Measurement assurance tools and potential application to alternative test methods

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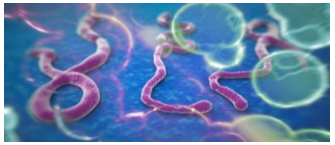
NIST Practices

- **Measurements**
  - Develop new measurement methods
  - Improve accuracy/precision of measurements
- **Reference Materials**
  - Well-defined materials for use as a reference when making measurements
  - Enables inter-lab comparability
  - Physical artifacts for calibrating instruments
- **Standards**
  - Documentary standards, ASTM, ISO
  - Reference data (chemical spectra)
  - Technical Notes: “Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results” (GUM)
- **Biology/biotechnology**
  - **Cell-related measurements and technology (~1990)**
  - **Cytotox measurements, organism measurements (~2005)**

Food-matrix reference materials to facilitate nutritional labeling

NIST Synthetic RNA controls (ERCCs) used in sequencing of Ebola virus genomes to characterize patterns of viral transmission



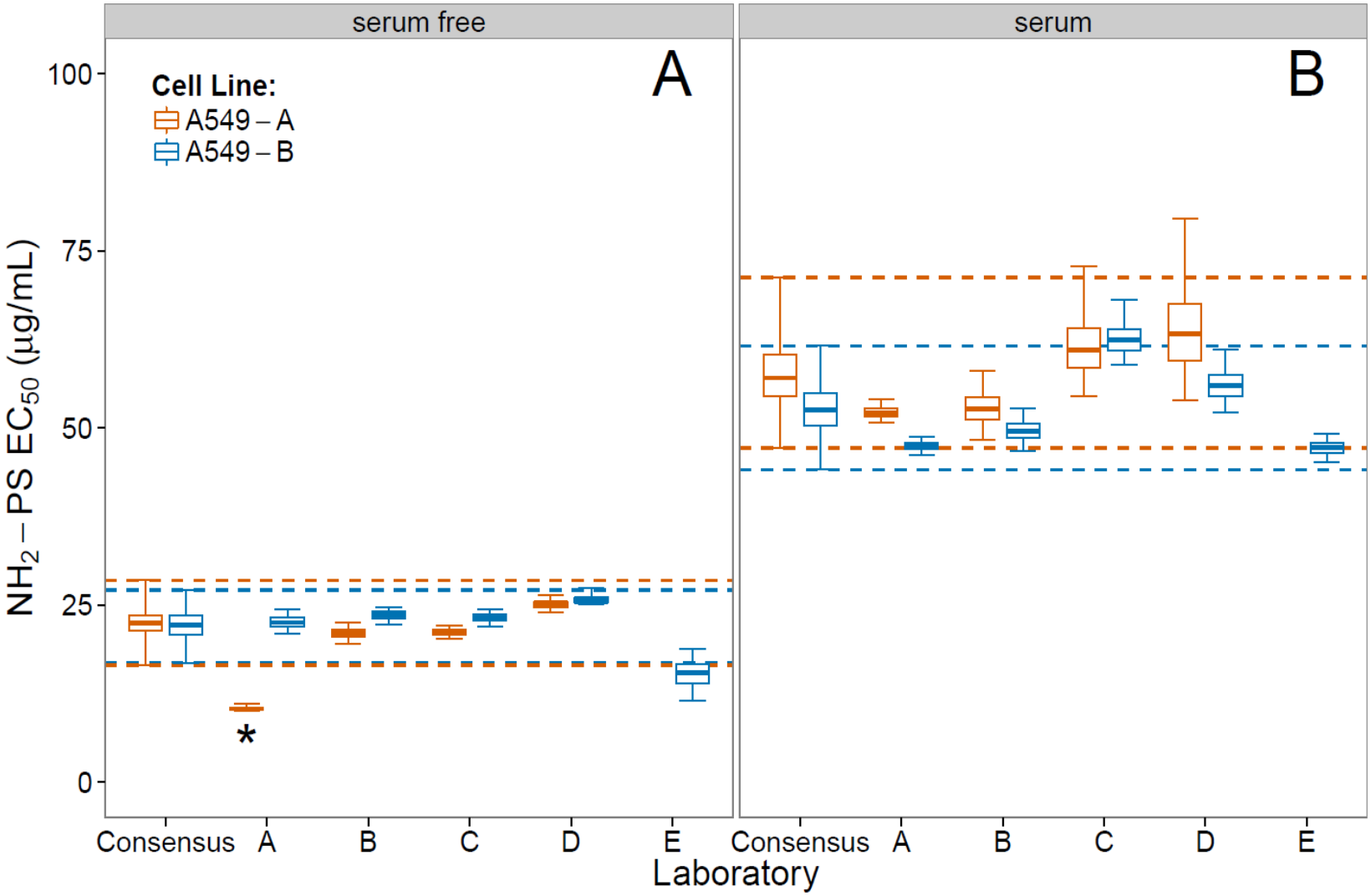
Interlaboratory comparison with MTS assay

- 5 national metrology institutes were involved in the interlaboratory comparison
- Experimental design:
  - Share two A549 cell lines from ATCC and EMPA
  - Serum from local provider
  - Reagents from local provider
  - Serum and serum-free tests
  - Multiple replicates
  - Share nanoparticles (+ve PS) and chemical control ($CdCl_2$)

Elliott et al., Altex, 2017, 34(2), 201-218.

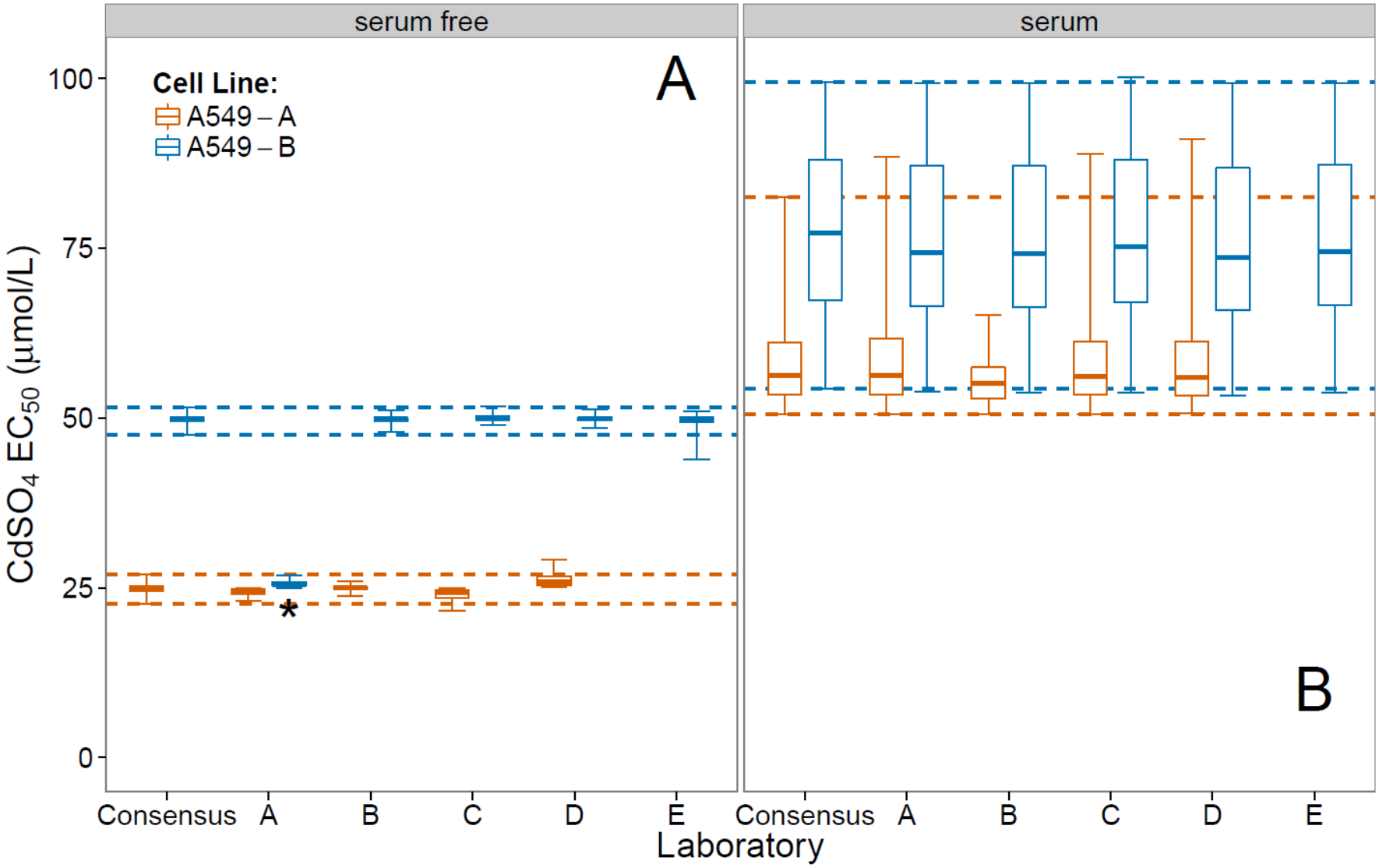
International standard (final draft international standard): ISO/DIS 19007: Nanotechnologies – In vitro MTS assay for measuring the cytotoxic effect of nanoparticles

NP EC_{50} values



-Looks like harmonization between the laboratories
-No cell line differences
-The serum conditions increases variability

$CdSO_4$ EC_{50} values



Serum free conditions, variability less than with NP

Differences between cell lines

Misidentified Cell Lines Remain a Problem

Cell Biology International

Explore this journal >

Retraction

Retracted: Knockdown of tumor protein D52-like 2 induces cell growth inhibition and apoptosis in oral squamous cell carcinoma

Yongchun He, Fengshan Chen ✉, Ying Cai, Sihui Chen

First published: 23 February 2016 Full publication history

DOI: 10.1002/cbin.10593 View/save citation

Cited by: 0 articles Citation tools

Am score 11

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Volume 40, Issue 3
March 2016
Page 361

Abstract

The above article, published online on 13 October 2014 in Wiley Online Library (http://onlinelibrary.wiley.com/doi/10.1002/cbin.10388/abstract), has been retracted by agreement between the authors, the journal Editor, Sergio Schenkman, and John Wiley & Sons Ltd. The retraction has been agreed because the authors discovered after publication that one of the cell lines described in the article had been unintentionally misidentified. The experiments described in the article as being conducted on Human Oral Squamous Cell Carcinoma cell line KB were in fact conducted on a Human Oral Epidermal-like Cancer cell line.

The authors and publisher apologise for any inconvenience.

References

He Y, Chen F, Cai Y and Chen S (2015) Knockdown of tumor protein D52-like 2 induces cell growth inhibition and apoptosis in oral squamous cell carcinoma. Cell Biology International 39: 264-271. doi: 10.1002/cbin.10388

A tale of two impostors

Christopher Korch estimated the impact of research on two cell lines, HEp-2 and INT 407. Due to contamination long ago, both are now widely acknowledged to be composed of cancer cells called HeLa.

5789
ARTICLES
in **1182** journals may have used HEp-2 inappropriately, producing an estimated **174,000** citations

1336
ARTICLES
in **271** journals may have used INT 407 inappropriately, producing an estimated **40,000** citations

$713
MILLION
Estimated amount spent on the original articles published on **INT 407** and **HEp-2**

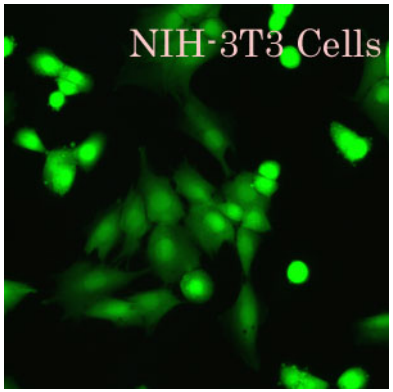
$3.5
BILLION
Estimated amount spent on subsequent work based on those papers

***Science*. 2015 Feb; 347(6225): 938-40.**

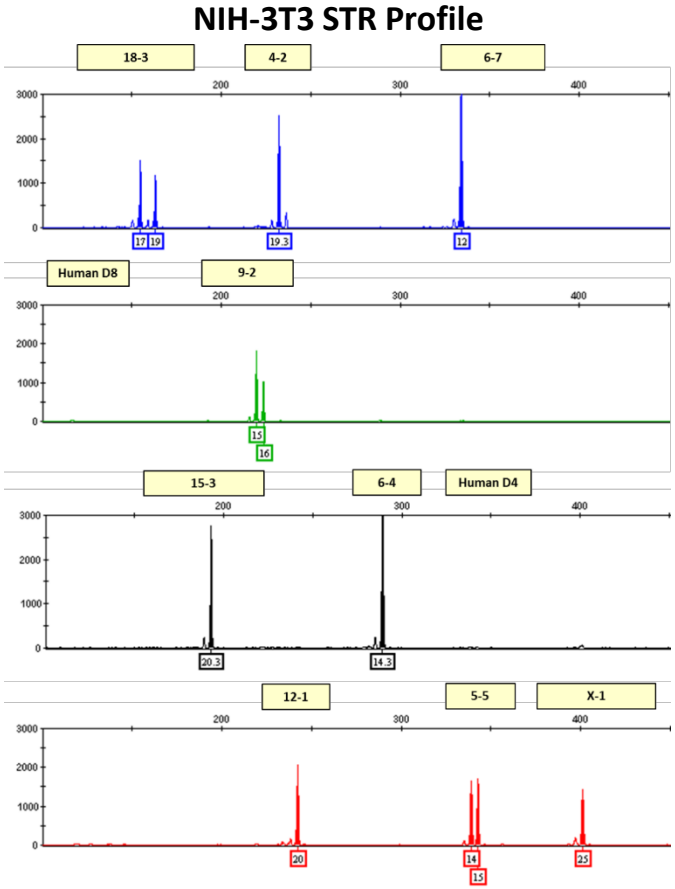
Assays Developed at NIST

STR genotyping

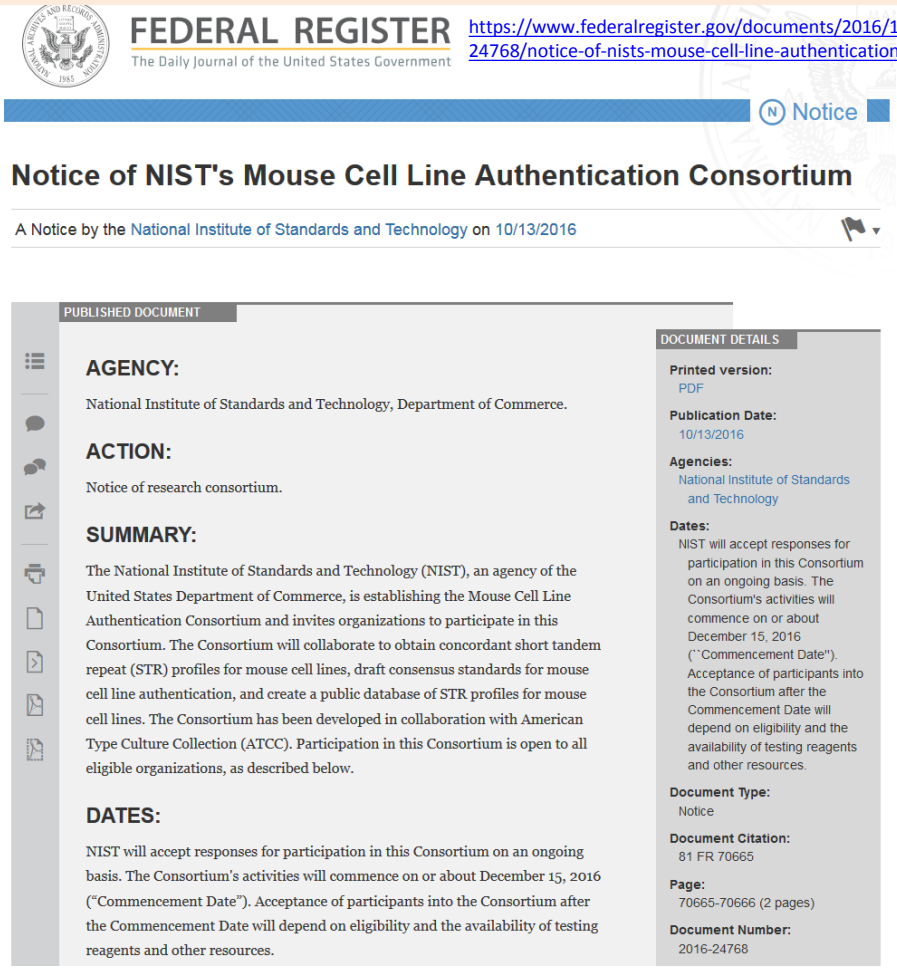
- **African green monkey** (*BMC Biotechnol*. 2011 Nov 7;11:102.)
- **Mouse** – US Patent (*Cytotechnology*. 2014 Jan;66(1):133-47.)
- **Chinese hamster (in progress)**
- **Rat (in progress)**



Multiplex PCR targeting mouse STR markers



Mouse Cell Line Authentication Consortium



FEDERAL REGISTER
The Daily Journal of the United States Government

https://www.federalregister.gov/documents/2016/10/13/2016-24768/notice-of-nists-mouse-cell-line-authentication-consortium

Notice

Notice of NIST's Mouse Cell Line Authentication Consortium

A Notice by the National Institute of Standards and Technology on 10/13/2016

PUBLISHED DOCUMENT

AGENCY:

National Institute of Standards and Technology, Department of Commerce.

ACTION:

Notice of research consortium.

SUMMARY:

The National Institute of Standards and Technology (NIST), an agency of the United States Department of Commerce, is establishing the Mouse Cell Line Authentication Consortium and invites organizations to participate in this Consortium. The Consortium will collaborate to obtain concordant short tandem repeat (STR) profiles for mouse cell lines, draft consensus standards for mouse cell line authentication, and create a public database of STR profiles for mouse cell lines. The Consortium has been developed in collaboration with American Type Culture Collection (ATCC). Participation in this Consortium is open to all eligible organizations, as described below.

DATES:

NIST will accept responses for participation in this Consortium on an ongoing basis. The Consortium's activities will commence on or about December 15, 2016 ("Commencement Date"). Acceptance of participants into the Consortium after the Commencement Date will depend on eligibility and the availability of testing reagents and other resources.

DOCUMENT DETAILS

Printed version: PDF

Publication Date: 10/13/2016

Agencies: National Institute of Standards and Technology

Dates: NIST will accept responses for participation in this Consortium on an ongoing basis. The Consortium's activities will commence on or about December 15, 2016 (``Commencement Date''). Acceptance of participants into the Consortium after the Commencement Date will depend on eligibility and the availability of testing reagents and other resources.

Document Type: Notice

Document Citation: 81 FR 70665

Page: 70665-70666 (2 pages)

Document Number: 2016-24768

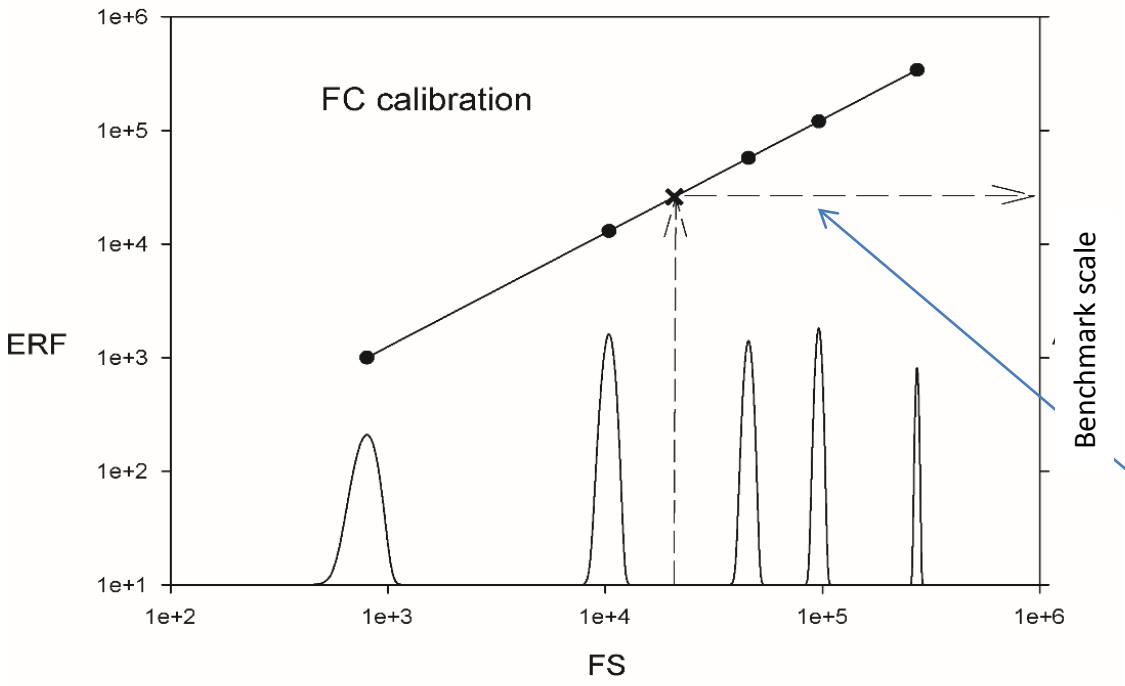
Consortium Members:

National Repositories
Academic Institutions (National and International)
Cancer Centers (National and International)
Commercial Service Providers (National and International)

Consortium Goals:

1. Perform preliminary testing to determine if 19 mouse STR loci are useful
2. Conduct an interlaboratory study to collect STR genotyping data for 50 of the most commonly used mouse cell lines
3. Deposit STR profiles for mouse cell lines into the NCBI BioSample database
4. Publish a written consensus standard for mouse cell line authentication

General Benchmarking of a Fluorescence Scale in Flow Cytometry



Two step process:

1. Establish linear range in fluorescence scale using beads with assigned "equivalent number of reference fluorophore value (ERF)

2. Anchor the fluorescence scale (FS) to a benchmark material with the sample fluorophore

Purpose:

Step 1: Provides evidence of linear range/proportionality on fluorescence scale

Step 2: Links relative intensity scale to a single reference material, provides reasonable instrument independent transferable scale.

Cytometry Part A 73A: 279-288, 2008;
Flow Cytometry Protocols: Third Edition, p53-65, 2011
Current Protocols in Cytometry, 75:1.29.1-14, 2016;
Flow Cytometry Protocols: Fourth Edition (in press)

Challenges in ERF assignments

- Interlaboratory comparisons between bead manufacturer's indicate this measurement is challenging

  Issues in background subtraction, spectral correction of fluorometer, light scattering, bead counting and characterization, low fluorescence signals

Table 7. ERF values assigned to the four surface labeled microsphere reference standards by four manufacturers in addition to NIST

	ERF^{MAJOR}				
MICROSPHERE	NIST	VENDOR A	VENDOR B	VENDOR C	VENDOR D
FITC	7.74×10^4	3.08×10^4	2.19×10^7	1.33×10^7	3.11×10^5
PE	7.94×10^5	5.01×10^4	1.89×10^{10}	1.81×10^7	1.58×10^6
APC	3.21×10^4	6.12×10^3	1.93×10^8	3.62×10^7	not done[a]
PB	1.59×10^6	3.36×10^4	4.12×10^9	8.00×10^6	7.12×10^6

[a] The assignment was not carried out due to the expiration of APC reference solution.

500-fold difference!

- NIST has now developed a ERF assignment service

Flow Cytometry Quantitation Consortium

81 Federal Register 136 (15 July 2016), pp. 46054-46055

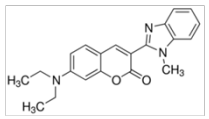
ERF Value Assignment to Cytometer Calibration Microspheres Submitted by Consortium Members

National Institutes of Health · FDA · Spherotech · Beckman Coulter · BD · Bangs Laboratories, Inc. Beads Above the Rest

How to assign ERF values to beads?

NIST SRM 1934

Fluorescein
Nile red
Allophycocyanin (APC)
Coumarin 30

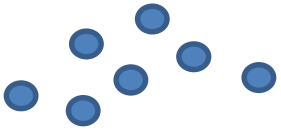


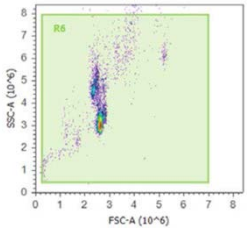
Reference fluorophore

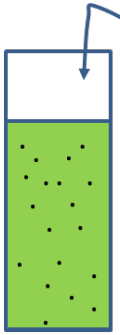
Calibrated fluorometer

Emission of RF

Different manufacturers' relative intensity beads



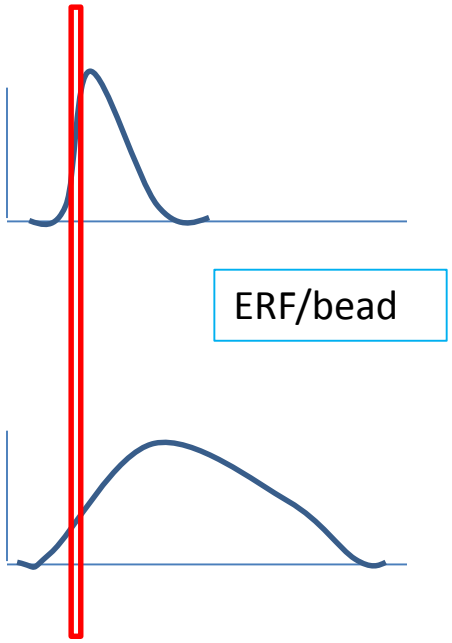




Count beads, stir

Calibrated fluorometer

ERF/bead



Emission of bead

Light obscuration and flow cytometry methods for accurate microsphere concentration, fraction of fluorescence signal in single spheres

ERF= Equivalent number of reference fluorophore

Conclusions

- Measurement assurance tools add robustness to a measurement process
- Interlaboratory comparisons, sensitivity analysis and process controls are valuable
- Robustness of Cell line ID, photoactivity testing and MTS viability assays have been validated with interlaboratory comparison
- Measurement assurance tools for flow cytometry including bead calibration underway

Collaborators

MTS assay interlaboratory comparison

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Cell line ID

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Photoactivity assay interlaboratory comparison

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Flow cytometer bead calibration

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