

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

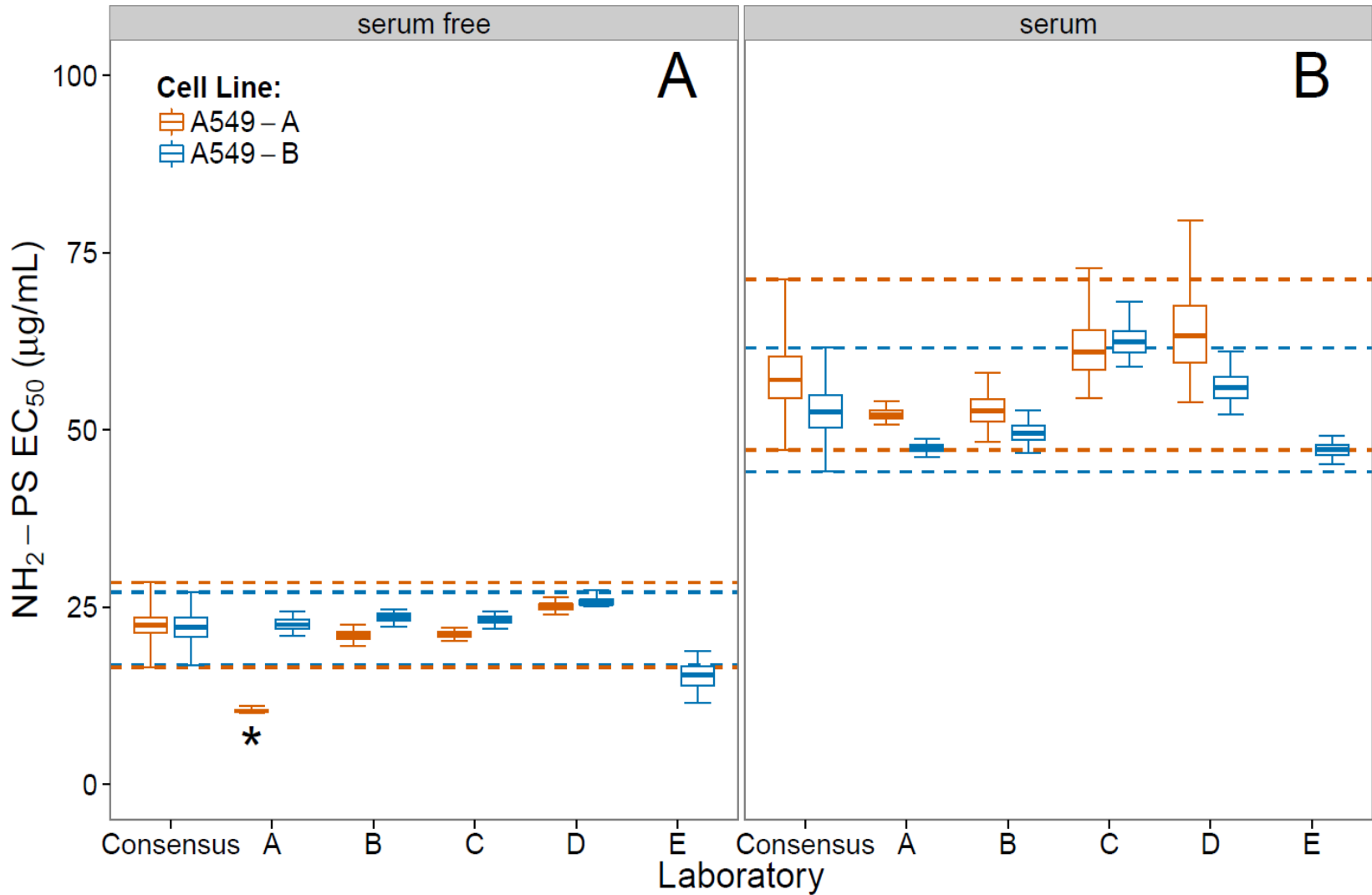
- 5 national metrology institutes were involved in the interlaboratory comparison
- Experimental design:
 - Share two A549 cell lines from ATCC and EMPA
 - Serum from local provider
 - Reagents from local provider
 - Serum and serum-free tests
 - Multiple replicates
 - Share nanoparticles (+ve PS) and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



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

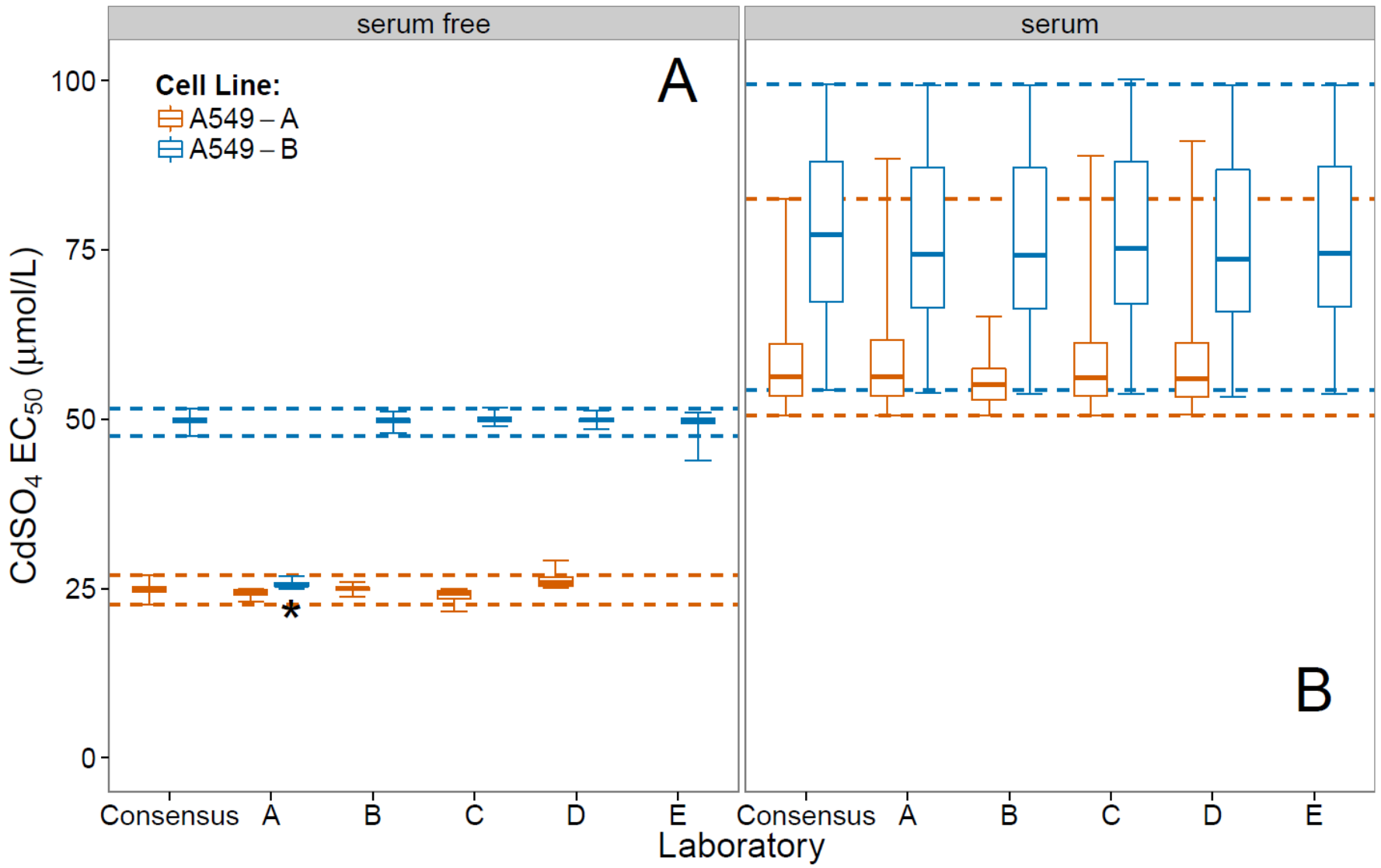
International standard (final draft international standard): ISO/DIS 19007: Nanotechnologies – In vitro MTS assay for measuring the cytotoxic effect of nanoparticles

NP EC₅₀ values



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

CdSO₄ EC₅₀ 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](#)



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



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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

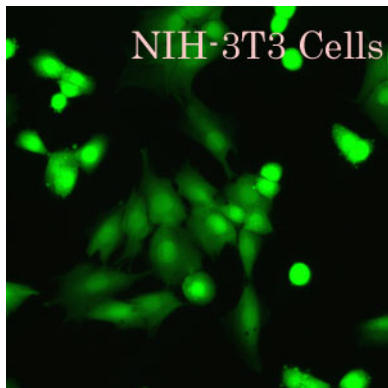
Estimated amount spent on subsequent work based on those papers

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

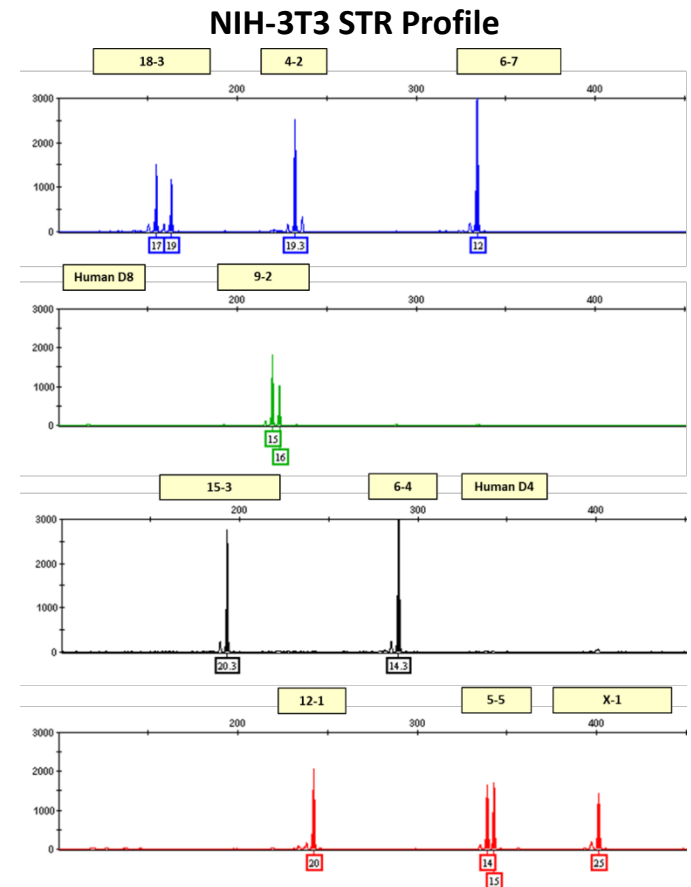
Assays Developed at NIST

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

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81 FR 70665

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70665-70666 (2 pages)

Document Number:
2016-24768

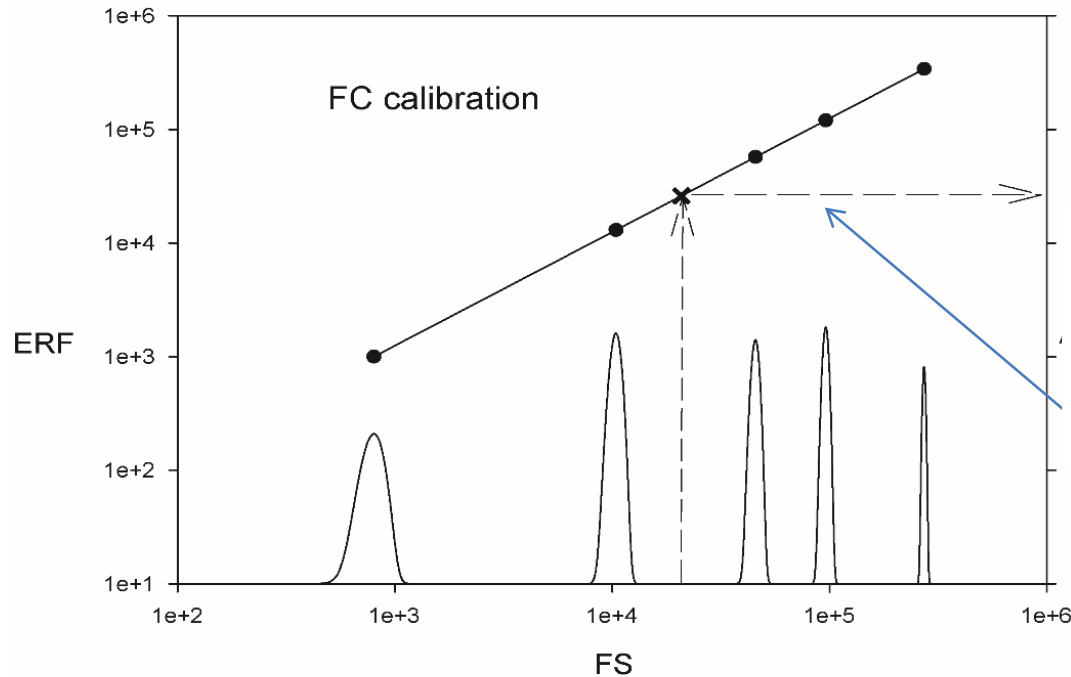
Consortium Members:

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.

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

MICROSPHERE	ERF ^{MAJOR}				
	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

500-fold difference!

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

- NIST has now developed a ERF assignment service

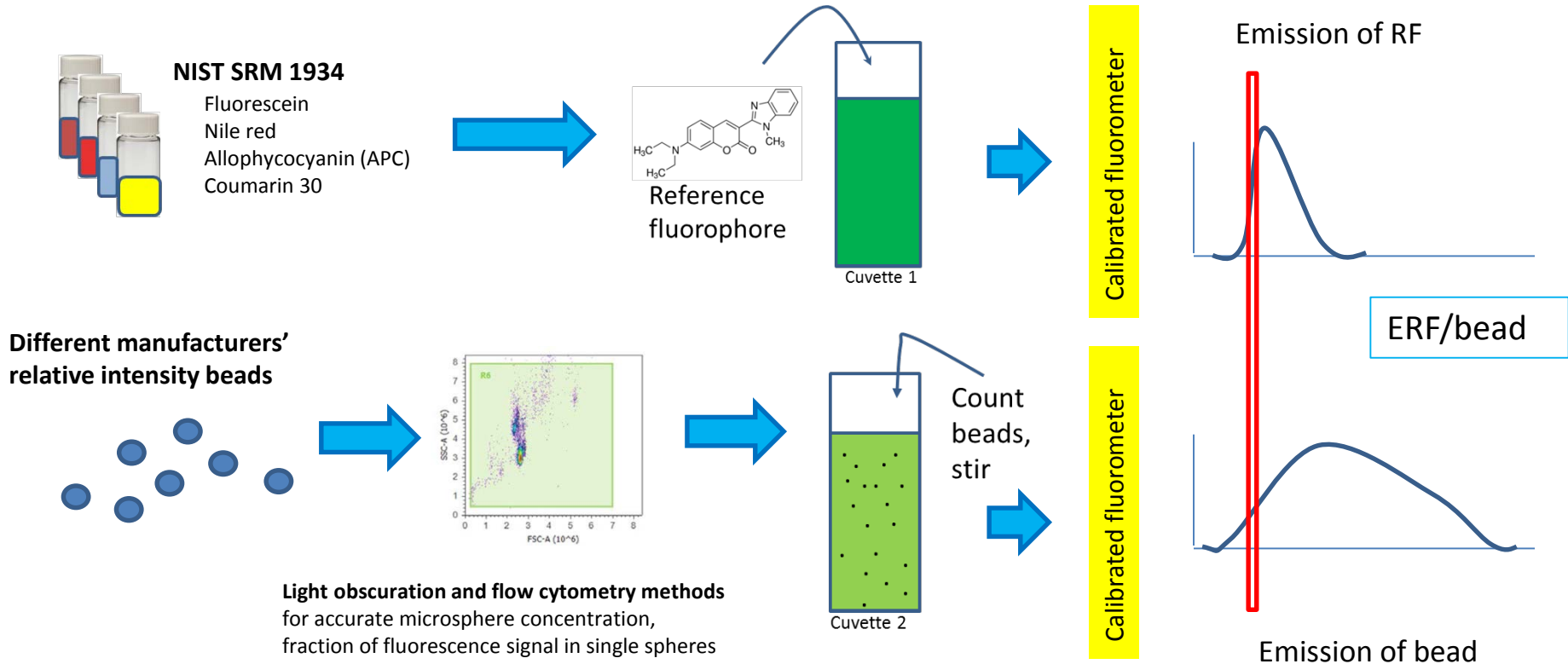
Flow Cytometry Quantitation Consortium

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

ERF Value Assignment to Cytometer Calibration Microspheres Submitted by Consortium Members



How to assign ERF values to beads?



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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Agnieska Kinsner-Ovaskainen

Blaza Toman

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Cell line ID

Jamie Almedia

Ken Cole

Photoactivity assay interlaboratory comparison

Vytas Reipa

Vince Hackley

Blaza Toman

Nam Woong Song

Paul Westerhoff

Haruhisa Kato

Kanokwan Nontapot

Jonghoon Choi

Flow cytometer bead calibration

Lili Wang

Paul DeRose

Adolfas Gaigalas