Masterclass Certificate in Leukemia Immunophenotyping

Principles of Leukemia Immunophenotyping

Antigen
Related terms: epitope, CD marker, antibody
An antigen is any molecular structure recognized by an antibody or a T-cell receptor. In leukemia immunophenotyping, antigens are typically surface proteins (e.g., CD33, CD19) that distinguish malignant from normal cells. Example: CD34 is expressed on hematopoietic stem cells and many acute leukemias, aiding in disease classification. Practical application includes selecting antibodies that target disease-specific antigens for flow-cytometric panels. A common challenge is antigen modulation after treatment, which can lead to false-negative results if the target is down-regulated.

Antibody
Related terms: monoclonal antibody, fluorochrome conjugate, isotype
An antibody is a protein produced by B-cells that binds a specific antigenic epitope. In immunophenotyping, monoclonal antibodies are conjugated to fluorochromes to detect antigens on leukemia cells. Example: a PE-conjugated anti-CD45 antibody highlights leukocyte common antigen for gating. Antibodies must be validated for specificity and lot-to-lot consistency; cross-reactivity can obscure interpretation, especially in multicolor panels where spectral overlap is common.

Back-gating
Related terms: forward scatter, side scatter, gating strategy
Back-gating is a verification step where a subpopulation identified on a fluorescence plot is re-examined on the scatter plot to confirm its physical characteristics. For instance, a $CD34^{+}$ blast gate is back-gated onto FSC/SSC to ensure blasts are appropriately sized and granulated. This technique improves confidence that the immunophenotypic gate corresponds to the intended lineage. Challenges arise when blasts overlap with mature cells, requiring careful adjustment of gate boundaries.

Compensation
Related terms: spectral overlap, spillover matrix, single-color controls
Compensation corrects for fluorescence spillover between detection channels. When a fluorochrome emits photons detected in more than one channel, a mathematical correction is applied using a spillover matrix derived from single-color controls. Example: FITC emits into the PE channel; proper compensation subtracts this contribution to avoid false positivity. Inadequate compensation can generate artificial antigen co-expression, leading to misclassification of leukemia subtypes. Routine verification of the compensation matrix is essential, especially after instrument maintenance.

CD Marker
Related terms: cluster of differentiation, surface antigen, lineage marker
CD markers are standardized nomenclature for cell surface molecules identified by specific antibodies. They provide a common language for describing immunophenotypes. Example: CD19 denotes B-cell lineage,

while CD3 marks T-cells. In leukemia, combinations such as $CD13^+/CD33^+$ suggest myeloid origin, whereas $CD7^+/CD5^+$ may indicate T-ALL. Interpretation must consider that some CD markers are expressed on both normal and malignant cells, requiring quantitative assessment (e.g., mean fluorescence intensity) to differentiate aberrant expression.

Clonality Assessment
Related terms: light-chain restriction, flow cytometric immunophenotyping, B-cell receptor
Clonality assessment determines whether a population of lymphoid cells originates from a single progenitor. In B-cell leukemias, the presence of a restricted κ or λ light-chain ratio (e.g., κ:λ >4:1) signifies clonality. Flow cytometry can simultaneously evaluate surface immunoglobulin and CD markers to establish a clonal pattern. Practical use includes distinguishing reactive lymphocytosis from malignant proliferation. The main difficulty is that early B-cell precursors often lack surface immunoglobulin, making light-chain analysis impossible; alternative markers such as CD10 and CD34 must then be relied upon.

Forward Scatter (FSC)
Related terms: cell size, scatter plot, gating
Forward scatter measures the amount of light diffracted by a cell, correlating with cell size. In leukemia immunophenotyping, blasts typically exhibit low FSC relative to mature lymphocytes. FSC is used together with side scatter to create a physical gate that excludes debris and aggregates. Example: a gate set on low FSC/low SSC isolates myeloblasts for subsequent antigen analysis. Limitations include variability in FSC due to instrument alignment or sample preparation, which can affect the reproducibility of blast gating across runs.

Fluorochrome
Related terms: fluorescent dye, excitation wavelength, emission spectrum
A fluorochrome is a molecule that absorbs light at one wavelength and emits at a longer wavelength. Conjugated to antibodies, fluorochromes enable detection of antigens by flow cytometry. Common fluorochromes include FITC, PE, APC, and PerCP. Choosing fluorochromes with minimal spectral overlap simplifies compensation. Example: pairing a bright PE-conjugated anti-CD33 with a dim APC-conjugated anti-CD45 maximizes discrimination of myeloid blasts. Fluorochrome stability can be compromised by light exposure or fixation, potentially reducing signal intensity and complicating longitudinal studies.

Gating
Related terms: population selection, hierarchical gating, Boolean logic
Gating is the process of defining a region on a plot to isolate cells of interest. In leukemia immunophenotyping, hierarchical gating often starts with FSC/SSC to select viable leukocytes, followed by CD45 versus side scatter to separate blasts, then specific antigen gates (e.g., $CD34^+$). Boolean logic can combine multiple gates to refine subpopulations (e.g., $CD34^+/CD13^+$ blasts). Incorrect gate placement can include contaminating cells or exclude relevant blasts, leading to inaccurate diagnosis. Regular training and use of standardized templates mitigate operator bias.

Hemoglobinopathies
Related terms: sickle cell disease, thalassemia, flow cytometric baseline
Hemoglobinopathies are inherited disorders affecting hemoglobin structure or synthesis. While not directly

part of immunophenotyping, they influence bone-marrow cellularity and may alter blast percentages. For instance, patients with sickle cell disease often present with extramedullary hematopoiesis, increasing circulating immature cells that can be misinterpreted as leukemic blasts. Awareness of the underlying hematologic context is essential when establishing normal reference ranges for antigen expression.

Immunophenotype
Related terms: antigen profile, flow cytometry, diagnostic algorithm
An immunophenotype is the pattern of antigen expression on a cell as determined by flow cytometry. In leukemia, the immunophenotype guides classification into AML, ALL, CML, or MPAL (mixed-phenotype acute leukemia). Example: a blast population expressing CD34, CD117, MPO, and CD13 is classified as AML. The immunophenotype also informs prognosis; certain antigen combinations (e.g., $CD56^+$ AML) are associated with adverse outcomes. Challenges include phenotypic plasticity, where leukemic cells change antigen expression over time or after therapy, necessitating repeat testing.

Instrument Calibration
Related terms: quality control beads, voltage settings, daily performance checks
Calibration ensures that the flow cytometer provides consistent, accurate fluorescence measurements. Calibration involves running standardized beads with known fluorescence intensities to set photomultiplier tube voltages and verify linearity. Example: daily QC with Rainbow Calibration Particles confirms that the PE channel remains within the target median fluorescence intensity range. Poor calibration can cause drift in antigen detection thresholds, leading to false-negative or false-positive results. Regular maintenance and documentation of calibration data are required for accreditation.

Minimal Residual Disease (MRD)
Related terms: sensitivity, quantitative flow cytometry, disease monitoring
MRD refers to the small number of leukemic cells that persist after treatment and are below the detection limit of conventional morphology. Flow cytometry can detect MRD at a sensitivity of 10^{-4} to 10^{-5} by identifying abnormal antigen patterns not present in normal marrow. Example: a post-induction sample showing $CD34^+$/$CD13^+$ blasts at 0.02% of nucleated cells indicates MRD positivity. MRD status informs therapeutic decisions and prognosis. Technical challenges include distinguishing rare leukemic cells from normal regenerative blasts and maintaining assay sensitivity across laboratories.

Monoclonal Antibody
Related terms: hybridoma, clone specificity, isotype control
A monoclonic antibody is produced by a single B-cell clone and recognizes one epitope with uniform affinity. In immunophenotyping, monoclonal antibodies provide reproducible binding to target antigens. Example: clone 8A3 (anti-CD33) is widely used in AML panels. Isotype controls help assess non-specific binding, although they are less informative than fluorescence minus one (FMO) controls. The main limitation is that a single clone may not recognize all allelic variants of an antigen, potentially missing certain leukemic subpopulations.

Multicolor Panel Design
Related terms: fluorochrome selection, antigen hierarchy, panel optimization
Designing a multicolor panel involves selecting fluorochromes and antibodies to maximize information

while minimizing spillover. The process starts with defining the diagnostic question, then assigning the brightest fluorochromes to low-density antigens (e.g., CD34) and dimmer fluorochromes to abundant markers (e.g., CD45). Example: an 8-color AML panel may include CD34-FITC, CD13-PE, CD33-PerCP-Cy5.5, MPO-APC, and a viability dye. Panel optimization requires iterative testing, compensation verification, and assessment of antigen-fluorochrome interactions. Over-crowding the panel can increase background and reduce resolution, especially for rare events like MRD.

Negative Control
Related terms: unstained sample, isotype control, background fluorescence
A negative control lacks the specific antigen or fluorochrome and establishes the baseline fluorescence of the assay. In flow cytometry, an unstained sample defines auto-fluorescence, while an isotype control assesses non-specific antibody binding. Example: a sample stained with an isotype-matched IgG-PE provides a reference for background PE fluorescence. Proper negative controls are vital for setting gates and interpreting dimly expressed antigens. However, isotype controls may not fully replicate the binding characteristics of the specific antibody, so FMO controls are often preferred.

Positive Control
Related terms: reference cell line, known antigen expression, assay verification
A positive control contains cells that definitively express the target antigen, confirming that the staining procedure and reagent are functional. Example: the HL-60 cell line expresses CD33 and MPO, serving as a positive control for myeloid panels. Running a positive control each day helps detect reagent degradation or instrument issues. The challenge lies in selecting a control that mirrors the antigen density of patient blasts; overly bright controls may mask suboptimal staining of low-level antigens in clinical samples.

Side Scatter (SSC)
Related terms: granularity, internal complexity, debris discrimination
Side scatter measures light reflected at a 90-degree angle, reflecting cellular granularity and internal complexity. Granular cells such as neutrophils produce high SSC, whereas lymphocytes and blasts have lower SSC. SSC is combined with FSC to create a physical gate that excludes debris and aggregates. Example: a low-FSC/low-SSC gate isolates blast cells from mature granulocytes. Variations in SSC can arise from sample fixation, affecting the ability to discriminate subpopulations, thus necessitating consistent processing protocols.

Specificity
Related terms: cross-reactivity, false-positive, diagnostic accuracy
Specificity is the ability of an assay to correctly identify negative cases, i.e., to avoid detecting antigen where it is absent. In immunophenotyping, high specificity means that an antibody does not bind unrelated cell types. Example: an anti-CD19 antibody that also binds a subset of NK cells would reduce specificity for B-cell leukemia. Ensuring specificity involves rigorous validation, including testing on normal bone-marrow repertoires. Low specificity can lead to over-diagnosis or inappropriate therapy.

Sensitivity
Related terms: detection limit, limit of detection, MRD
Sensitivity is the assay’s capacity to detect low-frequency events. In flow cytometry, sensitivity is influenced

by the number of events acquired, fluorochrome brightness, and background noise. For MRD, a sensitivity of 10^{-4} requires acquisition of at least 500,000 events and a well-optimized panel. Example: detecting 5 leukemic cells among 100,000 normal cells demonstrates a sensitivity of 5×10^{-5}. Enhancing sensitivity may compromise specificity if gating is too permissive, so a balance must be struck.

Standardization
Related terms: EuroFlow, SOPs, inter-laboratory comparability
Standardization refers to harmonizing protocols, reagents, and data analysis across laboratories. The EuroFlow consortium provides standardized panels, instrument settings, and analysis software to achieve comparable results worldwide. Example: using the EuroFlow AML panel with defined fluorochrome-to-antigen assignments enables consistent classification across sites. Challenges include variations in instrument models, reagent lot differences, and operator expertise, all of which can introduce variability despite published SOPs.

Surface Marker
Related terms: CD antigen, extracellular domain, flow cytometry
Surface markers are proteins expressed on the cell membrane that can be accessed by antibodies without permeabilization. They are the primary targets for immunophenotyping because they allow rapid, live-cell analysis. Example: CD45, a pan-leukocyte marker, is used to differentiate leukocyte subsets based on intensity. Surface markers may be down-regulated after chemotherapy, requiring intracellular staining (e.g., for MPO) to confirm lineage. Accurate quantification of surface marker density aids in distinguishing normal maturation from leukemic aberrancy.

Technical Replicate
Related terms: repeatability, intra-assay variation, precision
A technical replicate involves processing the same sample multiple times within a single run to assess assay precision. In leukemia immunophenotyping, replicates help identify stochastic variation in fluorescence intensity and gating. Example: staining two aliquots of the same bone-marrow aspirate with the same antibody panel and comparing median fluorescence intensity yields a coefficient of variation (CV) typically
Threshold Setting
Related terms: positivity cut-off, fluorescence intensity, ROC analysis
Threshold setting defines the fluorescence intensity above which a cell is considered positive for a given antigen. Determining thresholds may involve using normal donor samples to establish a baseline, then applying a statistical cut-off (e.g., mean + 2 SD). Example: CD7 expression on normal T-cells is bright; a dim $CD7^+$ population on myeloid blasts may be classified as aberrant when the threshold is set at the lower 95 % of normal T-cell intensity. Incorrect thresholds can either mask true positivity (if set too high) or generate false positives (if set too low).

Viability Dye
Related terms: live/dead discrimination, amine-reactive dye, fixable dye
Viability dyes distinguish live cells from dead or dying cells based on membrane integrity. Common dyes include 7-AAD, propidium iodide, and fixable amine-reactive dyes (e.g., Zombie NIR). Example: a fixable viability dye is added before surface staining; dead cells incorporate the dye and are excluded during

analysis, reducing background fluorescence. Failure to use viability dyes can inflate blast percentages due to non-specific binding on compromised membranes. Some dyes are incompatible with certain fluorochromes, requiring careful panel design.

Western Blot
Related terms: protein detection, immunoblotting, validation
Western blot is a laboratory technique for detecting specific proteins in a sample after electrophoretic separation. While not part of routine flow cytometric immunophenotyping, Western blot may be employed to validate the expression of intracellular antigens (e.g., BCR-ABL fusion protein) identified by flow cytometry. Example: confirming the presence of a BCR-ABL p210 band supports a diagnosis of chronic myeloid leukemia. The method is labor-intensive and less quantitative than flow cytometry, limiting its routine clinical use.

Fluorescence Minus One (FMO) Control
Related terms: gate setting, background fluorescence, multicolor panel
An FMO control contains all fluorochromes in the panel except the one of interest, providing a realistic measure of background fluorescence for that channel. FMOs are essential for accurate gate placement in complex panels where spectral spillover can elevate baseline levels. Example: an 8-color panel lacking the CD33-PE antibody yields an FMO for PE, allowing the analyst to set the $CD33^+$ gate just above the PE-only background. FMOs are more informative than isotype controls for multicolor assays but increase the number of tubes required.

Acquisition Time
Related terms: event rate, sample stability, flow rate
Acquisition time refers to the duration of sample collection on the flow cytometer. Longer acquisition enables collection of more events, improving statistical confidence for rare-cell detection. Example: acquiring 1×10^6 total events may take 5–10 minutes at a flow rate of 200 events/s. However, prolonged acquisition can lead to sample deterioration, especially if the sample is not kept cold or if the instrument's fluidics become clogged. Balancing acquisition time with sample integrity is crucial for reliable MRD analysis.

Aberrant Antigen Expression
Related terms: lineage infidelity, antigen modulation, diagnostic clue
Aberrant expression occurs when leukemic cells display antigens not typical for their lineage, such as myeloid markers on lymphoid blasts or vice versa. This pattern is a key diagnostic clue for mixed-phenotype acute leukemia (MPAL). Example: a blast population positive for both CD19 (B-cell) and MPO (myeloid) suggests MPAL. Recognizing aberrancy requires knowledge of normal maturation patterns; otherwise, normal developmental overlap may be misinterpreted as pathology. Aberrant expression can also evolve under therapy, demanding repeat immunophenotyping.

Blasts
Related terms: immature cells, nucleated cells, leukemia diagnostic criteria
Blasts are immature hematopoietic precursors that normally reside in the bone marrow. In leukemia, blasts proliferate and spill into peripheral blood, exceeding a threshold (typically >20 % of nucleated cells) for

diagnosis. Flow cytometry quantifies blasts by gating on low FSC/low SSC and evaluating antigen expression (e.g., CD34, CD117). Example: a peripheral blood sample with 30 % $CD34^+$/MPO^+ cells fulfills the morphological and immunophenotypic criteria for AML. Accurate blast enumeration is challenged by regenerative marrow after chemotherapy, where normal precursors may temporarily increase.

Chromatin Pattern

Related terms: DNA content, cell cycle analysis, nuclear staining

The chromatin pattern refers to the distribution of DNA within the nucleus, assessed by DNA-binding dyes (e.g., DAPI) in flow cytometry. While not a primary immunophenotypic parameter, DNA content analysis helps differentiate blast populations from mature cells based on cell-cycle status. Example: blasts typically have a 2N DNA content with a low side-scatter profile, whereas proliferating lymphocytes may show S-phase DNA content. Integrating DNA analysis with antigen panels can improve MRD detection specificity, though it adds complexity to the assay.

Data Analysis Software

Related terms: FlowJo, FCS Express, gating templates

Data analysis software processes raw flow cytometry files (.fcs) into quantitative reports. Modern packages offer automated gating, clustering algorithms, and MRD calculation tools. Example: using FlowJo's "Boolean gating" feature to create $CD34^+$/$CD13^+$/$CD33^+$ blast populations. Software must be validated for clinical use, with documented version control and secure data storage. A common challenge is the learning curve for advanced analytics, which can introduce user-dependent variability if not standardized.

Diagnostic Algorithm

Related terms: WHO classification, ELN recommendations, decision tree

A diagnostic algorithm integrates morphological, immunophenotypic, cytogenetic, and molecular data to classify leukemia. The WHO 2022 classification and ELN 2022 guidelines provide stepwise criteria: first assess blast percentage, then apply lineage-specific antigen panels, followed by cytogenetic testing for risk stratification. Example: a case with >20 % blasts, $CD34^+$/$CD13^+$/MPO^+ phenotype, and t(8;21) translocation is classified as AML with RUNX1-RUNX1T1. Implementing the algorithm requires coordinated laboratory workflows and clear communication with clinicians.

Event Count

Related terms: total events, rare event detection, statistical power

Event count is the total number of cells recorded during acquisition. Adequate event count is essential for detecting low-frequency populations such as MRD. For a target sensitivity of 10^{-4}, at least 500,000 total events are recommended to achieve a 95 % confidence interval. Example: acquiring 1×10^6 events yields sufficient data to identify a 0.01 % residual leukemic population. Insufficient event count reduces statistical confidence and may lead to false-negative MRD reports.

Fluorescence Intensity

Related terms: MFI, median fluorescence, antigen density

Fluorescence intensity quantifies the amount of fluorochrome bound to each cell, reflecting antigen density. Median fluorescence intensity (MFI) is commonly reported because it is less affected by outliers than mean intensity. Example: a blast population with an MFI of 2,500 for CD33 versus a normal myeloid cell MFI of 800 indicates over-expression. Comparing MFI across samples requires consistent instrument settings and compensation. Variations in MFI can result from reagent lot changes or instrument drift, emphasizing the need for regular calibration.

Gating Hierarchy
Related terms: sequential gating, logical flow, data reduction
Gating hierarchy describes the order in which populations are selected, starting from broad physical characteristics and moving toward specific antigen expression. A typical hierarchy: (1) FSC/SSC to exclude debris, (2) CD45 vs SSC to identify leukocyte subsets, (3) CD34 to isolate blasts, (4) lineage markers (e.g., CD13, CD19) to assign lineage. This systematic approach reduces analysis bias and ensures that each subsequent gate is applied to a defined parent population. Deviations from a standard hierarchy can lead to inconsistent results between analysts.

Hematopoietic Stem Cell (HSC) Marker
Related terms: CD34, CD117, progenitor cell
HSC markers identify early progenitor cells capable of multilineage differentiation. CD34 is the most widely used HSC marker; CD117 (c-Kit) is also expressed on early myeloid precursors. In leukemia, blasts often retain HSC markers, aiding in distinguishing them from mature cells. Example: a population expressing $CD34^{+}/CD117^{+}/MPO^{+}$ is indicative of AML. However, normal regeneration after chemotherapy can produce $CD34^{+}$ cells, requiring additional markers (e.g., HLA-DR, CD13) to differentiate benign from malignant progenitors.

Instrument Linearity
Related terms: dynamic range, calibration beads, fluorescence scaling
Linearity refers to the proportional relationship between fluorescence intensity and antigen density across the detector's dynamic range. A linear instrument accurately reflects changes in antigen expression from low to high levels. Calibration beads with known fluorescence units are used to assess linearity; deviations indicate detector gain issues or laser instability. Non-linear response can distort MFI measurements, compromising quantitative comparisons across samples. Regular verification of linearity is part of quality-control protocols.

Leukemia-Associated Immunophenotype (LAIP)
Related terms: MRD tracking, abnormal antigen pattern, patient-specific signature
A LAIP is a unique combination of antigen expression that distinguishes leukemic cells from normal counterparts in a given patient. LAIP identification at diagnosis enables sensitive MRD monitoring by focusing on the same aberrant pattern during follow-up. Example: a patient's AML cells may show $CD34^{+}/CD13^{+}/CD56^{+}$, a pattern not present in normal marrow, allowing targeted MRD detection. LAIP-based MRD can achieve sensitivities comparable to molecular methods, but antigen modulation during treatment may alter the LAIP, necessitating re-assessment.

Multiparametric Flow Cytometry
Related terms: multi-color analysis, simultaneous antigen detection, data complexity
Multiparametric flow cytometry (MFC) evaluates multiple fluorescence parameters per cell, enabling comprehensive immunophenotyping. Modern cytometers can measure 10–20 colors simultaneously,

allowing detailed lineage and maturation assessment. Example: an 10-color AML panel can resolve subpopulations such as $CD34^+/CD117^+/MPO^+$ blasts, $CD34^-/CD13^+/CD33^+$ mature myeloid cells, and residual normal progenitors. The increased data complexity requires robust analysis pipelines and careful panel design to avoid spectral overlap and compensation errors.

Normalization
Related terms: inter-run variability, bead-based scaling, data harmonization
Normalization adjusts fluorescence values across different runs or instruments to enable comparability. Bead-based scaling uses reference beads with known fluorescence to calculate a conversion factor applied to sample MFI values. Example: normalizing CD33 MFI from two different days using the same bead standard reduces inter-run variability from 15 % to Quality Assurance (QA)
Related terms: proficiency testing, SOP compliance, documentation
QA encompasses systematic activities to ensure that immunophenotyping results are reliable and reproducible. This includes participation in external proficiency testing programs, adherence to standard operating procedures, and thorough documentation of reagent lot numbers, instrument settings, and analyst signatures. Example: a laboratory that scores >90 % on EuroFlow proficiency panels demonstrates strong QA practices. QA challenges involve maintaining consistency amid staff turnover and evolving technology, requiring ongoing training and audit cycles.

Sample Preparation
Related terms: RBC lysis, fixation, permeabilization
Proper sample preparation preserves antigenicity while removing interfering components. Red blood cell (RBC) lysis removes hemoglobin that can quench fluorescence. Fixation stabilizes cell morphology for delayed analysis; however, some fluorochromes are sensitive to fixation, necessitating compatible reagents. Permeabilization enables intracellular staining (e.g., MPO, cytoplasmic CD3). Example: a standard protocol may use ammonium chloride lysis, followed by fixation with 1 % paraformaldehyde, then permeabilization with saponin for intracellular antigens. Inconsistent preparation can lead to variable antigen expression and compromised data quality.

Standard Operating Procedure (SOP)
Related terms: protocol, workflow, regulatory compliance
An SOP is a documented set of instructions that standardizes each step of the immunophenotyping process, from specimen receipt to data reporting. SOPs ensure that all personnel follow the same methodology, reducing variability. Example: an SOP may specify the exact antibody volumes, incubation times, washing steps, and instrument settings for an AML panel. Regular review and updating of SOPs are required to incorporate new reagents, instrument upgrades, and guideline revisions. Non-adherence to SOPs can jeopardize accreditation status.

Technical Sensitivity
Related terms: limit of detection, assay precision, event acquisition
Technical sensitivity defines the smallest number of leukemic cells that can be reliably detected by the assay under ideal conditions. It depends on factors such as fluorochrome brightness, number of events collected, and background noise. For flow cytometry MRD, a technical sensitivity of 10^{-4} is achieved by acquiring

≥500,000 events and using a bright panel. Example: a laboratory that consistently detects 0.01 % residual blasts demonstrates a technical sensitivity of 10^{-4}. Limitations include instrument stability and sample quality, which can reduce achievable sensitivity.

Turnaround Time (TAT)
Related terms: reporting latency, workflow efficiency, clinical impact
Turnaround time is the interval from sample receipt to result delivery. In acute leukemia, rapid TAT (Viability Assessment
Related terms: live/dead staining, apoptosis detection, data gating
Viability assessment determines the proportion of live cells in a specimen, influencing data quality. Viability dyes (e.g., 7-AAD) are applied before antibody staining; dead cells exhibit high fluorescence and are excluded during analysis. Example: a sample with >20 % dead cells may require repeat collection or additional washing steps. High dead-cell content can increase non-specific binding, elevate background, and obscure rare blast detection, making viability assessment an essential pre-analytic checkpoint.