Pre-analytical issues in a flow cytometry lab

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Conflict of Interest Disclosure

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It is estimated that more than 70% of clinical decisions are based on information derived from laboratory test results.

The process of blood testing, also known as the **"Total Testing Process,"** begins and ends with the patient. It includes the entire process from ordering the test to interpretation of the test results by the clinician.

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Total Testing Process

Can be subdivided into three stages:

- **Pre-analytical:** test request, patient and specimen identification, specimen collection, transport, accessioning and processing
- **Analytical:** specimen testing
- **Post-analytical:** reporting test results, interpretation, follow up, storage, retesting if needed.

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Additionally

➢The term **"pre-pre-analytical phase"** has been used for the initial part of the pre-analytical phase, focused on test selection and identification of test needed

➢The term **"post-post-analytical phase"** has been used for the interpretation of results by the clinician.

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➢**A Standard** is the minimum requirement for a procedure, method, staffing resource or laboratory facility that is required

➢**A Guideline** is a consensus recommendation for best medical laboratory practice for a procedure, method, staffing resource or facility

➢**A Commentary** may be provided to give clarification to the Guidelines

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IVD

A medical device test either it is a reagent, calibrator, control material, kit, Specimen receptacle, software, instrument, apparatus, equipment or system, whether used alone or in combination with other diagnostic goods for in vitro examination of Specimens derived from the human body.
For the purpose of giving information about a physiological or pathological state, a congenital abnormality or to determine safety and compatibility with a potential recipient, or to monitor therapeutic measures.

ACS Guideline for Clinical Flow Cytometry Laboratory Practice,2017

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Pre-analytical

- patient and sample identification
- sample collection
- transport of sample
- accessioning and processing of sample

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patient and sample identification

- collected samples should have the patients name and two identifiers (eg date of birth, medical record number, test/accession number), including collection date and sample type
- Secondary sample/assay tubes should have patient name and at least one identifier.
- Barcode alone is not acceptable.
- A protocol for return unlabeled/mismatch specimens according and criteria for rejection should be followed as described in your laboratory department manual.

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specimen collection/transport/process

Appropriate anticoagulant and storage according to sample type and disease investigation

Peripheral Blood
Collect into tube with appropriate anti-coagulant (K-EDTA; heparin)
Keep at ambient temperature
Process for analysis within 6-30hr of collection

Bone marrow
problems with fat, bone fragments, erythrocytes

CSF
Number and viability of cells depends on transportation time

FNA
Body Fluids
immunophenotyping depends on the cellularity of the specimen
No anticoagulant needed
Filter
Process within 6-8 hr of collection

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accessioning and processing

Specimen Viability

Non-viable cells are significant source of false positive staining. Viability testing is recommended for samples more than 24 hours after collection or if there is obvious deterioration of the sample

Dead/live staining
Viability can be tested using 7AAD or tryptophan blue.
7-AAD: minimal spectral overlap (excitation 488, emission 655) so can be combined with PE

DNA binding - PI
Propidium iodide is not recommended for immunophenotyping due to instrument contamination and carryover.

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accessioning and processing

- Laboratory (room)
- Instrument
- Panels (reagent – methods)

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Laboratory (room)

- Size
- Temperature
- Humidity
- Noise from other instruments
- Placement of flow cytometer

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Flow cytometer

Daily control of the instrument performance is mandatory
Flow cytometer maintenance (e.g QC,)

	Daily	weekly	monthly	bimonthly	When needed
Air filters					
Cleaning agent container					
Sample head and sample probe					
Sampling system					
Sheath fluid					
Vacuum trap					

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Panels (methods-reagent)

Selection of CD marker
- Choose antigens for identifying subsets
- Design staining combinations

  Keep in mind antigen density and expression pattern

Questions
Based on the flow cytometer that you have (lasers)
What fluorochromes can you detect
Match high Quantum Efficiency fluorochromes with low antigen density
Keep in mind that : same monoclonal antibody conjugated to FITC, PE, Cy5PE, APC, Cy7APC can show different distributions

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Panels (methods-reagent)

Panels should be evaluated and validated in multicenter studies

Normal references including different tissues and age-groups are needed

Sample preparation procedures have to be adapted to the clinical question

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Panels should be evaluated and validated in multicenter studies

- Euroflow (www.euroflow.org)
- IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)
- CLSI (Clinical Laboratory Standards Institute)
- ICCS (International Clinical Cytometry Society):
- ESCCA(European Society for Clinical Cell Analysis www.escca.eu)-
- OMIP
- ISAC : international society of advanced cytometry

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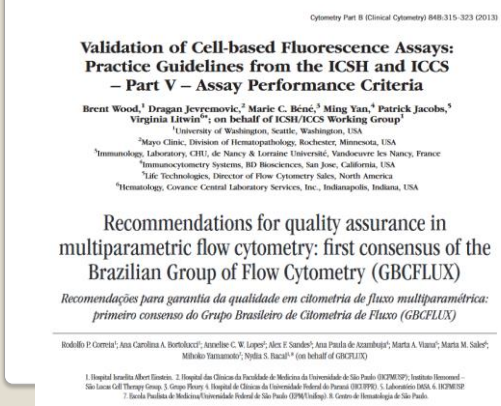

Cytometry Part B (Clinical Cytometry) 848:315–323 (2013)

Validation of Cell-based Fluorescence Assays: Practice Guidelines from the ICSH and ICCS – Part V – Assay Performance Criteria

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[5]Life Technologies, Director of Flow Cytometry Sales, North America
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Recommendations for quality assurance in multiparametric flow cytometry: first consensus of the Brazilian Group of Flow Cytometry (GBCFLUX)

Recomendações para garantia da qualidade em citometria de fluxo multiparamétrica: primeiro consenso do Grupo Brasileiro de Citometria de Fluxo (GBCFLUX)

Rodolfo P. Correia[1]; Ana Carolina A. Bortolucci[2]; Annelise C. W. Lopes[2]; Alex F. Sandes[3]; Ana Paula de Azambuja[4]; Marta A. Viana[5]; Marta M. Sales[6]; Mihoko Yamamoto[7]; Nydia S. Bacal[1,3] (on behalf of GBCFLUX)
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Normal references including different tissues and age-groups are needed

- What is normal
- What is pathological
- Children adults old

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Sample preparation procedures

- Proper sample
- Calibrated pipettes
- Pipetting and mixing techniques
- Sample processing (beads, gradient, whole blood)
- Incubation time and place
- Wash conditions (free Moabs and plasma contains Ig)
- Lysing and permeabilization reagents

Cytometry Part A

ISAC

Sample Preparation for Flow Cytometry Benefits From Some Lateral Thinking

Andrew Filby*

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Summary of pre-analytical issues

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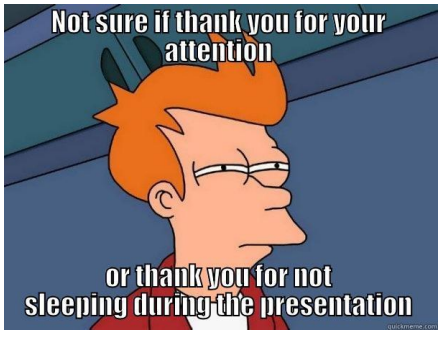

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