Strengthen R&D by Preventing Cell Line Contamination

Biotech & Pharma Cell Line Authentication Guide November 2021

IDEXX **BioAnalytics**

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I. Why Does CLA Matter? How does it affect research programs?

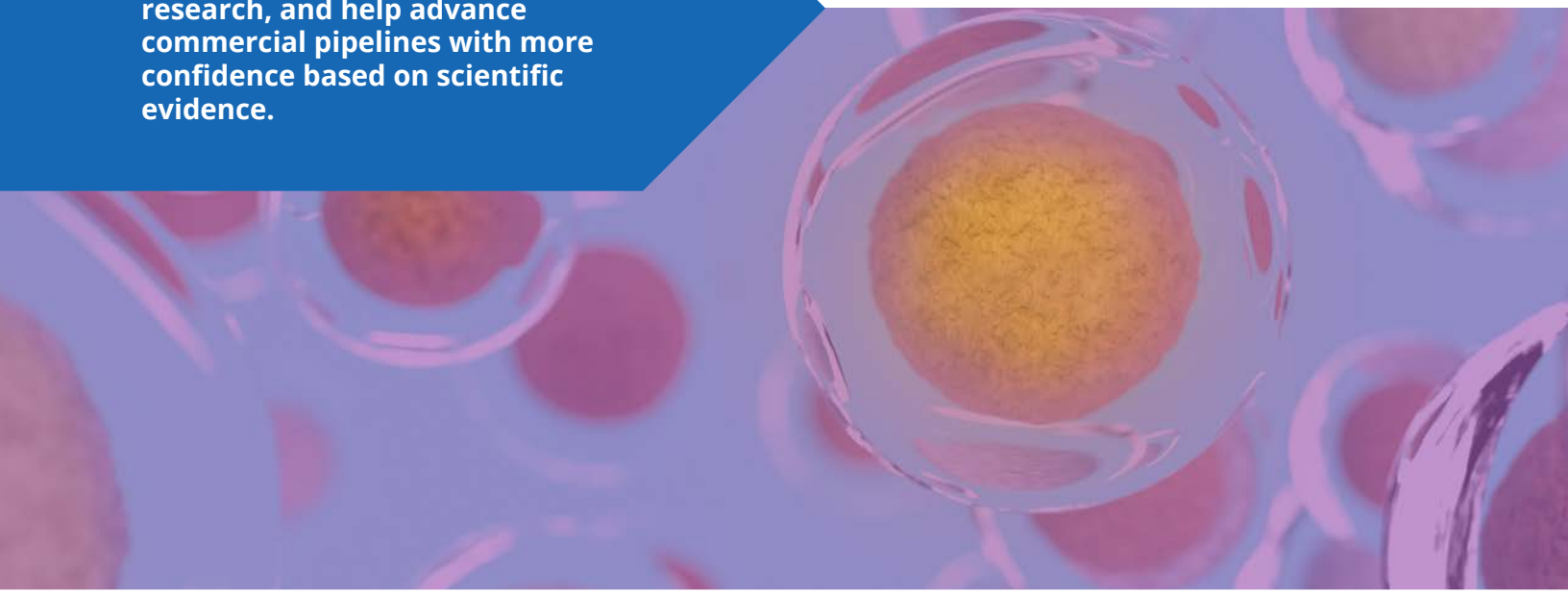
You and your colleagues spend years of time, energy, and resources researching a particular cellular process, disease state, or pharmaceutical intervention. Imagine the devastation if you discovered the cells weren't exactly what you thought they were. With the surge in popularity of cellular models and molecular techniques, cell line misidentification or contamination is an all-too-common occurrence in R&D labs around the world. If cells are invalidated, companies could experience significant setbacks, and in today's competitive pharma and biotech environment, the lost time and money could make the difference between progressing a program or not.

Cell line authentication (CLA) provides researchers with the data and confidence they need to replicate and reproduce their findings in pre-clinical testing and drug development pipelines.

Maintaining scientific integrity is the cornerstone of any research pursuit. Scientific activities conducted by research institutions, universities, or the private sector must ensure objectivity, clarity, and reproducibility. According to the USDA, these standards provide insulation from "bias, fabrication, falsification, plagiarism, inappropriate influence, political interference, censorship, and inadequate procedural and information security."

Cross-contamination of cell lines in scientific research is rampant. It is understood that the contamination or misidentification of cell lines is as high as 36%[1,2,3]. Such staggering contamination rates highlight the need to implement a routine cell line authentication monitoring program. An estimated **$10.1 Billion is spent annually on irreproducible research due to contamination or misidentification of key biological materials[4].**

This guide aims to help the biomedical and pharmaceutical industries prevent irreproducible research, and help advance commercial pipelines with more confidence based on scientific evidence.



II. The Impact of CLA in Research Labs

How big of a problem is cell line contamination during the research process?

The National Center for Biotechnology Information estimates that as many as 36% of all cell lines are contaminated, causing significant, high-impact losses to biomedical and pharmaceutical companies each year. The impact of contaminated cells is vast, often causing frustrating time delays, wasted budget in labor and other technoscientific salaries, and even unpleasant announcements to critical stakeholders. Contaminated or misidentified cells result in ruined experiments, poor quality control along internal process lines, and drained R&D resources.

"We receive contaminated cell lines each week, and it can be disconcerting news to deliver to a researcher when it represents years of research work. In many cases, it is countless hours of time and untold amounts of money all potentially washed away in an unfortunate case of mistaken identity."

- Robert S. Livingston, DVM, PhD, DACLAM
Director Operations & Scientific Affairs

There are at least 531 known cross-contaminated or misidentified cell lines in existence today. Contaminated or misidentified cell lines, pernicious in nature, may diminish therapeutic efficiency or create additional, unwanted side effects[5].

To maintain scientific integrity and protect the public from research misconduct, the National Institute of Health (NIH) began requiring all grant applicants to include a cell line authentication plan for biological materials. Adoption of the standards to authenticate cell lines and other critical biological materials are intended to "enhance the reproducibility of research findings through increased scientific rigor and transparency."

Given the outsized impact of contaminated cell lines in the biomedical field, NIH Notification #NOT-OD-16-011 directed grant applicants to include an authentication plan for critical biological materials with new and competitive renewal grant applications. Adoption of the new standards to authenticate cell lines and other vital natural materials were designed to enhance cell lines' reproducibility and decrease the prevalence of contaminated corporeality.

Custom-engineered cell lines, such as CRISPR-edited cells, add a truly modern aspect to the issue of cell line authentication. Researchers are now engineering proprietary disease-state mimicking models and establishing those in repositories or sharing with collaborators. Novel cell lines should be characterized and cataloged, then verified at the beginning, midway, and end of a research study, just like "standard" cell lines.

Nature Group, among other journals, have begun requesting that authors:

- Establish a profile of their cell lines to allow for future authentication
- Research and investigate any restrictions associated with the tissue they are using
- Report on the source of cells and provide proof of authentication

The world's scientific journals are also paying attention to cell line authentication. As of 2021, there are over 250 journals that have taken steps to address reproducibility and rigor, including asking for proof of authenticated cells. Currently, journals from the Nature Group, AACR, Endocrine Society, and other prestigious publications require cell line authentication with paper submission[7].

How do researchers know when contamination has occurred?

A fundamental underpinning of scientific research is the ability to describe your study to others and have it independently replicated using the same techniques. A frequent culprit of irreproducible science is cross-contaminated or misidentified cell lines. Often, a researcher does not have any verifiable evidence that contamination has occurred, further underscoring the need for routine monitoring to confirm cell line identity.

To identify contaminated cells (or confirm a valid line of cells), a consistent, standardized set of markers is used to generate a genetic profile for the analyzed sample. This genetic profile is compared to established reference profiles to confirm the cell lines are consistent. Using standardized markets allows for the comparison of global genetic profiles to profiles established in any laboratory.

NIH Notification #NOT-OD-16-011

In 2015, the National Institutes of Health (NIH) and Agency for Healthcare Research and Quality (AHRQ) issued an announcement entitled "Implementing Rigor and Transparency in NIH & AHRQ Research Grant Applications." Per the NIH, the notice informed the biomedical research community of "updates to application instructions and review language intended to enhance the reproducibility of research findings through increased scientific rigor and transparency." According to notice #NOT-OD-16-011, all grant applications must complete a cell line authentication assessment prior to award submission.

For a complete list of information released by the NIH, consult Notice Number: NOT-OD-16-011: Implementing Rigor and Transparency in NIH & AHRQ Research Grant Applications.

How can CellCheck™ support compliance standards?

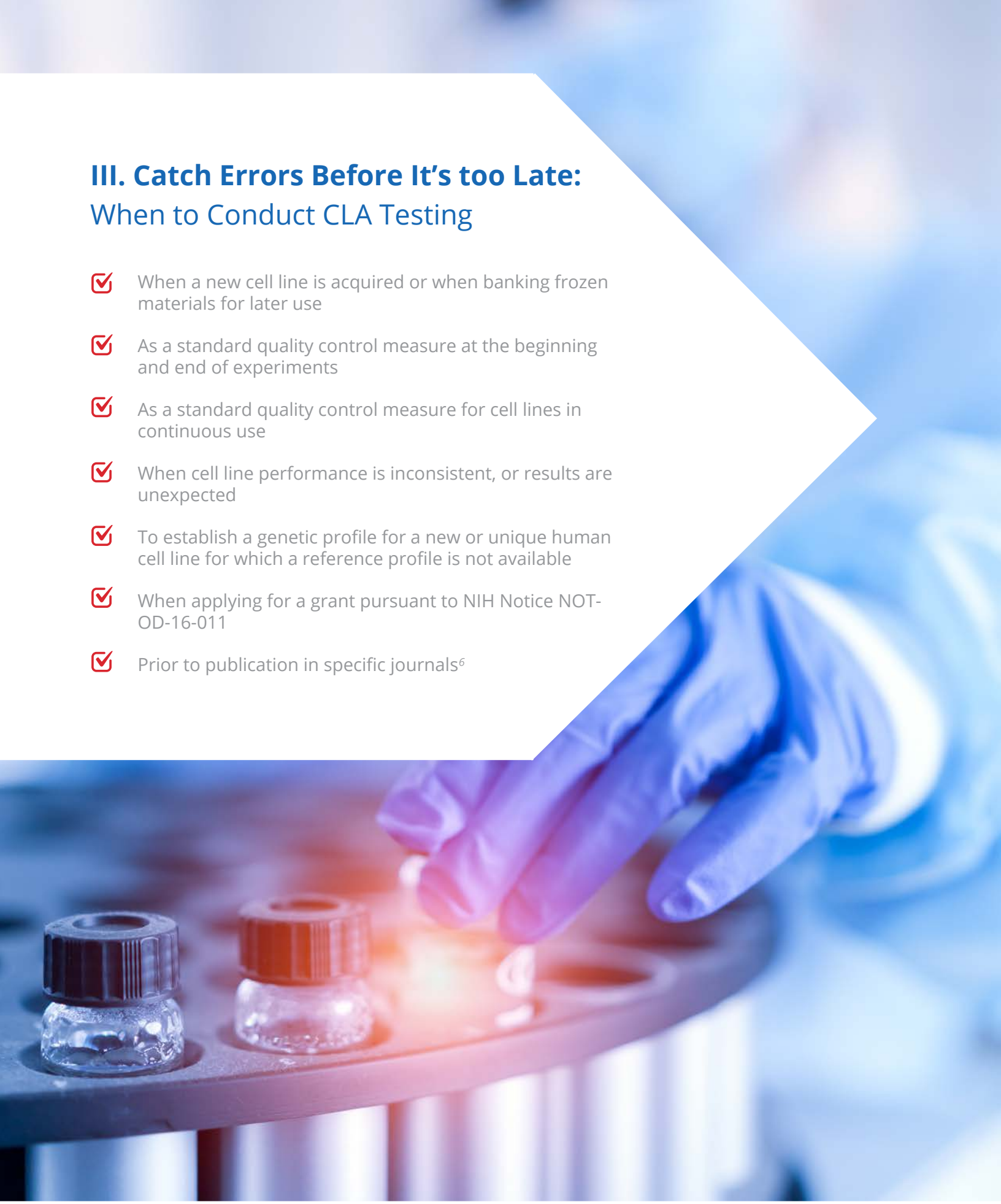
IDEXX BioAnalytics CellCheck™ authentication service utilizes methods recommended by the American National Standards Institute (ANSI ASN-0002-2011) to ensure your success in meeting authentication requirements. CellCheck™ cell line authentication service is the industry gold standard to ensure your success in meeting funding agency and top publication guidelines.

CellCheck™ combines STR-based DNA profiling and multiplex PCR to detect both contamination and misidentification. Additionally, CellCheck™ provides a comparative analysis and data interpretation analysis. In the event of contaminated cells, our team of experts will assist in developing a contamination recovery plan.

In the case of custom-engineered cells, IDEXX scientists can assist with generating a novel profile and will maintain that profile in our database. Repositories may then be distributed with certificates of authenticity, or collaborators can confidently employ these novel lines after receiving and testing them. Our scientists are happy to assist with this process.

III. Catch Errors Before It's too Late:
When to Conduct CLA Testing

- When a new cell line is acquired or when banking frozen materials for later use
- As a standard quality control measure at the beginning and end of experiments
- As a standard quality control measure for cell lines in continuous use
- When cell line performance is inconsistent, or results are unexpected
- To establish a genetic profile for a new or unique human cell line for which a reference profile is not available
- When applying for a grant pursuant to NIH Notice NOT-OD-16-011
- Prior to publication in specific journals[6]



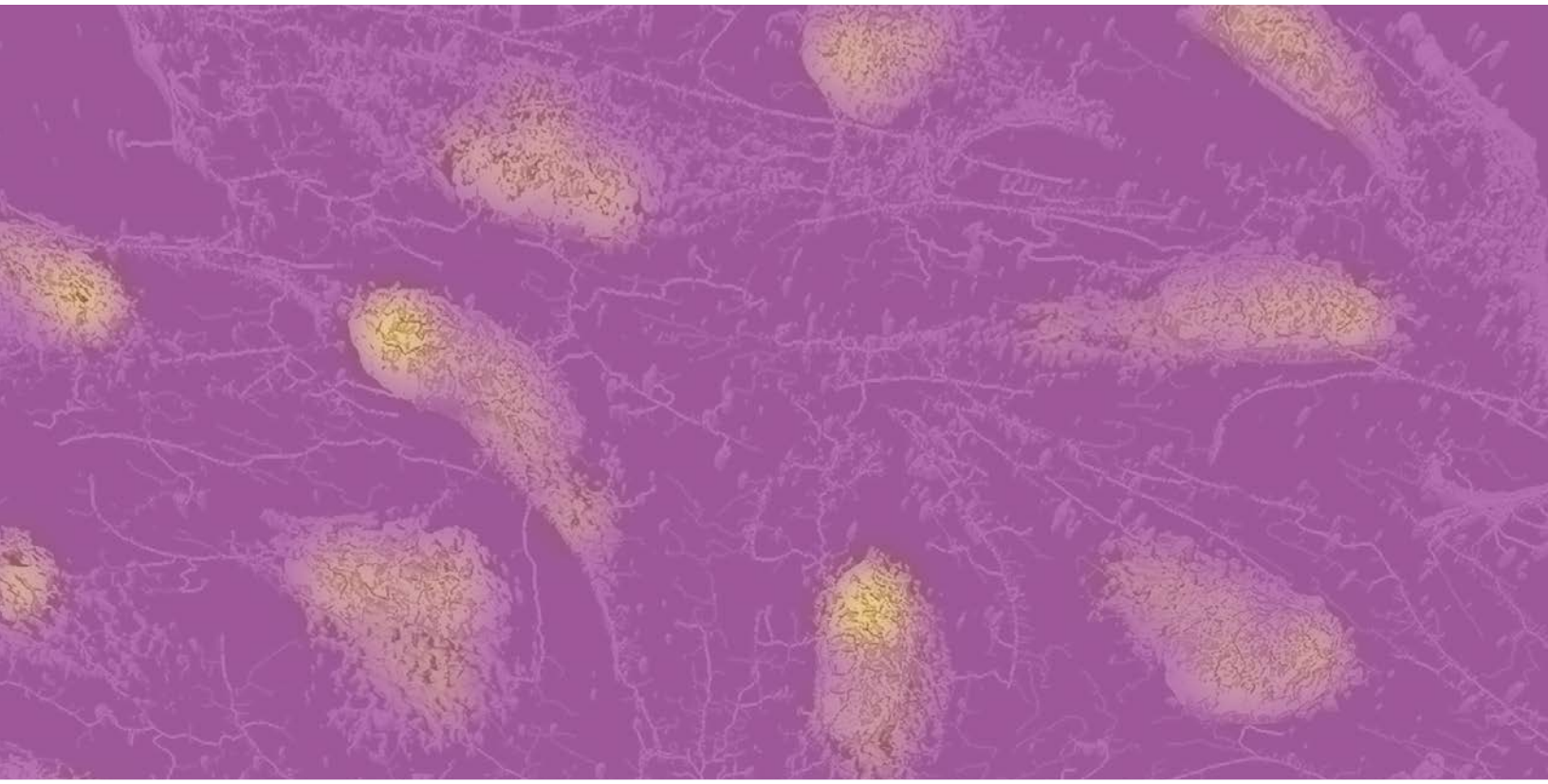
IV. Using CellCheck™ as Part of Cell Line Authentication Program

Your authentication solution starts here.

CellCheck™ is much more than an STR profile. It's a comprehensive cell line authentication service that utilizes STR-based DNA profiling and multiplex PCR to detect BOTH contamination and misidentification of human and mouse cell lines.

IDEXX BioAnalytics's gold standard CellCheck™ testing service provides comprehensive cell line authentication, consisting of both STR testing to confirm the identity of the human cell line coupled with species testing to ensure the sample is not cross-contaminated with another human cell line or another species of an origin cell line. Furthermore, important testing for mycoplasma and the presence of exogenous viral pathogens may be added - all using a single sample. Our scientists are ready to help you implement the testing routine that's right for your research program.

An easy and comprehensive service—we accept your cryovial, and there is no need for ordering special sample collection supplies. We will also provide data analysis and interpretation, along with a comparative analysis that can be run against a referenced profile or in comparison to your parental cells. Additionally, we'll provide you with a comparative analysis and data interpretation and even help you develop a contamination recovery plan if needed.



V. Sample Authentication Plan

Key Biologicals: Authentication Checklist

Recommended testing timepoints and testing tracker for easy reporting during the course of NIH grant performance.

Date Authenticated	Cell Line Name	Species	Sample ID	IDEXX Bioanalytics Case #
☐ Cell Line Acquired or Created				
☐ Established Baseline Genetic Profile For Unique Or Patient-Derived Material				
☐ Banking Frozen Materials For Later Use				
☐ Standard Quality Control Measure For Line(s) In Continuous Use				
☐ Initiated Use In Experiments				
☐ Concluded Use In Experiments				

VI. CellCheck™ Sample Collection and Shipping

To Submit Cells:

1. Send one cryovial (or other screw-top tube) of each sample with a minimum of $1x10^6$ cells/vial.

2. Cells may be in the form of a pellet or in growth media, freeze media or phosphate-buffered saline.

Note: Cells are preferred, but if DNA is high quality, it may be submitted.

To Submit Tissue/Solid Tumor Samples:

1. Send a 1.5 mL snap top or screw top tube of each sample with a minimum of 30 mg of tissue (2-3mm size fragment or larger).

To Submit DNA:

1. DNA must be submitted as high quality extracted DNA (crude lysate extractions are not suitable for STR analysis).

2. DNA needs to be submitted with a minimum of 100ng of DNA in a minimum volume of 15 µl.

3. Optimal concentrations submitted are preferred at >20 ng/µl. DNA concentration must be at least 10 ng/µl.

Note: Frozen cell cultures or tissue are preferred. Submission of cells allows extraction of the DNA in our facility for optimal DNA quality and test results.

4. Send DNA in a tube with screw top cap if available. If a snap cap top tube is used, secure the top with Parafilm™ before shipping. DNA samples can be shipped at room temperature by regular ground shipment. During hot weather, ship samples overnight with an ice pack to prevent exposure to excessive heat.

To Set Up Submission:

Online, visit the **Client Login** page.

Or visit us **HERE** to download a fillable PDF submission form.

Ship samples to our Columbia, Missouri facility by overnight courier with sufficient ice packs or dry ice so that samples remain frozen during shipment.

Ship Samples To:

IDEXX BioAnalytics
4011 Discovery Drive
Columbia, MO 65201

800-669-0825

idexxbioanalytics@idexx.com

Sources

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Need help getting started?

We are here with you to help you every step of the way. Connect with an Expert today to start creating your cell line authentication strategy

Ask Our Experts