INTERNAL TRAINING

21 CFR Compliance in Flow Cytometry

What the regulations actually require at the instrument, and how a flow result is made defensible before anyone asks for it.

Flow Cytometry Group | GLP and GMP Analytical Operations

ORIENTATION

Why this training exists

Data integrity findings are among the most frequently cited deficiencies in FDA inspections of regulated laboratories. Almost none of them are caused by bad science. They are caused by results that cannot be reconstructed.

1

The standard is reconstruction

An inspector does not ask whether you believe the result. They ask you to rebuild it from the record, without your memory.

2

Compliance is designed in, not added

Controls applied after acquisition cannot repair a record that was incomplete at the moment it was created.

3

The instrument is a regulated system

The cytometer workstation is a computerized system holding GxP records. It carries the same obligations as any other.

The test that matters

Pick any released result from the last twelve months.

- Who acquired it, and when
- Which instrument settings were active
- Which template and version analyzed it
- Every change made after acquisition
- Who reviewed it, and against what

If any line takes searching, that is the finding.

FRAMEWORK

Which regulation governs which run

The same instrument produces records under different regimes depending on what the run supports. Know which one applies before you acquire.

21 CFR Part 58

Good Laboratory Practice

Nonclinical safety studies. Study director accountability, protocol adherence, and an independent quality assurance unit.

21 CFR 210 / 211

Current Good Manufacturing Practice

Manufacturing and the QC testing that releases product. Complete laboratory records under 211.194, equipment controls under 211.68.

21 CFR Part 1271

Human Cells and Tissues

HCT/P requirements including donor eligibility and tracking, layered on cell therapy operations.

21 CFR Part 11

Electronic Records and Signatures

Cuts across all of the above. Applies whenever a record required by those rules is created, modified, or stored electronically.

Part 11 is not a separate obligation. It is how the predicate rule requirements apply once the record is electronic.

PART 11

Part 11, in one slide

What it covers

- Electronic records that a predicate rule requires you to keep
- Electronic signatures applied to those records
- The systems that create, modify, maintain, or transmit them

The trigger

Part 11 attaches to the record, not to the software. If a Part 58 or Part 211 record lives electronically, Part 11 controls apply to it. A vendor claim of compliance covers the tool, never your use of it.

Closed system: access controlled by the people responsible for the records. Open system: it is not, and encryption plus digital signature standards come into play.

1

Validation

The system does what you say it does, and detects invalid or altered records.

2

Audit trail

Secure, computer-generated, time-stamped. Records who, what, when, and prior values. Never obscures the original entry.

3

Access control

Unique accounts. Authority checks so only authorized people acquire, alter, or sign.

4

Accurate copies

You can produce complete copies in human-readable and electronic form for inspection.

5

Record retention

Records remain retrievable and readable for the full retention period.

APPLICATION

What that means at the cytometer

Every control above has a concrete form on the acquisition workstation. These are the ones that get cited.

1 Named accounts only

Each operator logs in as themselves. A shared workstation login makes every record on that instrument unattributable, which is the single fastest way to invalidate a data set.

2 Audit trail enabled and reviewed

Enabled is half the requirement. The trail has to be reviewed as part of record review, and the review has to leave its own evidence.

3 Controlled clock

System time is synchronized and users cannot change it. Contemporaneous means nothing if the timestamp is editable.

4 Signature meaning is explicit

An electronic signature carries the printed name, date and time, and what the signing means: authorship, review, or approval.

5 Authority checks

Acquisition, reanalysis, template modification, and approval are distinct permissions. Not everyone should hold all four.

6 Nothing local and undeclared

Data written to a desktop folder outside the controlled path is still a GxP record. Undeclared copies are findings waiting to happen.

DATA INTEGRITY

ALCOA+

The vocabulary regulators use to describe what a trustworthy record looks like. Learn it as nine questions asked of every data point.

A — Attributable
Who did it

L — Legible
Readable and permanent

C — Contemporaneous
Recorded as it happened

O — Original
The first capture, or a certified copy

A — Accurate
Correct, and errors corrected visibly

+ — Complete
Nothing omitted, including failures

+ — Consistent
Sequenced and dated coherently

+ — Enduring
Survives the retention period

+ — Available
Retrievable on demand

Reference: FDA guidance, Data Integrity and Compliance With Drug CGMP, Questions and Answers, December 2018.

APPLICATION

ALCOA+ applied to one flow run

Attributable	Named operator login on the acquisition workstation, not a shared instrument account.
Contemporaneous	Sample and reagent detail entered at the bench during the run, not reconstructed after the plate is done.
Original	The acquired FCS file is the raw record. An exported statistics table is a derivation of it, never a replacement for it.
Accurate	Instrument QC passing on the day of acquisition, with reference material and settings verified before samples are run.
Complete	Aborted, repeated, and failing runs stay in the record with a documented reason. Deleting a run is the finding.
Consistent	Acquisition, analysis, and review carry a coherent, controlled sequence of timestamps.

The record is the run. Everything downstream is derived from it and inherits its defects.

CONTROLS

Audit trails: enabled is not the same as compliant

What it must capture

- The identity of the person taking the action
- The date and time, from a controlled clock
- What was changed, and the value before the change
- The reason, where the change alters a reported result
- Creation, modification, and deletion events alike
- A form that never overwrites the original entry

Where it goes wrong

- Trail available but never reviewed, with no evidence of review
- Reviewed only when a result looks wrong, which is selective review
- Shared logins that make every entry unattributable
- Administrator rights held by the analysts who generate the data
- Reanalysis performed with no recorded reason for the change
- Trail retained for less time than the record it documents

Audit trail review belongs in the same procedure as data review, with a defined frequency and a documented reviewer.

RECORDS

Raw data: what it is, and what it is not

The FCS file is the raw record.

It holds the listmode events and the acquisition metadata: instrument, settings, operator, timestamp, and the parameters in force when the sample was collected. Everything reported downstream is an interpretation of that file, and cannot be more reliable than it is.

Retain	Listmode files, instrument QC records, settings and compensation or unmixing matrices, analysis templates, and the audit trail covering all of them.
Never delete	Aborted runs, repeats, and failures. A deleted run is an incomplete record, and completeness is not optional.
Justify in writing	Any exclusion of events, samples, or runs from a reported result, recorded at the time and reviewed by a second person.

Retention

Retention runs to the predicate rule, not to convenience or disk capacity.

1 **Readable**

The file must open in a qualified application for the whole retention period, including after a software upgrade.

2 **Backed up**

Backups verified by restore, not assumed from a green status light.

3 **Migrated with care**

A format conversion is a change. It needs assessment, verification, and a record.

THE HARD PART

Gating is where judgment enters the record

Acquisition is largely mechanical. Analysis is not. Gating is an interpretive decision applied to regulated data, which is exactly why it has to be controlled rather than trusted.

Control the template

- Analysis templates are controlled documents with versions.
- A template change is a change control event with a rationale.
- The template version is recorded against every result it produced.

Constrain the judgment

- Gate placement follows a defined, written strategy, not operator preference.
- Reference and control materials anchor placement objectively.
- Where a call is borderline, the basis is documented, not silently resolved.

Make reanalysis visible

- Reanalysis is permitted, concealment is not.
- Every reanalysis carries who, when, why, and what changed.
- The original analysis remains retrievable alongside the revision.

A result nobody else can reproduce from the same file is an opinion, and an opinion is not a release decision.

FIT FOR PURPOSE

Qualification and validation

A compliant record of an unfit method is still an unfit method. Part 11 governs the record. ICH Q2 governs whether the measurement deserves to be believed.

Specificity

The measured population is the intended one, resolved from what surrounds it.

Accuracy

Agreement with a reference or an orthogonal method.

Precision

Repeatability within a run, and intermediate precision across operators, days, and instruments.

Linearity and range

The interval over which the response tracks the true value, with limits stated.

Limits

Detection and quantitation limits, which matter most for rare populations.

Robustness

Behavior under small deliberate variations, including the ones that occur in practice.

Qualification asks whether the instrument is installed and operating as intended. Validation asks whether the method answers the question you are asking of it. An inspector will expect both, and will expect you to know which is which.

GOVERNANCE

Who may do what

Separation of duties under GLP

21 CFR 58.35(a) requires the quality assurance unit to be entirely separate from and independent of the personnel directing and conducting a given study.

In practice: the same person may serve as study director on one study and in the quality assurance unit on another. Never both on the same study. Say so plainly before anyone has to ask.

The reviewer cannot be the author.

Second-person review exists to catch what the first person could not see. Self-review satisfies a checkbox and nothing else.

1	**Acquire**	Trained operator with a named account and current qualification.
2	**Analyze**	Trained analyst applying a controlled template version.
3	**Modify a template**	Restricted role, under change control with recorded rationale.
4	**Review**	Independent of the person who generated the result.
5	**Approve or release**	Designated authority, signature meaning explicit.
6	**Administer the system**	IT or system owner. Never held by the analysts producing the data.

LEARN FROM OTHERS

Findings that recur in flow cytometry laboratories

1	**Shared instrument login**	Every record on that workstation becomes unattributable at once. The finding is rarely limited to one run.	High
2	**Audit trail never reviewed**	The control existed and nobody used it, which reads as a quality system that does not function.	High
3	**Undocumented reanalysis**	A result changed and the record does not say why. This is the pattern investigators treat as potential data manipulation.	High
4	**Uncontrolled analysis templates**	Templates edited freely, so two analysts produce two answers from one file and both are defensible.	Medium
5	**Orphan and deleted runs**	Aborted or failing acquisitions absent from the record. Completeness fails, and intent gets questioned.	High
6	**Local copies outside the controlled path**	Data on a desktop or a shared drive nobody declared, discovered by an inspector rather than disclosed.	Medium

None of these are scientific failures. Every one is a records failure, which is what makes them avoidable.

READINESS

What an inspector will actually ask

The requests, in the order they usually come

1. Show me the procedure that governs this assay.
2. Show me the training record for the person who ran it.
3. Show me the instrument qualification and the QC for that day.
4. Open the raw file and walk me from it to the reported number.
5. Show me the audit trail for this record, and your review of it.
6. Show me a run that failed, and what you did about it.

The honest standard

Readiness is not a state you enter before an inspection. It is whether the records you created on an ordinary Tuesday hold up when someone reads them two years later, without you in the room.

Before you leave the bench

- Entries made now, not later
- Deviations captured while fresh
- Failures recorded, not discarded
- Template version noted
- Nothing saved outside the controlled path

CLOSE

Five things to carry back to the bench

1	**Attribution is everything**	Named logins, always. Shared accounts undo an entire data set in one step.
2	**Write it as it happens**	Contemporaneous entries are not a formality. They are the only ones that can be defended.
3	**Keep the failures**	Completeness includes the runs you wish had gone differently. Deleting one changes the question from quality to integrity.
4	**Version the judgment**	Templates and gating strategies are controlled documents, because they decide the answer.
5	**Build for the reader in two years**	The record has to work without you standing next to it explaining what you meant.

Questions, and the procedures referenced here, through the Quality and Analytical Development groups.