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Comet-Assay Data Interpretation

Practical 25 - Biological teaching-data practical

Aim

To quantify supplied comet-assay images with a documented scoring rule.

Principle

Single-cell gel electrophoresis assesses migration of DNA under the selected assay conditions. The alkaline and neutral methods have different sensitivities; image metrics are assay-dependent and do not alone identify the damage mechanism.

Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Review supplied approved-system images and the exact assay variant, controls and image-quality criteria.
3. Select fields by a predeclared rule, blind scoring where possible and exclude artifacts only with documented reasons.
4. Measure the prescribed metrics consistently and summarise cells within independent biological replicates.
5. Compare groups with an analysis respecting the replicate structure and discuss cytotoxicity and handling artifacts.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation / control	Condition / method	Raw observation	Calculated / interpreted result
Negative / vehicle control	Specify	Record	Evaluate
Positive / reference control	Specify	Record	Evaluate
Test replicates	Specify	Record	Calculate

Calculations

- Tail DNA (%) = tail fluorescence / total comet fluorescence x 100

Worked Example

Illustrative total signal 1,000 units and tail signal 250 give 25% tail DNA.

Scoring 100 cells from one preparation does not create 100 independent biological replicates.

Result and Interpretation

- Provide representative full-field images, scoring rules, control results and replicate-level summaries.
- Report biological and technical replication separately and explain control performance.

Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

Viva Questions and Answers

What does tail DNA express?

The proportion of measured fluorescence in the defined tail.

Why avoid cell-level pseudoreplication?

Cells from one preparation are not independent experimental preparations.

Does the metric identify a mutation?

It measures assay-defined DNA migration, not a specific mutation.

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