

In flow cytometry, does results harmonization require the same equipment? Case of cell viability.



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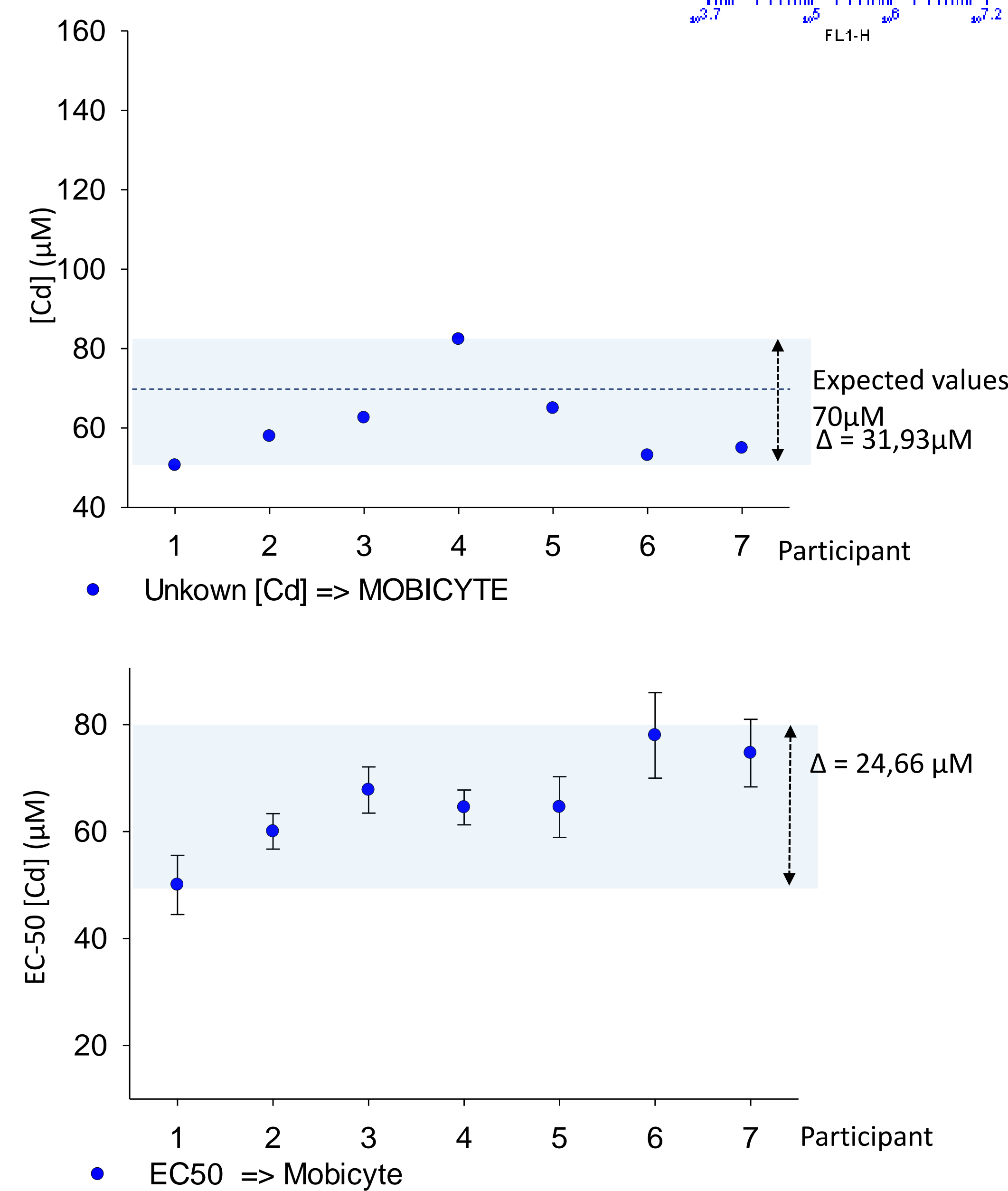
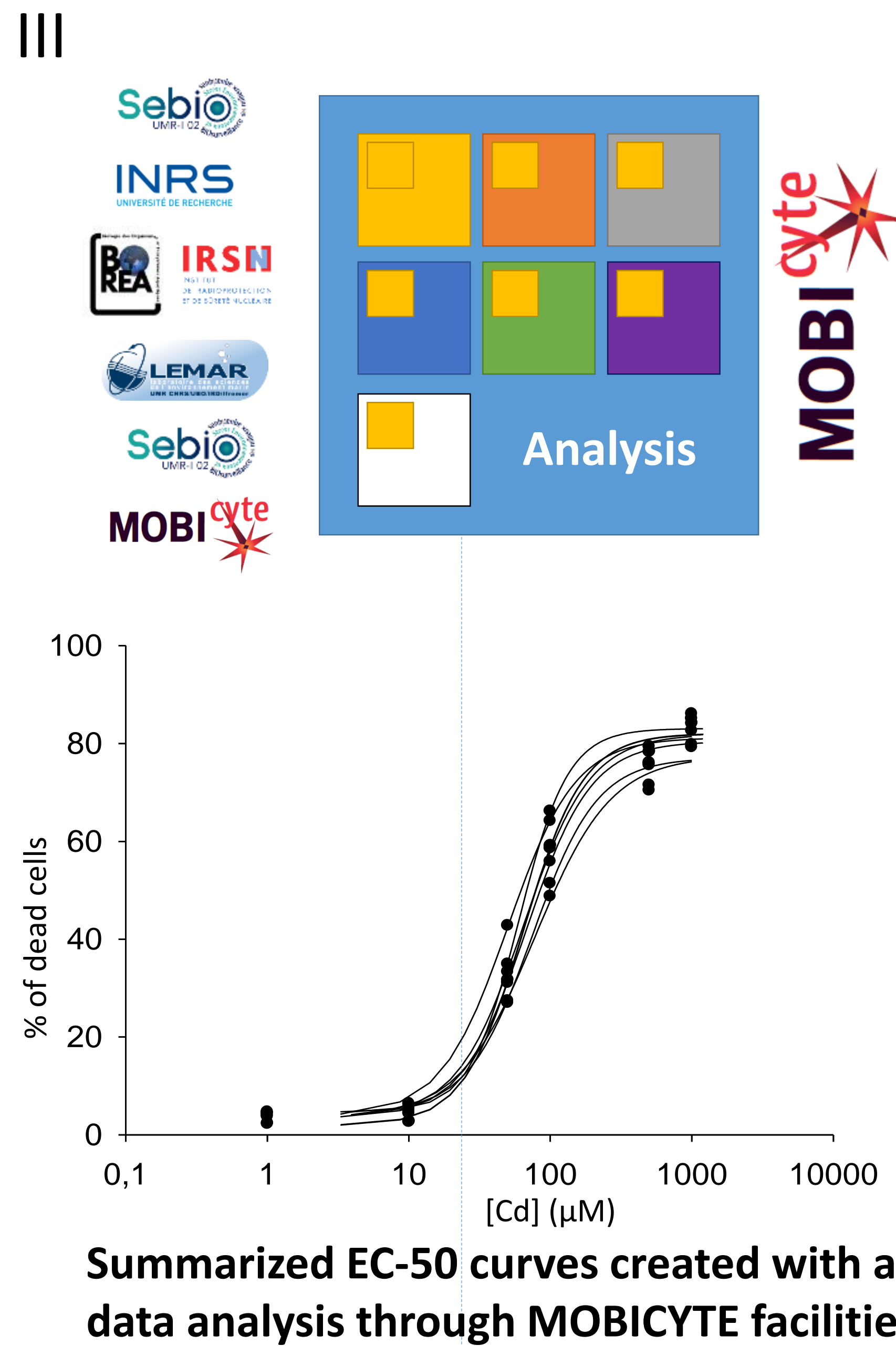
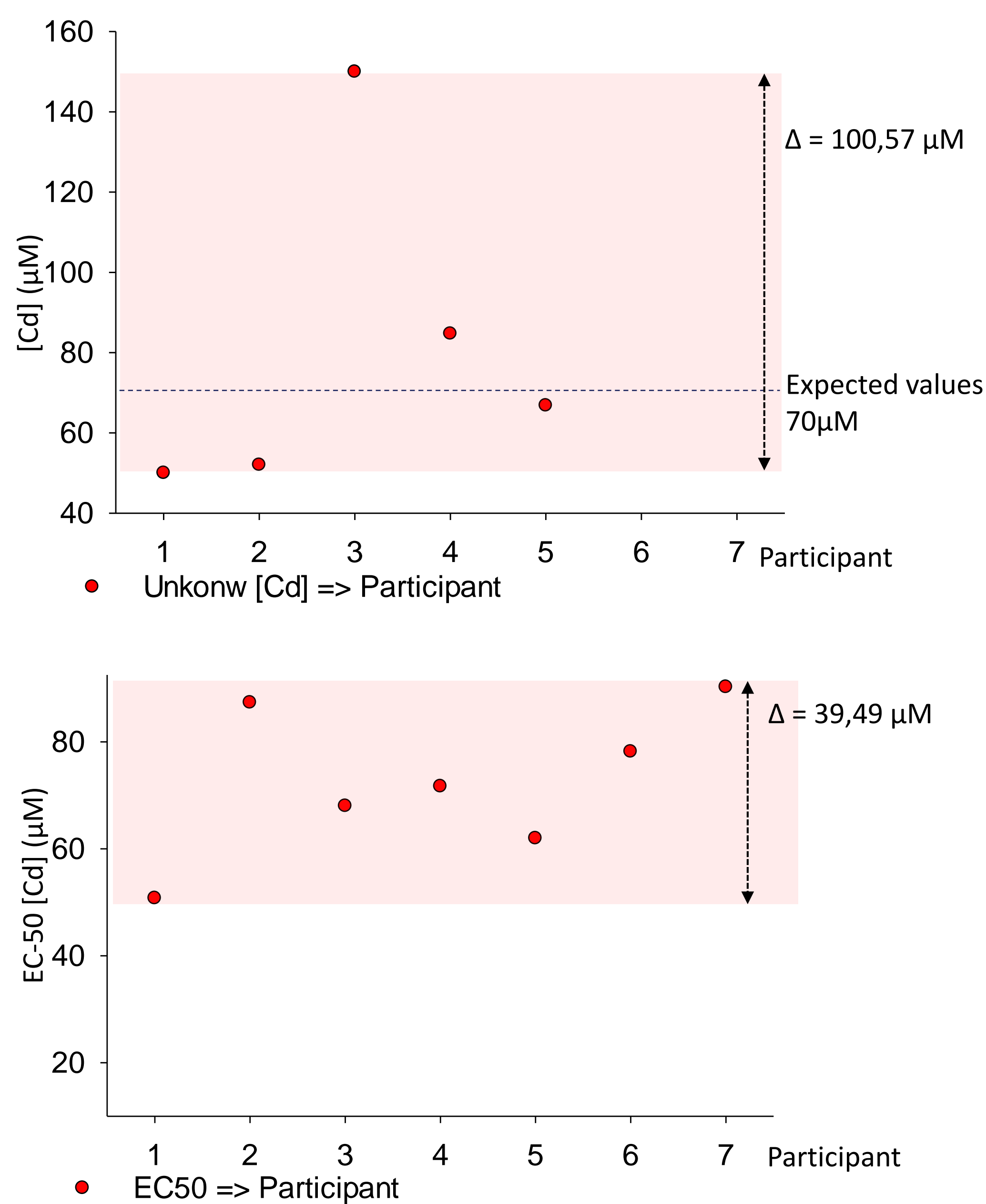
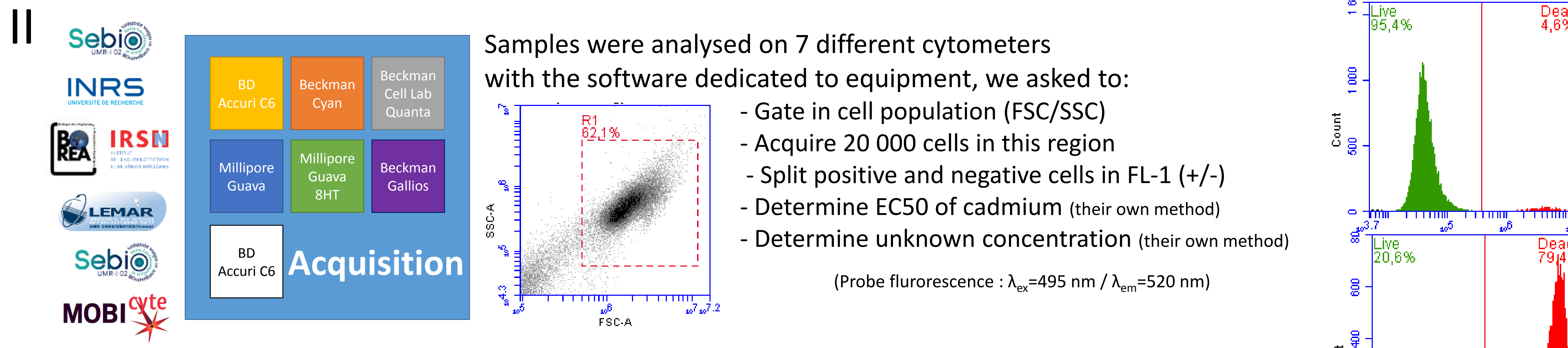
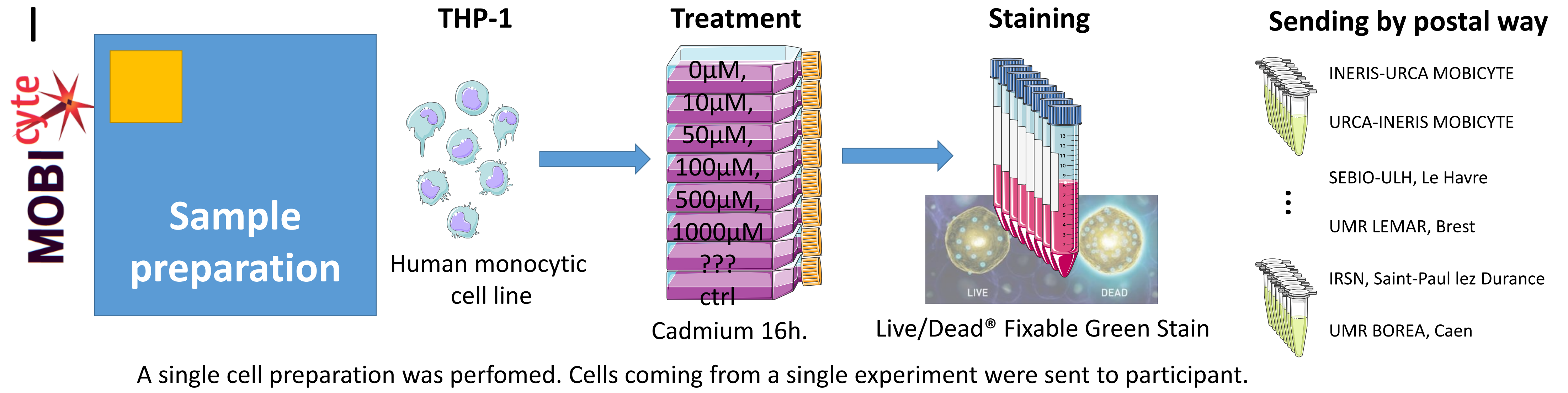
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Flow cytometry allows analysis of cellular parameters or functions in order to obtain single-cell and populational data with a statistical dimension. University of Reims and INERIS, have set up a mobile environmental flow cytometry facility: MOBICYTE. This platform is dedicated to applications in ecotoxicology, in functional ecology and in integrative ecophysiology.

Many cellular functions can be studied by flow cytometry by using a wide variety of commercialised fluorescent probes. As many research teams work on identical cellular responses (*i.e.* cytotoxicity tests), variations of protocols and methodologies for cytometric analyses can lead to misinterpretations with consequences for toxicological risk assessment.

In this context, we have estimated the ability of different groups to assess cadmium EC-50 with a same sample set by the use of flow cytometry technique. This inter-lab calibration implicated different flow cytometry equipment.



RAW data analyzed by each participant with their own equipment. Gating and data extraction were performed on flow cytometer dedicated software. In many cases, EC-50 values were determined with Excel™ macro RegTox.

RAW data analyzed by MOBICYTE. After acquisition in each lab, RAW data (FCS files) were sent to Mobicyte. Gating and analysis were performed with FlowJo© (FlowJo, LLC). EC50 and unknown concentration were determined with SigmaPlot© (Systat Software Inc.)

Some variabilities identified After discussion with partners, it turns out that the main source of variability was in post-acquisition analysis. This step is the one involving the most intervention of man.

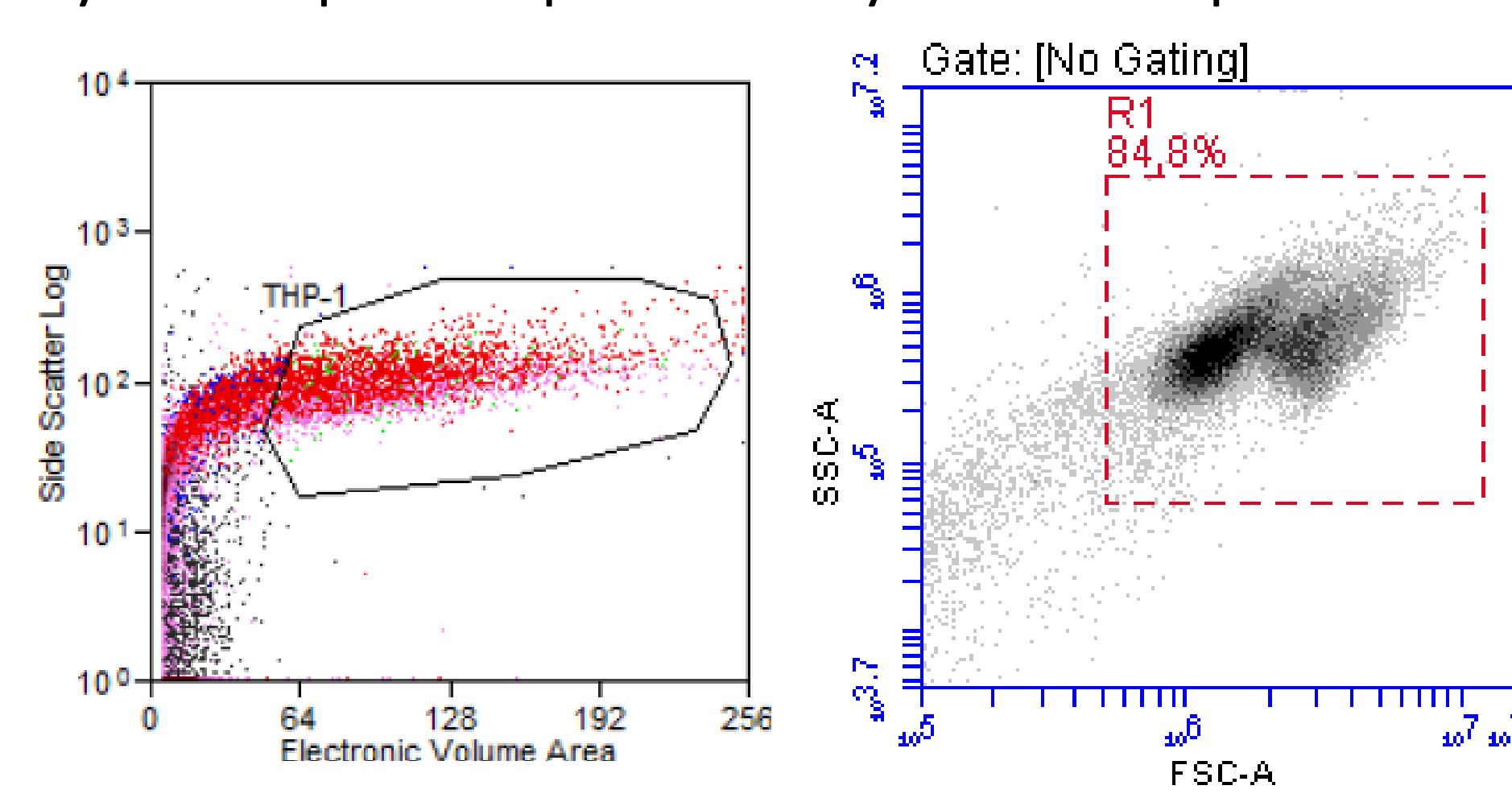
Excel™ Macro RegTox

| Participant | 1 | 2 | 4 | 5 | 6 | 7 |
|-------------------------|-------|--------|-------|-------|--------|--------|
| Reasoning on live cells | 51,54 | 111,34 | 85,21 | 97,43 | 161,08 | 203,84 |
| Reasoning on dead cells | 50,79 | 65,63 | 61,96 | 71,94 | 78,18 | 90,27 |

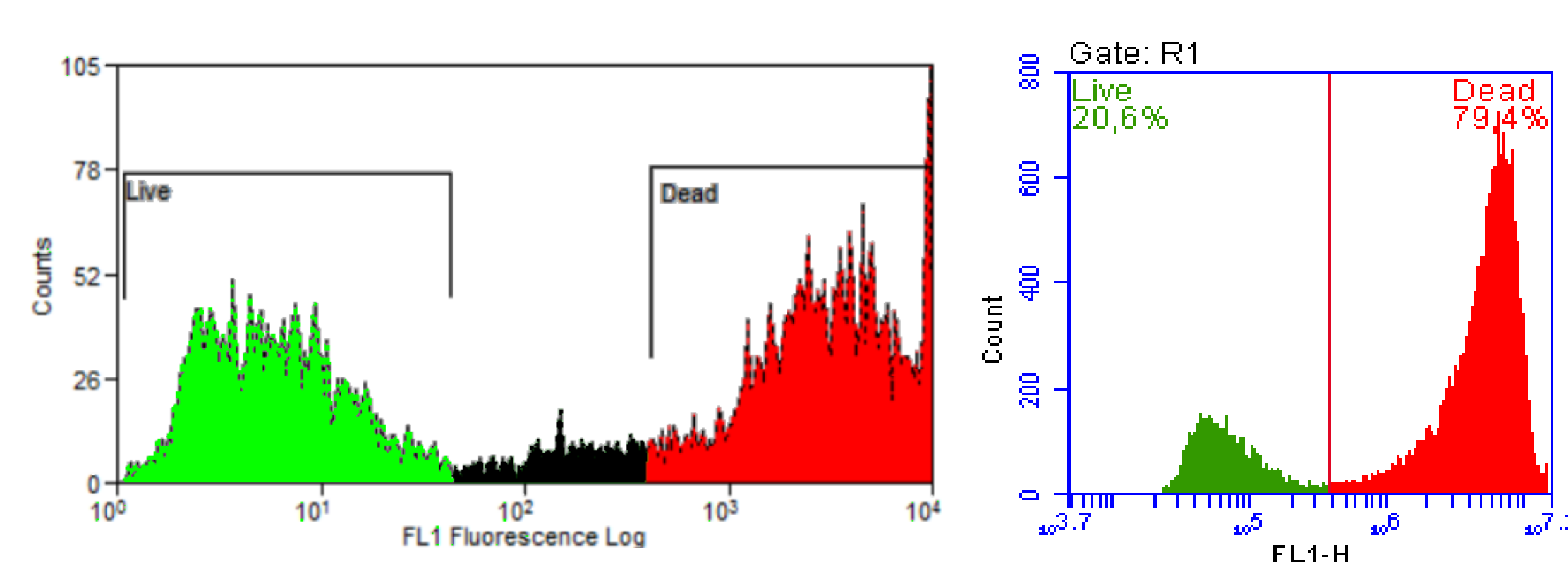
Values of EC-50 (μM) determined with RegTox. Application on live cells or dead cells give different values of EC-50.

Gating strategies on:

- Cell population (expressed in Log or Lin)



- Positive/negative cells (gaps between 2 regions)



Conclusion: Despite the diversity of flow cytometers, results were rather homogeneous. Variability lies mainly in the post-acquisition analysis (human intervention). To analyze cytotoxicity data, some conclusions and recommendations can be listed:

- No need of specific equipment
- Acquire enough cells (20 000 events on cells gate).
- Be binary and empirical in viability analysis.
- If probe detects dead cells, work only with dead cells percentage.

