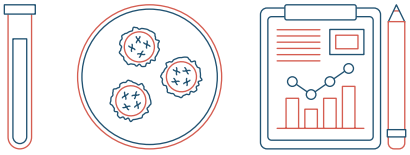
Development and evaluation of *in vitro* methods

When developing an *in vitro* method the process should include evaluation of reliability and relevance for the intended use

All *in vitro* methods benefit from a meticulous development and evaluation process with proper documentation to ensure reproducibility and facilitate use by others

Methods that are to be used in regulatory risk assessment need to be evaluated further through formal validation

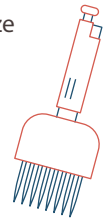


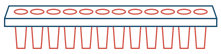
Guidances for method development

- GCCP and GCCP 2.0[1]
- OECD Guidance on GIVIMP[2]
- OECD Guidance on Validation[3]

Critical steps

- Evaluation of method relevance and ability to predict biological endpoint(s)
- Validation of measurement endpoints e.g. linearity, dynamic range, etc.
- Evaluation of possible interferences (over-bleeding, absorption, auto-fluorescence, etc.)
- Plate layout design to minimize possible errors and to include control and reference items
- Ensuring that all equipment is functional and calibrated/ verified to be fit-for-purpose





Describe the method carefully and in a detailed way so that others can use it

Evaluate method performance in another lab

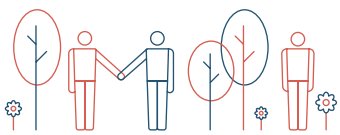
Increased confidence in method

Adapt the method for similar research questions

Evaluate method performance for additional test chemicals/ materials

Publish protocols for use within the research community

Methods useful for regulatory risk assessment



Methods that are to be used in regulatory risk assessment need to be formally validated to assess their reliability and relevance for the intended use

Development and evaluation of *in vitro* methods

Formal validation

Formal validation is a rigorous, science-based evaluation to establish performance, i.e. robustness and reproducibility, and fitness for a given purpose, i.e. scientific validity

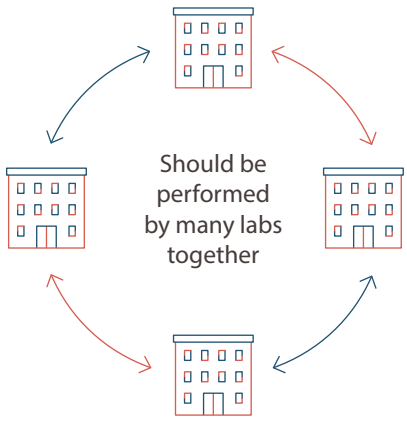
The validation study should be performed according to OECD Guidance on Validation[3]

EURL ECVAM[4] is responsible for validation within the EU

There are two steps in a validation study

Step 1

Experimental generation of quality assured data



Determines among other things:

- Within-laboratory reproducibility
- Transferability – that another lab can obtain the same results
- Between-laboratory reproducibility
- Predictive capacity – how well the method predicts the biological endpoint
- Applicability domain – what kind of samples can be evaluated with the method

Step 2

Independent scientific peer review

Publication of the results

OECD and ISO work globally to standardize and harmonize guidelines and ensure reliable data

International acceptance through adoption as OECD Guideline or ISO standard

Submission for uptake as an international guideline or standard

- Assessment
- Peer review
- Commenting

1. Good Cell Culture Practice from Coecke *et al.* 2005 ATLA and Pamies *et al.* 2021 ALTEX
2. OECD Guidance Document No. 286 on Good In Vitro Method Practices (GIVIMP)
3. OECD Guidance Document No. 34 on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment
4. EU Reference Laboratory for alternatives to animal testing