

EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS

GUIDELINES AND SUGGESTED EDUCATIONAL RESOURCES

www.escca.eu

1. BACKGROUND

The European Society for Clinical Cell Analysis (ESCCA) is committed to promote high quality education in Cytometry. Since its foundation, ESCCA has fostered its own continuous Educational Program, coordinated by ESCCA Board and Scientific Committees. In addition, ESCCA collaborates with other institutions in the diffusion of educational activities of relevance in Cytometry and related fields.

The ESCCA Education, Certification & Quality Assessment Committee is aware of the technical complexity and constant evolution of cytometry instrumentation and its applications, requiring constant training and education. These issues have been covered traditionally by the Education Activities incorporated in the ESCCA Conferences and, by the ESCCA International and Local Schools on Cytometry.

The final goal of the educational efforts of ESCCA is to help ESCCA members, and especially the young ones, to attain excellence in their work in cytometry, be it in the clinical or in the basic fields. In order to provide our members with a means to certify their knowledge and skill in clinical flow cytometry, the ESCCA Education, Certification & Quality Assessment Committee provides the European Cytometry Certificate.

2. THE EUROPEAN CYTOMETRY CERTIFICATE

The European Cytometry Certificate has two levels of certification:

A. The European Certificate for Cytometry Operators:

This first level of certification may be attained after evaluation of the candidate's knowledge about basic flow cytometry as further described in this document. Applicants should demonstrate a minimum of three years of experience in flow cytometry. The first level of ESCCA Certificate Examination is available online and open for applications.

B. The European Certificate for Cytometry Specialists - hemato-oncology:

This second level of certification may be achieved after evaluation of the candidate's skills and knowledge about clinical flow cytometry-hemato-oncology. Eligible candidates should satisfy the following requirements:

- a. Baccalaureate and/or Master degree and/or Doctorate from an accredited/approved educational institution in biological/life science, physics, engineering or an appropriately related field:
- b. European Certification for Cytometry Operators;

c. Minimum of three years acceptable laboratory experience in cytometry (clinical, research, industry or veterinary) in an ISO 15189, JCI, CAP accredited laboratory or laboratory authorized by a governing regulatory association or Ministry.

Certification consists of an online examination and revision of the candidate's CV by the ESCCA Education, Certification & Quality Assessment Committee.

3. THE EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS: RULES

- 1. The European Certificate for Cytometry Operators is available for ESCCA members only.
- 2. The examination language is English.
- 3. The fee for the examination and certification is € 100 (price level 2023).
- 4. Candidates should demonstrate a minimum of three years of experience in flow cytometry. The candidate's experience should be attested by the director of the employing facility or other entitled authority.
- 5. The examination consists of 100 multiple choice questions and will last a maximum of 2 hours.
- 6. The examination will be considered as passed if at least 60 questions are correctly answered.
- 7. The content of the examination and examples of questions are described later in this document.
- 8. Candidates who successfully pass the examination will receive a certificate and will be named on a dedicated page of ESCCA website and, when convenient, in the site of ESCCA-affiliated society.
- 9. Certification will expire after three years.
- 10. Reinstatement of certification before the date of expiration is free of charge upon proof of continuous education and practice in flow cytometry from an approved educational institution or other entitled authority. The proof should be addressed to the ESCCA Exam Committee and emailed to membership@escca.eu
- 11. For reinstatement of certification after the date of expiration but no more than 5 years, submission of an application for certificate reinstatement and a completed declaration form documenting all continuing education earned within the previous years is required. The reinstatement fee is 50 euros, for which an invoice will be sent. Reinstatement will take affect after receipt of the amount due
- 12. Reinstatement of expired certificate for more than 5 years is not possible. In such cases individuals will be required to retake and pass the certification examination in order to reinstate their certification.

4. THE EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS: PRACTICAL ASPECTS

- 1. The examination for the European Certificate for Cytometry Operators will be proceeded online through the Moodle platform of the ESCCA website anytime during the year, except during public holidays.
- 2. The application process is as follows:
 - The candidate should apply for the examination via the <u>ESCCA membership section</u> by completing the online registration form in the section 'ESCCA Certification Exam'.
 - An official attestation of the number of years of experience of the candidate, issued by the director of the employing facility or other entitled authority, should be uploaded in the online registration form.
 - The exam can take place from Monday Friday between 09.00 16.00 hrs. CET. All international public holidays are excluded.
 - Two dates must be selected in the registration form: the preferred date and a backup date. The exam can take place from 1 month after the submission of the application. Confirmation of the date is subject to the availability of the exam supervisors. The final date will be confirmed in the notification of acceptance.
 - The registration fee of €100 can be paid by iDeal (Dutch candidates only) or credit card.
 - After submission of the registration your application will be reviewed by the Exam Committee. The candidate will receive a notification of acceptance or rejection by email within 2 weeks after submission of the registration form.
 - In case the application is rejected, the registration fee will be reimbursed.
 - If a candidate fails the exam, the registration fee cannot be reimbursed.

Detailed instructions for the completion of the online registration form is available in the membership section.

5. THE EUROPEAN CERTIFICATE FOR CYTOMETRY OPERATORS: GUIDELINES

A. Examination content, relative weight of enlisted topics:

• Basic flow cytometry: 40%

• Sample preparation: 10%

Data acquisition and analysis: 10%Validation and quality control: 10%

Haematology: 10%Immunology: 10%

• Stem cell transplantation: 5%

Cell cycle analysis: 5%Functional analysis: 5%

Apoptosis: 5%

B. Examples of questions:

Basic flow cytometry

A 605 BP is an optical filter that:

- A) Allows passage of light with a wavelength longer than 605 nm
- B) Allows passage of light with a wavelength shorter than 605 nm
- C) Allows passage of a narrow range of wavelengths centred around 605 nm
- D) Blocks a narrow range of wavelengths centred around 605 nm

Sample preparation

Fluorescently-labelled antibodies are sensitive to:

- A) Temperature and light
- B) pH and vibrations
- C) All of the above
- D) None of the above

Data acquisition and analysis

According to Boolean criteria, the gate "A or (B and C)" encompasses:

- A) The events which are in the gate A, B, and C
- B) The events which are in the gate A and B, but not in C
- C) The events in the gate A, plus the events common to gate B and C
- D) The events in the gate A, with the exception of the events common to gate B and C

Validation and quality control

Clinical specificity measures:

- A) The proportion of positives that are correctly identified as such
- B) The proportion of negatives that are correctly identified as such
- C) The repeatability, or reproducibility of the measurement
- D) The proximity of measurement results to the true value

Haematology

The recommended markers to confirm the myeloid lineage in acute leukemias are:

- A) CD13, CD33
- B) CD34, CD117
- C) CD15, CD65
- D) MPO and ≥ 2 of the following markers: CD11c, CD14, CD64, lysozyme

Immunology

Which phenotype is correlated with cytotoxic T cells:

- A) CD3+, CD8+, CD5+
- B) CD3+, CD4+, Granzyme B+
- C) CD3+, CD8+, CD2+(dim)
- D) CD3+, CD4+, CD2+(dim)

Stem cell transplantation

Which of the parameters are used to enumerate viable CD34+ cells in a stem cell product?

- A) SSC and FSC
- B) 7-AAD and CD45
- C) All of these
- D) None of these

Functional analysis

Which of the following fluorochromes is used to detect intracellular superoxide ion:

- A) Dihydrodichlorofluorescein diacetate
- B) 4-amino-5-methylamino-2', 7'-difluorofluorescein diacetate (DAF)
- C) Dihydrorhodamine 123
- D) Dihydroetidine

Apoptosis

The monoclonal antibodies against the BCL-2 protein used in studies of apoptosis:

- A) Bind to a surface molecule expressed in apoptosis-resistant cells
- B) Bind to a surface molecule expressed in apoptosis-sensitive cells
- C) Bind to an intracellular molecule expressed in apoptosis-resistant cells
- D) Bind to an intracellular molecule expressed in apoptosis-sensitive cells

6. SUGGESTED BIBLIOGRAPHY AND RESOURCES AVAILABLE IN THE INTERNET

A. BOOKS

Shapiro, H. (2004) Practical Flow Cytometry, 4th Edition, Wiley-Liss http://www.beckman.com/coulter-flow-cytometry/practical

Ormerod, M.G. (2008) Flow Cytometry - A Basic Introduction http://flowbook.denovosoftware.com/

Longobardi-Givan, A. (2010) Flow Cytometry: First Principles, Second Edition, Wiley-Liss http://www.beckman.com/coulter-flow-cytometry/first-principles

Al-Ahmadi A, (2015) Quantum Dots - A Variety of New Applications. InTech Open Science https://www.intechopen.com/books/quantum-dots-a-variety-of-new-applications

Schmid, I, Ed. (2012) Flow Cytometry —Recent Perspectives. InTech Open Science http://www.intechopen.com/books/editor/flow-cytometry-recent-perspectives

Schmid, I, Ed. (2012) Clinical Flow Cytometry-Emerging Applications. InTech Open Science http://www.intechopen.com/books/clinical-flow-cytometry-emerging-applications

Schmid, I, Ed. (2016) Flow Cytometry - Select Topics. InTech Open Science http://www.intechopen.com/books/editor/flow-cytometry-select-topics

B. BASIC GUIDES

GENERAL ASPECTS

MDBioproducts, Flow Cytometry Guide www.mdbiosciences.com

Chromocyte, A Beginners Guide to Flow Cytometry www.chromocyte.com

Rahman M, Introduction to Flow Cytometry. AbD-Serotec https://www.abdserotec.com/introduction-to-flow-cytometry.html

FLUORESCENCE

Principles of fluorescence, Imperial College London http://www.imperial.ac.uk/media/imperial-college/medicine/facilities/film/Fluorophores-website.pdf

Chapter 2 - Principles of Fluorescence- AbD Serotec https://www.abdserotec.com/introduction-to-flow-cytometry.html#chapter2

AbCam Fluorochrome chart – a complete guide http://docs.abcam.com/pdf/secondary-antibodies/abcam-fluorochrome-chart.pdf

The Fluorescent Protein Color Palette http://www.microscopyu.com/pdfs/FPColorPalette.pdf

Tandem Dyes-Biolegend http://www.biolegend.com/tandem dyes

Introduction to Click Chemistry http://www.lumiprobe.com/click-chemistry

PANEL DESIGN, SETUP AND COMPENSATION

The Stain Index: What Is It and What Does It Tell You? http://www.biolegend.com/newsdetail/1245/

Biolegend Panel Selector https://www.biolegend.com/panelselector

Biolegend Panel Construction https://www.biolegend.com/custom panel construction

C. REVIEWS

GENERAL ASPECTS

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David A. Basiji, William E. Ortyn, Luchuan Liang, Vidya Venkatachalam, Philip Morrissey. Cellular Image Analysis and Imaging by Flow Cytometry Clin Lab Med. Author manuscript; available in PMC 2008 September 1. doi: 10.1016/j.cll.2007.05.008 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2034394/pdf/nihms29623.pdf

Bendall, SC; Simonds EF, Qiu P, Amir ED, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, Nolan GP (6 May 2011). "Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum". Science 332 (6030): 687–696 doi:10.1126/science.1198704

Di Palma, S; Bodenmiller, B. (2015). "Unraveling cell populations in tumors by single-cell mass cytometry". Current Opinion in Biotechnology 31: 122–129 doi:10.1016/j.copbio.2014.07.004

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Erika A O'Donnell, David N Ernst, Ravi Hingorani. Multiparameter Flow Cytometry: Advances in High Resolution Analysis. Immune Netw. 2013 April; 13(2): 43–54. Published online 2013 April

30. doi: 10.4110/in.2013.13.2.43

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FLUORESCENCE

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Alejandro Sosa-Peinado and Martín González-Andrade (2011). Fluorescent Biosensors for Protein Interactions and Drug Discovery, Biosensors for Health, Environment and Biosecurity, Prof. Pier Andrea Serra (Ed.), ISBN: 978-953-307-443-6, InTech, DOI: 10.5772/17431.

http://www.intechopen.com/books/biosensors-for-health-environment-and-biosecurity/fluorescent-biosensors-for-protein-interactions-and-drug-discovery

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http://www.intechopen.com/books/macro-to-nano-spectroscopy/current-achievement-and- future-potential-of-fluorescence-spectroscopy

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SAMPLE PREPARATION

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Clinical and Laboratory Standards Institute. Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline, 2nd ed. CLSI document H43-A2. Clinical and Laboratory Standards Institute; 2007 http://www.techstreet.com/standards/clsi-h43-a2?product_id=1510821

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Chow S, Hedley D, Grom P, Magari R, Jacobberger JW, Shankey TV (2005) Whole Blood Fixation and Permeabilization Protocol with Red Blood Cell Lysis for Flow Cytometry of Intracellular Phosphorylated Epitopes in Leukocyte Subpopulations. Cytometry Part A 67A: 4–17

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PANEL DESIGN, SETUP AND COMPENSATION

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Mahnke Y, Chattopadhyay P, Roederer M. Publication of optimized multicolor immunofluorescence panels. Cytometry, 77A: 814–818. doi: 10.1002/cyto.a.20916. Spidlen J, Breuer K, Rosenberg C, Kotecha N, Brinkman RR. (2012) FlowRepository - A Resource of Annotated Flow Cytometry Datasets Associated with Peer-reviewed Publications. Cytometry A. 81:727-731

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DATA ANALYSIS

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BIOSAFETY

Flow cytometry: Biosafety recommendations and protective measures http://www.biosafety.be/CU/FlowCytometry/FCMMenu.html

Biosafety in Flow Cytometry – To Be or Not to Be... http://bitesizebio.com/21608/biosafety-in-flow-cytometry-to-be-or-not-to-be/

D. WEBINARS, VIDEOS AND ANIMATIONS

GENERAL ASPECTS

History of Flow Cytometry-BioLegend https://www.biolegend.com/historyofflow

Molecular Probes—Introduction to Flow Cytometry https://youtu.be/sfWWxFBltpQ

Introduction to Flow Cytometry Webinar https://youtu.be/o2joszUiVhM

MCBC Flow Cytometry Training Course - Session 1 https://youtu.be/fMNNXlh4OkQ

Flow Cytometry-Genesync https://youtu.be/6j-AzBocWKw

Flow Cytometry lecture part 1 https://youtu.be/YPb9Pfpm66c?list=PL1DA0F59A86AFCE59

Flow Cytometry lecture part 2 https://youtu.be/YEl96A7L1rU?list=PL1DA0F59A86AFCE59

Flow Cytometry lecture part 3 https://youtu.be/JXovmJJOjs8?list=PL1DA0F59A86AFCE59

FLUORESCENCE

Fluorescence Tutorials-Thermofisher http://www.thermofisher.com/es/en/home/support/tutorials.html

Chemwiki

http://chemwiki.ucdavis.edu/Core/Physical Chemistry/Spectroscopy/Electronic Spectroscopy/Fluorescence

Molecular Probes Tutorial Series—Introduction to Fluorescence https://youtu.be/SGFlr1jFNBM

Lecture 4 part 1 (fluorescence, Jablonski diagram): https://youtu.be/5KLBrnauilg

Lecture 4 part 2 (fluorescence spectral distribution, parameters) https://youtu.be/PYmjrL 80Y0

Lecture 4 part 3 (fluorescence microscope, applications of fluorescence, photobleaching) https://youtu.be/ywE6VaVm5kg

Lecture 4 part 4 (FRET)

https://youtu.be/JH2LIffu 71

Microscopy: Introduction to Fluorescence Microscopy (Nico Stuurman)

https://youtu.be/AhzhOzgYoqw

Microscopy: Fluorescent Proteins (Roger Tsien)

https://youtu.be/qK9aYnkIr3w

The expanding palette of fluorescent proteins

https://youtu.be/n7f1-PttVcs

Nobel Laureate Martin Chalfie - "Green Fluorescent Protein: Lighting up Life"

https://youtu.be/YCY0Inhb4ol

What are Quantum Dots?

https://youtu.be/LIPDyl53rZA

SAMPLE PREPARATION

Techniques of Breast Biopsy - Manipal Hospital https://youtu.be/ZcWOPmyPj68

Dividing the FNA aspirate sample for ancillary testing

https://youtu.be/J3gBCqAu3GM

DNA content cell cycle analysis using flow cytometry

https://youtu.be/MIE0Xnr9oz

DATA ANALYSIS

Molecular Probes Tutorial Series—Analyzing Flow Cytometry Data https://youtu.be/ccR5snuCE80

Basics of flow cytometry, Part I: Gating and data analysis

https://youtu.be/y9-mojIXU_I

INSTRUMENTATION

Beckman Coulter Flow Cytometry https://www.youtube.com/playlist?list=PL1DA0F59A86AFCE59

Gallios Flow Cytometer https://youtu.be/5TtOpfYwqoQ

Gallios Cytometer Tour Guides

http://beckman.eu/assets/training/flowcytometry/flowcytometer/index.html

FC500 Fow Cytometer

https://youtu.be/kLcf7QsSfrQ

Modifying the FC500 Flow Cytometer with Multiple Lasers https://youtu.be/XygjLak2BUM

Flow Cytometry -Beckman Coulter Life Sciences https://www.youtube.com/playlist?list=PLzfAZrs5hqGdSCbWAv5Sjzm_J7761PoyA

"The BD Accuri C6 Flow Cytometer"-David Lee, BD Biosciences https://youtu.be/k0QhLWk3RO4

Accuri C6 Flow Cytometer.mp4 https://youtu.be/gz090i3ci8A

Accuri Cytometers

https://youtu.be/6lqvpykoqjl

Displaying Accuri CFlow Data and Using the CFlow Importer in FCS Express https://youtu.be/9HTSgzBJ4v4

Becton Dickinson Flow cytometry

https://www.youtube.com/playlist?list=PLrTm-FBR3jxT9sJ0H8BTakbhokek1X2fc

Attune-Thermo Fisher Scientific Flow Cytometry https://www.youtube.com/playlist?list=PLGlvFEwL2wDHYu3pyBrrkClt_0jRuRcao

Milteny Biotec Flow Cytometry

https://www.youtube.com/playlist?list=PL5EpKG- c5XfrPax8A-Oh3sHy4CLuJRkQ

ADVANCES IN CYTOMETRY

ACOUSTIC PRE-FOCUSING CYTOMETRY

The Discovery of Acoustic Focusing & the Attune® Flow Cytometer https://youtu.be/b2ilHEnugE0

Attune® Acoustic Focusing Cytometer Tutorial https://youtu.be/kpkL2EEJDsU

The Next Generation in Acoustic Cytometry https://youtu.be/Q1PIICS5VnM

HyperCyt revisited https://youtu.be/jf-1Q3QZ6Oc

MASS-SPECTROMETRY CYTOMETRY

National CyTOF Meeting 2014: Scott Tanner, PhD, Fluidigm Corp https://youtu.be/HnUVWIhKA3k

SPECTRAVIEWERS

eBioscience

http://www.ebioscience.com/resources/fluorplan-spectra-viewer.htm

Thermofisher

https://www.thermofisher.com/es/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html

BioLegend

http://www.biolegend.com/spectraanalyzer

Becton Dickinson

http://m.bdbiosciences.com/us/s/spectrumviewer

E. OTHER RECOMMENDED SITES FOR EDUCATIONAL RESOURCES

Chromocyte

https://www.chromocyte.com/

Purdue University Cytometry Labs (PUCL)

http://www.cyto.purdue.edu/

Cytobank

www.cytobank.org

FlowRepository

www.flowrepository.org

Bitesize Bio

http://bitesizebio.com/category/technical-channels/flow-cytometry/

Websites of Cytometer-Manufacturing Companies

ACEA Biosciences, Inc.

http://www.aceabio.com/

Apogee Flow Systems

http://www.apogeeflow.com/

Beckman Coulter

http://beckman.es/coulter-flow-cytometry

Becton Dickinson Biosciences

http://www.bdbiosciences.com/eu/applications/s/flowcytometry?WT.srch=1&gclid=

CP6gi- ql8swCFU4o0wodSOsLxg

Bio-Rad

http://www.bio-rad.com/es-es/category/flow-cytometry

Standard Biotools

https://www.standardbio.com/products/instruments/helios-a-cytof-system

Merck-Millipore

http://www.merckmillipore.com/ES/es/products/life-science-research/cell-analysis/yjSb.qB.uBwAAAE 3S53.M6W,nav

Miltenyi Biotec

http://www.miltenyibiotec.com/en/products-and-services/macs-flow-cytometry.aspx Propel Labs

Sony Biotechnology

http://www.apogeeflow.com/

Stratedigm

https://stratedigm.com/

Sysmex

http://www.sysmex-europe.com/products/flow-cytometry.html

Thermo-Fisher

https://www.thermofisher.com/es/es/home/life-science/cell-analysis/flow-cytometry.html

Websites of Companies Manufacturing Antibodies and Fluorescent Probes

Abcam

http://www.abcam.com/

Antibody BCN

http://www.antibodybcn.com/

Beckman Coulter

http://beckman.es/coulter-flow-cytometry/reagents

Becton Dickinson

http://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/c/744843

BioLegend

http://www.biolegend.com/

Bio-Rad

https://www.bio-rad-antibodies.com/

Cell Signaling Technology

http://www.cellsignal.com/

Cytognos

http://www.cytognos.com/index.php/es

Agilent

https://www.agilent.com/?gclid=Cj0KCQiAxbefBhDfARIsAL4XLRodJy034rMWT7f7VaBRPHCQYcqvPzC1RNUfgM35YZqbbdPDYwTl10MaApCPEALwwcB&gclsrc=aw.ds

Enzo Life Sciences

http://www.enzolifesciences.com/

ExBio

http://www.exbio.cz/

Hycult Biotech

http://www.hycultbiotech.com/

Immunostep

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http://www.labclinics.com/

Miltenyi Biotec

http://www.miltenyibiotec.com/en/products-and-services/macs-flow-cytometry/reagents.aspx

Santa Cruz Biotechnology

http://www.scbt.com/

Thermo-Fisher

https://www.thermofisher.com/es/es/home/life-science/antibodies.html

Tonbo Biosciences

http://www.tonbobio.com/

Websites of Companies Producing Cytometry Software

De Novo Software

https://www.denovosoftware.com/

FlowLogic Software

http://www.inivai.com/flowlogic

FlowJo Software

http://www.flowjo.com/

Infinicyt Software http://www.infinicyt.com/

Kaluza Software

http://beckman.es/coulter-flow-cytometry/software/kaluza-analysis-software

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