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Journal of Stem Cell Research & Therapeutics

Mini Review

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Cell line cross-contamination and accidental co-culture

Abstract

The cell line cross contamination and co-culture is a major issue in animal cell culture that invalidates the research results, compromises the comparison of results in different laboratories and diminishes the use of animal cell culture for medical purpose and as a viable alternative and an effective tool in understanding the fundamental cell processes. It reduces the quality of the research and may lead to unusable therapeutic products. In stem cell therapy, the engraftment of undifferentiated or incorrectly differentiated cells has been reported to cause substantial tumorigenic or immunogenic risks to the recipient. However, the problem of the undesired or accidental co-culture can be resolved by increasing awareness and following standard procedures, including inspecting regularly the quality of cell lines used in cell culture laboratories. This review provides an insight into accidental co-culture as a result of cross contamination with a brief account of common cross contaminating cell lines and appropriate measures to diminish the chances of cross contamination and accidental co-culture.

Keywords: cell line, cross-contamination, authentication, methods, stem cell, database, cell culture

Volume 1 Issue 5 - 2016

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Received: August 29, 2016 | **Published:** September 28, 2016

Introduction

Contamination of a cell line with another cell in a culture medium, besides infection with microorganisms such as molds, yeast, viruses, protozoa, mycoplasma and bacteria, is a major issue in cell culture experiments. Undesired co-culture or growth of more than one distinct cell types together in a culture medium can be problematic, seriously compromising the quality of results and diminishing the use of cell line as a model system. It may lead to unusable therapeutic product in biotech industry, where a cell line or a manufactured cell product must be fully free from all sorts of contaminations, including the presence of another cell line or its product. Culturing cells together in a combined medium may affect the genotypic and phenotypic stability of the desired cell.[1] The genetic instability due to accidental co-culture is a serious issue and this inherent instability in cells like embryonic stem cells and pluripotent stem cells makes it imperative to perform detailed genetic analysis of cells in culture.[2] Acceptable degrees of genetic change must be established through chromosomal aberration and karyotyping, cell surface markers and expression of transcription factors, as well as proliferation capacity and differentiation propensity of the cell.[3] The accidental co-culture is a particularly serious problem in stem cell therapy, where the engraftment of undifferentiated or imperfectly differentiated cells may cause a substantial tumorigenic or immunogenic risk to the recipient.[4,5] This mini review provides a quick insight into the problem of common cross contaminations with undesirable cell line and their co-culture and describes appropriate measures to diminish the chances of contamination of the desired cell line with another cell line and accidental co-culture.

Discussion

On every occasion, a cell that is maintained in a laboratory faces the risk of contamination with another cell, particularly a rapidly growing and continuous cell line. The first case of cross-contamination in cell culture was reported in 1950s and since then cross contamination (and accidental co-culture) remains a disturbing issue in cell culture laboratories, as contamination of a cell line with another cell line is not readily detectable like the bacterial and fungal contamination.

Among the various cell contaminants, HeLa, the oldest and most commonly used human cell line that was derived from the cervical cancer cells from Henrietta Lacks, taken by George Gey, is perhaps the commonest contaminating cell in cell culture laboratories. The International Cell Line Authentication Committee (ICLAC) database of 475 Cross-Contaminated or Misidentified Cell Lines - originally developed by Amanda Capes-Davis and Ian Freshney (published in 2010) and curated by ICLAC - lists 138 different cell contaminants, with HeLa being the most common, with 113 entries (24%) (http://iclac.org/databases/cross-contaminations/). In an early 1976 study on 246 cell lines over 18 months at The Child Research Center of Michigan, 25% human cell lines were HeLa cells; out of this (246), 30% were reported to be incorrectly designated and 14% were wrong species.[6]

HeLa has been recognized to be not only the commonest contaminant, but to contaminate a number of cell lines. A PubMed database search for the period between 1969 and 2004 revealed over 60 cell lines that were actually contaminated with HeLa. These HeLa-contaminated cell lines included HEp-2 (laryngeal cancer), KB (oral cancer) and D98/AG. About16 others were contaminated by non-HeLa human cells, while almost 12 cases were that of interspecies contamination.[7] According to data published by Capes-Davis and coworkers,[8] and used in this review with permission Figure 1, the maximum number of affected cell lines (106) were found to be contaminated by the human cervical adenocarcinoma (HeLa), followed by human bladder carcinoma (T-24) (18) and human colon carcinoma (HT-29) (12). The other contaminating cell lines in this study included human acute lymphoblastic leukemia (CCRF-CEM) and human chronic myeloid leukemia (K-562), nine each, human lymphoma (U-937) and human acute myeloid leukemia (OCI/AML2), eight each, human esophageal carcinoma (Hcu-10) and human melanoma (M14), seven each, and human acute myeloid leukemia (HL-60), human

colon carcinoma (SW-480, SW620) and human prostate carcinoma (PC3), six each. The most frequent contaminants were HeLa, T24 and H29. HeLa has also been reported to be a common contaminating cell in several other studies.[9] According to an estimate, HeLa cell contamination is reported to produce financial loss of about 10 million US$.[10]

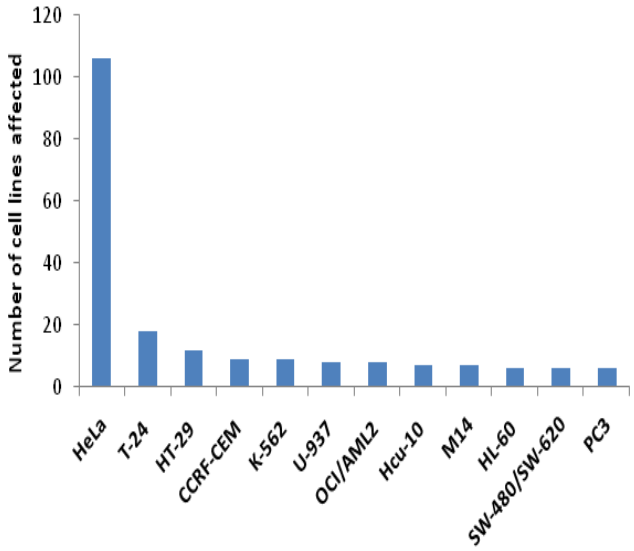

Figure 1 12 most frequently contaminating cell lines (Data reproduced with permission of the copyright holder, Capes-Davis © 2010).

Reasons for cross-contamination

The cross-contamination in a cell culture laboratory generally occurs due to failure in adopting good cell culture practices. It may occur at various levels which can be summarized as follows:

a. Accidental inoculation of a cell line with another cell line - a single drop of another cell suspension or accidental reuse of pipette in a faster growing cell line may soon completely displace the original culture.

b. Mislabeling a flask or if labels are misread.

c. Thawing a wrong frozen stock.

d. Keeping more than one cell lines in a safety cabinet at the same time and using same tools when working with different cell lines.

e. Using one reservoir of medium for more than one cell line.

f. Accidental transfer of cells to stock bottle.

g. Use of cell samples from colleagues and other labs which are not tested thoroughly.

Cell line authentication: Methods for the identification of cross contamination

In co-culture, it may not be easy to readily identify different types of cells that grow together unless a specifically designed medium is used to encourage balanced growth Figure 2. In other instances, for example the cross-contamination of normal human fibroblasts (orbital shaped) with HeLa cells (rounded cell colonies), contaminating cell line is easy to identify Figure 3. An important practical approach to learn if a researcher's own cell line is cross contaminated is to first check previous literature, looking for possible contaminating cells in prior work. In the absence of a prior publication, it is important to check a culture for cross contamination by authenticating testing.[9] The Giesma-banded karyotyping and isoenzyme analysis to detect intra- and inter-species contamination of cell lines are two old classical methods to identify contaminating cells.[11–14] DNA fingerprinting is a more recent technique that provides valuable input on cell line contamination.[15] Cross-contaminated Leukemia cells with three different cell lines (leukemia cell line, acute lymphoblastic leukemia cell line SPI-801, SPI-802, and chronic myeloid leukemia cell line K-562) have been reported to be identified by a multi parameter approach involving cytogenetic examination, DNA fingerprinting and bcr-abl genotyping. The study concluded that the three cell lines were related and had a common origin.[14] A list of some authenticated cell line suppliers/authenticating test centers, both stem and non stem cells, and common methods used for authentication is provided Table 1. With regard to stem cell, the NIH Human Embryonic Stem Cell Registry (http://stemcells.nih.gov/Pages/Default.aspx or http://grants.nih.gov/stem_cells/registry/current.htm), beside others, is one of the authenticated sources of stem cells. NIH (http://stemcells.nih.gov) has also formulated guidelines that govern the conduct of stem cell research, particularly the NIH-funded stem cell research.

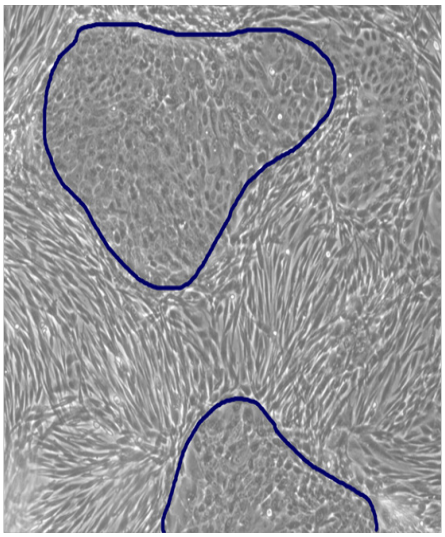

Figure 2 Co-culture of primary human keratinocytes with fibroblasts in CnT-Prime Co-Culture medium specifically designed to encourage balanced growth of keratinocytes and fibroblasts. At confluency, the cultures display the characteristic keratinocyte progenitor cell colonies (small and tight cobblestone morphology) surrounded by fibroblasts (Reproduced with permission from CELLnTEC, http://cellntec.com/products/cnt-pr-cc/#features).

Precautions to check cross-contamination and accidental co-culture

Utmost care is required while handling cell lines, especially when managing several cell lines together. The precautionary measures include handling one cell line at a time, proper labeling of each culture flask with name of the cell line, passage number and transfer date. The media for each cell line should be kept in separate tubes. Serological pipettes must be discarded after each operation. Used media and fluids must be discarded in separate containers. The hands and workbench must be cleaned with 70% alcohol before and after each operation, particularly while starting with the second cell line. Bottles, aliquots of the medium and other reagents should be dedicated for single cell

Citation: Routray I, Mahmood A, Ngwa NE, et al. Cell line cross-contamination and accidental co-culture. *J Stem Cell Res Ther.* 2016;1(5):179–185.
DOI: 10.15406/jsrt.2016.01.00031

line. These regents and aliquots of the medium should be labelled properly and never be shared between cell lines. The used waste pots should be replaced with disinfected ones. The cell line should be purchased from reputed cell bank and a periodic check of its morphology and growth characteristic should be conducted in phase contrast microscope. Good aseptic technique and lab practices should be practiced and techniques such as the short tandem repeat profiling, DNA fingerprinting, karyotype analysis and isoenzyme analysis through electrophoresis should be employed to periodically check cross contamination and reduce the risk of accidental co-culture. The cross contamination, if detected, should be reported to cell repository data bank to avoid use of suspected cell lines.[15]

Table 1 List of some cell line suppliers/authentication test centres, both stem cell and non stem cell, and common methods used for authentication

Cell line supplier / authentication test centre	Web link	Method used for authentication
American Type Culture Collection (ATCC), USA	www.atcc.org	STR profiling
Applied biological materials (Abm), Canada	https://www.abmgood.com/Custom-Cell-Authentication-Service.html	STR Profiling
Analytical Biological Services Inc, USA	http://www.absbio.com/products/cellQC/celllineauthen.html	STR profiling
AllCells, California	www.allcells.com	-
Asterand, Bioscience, USA	www.asterand.com	-
Amsbio, Cambridge	www.amsbio.com/	-
BioReliance, USA	http://www.bioreliance.com/in/services/biopharmaceutical-services/cell-line-characterization/cell-line-authentication	CO1 barcode assay, DNA fingerprinting, DNA sequencing, Karyotyping, Random amplification of polymorphic DNA
Bio-Engineering (I), India	http://www.tradekeyindia.com/bio-engineering-india/cell-line-identification-service.htm	STR profiling
Biocompare, USA	www.biocompare.com	-
Biosynthesis, Texas	http://www.biosyn.com/cell-line-services.aspx	DNA extraction, Unknown sample and known reference standard profiling using STR, Comparison analysis to detect contamination or mutation occurred relatively to the known sample, Bioinformatics data analyses, Electropherogram, Species-specific authentication
B-Bridge International, USA	lifesciences.b-bridge.com/	-
Cell Line Genetics®, Wisconsin	https://www.clgenetics.com/our-services/short-tandem-repeat-analysis/	STR profiling
Cell Culture and Cytogenetics Facility, University of Pittsburgh	https://www.scienceexchange.com/labs/cell-culture-and-cytogenetics-facility-pitt	DNA Fingerprinting, STR Loci identification by AmpFlSTR® Identifiler®
Children's Medical Research Institute, Australia	www.cellbankaustralia.com/	Human cell line authenticating testing through STR profiling, Non-human cell line authenticating testing through amplification and sequencing of CO1
Cell Applications, California	www.cellapplications.com	-
Cell Engineering Division, Japan	http://cell.brc.riken.jp/en/	-
DDC Medical, Ohio	http://ddcmedical.com/	Human, mouse and canine authentication through STR DNA profiling, Human and mouse pathogen testing, Mycoplasma PCR detection assay

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Table Continued..

Cell line supplier / authentication test centre	**Web link**	**Method used for authentication**
Dana-Farber Cancer Institute, Massachusetts	http://moleculardiagnosticscore.dana-farber.org/human-cell-line-identity-verification.html	Human cell line cross contamination identified using DNA fingerprinting with STR
Duke Clinical & Translational Science Institute, USA	https://www.ctsi.duke.edu/news/free-human-cell-line-authentication-assays-short-time	STR profiling to detect misidentified, cross-contamination or genetically drift cell
DiagCor, Hongkong	http://www.diagcor.com/en/mdx-consulting-services	STR profiling
DSMZ, Germany	https://www.dsmz.de/home.html	STR profiling
DNA Analysis Facility on Science Hill (Yale University), USA	http://dna-analysis.yale.edu/human-cell-line-authentication-service	STR profiling
DNA Sequencing Facility, University of California, USA	https://mcb.berkeley.edu/barker/dnaseq/node/24	Human cell line authentication through STR DNA profiling
Dkfz. German Cancer Research Center, Germany	https://www.dkfz.de/gpcf/cell_line_auth0.html	STR profiling
EMD Millipore, USA	www.emdmillipore.com	-
Eurofins Genomics, Germany	https://www.eurofinsgenomics.eu/en/genotyping-gene-expression/applied-genomics-services/cell-line-authentication.aspx	STR profiling
European Collection of Authenticated Cell Cultures (ECACC), UK	www.hpacultures.org.uk/collections/	-
ESI Bio Stem Cell Solution	www.esibio.com/	-
Fluidigm, France	https://www.fluidigm.com/	SNP Type Assays
Gentica Cell Line Testing - a LabCorp brand, USA	http://www.celllineauthentication.com/services.html	STR profiling analysis, Mycoplasma contamination detection
Genetic Resources Core Facility (grcf) - Johns Hopkins School of Medicine, Institute of Genetic Medicine, USA	http://grcf.med.jhu.edu/	STR profiling, Mycoplasma detection
GenoSeq UCLA Genotyping & Sequencing, California	http://www.genoseq.ucla.edu/action/view/Other_Services	GenePrint® 10 system-Promega (STR profiling analysis)
Garvan Institute of Medical Science, Australia	http://www.garvan.org.au/research/capabilities/molecular-genetics/cell-line-identification	STR profiling, includes comparing results to cellbank and ATCC, Authentication reliability, Matching percentage, Detecting genetic drift, Allelic dropouts, Off-ladder alleles, Checking cross-contamination, Mycoplasma testing
Global Biological Standards InstituteTM (GBSITM), USA	https://www.gbsi.org/about/	STR profiling
Human Embryonic Stem Cell Registry, USA	http://stemcells.nih.gov/	-

Table Continued..

Cell line supplier / authentication test centre	**Web link**	**Method used for authentication**
IDEXX BioResearch, USA	http://www.idexxbioresearch.com	STR profiling, inter- and intra-species contamination check, Comparative analysis of published profile, Genetic profiling using microsatellite markers, Mycoplasma testing
IdentiCell, Denmark	http://www.identicell.eu/	STR profiling
Institute for Regenerative Medicine at Scott & White Texas A&M University College of Medicine, USA	https://medicine.tamhsc.edu/irm/msc-distribution.html	-
InvivoGen, California	www.invivogen.com	-
Japanese Collection of Research Bioresources (JCRB) Cell Bank	http://cellbank.nibiohn.go.jp/english/cellinfo_e/	-
Korean Cell Line Bank, Korea	cellbank.snu.ac.kr	STR profiling
Laragen Sequencing & Genotyping, California	http://www.laragen.com/laragen_cellline.html	STR profiling
Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany	www.dsmz.de/	-
Lonza, India	www.lonza.com	-
Microsynth The Swiss DNA Company, Switzerland	http://www.microsynth.ch/index.php?TPL=10581	STR profiling, Mycoplasma contamination testing of cell culture supernatant
MHTP Medical Genomics Facility, Australia	http://www.mhtpmedicalgenomics.org.au/index.php/services/cell-line-service	STR profiling
Multiplexion, Germany	http://www.multiplexion.de/en/cell-line-testing-service/multiplex-human-cell-line-authentication	Identification of human cell lines by SNP profiling, STR profiling
Miltenyi Biotec, California	www.miltenyibiotec.com	-
Mirus, USA	www.mirusbio.com	-
MTI-GlobalStem, USA	www.mti-globalstem.com	STR profiling
NIH AIDS Reagent Program, USA	https://www.aidsreagent.org/index.cfm	-
Northgene, UK	http://www.northgene.co.uk/	DNA STR analysis
Provitro, Germany	http://www.provitro.com/	-
Public Health England (PHE), UK	https://www.phe-culturecollections.org.uk/	STR profiling
Promocell, India	www.promocell.com	-

Table Continued...

Cell line supplier / authentication test centre	Web link	Method used for authentication
Promega, Sweden	http://www.promega.in/	STR profiling
RIKEN Bioresource Center Cell Bank, Japan	www.brc.riken.go.jp/lab/cell/english/	STR profiling
Reachbio Research Lab, USA	reachbio.com/	-
ScienCell Research Laboratories, California	www.sciencellonline.com	-
Sigma-Aldrich, USA	www.sigmaaldrich.com	-
Stemgent, Massachusetts	www.stemgent.com	-
The Translational Research Initiatives in Pathology (TRIP) Lab, Wisconsin	https://www.pathology.wisc.edu/research/trip	STR profiling
Thermo Fisher Scientific, USA	www.thermofisher.com	-
The National Centre for Cell Science (NCCS), India	www.nccs.res.in/	-
University of Arizona Genetics Core, Arizona	http://uagc.arl.arizona.edu/services/cell-line-authentication-human	STR profiling
University of Florida Interdisciplinary Center for Biotechnology Research (ICBR), USA	http://www.biotech.ufl.edu/now-offering-human-cell-line-authentication/	STR profiling analysis performed using GenePrint 10 STR system
Uppsala Universitet, Sweden	http://www.igp.uu.se/	STR profiling
WiCell, USA	http://www.wicell.org/home/stem-cell-lines/stem-cell-lines.cmsx	-

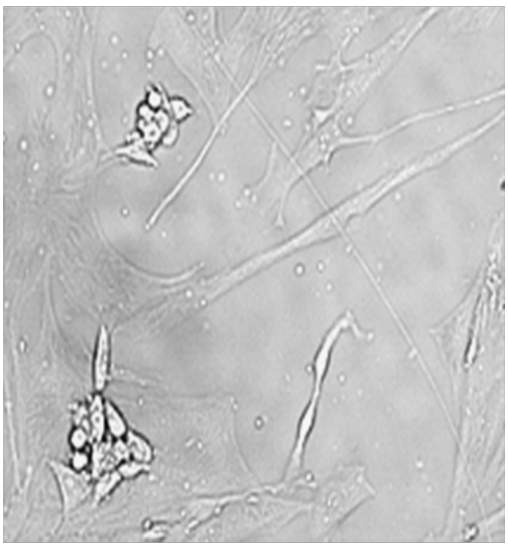

Figure 3 Normal human fibroblasts (orbital shaped) cross-contaminated with HeLa (rounded cell colonies).[9]

Conclusion

Cell line cross-contamination and co-culture is a serious problem in cell culture laboratories as it is difficult to identify when compared with microbial contamination. The contamination of a cell line with another cell line and accidental co-culture invalidates the research results and has serious implications in terms of therapeutic and industrial use of cell lines or their products. In this review, we attempted to provide a quick insight with regard to cross contamination with common contaminating cells, reasons for cross-contamination, most common contaminating cell lines and methods to detect contamination and check accidental co-culture.

Acknowledgements

Authors acknowledge the UGC SAP grant and DBT supported Bioinformatics Infrastructure Facility at JH. NEG acknowledges ICGEB SMART Fellowship.

Conflict of interest

The author declares no conflict of interest.

Citation: Routray I, Mahmood A, Ngwa NE, et al. Cell line cross-contamination and accidental co-culture. *J Stem Cell Res Ther.* 2016;1(5):179–185. DOI: 10.15406/jsrt.2016.01.00031

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