell line characterization and is a provider of fully-characterized human and mouse ESCs and qualified media and sera.

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What determines the biosafety level of a culture at ATCC?

ATCC has classified cultures and related products by biosafety level (BSL) based on an assessment of potential risk. Each item is assessed according to guidelines established by the U.S. Public Health Service, with assistance from ATCC scientific advisory committees. These levels are assigned for packaging and shipping purposes only; the BSL for use of the material may be higher. To assess the BSL for each item, the origin and authentication of the material are carefully reviewed as are the guidelines set in the 4th edition of the publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL)¹. In some cases, the ATCC BSL level for an item is more restrictive than what is recommended in BMBL guidelines. This may be the situation for organisms that are not normally associated with a disease, but were isolated from a clinical sample or for cloned DNA that has the potential to be hazardous with some manipulation. The requirements for each BSL level are outlined briefly below:

- BSL-1 – Organisms not known to cause disease in healthy adult humans and pose minimal hazard to people and the

environment.

- BSL-2 – Organisms that pose a moderate risk and are associated with human disease through skin breaks, ingestion or mucous membrane exposure.
- BSL-3 – Indigenous or exotic agents with potential for aerosol transmission and potential for serious and even lethal effects.
- BSL-4 – Dangerous/exotic agents which pose high risk of life threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission.

¹(HHS Publication No. 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health; U.S. Government Printing Office: Washington DC; 1999). This manual is also available in its entirety on line <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>.

I've worked with BSL-2 organisms in the past but now ATCC is asking me to complete a form before I can order any BSL-2 cultures. Why?

Before any BSL-2 item can be sent to a lab, ATCC is required to ensure that the lab is suitable for BSL-2 work. The same is true for

any BSL-3 organisms. ATCC does not distribute any BSL4 items. The lab must comply with the laboratory biosafety level criteria listed in the BMBL handbook. Refer to that manual for more information on the specific details required for working with organisms in each of the different BSLs.

A form can be obtained from the ATCC on the New Accounts Application page (<http://www.atcc.org/Order/NewAccountForm.cfm>) that outlines what type of information is needed for ordering. The completed form must be returned and reviewed by ATCC before cultures can be ordered. It is the responsibility of each lab receiving this type of material to fully assess the potential risk of working with the material in their laboratory. The actual risk associated with handling a biological agent depends not only on the nature of the agent but also on the laboratory manipulations employed during its handling. For further information, contact New Accounts at sales@atcc.org.

TechQs are intended for informational purposes only. ATCC is not responsible for results obtained from the use of this information.

Biodefense Experts Chart Path Forward On Consensus Standards

The development of standards for biodefense R&D is critical to the health, safety and welfare of the public and the viability of the biodefense industry. The ATCC Expert Panel on the Development of Standards for Biodefense convened on April 5 & 6 at the Hay-Adams hotel in Washington, DC. Leaders in the biodefense R&D community met to identify and frame key issues in the establishment of voluntary consensus standards for biodefense-related materials and processes. The summit brought together academic, industry and government stakeholders to address the current lack of standards in biodefense research and product development. In addition to establishing the expert panels to identify key

questions and issues, the meeting defined the scope for biodefense standards and outlined specifications that biodefense standards must address. The meeting represents a launching point for a wider effort intended to produce consensus standards that will improve the quality and productivity of biodefense R&D.

The meeting agenda included a keynote address by Major General Eric Schoomaker, MD, PhD, commander of the U.S. Army's Fort Detrick, a review of the Bush Administration's view of biodefense from Lawrence Kerr, PhD, director of biodefense policy for the President's Homeland Security Council, as well as working sessions where the panelists cultivated agreement on the major hurdles

that need to be negotiated in biodefense standards development as the process moves forward.



Conference Chair Joe Perrone, ATCC

Coming Soon

ATCC is pleased to announce that a searchable web-based standards resource will soon be available by accessing the ATCC website at <http://www.atcc.org>. The ATCC Standards Program has established the resource so that users will be able to search for ATCC strains that are specified in a standard test method or commercial quality control protocol.

The web-based search will enable a user to search standard test methods by name of the organization or company, name of the test or identification system and/or the number of the test that calls for ATCC strains and access product page descriptions for the cultures specified. ATCC strains used by commercial firms as controls for rapid identification, minimum inhibitory concentration of antibiotics and antibiotic susceptibility panels can also be searched by manufacturer's name or instrument/test name. Links to organizations and commercial firms may also be available.

Many professional organizations and government agencies draft standards for assays and tests used in industrial quality control, environmental monitoring and medicine. Many of the organizations form committees who draft specific standards and build a voluntary consensus of interested parties before final adaptation and publication of the approved text. For over eighty years,

ATCC's primary role in the standards process has been to authenticate and maintain the standard strains in a manner that permits the user to obtain intended results, and ensure that the strains maintain characteristics. We also work with the organizations and agencies to recommend strains and protocols using those strains, when the standards agencies require the use of a microorganism, cell line, virus or genomic material. Most of the standards test one of the following:

efficacy of an antibiotic or technique (e.g., handwashing technique, steam sterilization)
presence of some substance or characteristic (e.g., enterotoxins in food, antibiotics in feed)
resistance of something to microbial attack (e.g., paint, wallpaper)

Copies of the standards can be obtained from the organization or agency responsible for them. ATCC does not distribute copies of the standards.

ATCC encourages deposits of new strains by standards organizations, regulatory agencies, and manufacturers of microbial identification systems. Deposits are accepted as part of ATCC's role as a global nonprofit bioresource center that provides biological products, technical services, and educational programs to private industry, government, and academic organizations around the world.

Meetings and Conferences

ATCC will be attending the following meetings in 2006. Stop by and talk to an ATCC representative about how we can help your research.

Biomedical Research Equipment & Supplies Exhibit
 May 17-18, Fort Detrick, MD

American Society for Microbiology
 May 21-25, Orlando, FL

NIH Post Baccalaureate Research Festival Exhibit
 May 25-24, Bethesda, MD

International Society for Stem Cell Research
 June 29-July 1, Toronto, Canada

American Society of Virology
 July 15-19, Madison, WI

University of Wisconsin Exhibit
 August 3, Madison, WI

International Association for Food Protection
 August 13-15, Calgary, Alberta

Texas Medical Center Exhibit
 August 17, Houston, TX

Ohio State University Exhibit
 September 14, Columbus, OH

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