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Accreditamento delle Biobanche

Webinar di approfondimento

On Line, 18 marzo 2022



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Focus sulla riferibilità metrologica

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On Line, 18 marzo 2022

Sommario

- Obiettivi della Metrologia
- Ruolo degli NMI
- INRIM in breve
- Confronti interlaboratorio
- Materiale di riferimento in biologia
- Esempi di riferibilità metrologica per misurazioni biologiche

→ BBK

Gli obiettivi della Metrologia

Metrology and worldwide uniformity of measurements

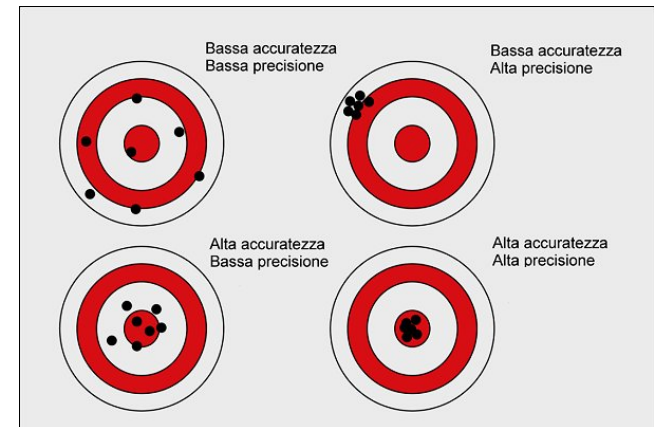
What is metrology?

Measurement is the process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity (JCGM, 2012). Ordinary measurements take place everywhere and every day in our life, be it checking the time, measuring body temperature, or weighing fruit in a supermarket. However, when it comes to manufacturing, trade, safety, regulatory administration, and quantitative research in many disciplines, measurements need to be highly accurate and consistent worldwide.

Metrology is the science of measurement and its application. It includes all theoretical and practical aspects of measurement, whatever the measurement uncertainty and field of application (JCGM, 2012). The objectives of the metrology community are to ensure that:

- Measurements are **accurate**
 - An expressed value can be as close to the true value as possible
- Measurements are **stable**
 - Long-term trends can be used for decision-making
- Measurements are **comparable**
 - Results from different laboratories can be brought together
- Measurements are **coherent**
 - Results from different methods can be brought together.

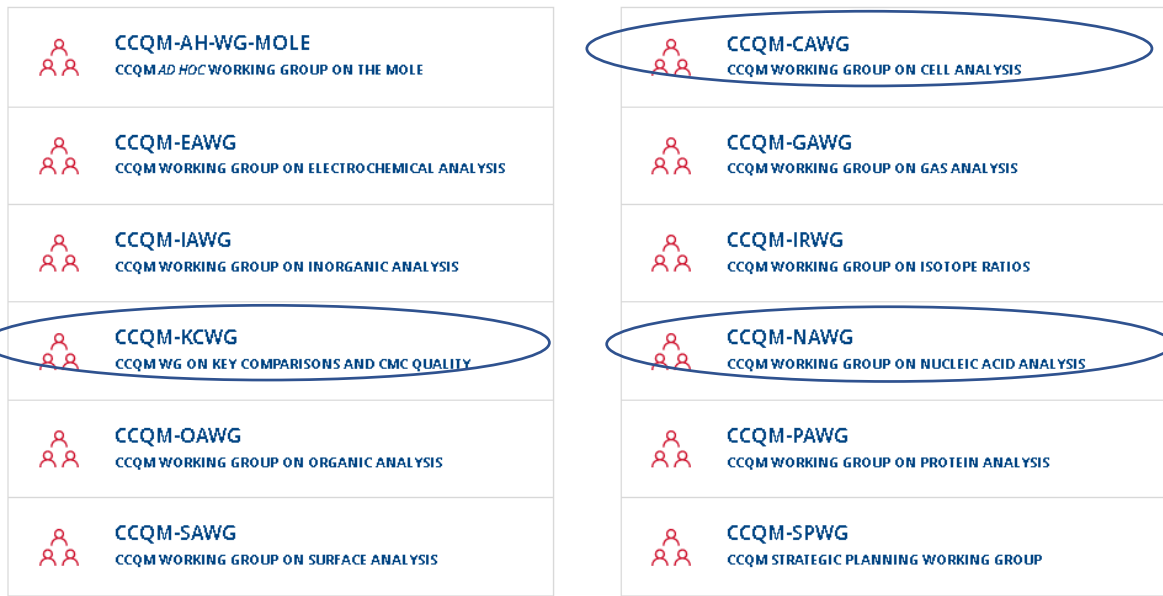
OECD/BIPM (2020), “International Regulatory Co-operation and International Organisations: The Case of the International Bureau of Weights and Measures (BIPM)”, OECD and BIPM.



Piramide della riferibilità metrologica



BIPM

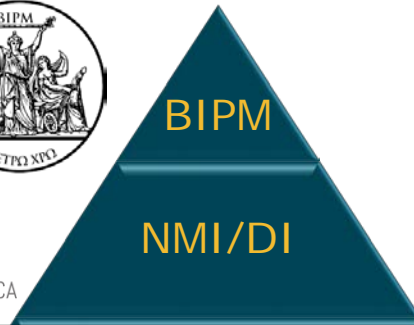


Definizione delle unità SI e loro realizzazioni primarie



Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM)

Piramide della riferibilità metrologica

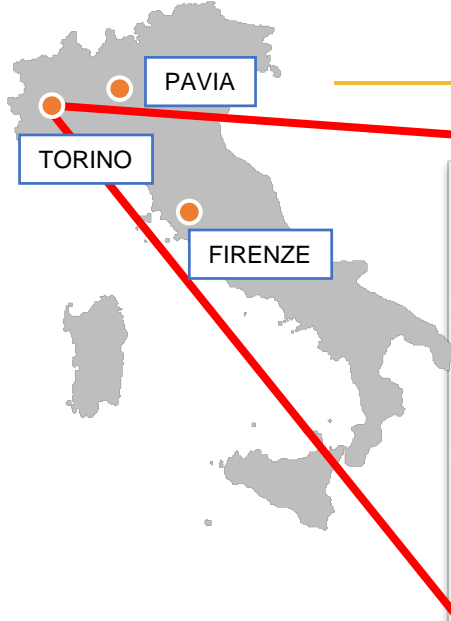


National Metrology Institutes (NMIs)

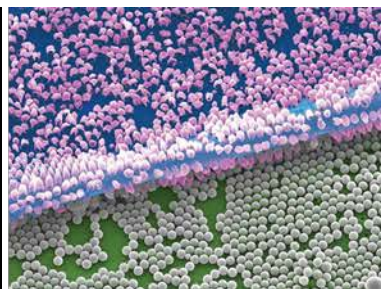
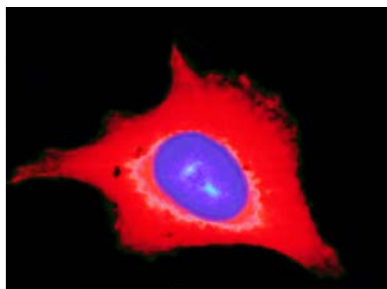
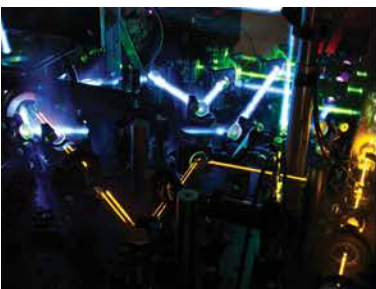
An NMI has the responsibility of developing and maintaining national measurement standards and disseminating the SI units. To aid international recognition of national measurement standards and the associated measurement capabilities, NMIs participate in international comparisons of measurement standards under the CIPM MRA (Mutual Recognition Arrangement) framework (see next chapter). In some

OECD/BIPM (2020), "International Regulatory Co-operation and International Organisations: The Case of the International Bureau of Weights and Measures (BIPM)", OECD and BIPM.

INRiM in breve



- **Istituto Metrologico italiano** nella Convenzione del Metro
- **250 dipendenti**, 30 M€ bilancio annuale
- Campus di 120.000 m²
- **4° Istituto Metrologico Europeo** (dipendenti/bilancio)
- **5° Ente Pubblico di Ricerca in Italia** (vigilato dal MUR)
- Forte legami con l'Università e centri di ricerca



Tre Divisioni Scientifiche

Metrologia Applicata e Ingegneria

Metrologia Quantistica e Nanotecnologia

Metrologia per Materiali Avanzati e Scienze della Vita



Biologia cellulare

Colture cellulari



Spettrofotometria



PCR RealTime PCR



PCR digitale



Conservazione



Microscopia



CARATTERIZZAZIONE METROLOGICA

Working Group del BIPM



CCQM Working Group on **Nucleic Acid Analysis (CCQM-NAWG)**



CCQM Working Group on **Cell Analysis (CCQM-CAWG)**



CCQM WG on **Key Comparisons and CMC Quality (CCQM KCWG)**



EMN FOR TRACEABILITY IN LABORATORY MEDICINE

Table 1. RMOs recognised within the framework of the CIPM MRA

| | |
|------------|--|
| • AFRIMETS | Intra-Africa Metrology System |
| • APMP | Asia Pacific Metrology Programme |
| • COOMET | Euro-Asian Cooperation of National Metrological Institutions |
| • EURAMET | European Association of National Metrology Institutes |
| • GULFMET* | Gulf Association for Metrology |
| • SIM | Inter-American Metrology System |

Note: *At the time of writing, the recognition of GULFMET by the CIPM is "provisional".

Source: BIPM, 2020

BioMetrologia all'INRIM

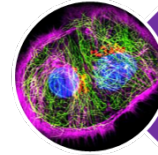
Microscopia ottica a fluorescenza



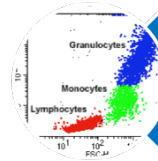
Citofluorimetro



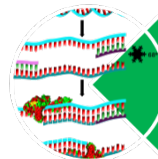
digital PCR



Microscopia



Citofluorimetria



PCR

Laboratorio di BioMetrologia

RealTime PCR



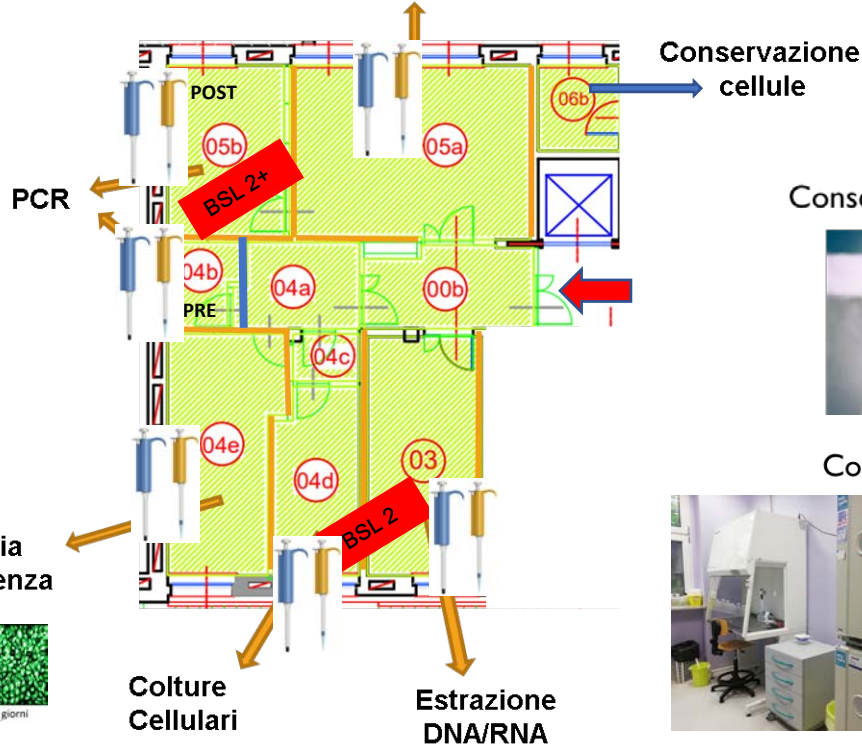
digital PCR



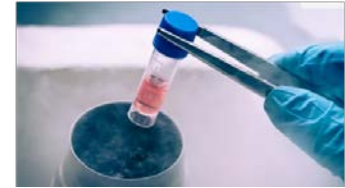
Spettrofotometria



DNA / RNA / test biologici



Conservazione cellule e tessuti



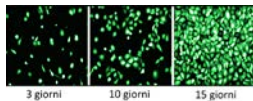
Colture Cellulari Umane



Microscopia ottica a fluorescenza



Microscopia a fluorescenza



3 giorni 10 giorni 15 giorni

Concetti importanti

Misurazione **ACCURATA**

Materiali di Riferimento

Metodi di Riferimento

Incertezza di misura associata al risultato

Sorgenti di Incertezza

Calibration and Measurements Capabilities (CMC)

Valore di Riferimento

Valore di Consenso

Valore assegnati al RM

Risultati

ACCURATI

STABILI

CONFRONTABILI

COERENTI

Confronti Internazionali



CCQM Working Group on Nucleic Acid Analysis (CCQM-NAWG)

CCQM Working Group on Cell Analysis (CCQM-CAWG)

Materiali di Riferimento

stabilità

omogeneità

commutabilità

Valore di Riferimento/Consenso

Valore assegnati al RM

Metodi di Riferimento

Misurazione **ACCURATA**

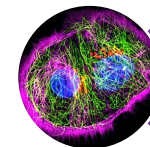
Incertezza di misura associata al risultato

Sorgenti di Incertezza

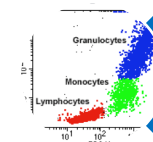
Risultati

ACCURATI
STABILI
CONFRONTABILI
COERENTI

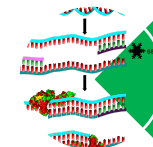
Calibration and Measurements Capabilities (CMC)



Microscopia



Citofluorimetria



PCR

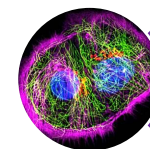
Confronti Internazionali



CCQM Working Group on Nucleic Acid
Analysis (CCQM-NAWG)

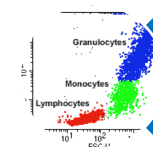
CCQM Working Group on Cell Analysis
(CCQM-CAWG)

- **Cellule A549** in adesione: quantificazione di **densità** e **confluenza cellulare**
- **Cellule 3D** di hMSC adese: **quantificazione differenziale** di **cellule** staminali
- **Cellule PBMC** in sospensione: **quantificazione** del **numero assoluto** di **cellule**



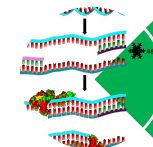
Microscopia

- **Cellule CD4+**: **conteggio cellule** per monitoraggio dell'AIDS
- **Cellule CD34+**: **conteggio cellule** per efficacia del trapianto di staminali



Citofluorimetria

- *KRAS*
 - *BRAF*
 - *EGFR*
 - *HER2*
- Geni – **quantificazione DNA** per diagnosi e monitoraggio di tumori



PCR

- SARS-CoV-2: **RNA** – **quantificazione RNA virale** per diagnosi e monitoraggio di COVID 19

Confronti Internazionali



CCQM Working Group on Nucleic Acid
Analysis (CCQM-NAWG)

CCQM Working Group on Cell Analysis
(CCQM-CAWG)

Indicatori di qualità

Proprietà fisica, chimica, biologica, microbiologica

Conteggio delle cellule

Vitalità cellulare

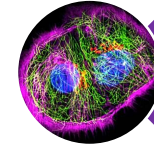
Misurazione della concentrazione

purezza

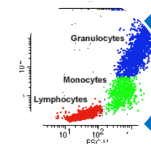
Espressione genica

stabilità

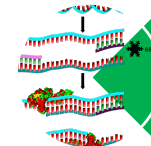
omogeneità



Microscopia



Citofluorimetria



PCR

Densità e confluenza di cellule adese

The measurement claims for the CCQM-P123 study are:

1. number of cells in monolayer adhesion on a defined area¹, in the range 0 to 5000 cell/mm², defined as cell density and calculated as:

$$\text{Cell density} = \frac{\text{number of cells}}{\text{area}} \quad [\text{unit count value/mm}^2]$$

2. confluency fraction of cells in monolayer adhesion on a defined area¹, in the range 0 % to 100 %, defined as cell confluency and calculated as:

$$\text{Cell confluency} = \frac{\text{area occupied by cells}}{\text{area}} \times 100 \quad [\text{mm}^2/\text{mm}^2 \quad \%]$$

3. average cell area of cells in monolayer adhesion on a defined area¹, derived from the previous measurements and calculated as:

$$\text{Average Cell Area} = \frac{\text{area occupied by cells}}{\text{number of cells}} \quad [\mu\text{m}^2]$$

¹ the defined area was intended on a planar (2D) surface. The area of measurement defined for the comparison was $S = (0.2098 \pm 0.0006) \text{ mm}^2$ and its definition was described in the protocol and reported within this document in Appendices 1 and 2.

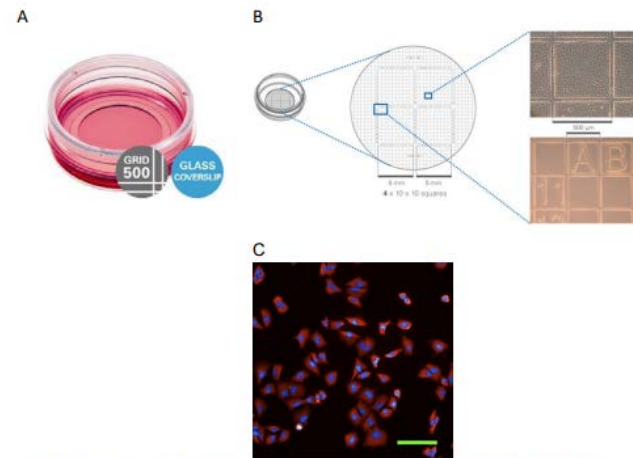


Fig. 1. Imaging dish (A and B) and example of measurement standard (C) of the CCQM-P123 pilot study. Images in A and B is taken from <https://www.ibidi.com/>



Fig. 2. Dual Axis Linear Scale Micrometer (Edmund Optics) namely "ruler".

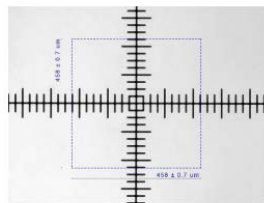


Fig. 4. XY ruler, traceable to the SI. In the image on the left,

Densità e confluenza di cellule adese

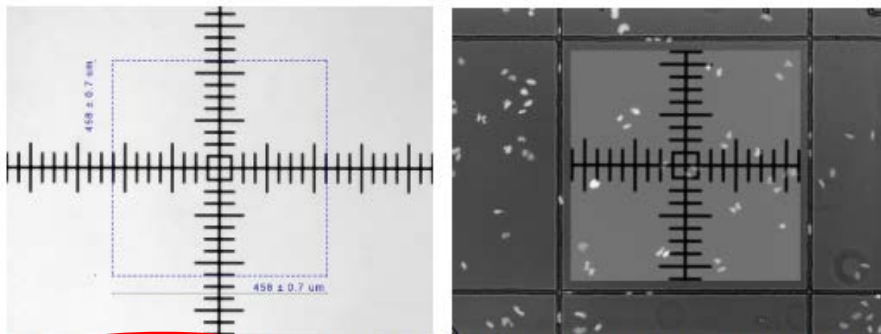
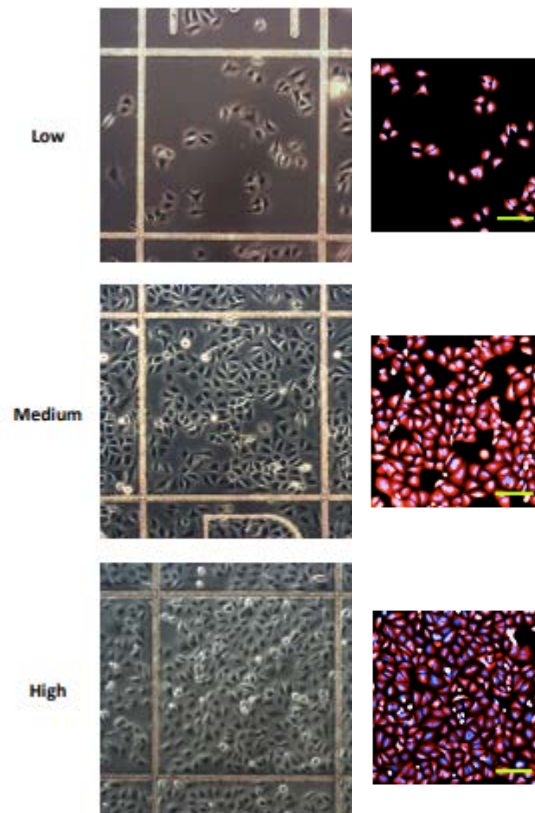


Fig. 4. XY ruler, traceable to the SI. In the image on the left, dashed lines indicate the ruler pitches to be used to draw the area of measurement on the square, as shown in the image on the right.

| Level | Confluency ranges | cells per square | n. of cells per dish (nominal) |
|---------------|-------------------|------------------|--------------------------------|
| LOW (l.c.) | 10% - 30% | 40 - 70 | 1×10^5 |
| MEDIUM (m.c.) | 40% - 60% | 100 - 200 | 2×10^5 |
| HIGH (h.c.) | 70% - 90% | 300 - 550 | 4×10^5 |

Table 4. Nominal values of confluency, cells per square and cells per dish for each P123 reference material



Densità e confluenza di cellule adese

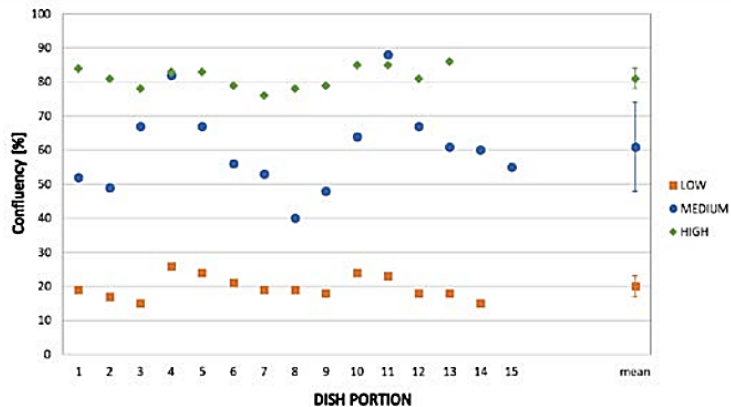


Fig 2. Homogeneity calculated for the three ranges of confluency

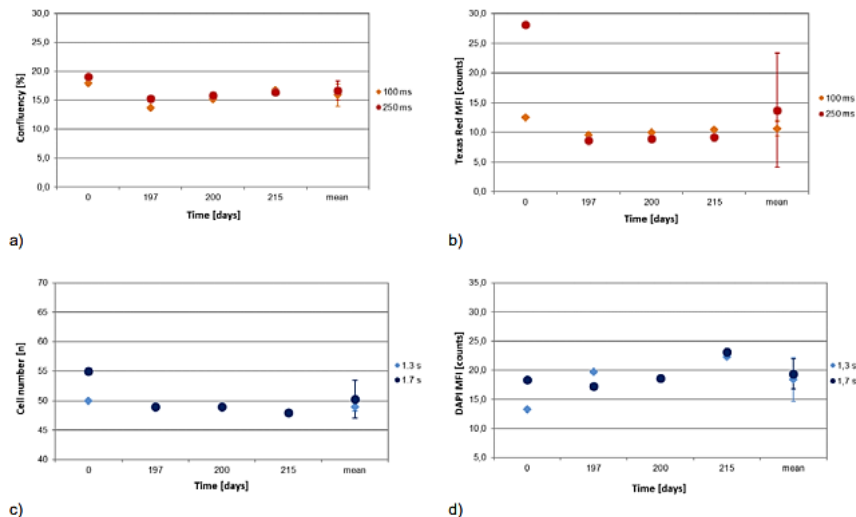


Fig 4. Results of fluorescent dyes brightness decay analysis. Confluency (a), MFI of Texas Red (b), cell number (c) and MFI of DAPI (d) are reported as mean values over time (7 months).

Densità e confluenza di cellule adese



| Component | Source | Description |
|--------------------------------|--|--|
| uncertainty due to the sample | cell proliferation | dividing cells (e.g. two nuclei sharing the cytoplasm) |
| | omogeneity / distribution | cells agglomerates or overlapped cells |
| | edge error | cells on the edges of the area of measurement |
| | dyes stability | decay of fluorescence intensity |
| | sample quality | dust, scratches |
| uncertainty due to acquisition | image assembly | automatic image overlapping |
| | instrument set-up | brightness, contrast, illumination distribution over the image field of view, detection efficiency distribution, focus plane adjustment, image exposure time |
| uncertainty due to analysis | measurement repeatability | standard deviation of the means of repeated measurements |
| | reproducibility regarding operator / automatic machine | mean of the standard deviation of the values of repeated measurements for every operator / automatic machine |
| | image quality | image and background noise |
| | threshold setting | manual setting of threshold for image analysis |
| | algorithms | algorithm selected for image analysis |

Table 3. Uncertainty sources considered by participants.

$$U_{N,k=2} = 2 \sqrt{\left(\frac{u_A}{S}\right)^2 + \left(\frac{u_B}{S}\right)^2 + \left(\frac{u_C}{S}\right)^2 + \left(-\frac{N}{S} u_s\right)^2}$$

Densità e confluenza di cellule adese

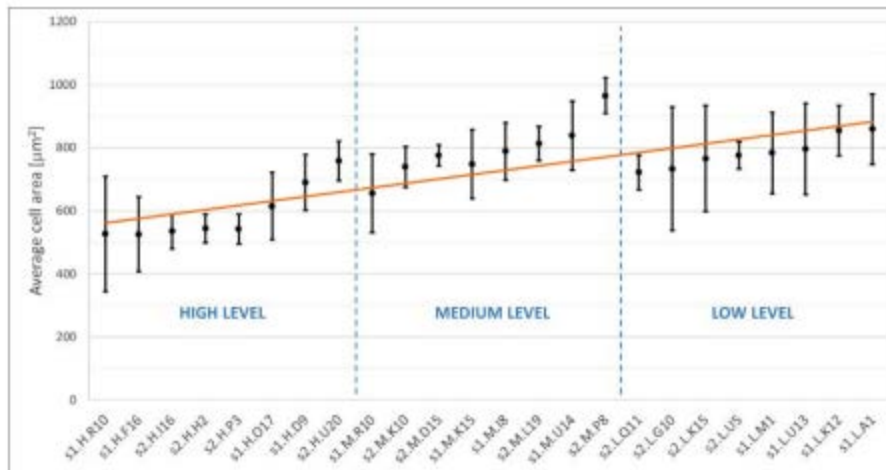


Fig. 18. Plot of average cell area with best-fitting line (orange). Error bars shows standard deviations.

Conteggio di cellule in sospensione

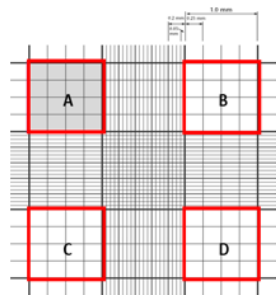


Figure 1: Haemocytometer counting.



Figure 3: Neubauer chamber image.

$$\text{Cell concentration} = \frac{\text{cell count A} + \text{cell count B} + \text{cell count C} + \text{cell count D}}{4} \times 10^4 \left[\frac{C}{ml} \right]$$

Conteggio di cellule in sospensione



| Dilution series generation method | | |
|-----------------------------------|-----------------------------------|----------------------------------|
| Target Dilution fraction (df) | Cell suspension (μl) | Diluent volume (μl) |
| 1 | 150 | 0 |
| 0.7 | 105 | 45 |
| 0.5 | 75 | 75 |
| 0.3 | 45 | 105 |
| 0.1 | 15 | 135 |
| 0.05 | 10 | 190 |

Table 3: Volume used for dilution preparation.

| Operation | Pipette code | Nominal volume (μl) | Conversion in μl | Standard deviation (%) | Pipette resolution (μl) |
|-----------------|--------------|----------------------------------|-----------------------------|------------------------|--------------------------------------|
| vial reconstit. | 04e-P1000-S | 1000 | 992.6 | 0.09 | 1 |
| dilution | 04e-P20-S | 10 | 9.03 * | 1.53 | 0.02 |
| dilution | 04e-P20-S | 15 | 13.17 ** | 1.42 | 0.02 |
| dilution | 04-e-P200 | 45 | 45.43 | 0.32 | 0.2 |
| dilution | 04-e-P200 | 75 | 74.36 | 0.60 | 0.2 |
| dilution | 04-e-P200 | 105 | 104.24 | 0.16 | 0.2 |
| dilution | 04-e-P200 | 135 | 134.23 | 0.47 | 0.2 |
| dilution | 04-e-P200 | 150 | 149.39 | 0.17 | 0.2 |
| dilution | 04-e-P200 | 190 | 189.14 | 0.17 | 0.2 |

Table 7: Micropipettes used of the comparison performances.

SI traceable analytical scale at INRIM

to assess the performance of each pipette, employed to generate the dilutions, at the working volumes



Conteggio di cellule in sospensione

| Pipettes code | 04e-P100-S | 04e-P1000-S | 04e-P200 | |
|---------------|------------|-------------|------------|----------------------------------|
| n.1 | 0.10004 g | 0.10004 g | 0.10016 g | Recorded values |
| n.2 | 0.19999 g | 0.19941 g | 0.19987 g | |
| n.3 | 0.29986 g | 0.29899 g | 0.29989 g | |
| n.4 | 0.39963 g | 0.39918 g | 0.39950 g | |
| n.5 | 0.49939 g | 0.49922 g | 0.49915 g | |
| n.6 | 0.59902 g | 0.59948 g | 0.59889 g | |
| n.7 | 0.69676 g | 0.69940 g | 0.69866 g | |
| n.8 | 0.79658 g | 0.80019 g | 0.79828 g | |
| n.9 | 0.89615 g | 0.90067 g | 0.89783 g | |
| n.10 | 0.99575 g | 1.00093 g | 0.99754 g | |
| Start | 10:06 | 10:13 | 9:56 | |
| End | 40:08 | 10:16 | 10:03 | |
| T (°C) | 24.8-24.9 | 24.7-24.8 | 24.8-24.7 | |
| n.1 | 0.10004 g | 0.10004 g | 0.10016 g | Data elaborated |
| n.2 | 0.09995 g | 0.09937 g | 0.09971 g | |
| n.3 | 0.09987 g | 0.09958 g | 0.10002 g | |
| n.4 | 0.09977 g | 0.10019 g | 0.09961 g | |
| n.5 | 0.09976 g | 0.10004 g | 0.09965 g | |
| n.6 | 0.09963 g | 0.10026 g | 0.09974 g | |
| n.7 | 0.09774 g | 0.09992 g | 0.09977 g | |
| n.8 | 0.09982 g | 0.10079 g | 0.09962 g | |
| n.9 | 0.09957 g | 0.10048 g | 0.09955 g | |
| n.10 | 0.09960 g | 0.10026 g | 0.09971 g | |
| Mean | 0.099575 g | 0.100093 g | 0.099754 g | |
| Std dev | 0.000662 g | 0.000411 g | 0.000192 g | |
| Std dev % | 0.67 % | 0.41 % | 0.19 % | |
| | 99,77 µl | 100,29 µl | 99,95 µl | Conversion in µl |
| | -0,23 µl | 0,29 µl | -0,05 µl | Difference between nominal value |

Conteggio di cellule in sospensione



| Df | F _c |
|------|----------------|
| 1 | 1.007 |
| 0.7 | 1.013 |
| 0.5 | 1.007 |
| 0.3 | 0.996 |
| 0.1 | 1.006 |
| 0.05 | 1.000 |

Table 30: Correction factors of each dilution.

The pipetting systematic errors were evaluated by weighing the pipetting volume, used to generate the dilutions, with a **SI traceable** analytical scale.

$$\text{Cell concentration} = \frac{\text{cell count A} + \text{cell count B} + \text{cell count C} + \text{cell count D}}{4} \times 10^4 \left[\frac{C}{ml} \right] \quad [1]$$

$$\text{Cell count corrected} = [\text{cell count} - (\text{cell count} \times C_{\text{recon}})] \times \frac{FD_n}{FD_r}$$

Where:

Cell count = value of cell obtained by [1]

C_{recon} = correction for the reconstitution

FD_n = nominal dilution factor

FD_r = real dilution factor

Conteggio cellule CD4+: monitoraggio AIDS



- L'HIV porta allo sviluppo della sindrome da immunodeficienza acquisita (AIDS)
- AIDS caratterizzata da una perdita di cellule CD4+ necessarie per una risposta immunitaria attiva contro le infezioni
- La misurazione accurata del numero di cellule CD4+ assicura che i pazienti ricevano una terapia antiretrovirale appropriata contro l'HIV-1
- Requisiti OMS: valutare la stabilità di un materiale di riferimento per standardizzare il conteggio diagnostico delle cellule CD4+

Collaborazione con Dip. Scienze
Cliniche e Biologiche UNITO



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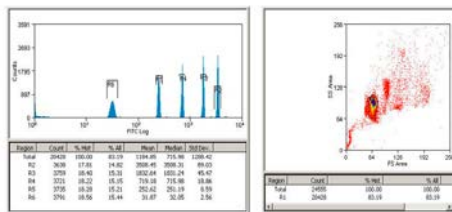
Focus sulla riferibilità metrologica



Cellule cellule CD4+: confronto internazionale



CCQM Working Group on Cell Analysis
(CCQM-CAWG)



ORIGINAL ARTICLE

Cytometry

Quantification of Cells with Specific Phenotypes I: Determination of CD4+ Cell Count Per Microliter in Reconstituted Lyophilized Human PBMC Prelabeled with Anti-CD4 FITC Antibody

Richard Stebbings,^{1*} Lili Wang,² Janet Sutherland,³ Martin Kammel,³ Adolfo K. Gaigalas,² Manuella John,⁴ Bodo Roemer,⁵ Marcus Kuhne,⁶ Rudolf J. Schneider,⁷ Michael Braum,⁸ Andrea Engel,⁹ Dinesh K. Dikshit,⁹ Fatima Abbasi,⁹ Gerald E. Marti,⁹ Maria Paola Sassi,¹⁰ Laura Revel,¹⁰ Sook-Kyung Kim,¹¹ Marc-Olivier Baradez,¹² Tamara Lekishvili,¹² Damian Marshall,¹² Liam Whitty,¹³ Wang Jing,¹⁴ Volker Ost,¹⁵ Maxim Vonsky,¹⁶ Jörg Neukammer¹⁷

ORIGINAL ARTICLE

Cytometry

Quantification of Cells with Specific Phenotypes II: Determination of CD4 Expression Level on Reconstituted Lyophilized Human PBMC Labeled with Anti-CD4 FITC Antibody

L. Wang,^{1*} R. Stebbings,² A. K. Gaigalas,¹ J. Sutherland,² M. Kammel,² M. John,³ B. Roemer,⁴ M. Kuhne,⁵ R. J. Schneider,⁷ M. Braum,⁸ A. Engel,⁹ D. Dikshit,⁹ F. Abbasi,⁹ G. E. Marti,⁹ M. Sassi,¹⁰ L. Revel,¹⁰ S. K. Kim,¹¹ M. Baradez,¹² T. Lekishvili,¹² D. Marshall,¹² L. Whitty,¹³ W. Jing,¹⁴ V. Ost,¹⁵ M. Vonsky,¹⁶ J. Neukammer¹⁷

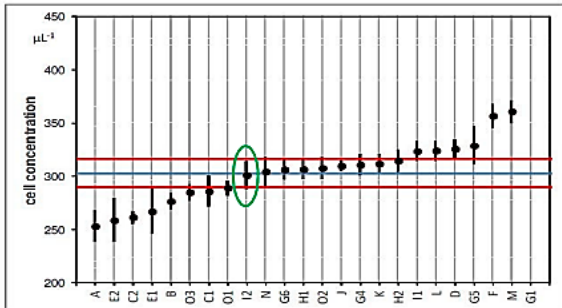


Figure 1. Concentrations of PBMC standard measured by the participants for dilution 1:20. I2 is INRiM data.

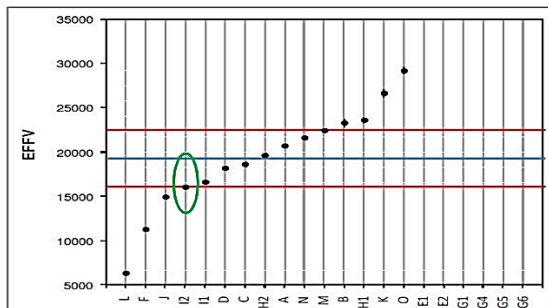


Figure 2. Quantification of the level of CD4 expression on the CD4+ cells, in terms of EFFV. I2 is INRiM data

The comparison aimed to:

- evaluate **stable reference material** for the harmonization of diagnostic CD4+ cell counting and satisfy the requirements of the World Health Organization (WHO);
- demonstrate **participants capability** in measuring CD4+ cell concentrations (number of events) and CD4 level of expression on surface of cells (fluorescence intensity of each event) by evaluating the measurement variability and uncertainty

Collaborazione con Dip. Scienze Cliniche e Biologiche UNITO

Cellule CD34+: efficacia del trapianto di staminali

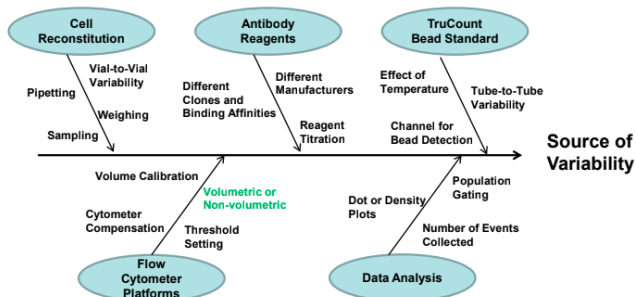
- CD34: proteina di superficie di cellule staminali ematopoietiche
- Capacità di ricostituire il sistema immunitario dopo la chemioterapia
- Il numero di cellule CD34+ vitali infuse per kg di peso corporeo è un biomarcatore predittivo dell'adeguatezza e del successo del trapianto
- Bassa frequenza del numero di CD34+ nel sangue
- Citometria a flusso + materiali di riferimento: gold standard
- Quantificazione volumetrica: nuovo metodo di riferimento
- Confronto tra citometri a flusso tradizionali e volumetrici



Collaborazione con Laboratorio di Analisi
dell'Ospedale San Luigi di Orbassano (Torino)



Cellule CD34: confronto internazionale

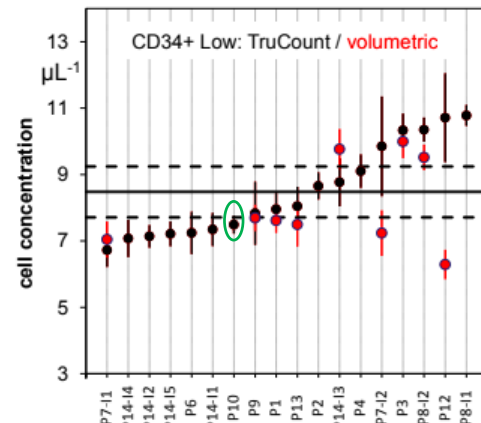
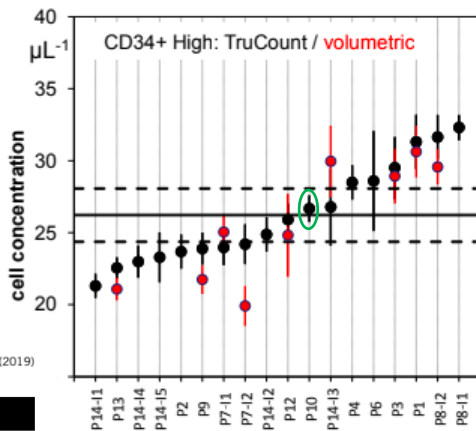


Cytometry Part B (Clinical Cytometry) (2019)

Original Article

Comparison of Volumetric and Bead-Based Counting of CD34 Cells by Single-Platform Flow Cytometry

Luisa Saraiva,^{1*} Lili Wang,² Martin Kammel,³ Andreas Kummrow,³ Eleanor Atkinson,⁴ Ji Youn Lee,⁵ Burhanettin Yalcinkaya,⁶ Muslum Akgöz,⁶ Jana Höckner,⁷ Andreas Ruf,⁷ Andrea Engel,⁸ Yu-Zhong Zhang,⁹ Orla O'Shea,¹⁰ Maria Paola Sassi,¹¹ Carla Divieto,¹¹ Tamara Lekishvili,¹² Jonathan Campbell,¹² Yingying Liu,¹³ Jing Wang,¹³ Richard Stebbings,¹ Adolfo K. Gaigalas,² Peter Rigsby,³ Jörg Neukammer,³ and Sandrine Vessillier¹



| | weighted mean value | uncertainty of mean value | standard deviation | number of measurement | expansion factor | 159.3 | consistency test |
|-----------------|---------------------|---------------------------|--------------------|-----------------------|------------------|-------|------------------|
| high, TruCount | 26.2 | 0.9 | | 18 | 2.101 | 159.3 | negative |
| Low, TruCount | 8.5 | 0.4 | | 18 | 2.101 | 167.7 | negative |
| igh, volumetric | 25.7 | 1.5 | 4.2 | 9 | 2.262 | 60.9 | negative |
| ow, volumetric | 8.1 | 0.5 | 1.3 | 9 | 2.262 | 60.9 | negative |

good agreement

Incertezze di misura estese inter-laboratorio: 30%

Collaborazione con laboratorio di analisi dell'Ospedale San Luigi di Orbassano (Torino)

Biomarcatori tumorali

Genomics Proteomics Bioinformatics 15 (2017) 220–235

HOSTED BY



Genomics Proteomics Bioinformatics

www.elsevier.com/locate/gpb
www.sciencedirect.com



REVIEW

Exploitation of Gene Expression and Cancer Biomarkers in Paving the Path to Era of Personalized Medicine



Hala Fawzy Mohamed Kamel^{1,2,*,3}, Hiba Saeed A. Bagader Al-Amodi^{1,4}

Current Pathobiology Reports (2018) 6:265–274
<https://doi.org/10.1007/s40139-018-0179-5>

MOLECULAR PATHOLOGY OF TUMOR PRE-ANALYTICS (D HICKS, SECTION EDITOR)



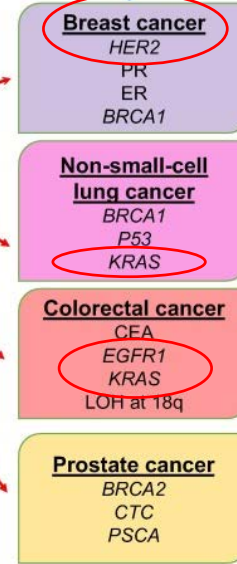
Tumor Pre-Analytics in Molecular Pathology: Impact on Protein Expression and Analysis

Veronique M. Neumeister¹ · Hartmut Juhl¹

Targeted therapy



Biomarkers



Biomarcatori tumorali

KRAS

*v-Ki-ras2 Kirsten rat sarcoma
viral oncogene homolog*

- Carcinoma del colon-retto
- Carcinoma polmonare non a piccole cellule

- Errori di quantificazione nel 20% dei laboratori.
- Inadeguata sensibilità del metodo.

BRAF

*v-raf murine sarcoma viral
oncogene homolog B1*

- Melanoma cutaneo
- Carcinoma del colon-retto
- Carcinoma papillare della tiroide

EGFR

Epidermal growth factor receptor

- Carcinoma polmonare non a piccole cellule

HER2

*human epidermal growth factor
receptor 2*

- Tumore al seno
- Tumore gastrico
- Tumore esofageo

Apparecchiature: digital PCR

Clinical Chemistry 64:9
1296-1307 (2018)

Special Report



Assessment of Digital PCR as a Primary Reference Measurement Procedure to Support Advances in Precision Medicine

Alexandra S. Whale,^{1†} Gerwyn M. Jones,^{1†} Jernej Pavšič,^{2,3} Tanja Dreo,² Nicholas Redshaw,¹ Sema Akyürek,⁴ Müslüm Akgöz,⁴ Carla Divieto,⁵ Maria Paola Sassi,⁵ Hua-Jun He,⁶ Kenneth D. Cole,⁶ Young-Kyung Bae,⁷ Sang-Ryoul Park,⁷ Liesbet Deprez,⁸ Philippe Corbisier,⁸ Sonia Garrigou,⁹ Valérie Taly,⁹ Raquel Larios,¹⁰ Simon Cowen,¹¹ Denise M. O'Sullivan,¹ Claire A. Bushell,¹ Heidi Goenaga-Infante,¹⁰ Carole A. Foy,¹ Alison J. Woolford,¹ Helen Parkes,¹ Jim F. Huggett,^{1,12††} and Alison S. Devonshire^{1††}



- Altamente sensibile
- Quantificazione assoluta
- Metodo di riferimento primario

sample and reaction mix preparation

droplets generation

PCR amplification

droplet reading

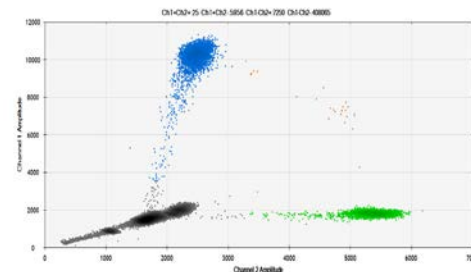
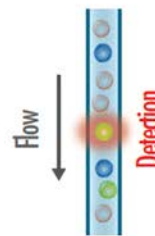
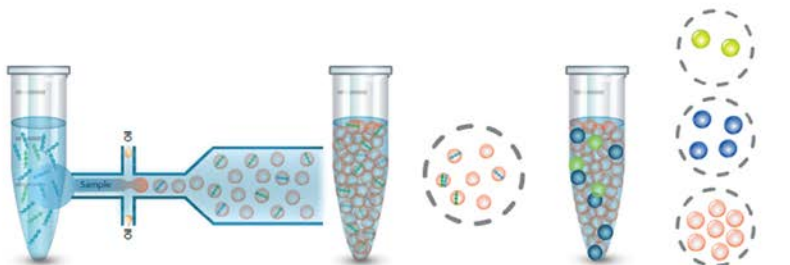


Immagine disegnata da C.Divieto e Studio Makemood

Digital PCR: caratterizzazione metrologica

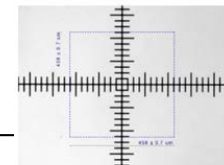
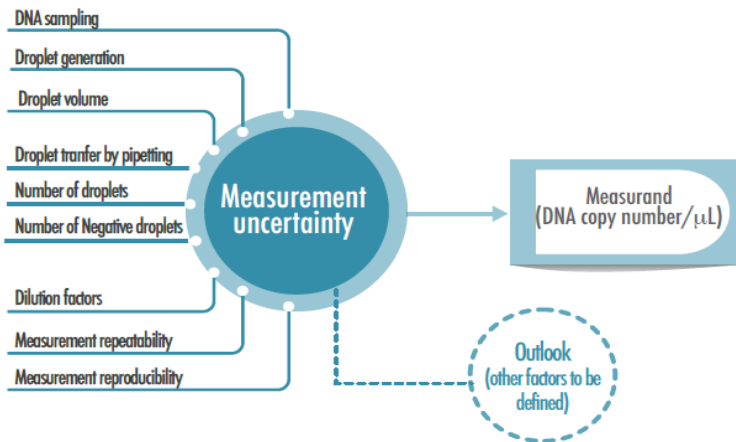


Fig. 4. XY ruler, traceable to the SI. In the image on the left,



ANALYTICAL AND BIOANALYTICAL
CHEMISTRY

springer.com

Anal Bioanal Chem. 2017; 409(28): 6689–6697.

PMCID: PMC5670190

Published online 2017 Sep 18. doi: [10.1007/s00216-017-0625-y](https://doi.org/10.1007/s00216-017-0625-y)

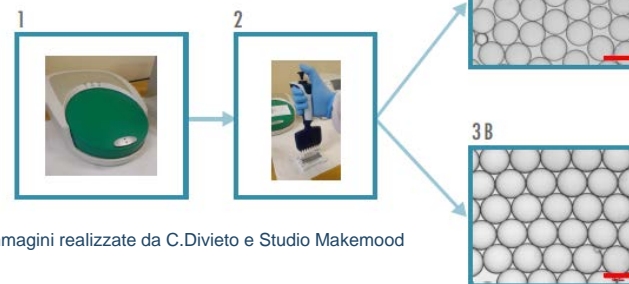
PMID: [28921124](https://pubmed.ncbi.nlm.nih.gov/28921124/)

Droplet volume variability as a critical factor for accuracy of absolute quantification using droplet digital PCR

Alexandra Bogožalec Košir,^{1,2} Caria Divieto,³ Jernej Pavšič,^{1,2} Stefano Pavarelli,³ David Dobnik,¹ Tanja Dreo,¹ Roberto Bellotti,³ Maria Paola Sassi,³ and Jana Žel¹

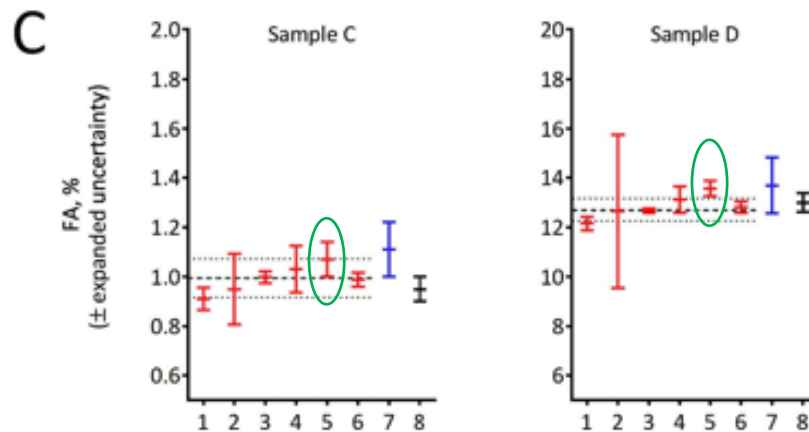
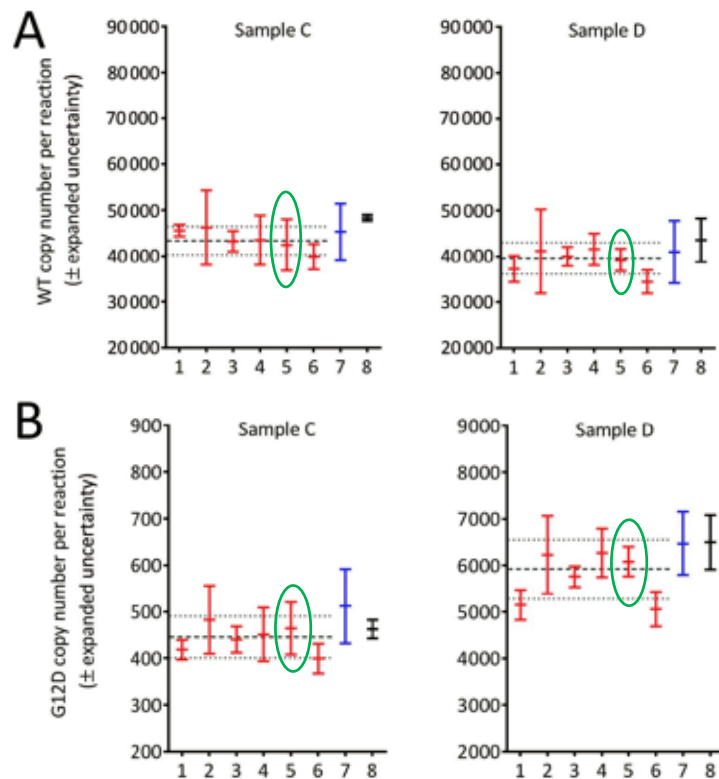
| Component (y) | Uncertainty u(y) | | Sensitivity coefficient $c_y = \frac{\partial x}{\partial y}$ | Contribution to $u(C_{\text{BRAF mut}})$ $c_y \times u(y)$ |
|---|-------------------------------------|--|--|---|
| | Source | Standard uncertainty | | |
| D_f | Pipette calibration | 0,268 μL | $-\frac{\ln(\frac{N_{\text{neg}}}{N})}{V_d}$ | 1,221 |
| N_{neg} / N | Measurement reproducibility | 24,304 | $-\frac{D_f}{(\frac{N_{\text{neg}}}{N}) \times V_d}$ | -0,070 |
| V_d | Volume variability* | $6,5 \times 10^{-6}$ | $\frac{D_f \times \ln(\frac{N_{\text{neg}}}{N})}{V_d^2}$ | -0,164 |
| $u(C_{\text{BRAF mut}}) = \sqrt{(1,221)^2 + (-0,070)^2 + (-0,164)^2}$ | | | | |
| $C_{\text{BRAF mut}}$ [cp/μL] | $u(C_{\text{BRAF mut}})$ [cp/μL] | $U(C_{\text{BRAF mut}})$ [cp/μL], k=2 | $U(C_{\text{BRAF mut}})$ [%], k=2 | |
| 18,22 | 1,24 | 2,48 | 13,6 | |

Real cases of droplet generation and pipetting at Inrim lab.



Immagini realizzate da C.Divieto e Studio Makemood

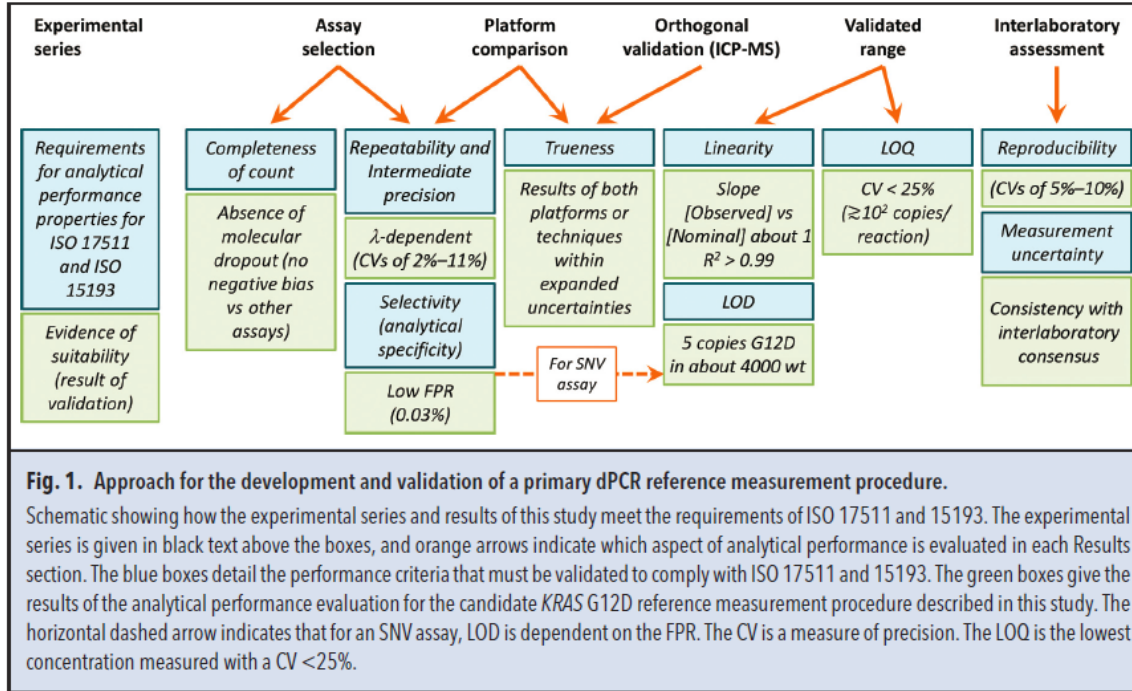
Confronto internazionale KRAS: risultati



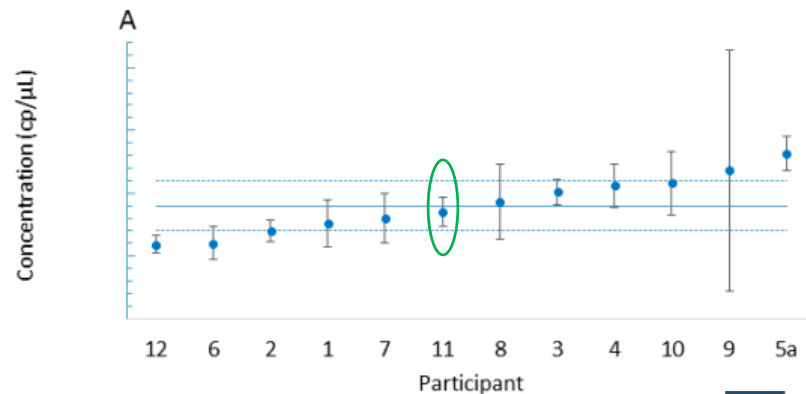
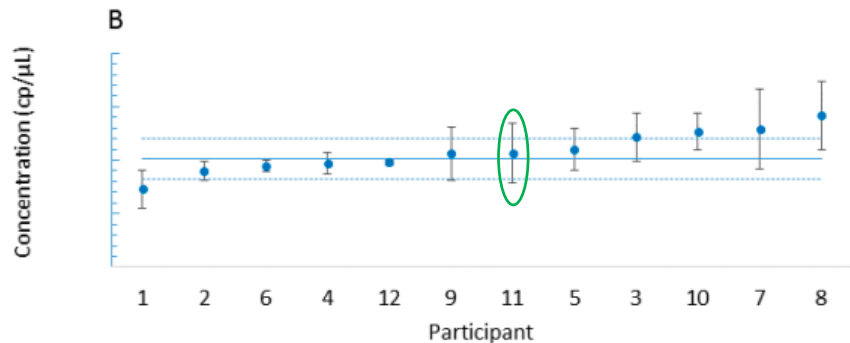
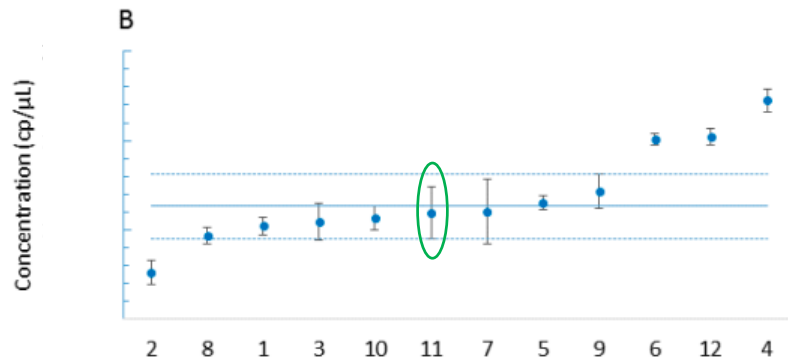
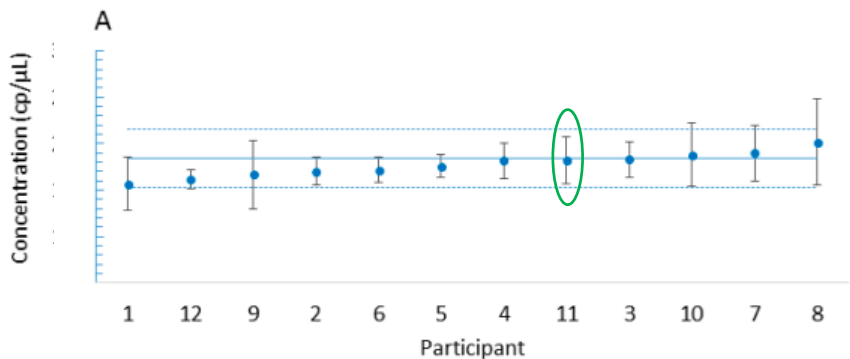
Incertezze di misura estese inter-laboratorio: 5% - 10%

Confronto internazionale KRAS: risultati

Whale et al 2018



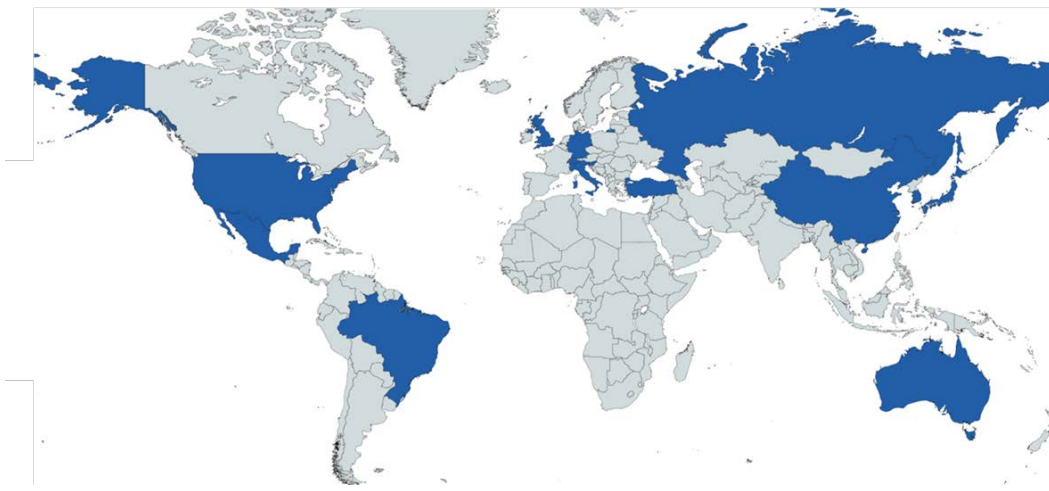
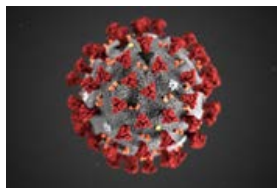
Confronto internazionale BRAF-EGFR: risultati



Confronto internazionale SARS-CoV-2

Scopo:

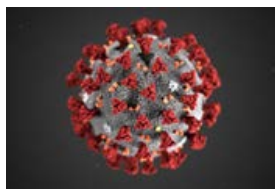
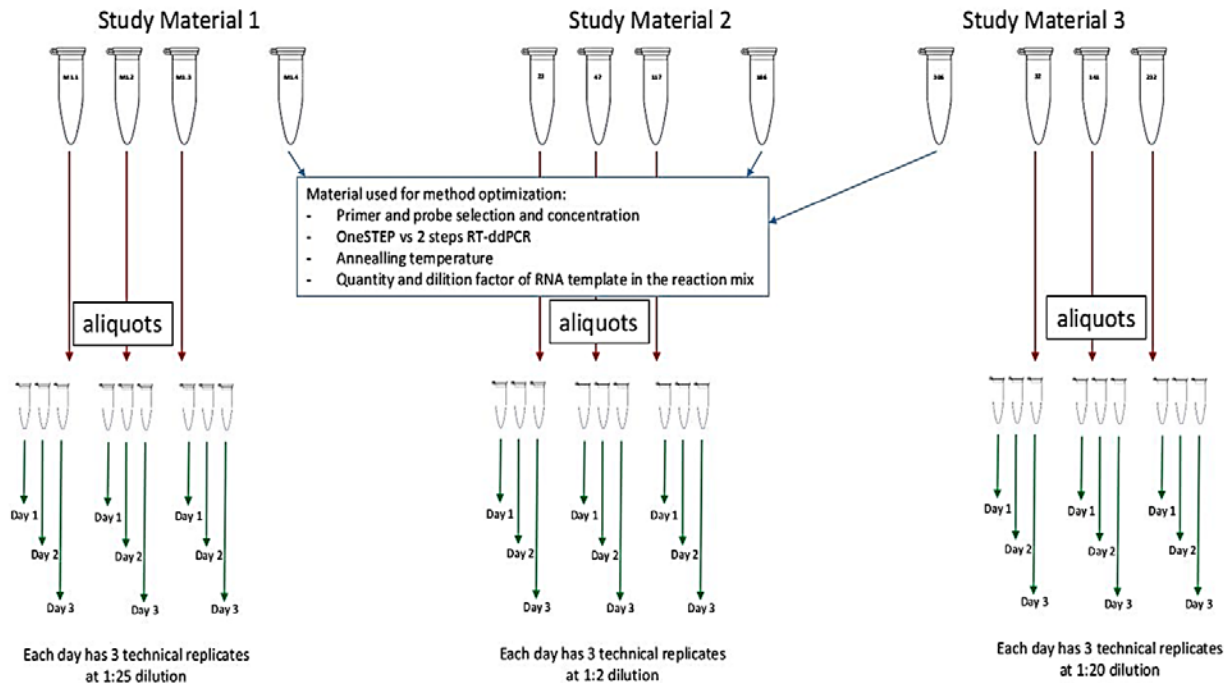
- quantificare copie di RNA del SARS-CoV-2
- 10 copie/ μL (limite di rilevamento) - 10^4 copie/ μL (massimo rilevabile)
- in matrici rappresentative di RNA umano
- sviluppo del protocollo di misura o adozione protocolli WHO



Partecipanti:

21 enti tra NMI,
DI
e altri enti di
ricerca e
sviluppo
di materiali di
riferimento

Confronto internazionale SARS-CoV-2



Confronto internazionale SARS-CoV-2: risultati

Uncertainty budget – material 2, gene N

| Variable x_j | | | statistical | non statistical | | parameters | | | | | rank | AI [%] | SI [%] |
|----------------|--------|---------------------|-------------|-----------------|-------|------------|----------|------------------------------------|--------------------|----------|------|--------|--------|
| Symbol | Value | note | s_j | a_j | k_j | DoF | n sample | $u^2(x_j)$ | $q=Dy_j/Dx_j$ | $u^2(y)$ | | | |
| Df | 4.21 | pipette calibration | 8.8E-02 | | | 39 | 40 | 1.9E-04 | 3.4E+00 | 0.002 | 6 | 0.3 | 0.6 |
| | | resolution | | 2.0E-01 | 3 | 100 | 1 | 1.3E-02 | 3.4E+00 | 0.15 | 3 | 185 | 38.6 |
| Nneg/N | 0.9974 | reproducibility | 0.001 | | | 8 | 27 | 1.3E-08 | -5.6E+03 | 0.39 | 1 | 47.9 | 100.0 |
| | | repeatability | 0.0004 | | | 18 | 27 | 6.0E-09 | -5.6E+03 | 0.18 | 2 | 22.5 | 46.9 |
| Vd | 7.60E- | volume estimation | 5.0E-05 | | | 99 | 100 | 2.5E-11 | -1.9E+04 | 0.01 | 5 | 1.1 | 2.2 |
| | | pixel calibration | 1.5E-05 | | | 30 | 1 | 2.3E-10 | -1.9E+04 | 0.08 | 4 | 9.8 | 20.6 |
| | | | | | | | | | | | | | |
| C | 14.20 | cp/ μ l | | | | | | | Variance, $u^2(y)$ | 0.82 | | | |
| | | | | | | | | Standard uncertainty, $u(y)$ | 0.91 | | | | |
| | | | | | | | | DoF | 31 | | | | |
| | | | | | | | | Confidence level | 95% | | | | |
| | | | | | | | | Coverage factor | 2.04 | | | | |
| | | | | | | | | Expanded uncertainty [cp/ μ l] | 1.85 | | | | |
| | | | | | | | | Relative expanded uncertainty [%] | 13.00 | | | | |

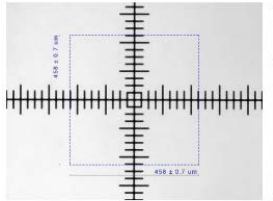


Fig. 4. XY ruler, traceable to the SI. In the image on the left,

Riferibilità metrologica all'SI



Diagnosi di Covid-19: bisogno di misure quantitative accurate

- ELITechGroup: agosto e dicembre 2021
- quantificazione in digital PCR di copie/ μ l di un materiale di riferimento di SARS-CoV-2 fornito dal WHO
- utilizzato per eseguire i test di verifica e validazione di un nuovo kit per la diagnosi di infezioni di SARS-CoV-2 mediante Real-Time PCR



The first PCR kit that differentiates the
SARS-CoV-2 Omicron Variant

🕒 December 9, 2021 📄 News, Press Releases

📍 ITALY, Torino, December 9th, 2021.

KCDB

← to BIPM.org

🌐 CIPM MRA PARTICIPANTS

🔑 Login



All data listed in the KCDB have been reviewed and approved within the CIPM Mutual Recognition Arrangement



CMCS

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Calibration and Measurement Capabilities - CMCS

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Key and supplementary comparisons

SEARCH



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<https://www.bipm.org/kcdb/>



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