Promega Corporation

Know Thy Cells: How to Avoid an Identity Crisis with Cell Line Authentication

Lisa M. Ortuno, Ph.D.
Thank you to our sponsors!!

Linda Green
Associate Director for Science
Scientific Director, Monoclonal Antibody
What is in your cell lines?

The word is out!

National Public Radio is reporting today on the prevalence of contaminated and mis-identified cell lines being used in research. The Genomics core is hosting a seminar about this today. Find out how to verify your cell lines, join us TODAY at 1pm.
Scientists Often Skip A Simple Test That Could Verify Their Work

There's a simple test that scientists could use to make sure the cells they're studying in the lab are what they think they are. But most of the time, academic scientists don't bother.

That omission is a problem. One study found that between 18 percent and 36 percent of all cell lines have been misidentified. And this kind of mistaken identity is one reason that many results from experiments run in scientific labs can't be reproduced elsewhere.
SCOPE

• a standardized procedure for unambiguous authentication and identification of human cell lines using STR profiling.

• specification of the methodology for
  - DNA extraction
  - STR profiling
  - data analyses
  - quality control of the data
  - interpretation of results
  - and implementation of a searchable public database.

ANSI: American National Standards Institute
The legend of HeLa

- HeLa - first immortal cancer line to be established in 1951 by George Gey
- Cervical cancer derived from patient Henrietta Lacks
- Widely used in research. According to Rebecca Skloot “More than 60,000 scientific articles had been published about research done on HeLa, and that number was increasing steadily at a rate of more than 300 papers each month.”
- Rapid growing; can contaminate and overtake other cell types

Feb 2010
## Establishment of other human cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Tissue Type</th>
<th>Year</th>
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<tbody>
<tr>
<td>AV3</td>
<td>amnion</td>
<td>1956</td>
</tr>
<tr>
<td>Minnesota EE</td>
<td>esophagus</td>
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<tr>
<td>Chang liver</td>
<td>liver</td>
<td>1954</td>
</tr>
<tr>
<td>Detroit 98</td>
<td>bone marrow</td>
<td>1969</td>
</tr>
<tr>
<td>Giardi heart</td>
<td>heart</td>
<td>1956</td>
</tr>
<tr>
<td>HBT3</td>
<td>breast</td>
<td>1972</td>
</tr>
<tr>
<td>Hep 2</td>
<td>larynx</td>
<td>1955</td>
</tr>
<tr>
<td>Intestine 407</td>
<td>intestine</td>
<td>1957</td>
</tr>
<tr>
<td>J-111</td>
<td>mon. leukemia</td>
<td>1955</td>
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<tr>
<td>KB</td>
<td>oral carcinoma</td>
<td>1955</td>
</tr>
<tr>
<td>WISH</td>
<td>amnion</td>
<td>1961</td>
</tr>
</tbody>
</table>
**Discovery of HeLa Cell Contamination**

1968: Gartler publishes first evidence of HeLa contamination based on G6PD isoenzymes

1974: Nelson-Rees et al. publish evidence of HeLa contamination based on marker chromosomes

*From Nature Reviews 10:441, 2010*

*Courtesy of Jim Coran*
ROLAND NARDONE, author

“An open letter regarding the misidentification and cross-contamination of cell lines: Significance and recommendation for correction”

July 11, 2007

Secretary Michael O. Leavitt
U.S. Department of Health and Human Services
20 Independence Avenue, S.W.
Washington, DC 20201

Dear Secretary Leavitt,
## ANSI/ATCC Standards Development Organization Guidelines Workgroup Participants

<table>
<thead>
<tr>
<th>Name and Title</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>John R. W. Masters, PhD, FCR Path</td>
<td>University College London, UK</td>
</tr>
<tr>
<td>Yvonne A. Reid, PhD</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>Raymond W. Nims, PhD</td>
<td>RMC Pharmaceutical Solutions, Inc.</td>
</tr>
<tr>
<td>Steven R. Bauer, PhD</td>
<td>FDA/Center for Biologics Evaluation and Research</td>
</tr>
<tr>
<td>Manohar Furtado, PhD</td>
<td>Applied Biosystems</td>
</tr>
<tr>
<td>Jaiprakash G. Shewale, PhD</td>
<td>Applied Biosystems</td>
</tr>
<tr>
<td>Anton F. Steuer, PhD</td>
<td>BioReliance Corporation</td>
</tr>
<tr>
<td>Douglas R. Storts, PhD</td>
<td>Promega Corporation</td>
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<tr>
<td>Arihiro Kohara, PhD</td>
<td>National Institute of Biomedical Innovation, Japan</td>
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<tr>
<td>Roland M. Nardone, PhD</td>
<td>Catholic University of America</td>
</tr>
<tr>
<td>Georgyi V. Los, MD, PhD</td>
<td>Thermo Fisher Scientific</td>
</tr>
<tr>
<td>Wilhelm G. Dirks, PhD</td>
<td>DSMZ, Germany</td>
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<tr>
<td>Margaret C. Kline</td>
<td>NIST</td>
</tr>
<tr>
<td>Paul J. Price, PhD</td>
<td>ThermoFisher Scientific</td>
</tr>
<tr>
<td>Jim Thomson, PhD</td>
<td>Life Sciences</td>
</tr>
</tbody>
</table>
Why Authenticate Cell Lines?

• Estimated that 15-20% of the cells are misidentified or cross-contaminated

• Tissue culture labs susceptible to misidentification of cell lines
  • Survey that profiled active cell culture workers indicates that out of 483 respondents, 32% used HeLa cells, 9% unwittingly using HeLa contaminants, 33% tested authenticity, and 35% obtained cell lines from other labs rather than from a repository

• Cell repositories authenticate cell line submissions and monitor cross-contamination
  • Regardless the original source, cultures can be cross-contaminated or mislabeled while being maintained
  • ATCC focussing on cell line authentication

• Validation of cells and cell components (gDNA, RNA, proteins) used as standards in MDx
### HeLa Contaminated Cell Lines

<table>
<thead>
<tr>
<th>Name</th>
<th>Tissue</th>
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</thead>
<tbody>
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</table>
**Consequences: False species based conclusions**

<table>
<thead>
<tr>
<th>ORIGIN/PRESUMED TISSUE</th>
<th>ACTUAL TISSUE</th>
<th># FALSE PUBLICATIONS</th>
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<tbody>
<tr>
<td>Human salivary gland carcinomas</td>
<td>Mouse, rat cells</td>
<td>12</td>
</tr>
<tr>
<td>Human Hodgkin lymphomas</td>
<td>Brown-footed owl monkey</td>
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<tr>
<td>Bovine mammary epithelial cells</td>
<td>Mouse mammary epithelial cells</td>
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</table>
## Consequences: False Tissue Based Conclusions

<table>
<thead>
<tr>
<th>DESIRED TISSUE</th>
<th>ACTUAL TISSUE</th>
<th># FALSE PUBLICATIONS</th>
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<tbody>
<tr>
<td>Human breast</td>
<td>Cervix (HeLa), ovary, melanoma</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Human esophageal cancer</td>
<td>Lung, colon, gastric carcinomas</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Human salivary gland carcinoma</td>
<td>Cervix (HeLa), bladder carcinoma</td>
<td>75</td>
</tr>
</tbody>
</table>
Inappropriate use of HeLa contaminants: frequency of published reports

A retraction resulting from cell line contamination


After nine years in print, *Nature Methods* today published its first retraction; one that could have been prevented by cell line authentication. What does this mean for journal-mandated cell line testing?

In a *Nature Methods* paper published in 2010, Ivan Radovanovic and colleagues described a method to isolate cancer-initiating cells in human glioma without the need for molecular markers. Based on morphology and on a green autofluorescence, the authors reported they could use FACS to sort cancer-initiating cells from gliomasphere cultures (which had been derived from primary tumors). They also detected autofluorescence in cells from fresh glioma specimens, but at a much lower level.

Cells from the autofluorescent fraction could self renew clonogenically in vitro and were tumorigenic when transplanted into mouse brains, the authors reported, and in both cases performed better than non-autofluorescent cells from the rest of the culture or tissue. The origin of this autofluorescent signal was not understood at the time. The authors speculated it may have been related to the unique metabolism of the cancer-initiating cells.
It is hardly surprising that the first retraction in *Nature Methods* is due to cell line contamination, a well
acknowledged problem. A 2009 *Editorial* in *Nature* pointed to the disturbing results of cell testing by
repositories which indicated that 18-36% of cultures were misidentified. It called on repositories to
authenticate all of their lines, and for major funders to provide testing support to grantees. At that point
funders could require cell line validation for investigators to retain funding, and *Nature* would require that all
immortalized lines used in a paper were verified before publication. Unfortunately, it is now 2013 and we are
still far from this goal.

But progress is being made. Community-based efforts are alerting researchers to this problem and providing
resources to help them avoid being misled by erroneous results caused by cell line contamination. A 2012
*Correspondence* in *Nature* by John R. Masters on behalf of the International Cell Line Authentication
Committee (ICLAC) pointed to the following resources available to researchers:

- An STR procedure available from the American National Standards Institute webstore.
- Two helpful PDF documents from the ICLAC with information and tips on authentication
  including ‘Advice to Scientists’ and an ‘Authentication SOP’.
- A continuously curated list of more than 400 misidentified cell lines (*Database of Cross-
  Contaminated or Misidentified Cell Lines, Version 7.1*).

Please go to the ICLAC website for the most recent version of each of these documents.

Meanwhile in early 2013, at the publication end of the process, the Nature journals published coordinated
editorials announcing a *reproducibility initiative* and stating that “…authors will need to […] provide precise
characterization of key reagents that may be subject to biological variability, such as cell lines and
antibodies.” In practice, the Nature journals are currently requiring all authors to state whether or not testing
was done but are only requiring testing in cases where it makes particular sense.
Articles supporting cell line authentication

Recommendation of short tandem repeat profiling for authenticating human cell lines, stem cells, and tissues


Match criteria for human cell line authentication. Draw the line?

Amanda Capes-Davis, Yvonne A. Reid, Margaret, Hans G. Drexler, Roderick A.F. MacLeod, Greg, Raymond W. Nims, Christine Alston-Roberts, Anton Steuer, John R.W. Masters

Check your cultures! A list of cross-contaminated or misidentified cell lines

Amanda Capes-Davis, George Theodosopoulos, Isobel Atkin, Hans G. Drexler, Arihiro Kohara, Roderick A.F. MacLeod, John R. Masters, Yukio Nakamura, Yvonne A. Reid, Roger R. Reddel, and R. Ian Freshney

Cell line misidentification: the beginning of the end

American Type Culture Collection Standards Development Organization
Workgroup ASN-0002

Eradication of Cross-Contaminated Cell Lines: A Call for Action
by
Roland M. Nardone, PhD
How does false cell line identity occur?

- Cross-contamination during original cell line development
- Cross-contamination in the lab from which you received the cell line
- Cross-contamination in your lab
- Mislabling
Watch out for the **morphing cell line name**:

- LLC-MK2
- LLCMK2
- LLCMK₂

Watch out for **two cell lines with the same name**

- 15C6 (ATCC CRL-2431) mouse myeloma
- 15C6 (ATCC HB-326) rat/mouse fusion

What investigators can do to prevent cell line misidentification

- Become aware of the magnitude of the problem
- Authenticate all cell lines they are working with!
- Eliminate laboratory practices that result in misidentification
- Notify recipients of misidentified cell lines
- Publish retractions/explanations of “false” publications
What organizations can do to prevent cell line misidentification

- Repositories: Discontinue sale of misidentified cell lines
- FDA: Require cell line authentication as part of research and development
- Funding agencies: Require authentication before releasing funds
- Journals:
  - Require authentication before accepting manuscripts for publication
  - Encourage publication of retractions
- Literature databases: Use the word “retraction” in publication titles and as a publication category
- All of the above: Promote education to increase awareness
Instructions to Authors

Journals moving to require cell line authentication as a prerequisite for publication
GUIDELINE


Keywords: cell culture; mycoplasma contamination; Human Tissue Act; cell line; cell line misidentification; cryostorage; Human Tissue Authority; STR profiling; human tissue; Human Fertilisation and Embryology Act

Guidelines for the use of cell lines in biomedical research

R J Geraghty*,1, A Capes-Davis2, J M Davis3, J Downward4, R I Freshney5, I Knezevic6, R Lovell-Badge7, J R W Masters8, J Meredith9, G N Stacey10, P Thraves11 and M Vias1
Cell Authentication is more than just identity

- It consists of a number of orthogonal endpoints (identity, morphology, phenotype, ploidy, purity)

- Look for increasing demand for authentication of cells used in research and in biological activity assays
New ANSI/ATCC Standards Development Organization Guidelines

- ASN-0002 consensus standard describing the use of **short tandem repeat (STR) analysis** for cell line authentication
  - Also recommend assessing cell behavior such as doubling time and morphology
- Plans to create a database containing STR genotypes of validated cell lines
  - Hosted by the National Center for Biotechnology Information (NCBI)
- Public database available on ATCC website

**ANSI:** American National Standards Institute
**ANSI Authentication Points**

With STR profiling authentication will may include:

(i) verifying the cells are of human origin
(ii) evaluating the consistency of profiles between isolates/pasages
(iii) comparing STR profile to a database
(iv) detecting contaminating human DNA*

*Note that commercial STR kits are designed to detect human DNA, so other species’ contaminating DNA will not be detected.
**STR Analysis Workflow**

**Multiplex PCR through Data Analysis**

**DNA Sample**
- Amplification of select STR loci
- Simultaneous fluorescent labeling

**Multiplex PCR**
- Add Internal Lane Standard
- CE to size separate
- Fluorescent detection
- Also run Allelic Ladder in parallel

**Capillary Electrophoresis (CE)**

**Data Analysis**
- Calculate sizes based on ILS
- Compare fragment sizes to allelic ladder to determine STR alleles present in sample
- Compare to databases

**Internal Lane Standard**
- Labeled size standards in a different color than STR fragments

**Allelic Ladder**
- Labeled fragments of all possible alleles for each STR locus
The **LOCUS** identifies the specific physical location of a gene or STR on a chromosome. Both chromosomes of a homologous pair contain this locus.

The **ALLELE** for the gene or STR contained at that locus may be the same on both chromosomes (**homozygous**), or different on each chromosome (**heterozygous**).
**STR (Short Tandem Repeat) Genotyping**

**STR = Short Tandem Repeats:** Multiple copies of a short (2-6bp), identical DNA sequence arranged in direct succession within a chromosome.

**STR Examples**

- **Dinucleotide:** ---AGAGAGAGAG---
- **Trinucleotide:** ---CTTCTTCTTCTTCTT---
- **Tetranucleotide:** ---AAAGAAAAAAG---

**STR Variation in an Individual**

[Diagram showing STR Locus and alleles for mom and dad with allele 4 and allele 3 variations]
Amplifying STRs

DNA is extracted from source material

Extracted DNA is template for PCR

Size of product based on repeat region length (varies with number of repeats) plus length from repeat to primer
Multiplexing: Both Size & Color are Used

Size of the PCR product can be changed by moving primers closer or further from repeat region

**SIZE**

![Diagram showing size change by moving primers]

**COLOR**

![Diagram showing color change by using different dyes]

Different primers can be labeled with different fluorescent dyes
Multiplex STR Analysis Combines Size & Color Differences to Analyze Multiple Loci

Locus 1
// ← CTTCTTCTTCTT ← //
// ← CTTCTTCTT ← //
Locus 2
// ← CTTCTTCTTCTT ← //
// ← CTTCTTCTT ← //
Locus 3
// ← AGAG ← //
// ← AGAGAGAG ← //

Multiplex PCR amplification/labeling

Locus 1 PCR Products

Locus 2 PCR Products

Locus 3 PCR Products

= Different chromosome constant regions
= Fluorescently labeled forward PCR primers
= Unlabeled reverse PCR primers
Separating and Detecting Amplified STRs

Capillary Electrophoresis
Separates amplification products based on size using Applied Biosystems’ Genetic Analyzers
K562 Profile from GenePrint 10
### Table of Genotypes

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Marker</th>
<th>Dye</th>
<th>Allele 1</th>
<th>Allele 2</th>
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<td>TH01</td>
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<td>9</td>
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## Sample Comparison

<table>
<thead>
<tr>
<th>Loci</th>
<th>Query Profile</th>
<th>ATCC Profile or Reference Profile</th>
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<tr>
<td><strong>Test Results for Submitted Sample</strong></td>
<td><strong>Database (or Reference) Profile: A549 (CCL-185)</strong></td>
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<tr>
<td>D3S1358</td>
<td>16</td>
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<td>TH01</td>
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<tr>
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<td>7</td>
<td>11</td>
</tr>
<tr>
<td>D5S818</td>
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<tr>
<td>D2S1338</td>
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</table>

Number shared alleles between reference and test profile: **15**

Total number of alleles in the database/reference profile: **15**

Percent match between the submitted sample and the database/reference profile: **100**
**Match Criteria**

**Authentic cell line**: a match at $\geq80\%$ of alleles across the eight (8) core STR loci

**Match**: when two STR profiles show identical alleles. This is describes as a percentage as shown above. Cell line samples matching at $\geq80\%$ of alleles across the eight (8) core loci are said to be related.

**Unrelated STR profiles**: when STR profiles match at $<55\%$ of alleles. STR profiles with alleles matching at 55-80% may be related and require further investigation
Software Options
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Price 1</th>
<th>Price 2</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Standalone Program and single user license</td>
<td>$4,900.00</td>
<td>$3,995.00</td>
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</tr>
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<td>GMHID002</td>
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<td>GMHID002N</td>
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<td>$4,590.00</td>
<td>$3,675.00</td>
</tr>
</tbody>
</table>
GeneMapper® Software*

GeneMapper® ID-X Software

*There are several versions. Some instrument files are not compatible with some versions. Additional files may be needed to perform full genotyping analysis depending upon the version used.
HEK293 STR Profile Demonstrates Triallelic Pattern Often Found in Immortalized Cell Lines
STR Profile Illustrates HeLa Characteristic D13 Locus 13.3 Allele
Detection of Contaminating HeLa Cells in HEK293 Culture using GenePrint® 10 System
Summary
Cell Line Authentication is Growing in Importance

✓ The number of misidentified(contaminated) cells continues to grow
  • Invalidates published and unpublished data leading to wasted time, effort and money

✓ Granting agencies and journals are strongly recommending cell authentication
  • ANS-0002 standards for cell authentication published by ANSI/ATCC
  • Check journal Instructions to Authors for requirements/recommendations

✓ STR profiling is the gold standard for cell authentication
  • Genotyping method that assays across highly variable short tandem repeat loci to create a DNA fingerprint of a sample
  • Also important to observe phenotype of cells to detect possible changes

✓ GenePrint® 10 System is optimized for human cell authentication
  • Covers all loci recommended in ANS-0002 standards and available in public databases
  • Covers two additional loci for greater discriminatory power
  • Easy-to-use, complete kit compatible with multiple PCR & CE instruments
Cell Line Authentication Resources

General Resources

Cell Line Authentication Resources
Short Tandem Repeat Analysis in the Research Laboratory
Doing Good Science: Authenticating Cell Line Identity
Frequently Asked Questions About STR Uses

These links will redirect to the ATCC website:

Maintaining High Standards in Cell Culture
Misidentified cell lines
Animal Cell Culture guide
Cell Line Authentication Test Recommendations
Passage Number Effects in Cell Lines

ATCC STR Profile Database

Compare STR profiles to the ATCC database to confirm cell line identity
Ugh – Any Questions?

Everything was going along fine until they discovered their HeLa cell line expressed Y chromosome markers.
PURPOSE
The contamination or misidentification of cell cultures can seriously compromise research performed with such cell lines. The purpose of this policy is to ensure that information generated using cultured cells at The University of MD Anderson Cancer Center (MD Anderson) is obtained from samples that have been authenticated by DNA validation techniques, to rule out misidentification and inter- or intra-species contamination. This will prevent the necessity for retraction of papers in which cell lines are found not to be of the reported lineage. It has been indicated by NIH that grant applications that fail to use acceptable experimental practices “would not fare well in the review process.”

POLICY STATEMENT
Validation by DNA analysis will be performed on cell lines used to produce scientific information at MD Anderson. This validation process will enhance the quality of research at MD Anderson and maintain the scientific integrity and reputation of the institution.

SCOPE
This policy applies to all workforce members utilizing cell lines for research.