Chapter 11
Quality assurance and quality control (QA/QC)

This presentation accompanies Chapter 11 of
“Bioanalytical Tools in Water Quality Assessment”
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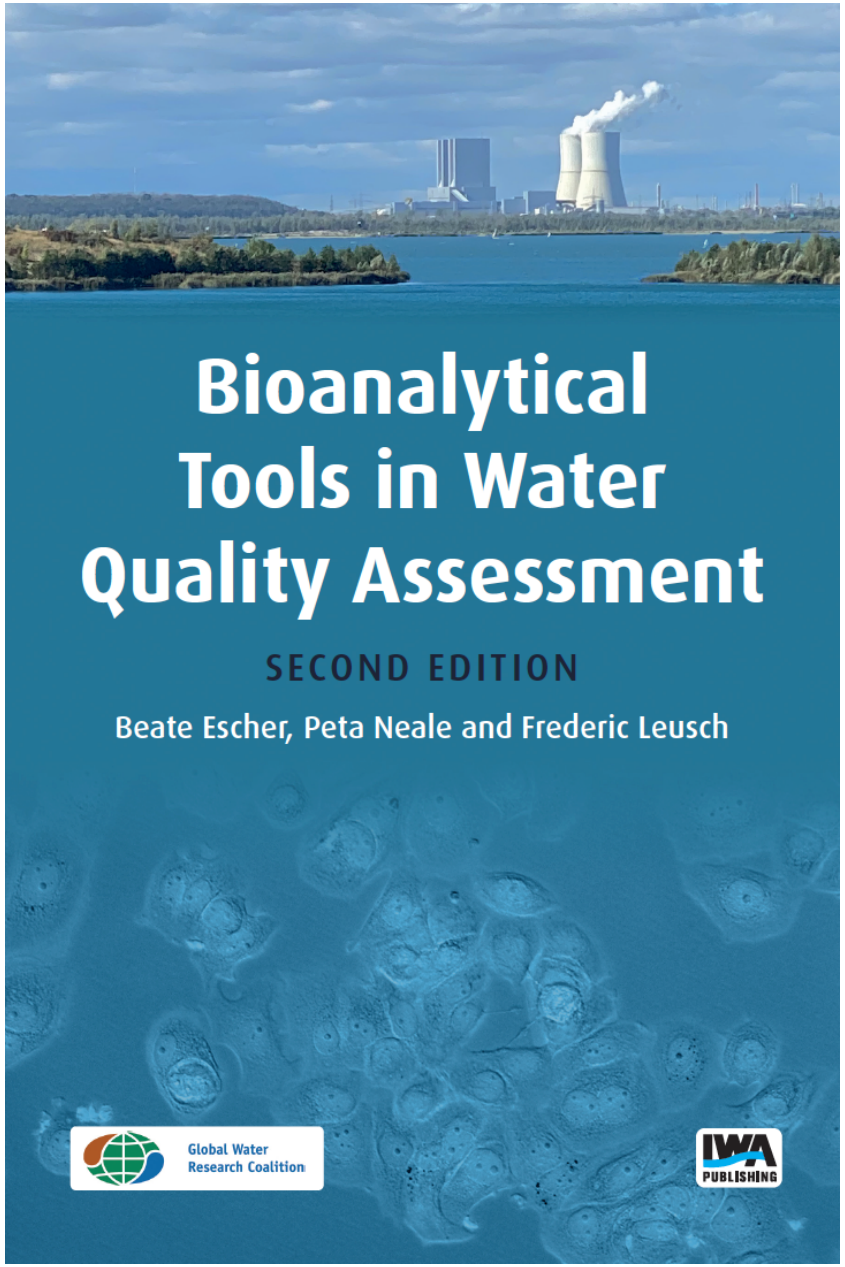
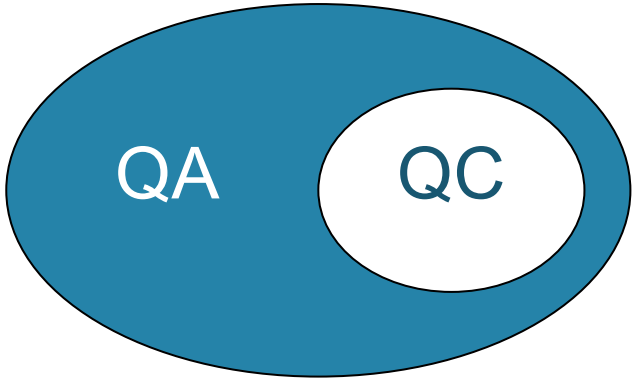
Exercises and more material can be found at
www.ufz.de/bioanalytical-tools.

For questions please send e-mail to bioanalytical-tools@ufz.de

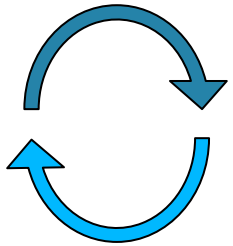


Quality assurance & quality control



QA … Quality Assurance

Develop and apply protocols for method validation



Permanent improvement required

QC … Quality Control

Confirm previously defined requirements and give feedback in case of failure

Learning goals QA/QC

- initially developed in the manufacturing industry as a set of documents and procedures to ensure consistent product quality
- In the laboratory, the application of QA/QC principles ensures that bioassay results are accurate and consistent
- Minimum laboratory QA/QC:
  - Replicate analysis,
  - inclusion of adequate positive + negative controls
  - verification of assay performance with control charts and fixed control criteria
  - good record-keeping
  - written SOPs in place

- Accuracy
- Precision
- Robustness
- Quality
- Matrix interference
- Sensitivity

Accuracy and precision

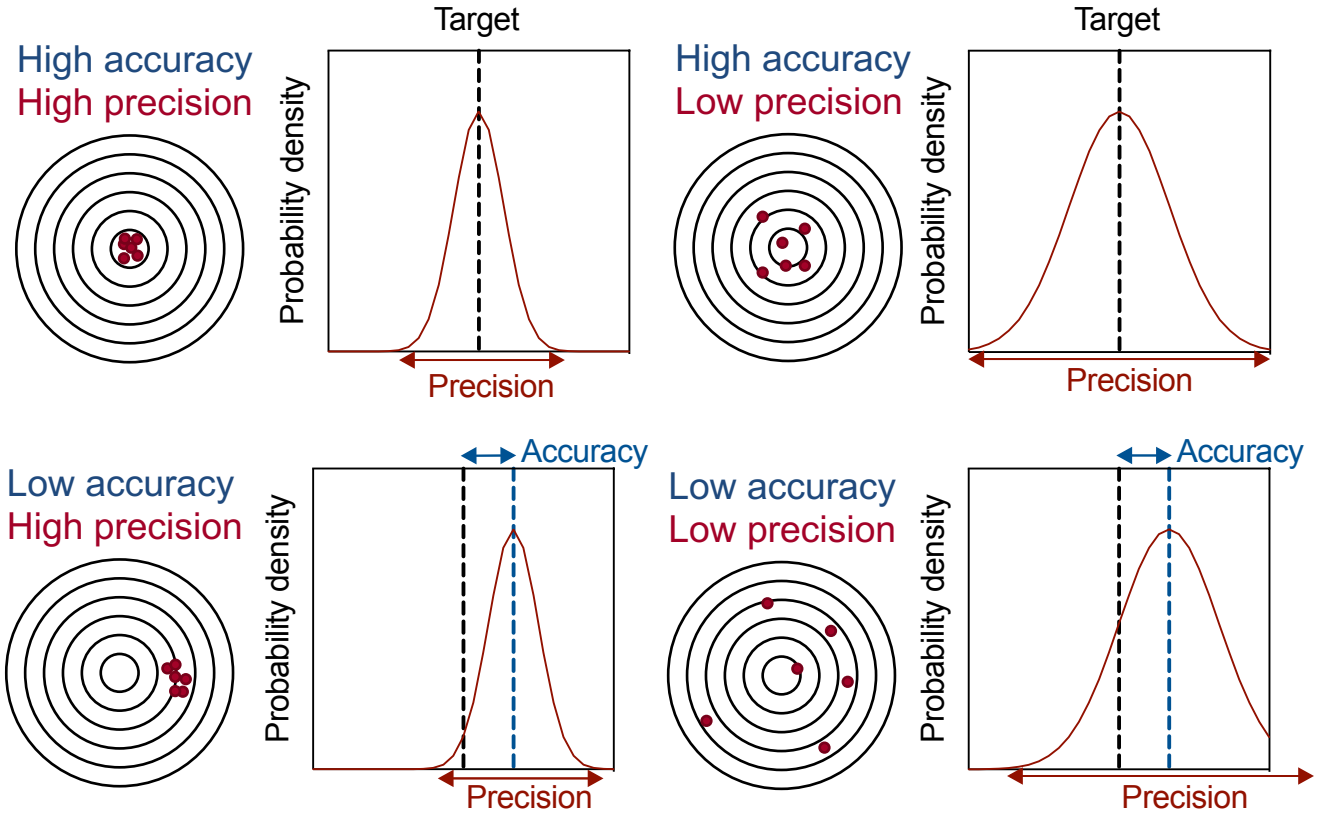
Accuracy in bioassays

How close a bioassay result is to the true value ?

- Replicate analysis of samples (reference compound) including at least three different concentrations
- mean value in an accurate *in vitro* assay should be within 15-20% of the actual value

Precision in bioassays

= closeness of repeated individual measures of the same sample



Precision

Repeatability = when the same person taking multiple measurements on the same item or characteristic gets the same result every time

Coefficient of variation for repeatability $CVr = (\sigma_r / \mu_r)$

Reproducibility = when other people (or other instruments or labs) get the same results you get when measuring the same item or characteristic

Coefficient of variation for reproducibility $CVR = (\sigma_R / \mu_R)$

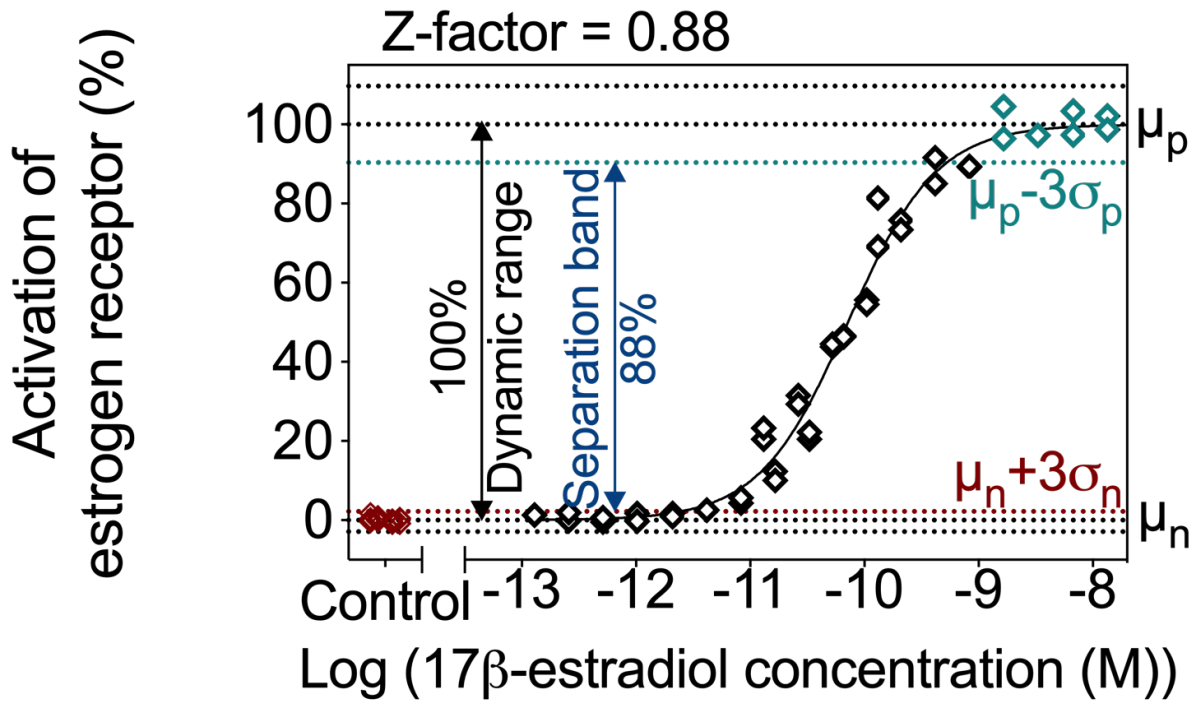
Robustness characterises the sensitivity of a method to operational variations and is a measure of how transferrable the method is to other operators and/or laboratories (ideally close to 1)

$$\text{Robustness index} = \frac{CVr}{CVR}$$

Quality

$$Z\text{-factor} = 1 - \frac{3 \times (\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

μ_p is the mean and σ_p is the standard deviation of the positive control, and μ_n is the mean and σ_n is the standard deviation of negative controls.



Z-factor between 0.5 and 1.0 is an excellent assay
A Z-factor between 0 and 0.5 is marginal

Selectivity

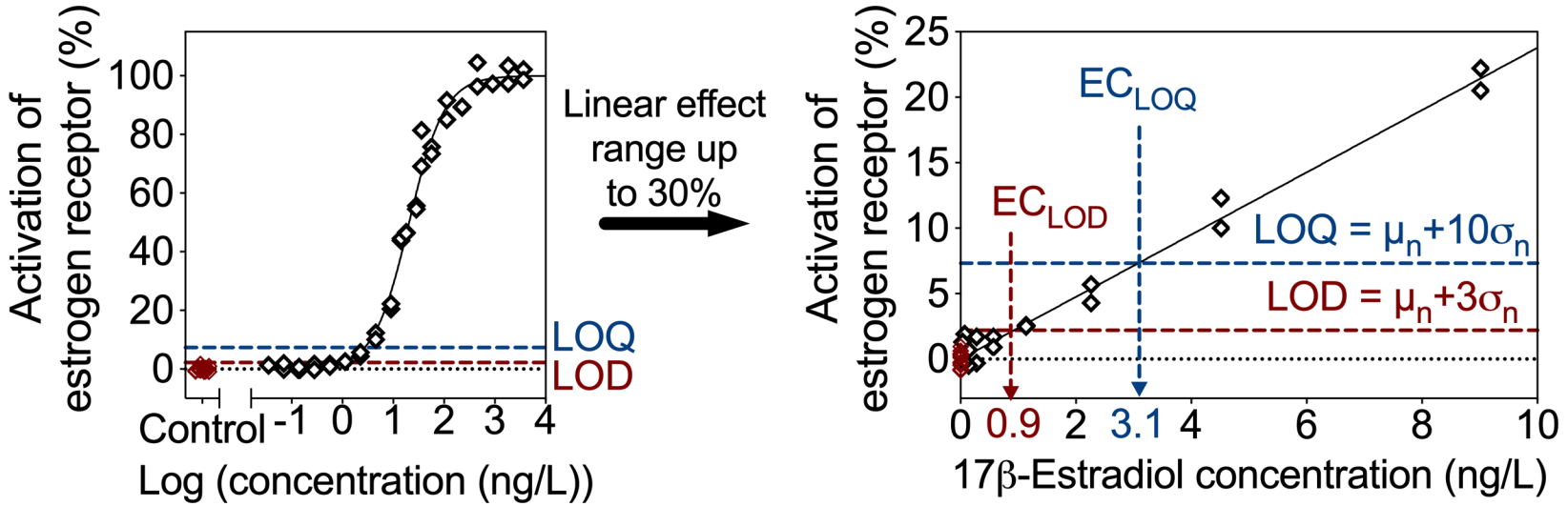
= measure of matrix interference

- characterises how much the analysis is affected by the presence of other components in a sample (*e.g.*, dissolved organic carbon DOC)
- includes extraction-specific (*e.g.*, solvent) parameters
- determined with a variety of reconstitution solvents (*e.g.*, ethanol, methanol, DMSO) and water matrices, including treated and untreated municipal, industrial and agricultural wastewater, surface waters, reclaimed water and drinking water

Sensitivity

- Limit of detection LOD = $\mu_n + (3 \times \sigma_n)$
- Limit of quantification LOQ = $\mu_n + (10 \times \sigma_n)$

μ_n is the mean and σ_n is the standard deviation of negative controls

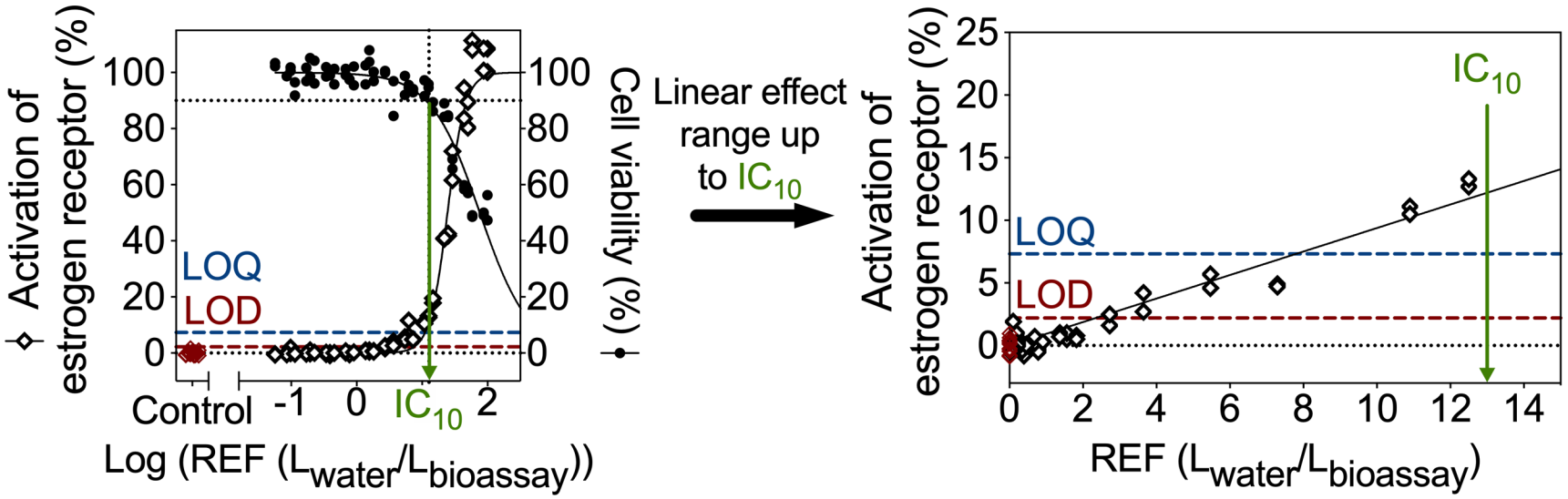


$$\log EC_{LOD} = \log EC_{50} - \frac{1}{slope} \cdot \log\left(\frac{max - min}{LOD - min} - 1\right)$$

$$EC_{LOD} = \frac{LOD}{slope}$$

Sensitivity

- Limit of detection LOD = $\mu_n + (3 \times \sigma_n)$
- Limit of quantification LOQ = $\mu_n + (10 \times \sigma_n)$
- Mind the cytotoxicity!



Solvent effects

- DMSO is a popular solvent but even below cytotoxicity it cause many side effects
- At 0.1% DMSO, >2000 genes were differentially expressed in cardiac and hepatic microtissue and methylation pattern indicated epigenetic changes (Verheijen *et al.*, 2019)

DMSO cytotoxicity

Cell line	IC_{10} (mM)	IC_{10} (%)
AREc32	92±4	0.7±0.1%
AhR CALUX	149±19	1.1±0.1%
PPARg-BLA	303±50	2.2±0.4%
AR-BLA	430±159	3.1±1.1%
ERa-BLA	172±14	1.2±0.1%
PR-BLA	128±8	0.9±0.1%
GR-BLA	63±2	0.5±0.1%
ARE-BLA	553±112	3.9±0.8%

Solvent effects

Non-volatile solvents (DMSO)

+ no loss of extract during storage

-subject to (photo)oxidation

+limited enrichment of sample (max 0.1% DMSO)

Volatile solvents

(methanol, ethanol, ethyl acetate, MTBE)

- loss of extract during storage

+ weight control circumvents the problem

+ less toxic (evaporates during testing, but little reproducible)

No solvents

+ blow down solvent extracts of water samples prior to addition of medium

+very high enrichment possible

- difficult for single compounds and sediment/tissue samples

Blanks

- Field blanks
- Laboratory blanks

- Avoid blank contamination (high purity solvents, solvent-cleaning of all materials the sample touches)
- Subtraction should only be performed if the blank has an EU_{blank} or TU_{blank} that is less that 50% of the EU_{sample} or TU_{sample}

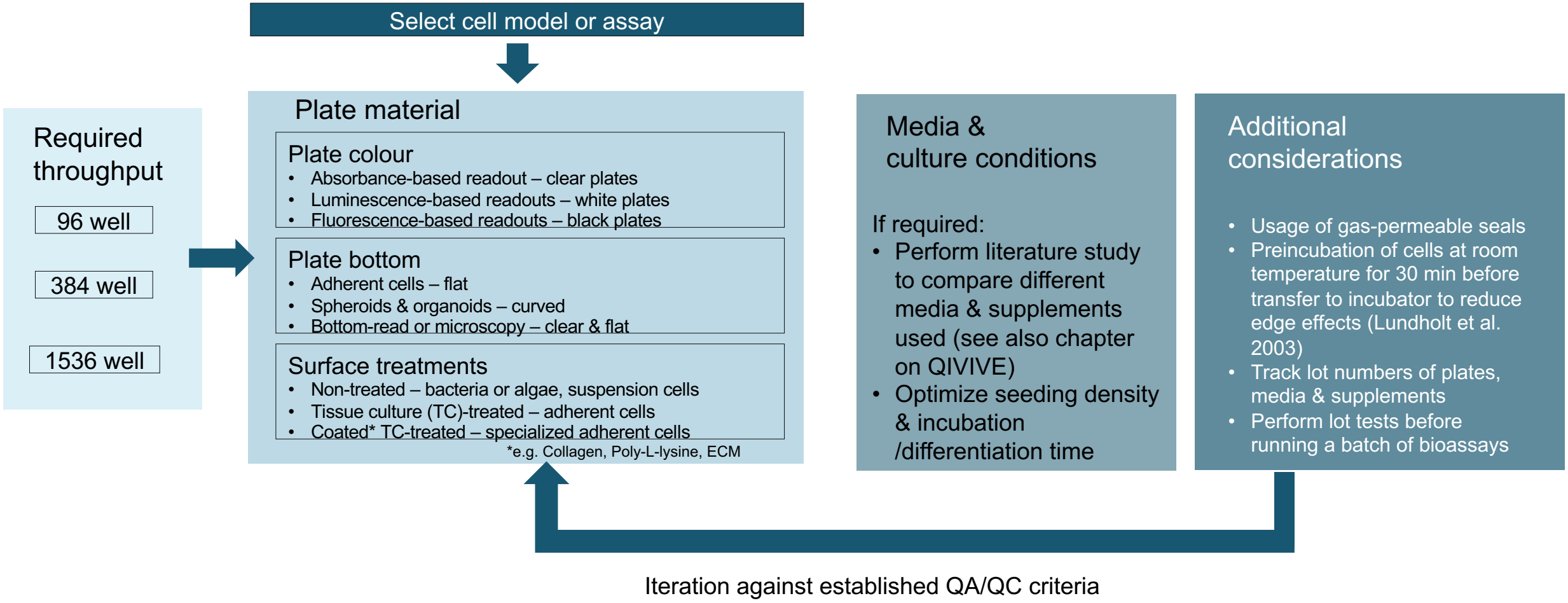
$$EU_{blank\text{-}corrected} = EU_{sample} - EU_{blank}$$

$$TU_{blank\text{-}corrected} = TU_{sample} - TU_{blank}$$

QA/QC in the laboratory

- Practical considerations for bioassay optimisation
- Replication
- Quality control samples
- Standardisation and documentation
- Guidelines
- FAIR principles – **F**indability, **A**ccessibility, **I**nteroperability, **R**eusability
  - documents such as chain of custody
  - field observation forms for sampling
  - information on sample origin and manipulation in centralised sample tracking databases
  - well-maintained laboratory books with details of experimental manipulations (*e.g.*, cell passage number, operator, observations)
  - archives of raw and analysed data in a safe location

Bioassay set up



Replication

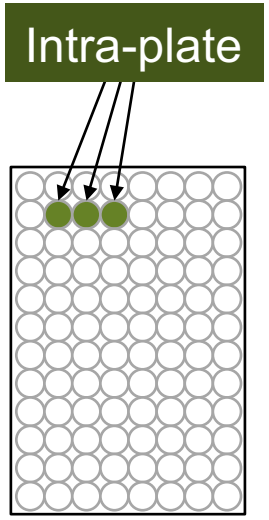
Intra-plate

- usually duplicate or triplicate on one plate
- depending on the inherent variability of the assay

Example

- uneven distribution of cells in 384-well plates

→ pre-incubation of newly seeded plates in ambient conditions (air at room temperature) resulted in even distribution of the cells in each well



Day 1

J Biomol Screen. 2003 Oct;8(5):566-70.

A simple technique for reducing edge effect in cell-based assays.

Lundholt BK[1], Scudder KM, Pagliaro L.

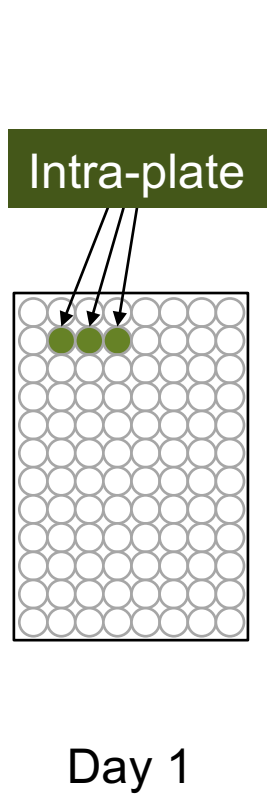
Replication

Inter-plate /Intra-assay

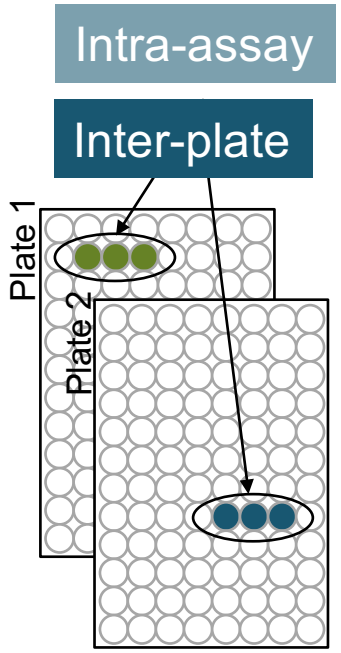
- Intra-assay replication verifies that there is no temporal drift during the assay run
- Intra-assay replication is only necessary for a subset of randomly selected samples (but at least one per assay run, *e.g.*. reference compound)

Example

- environmental factors (*e.g.*, an increase in temperature during experiment)
- instrumental issues (*e.g.*, a spectrophotometer that loses its sensitivity during experiment)



Day 1

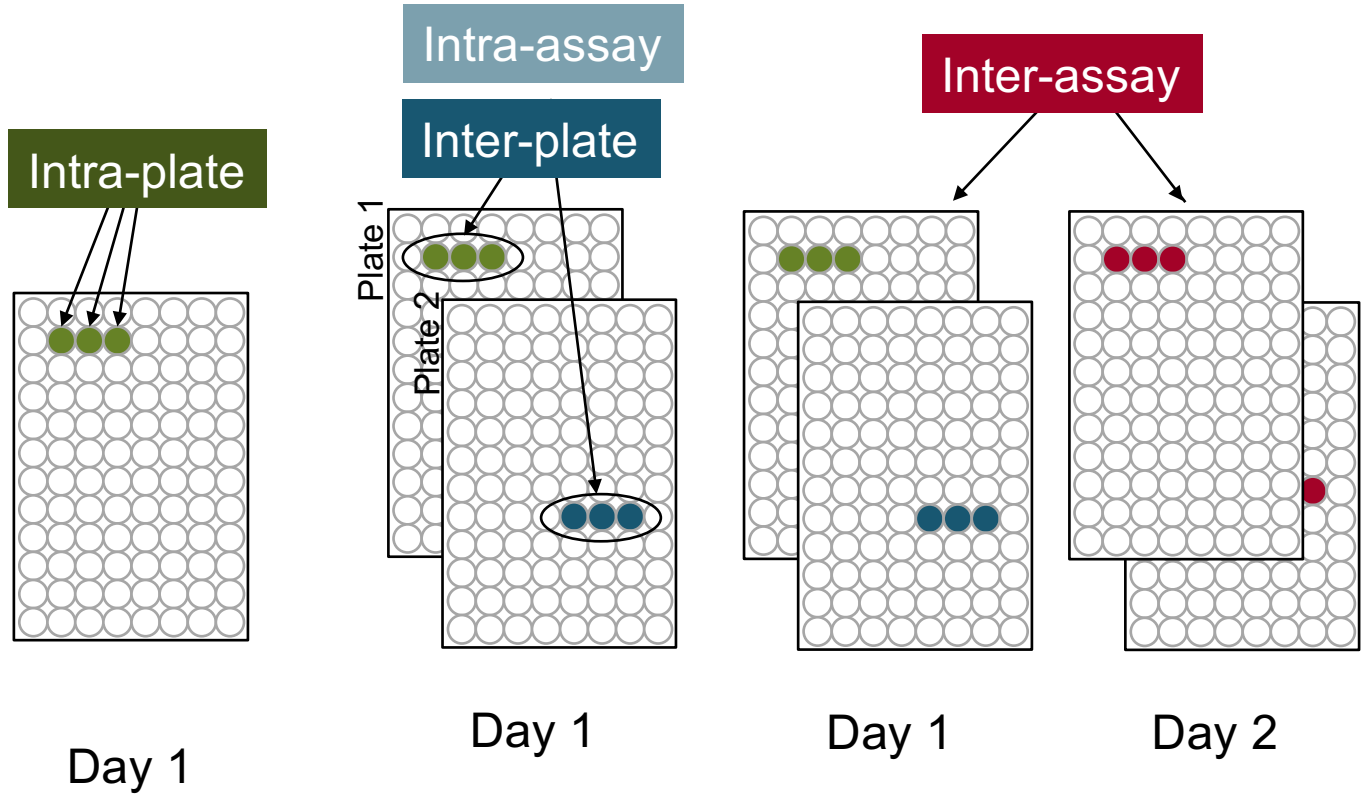


Day 1

Replication

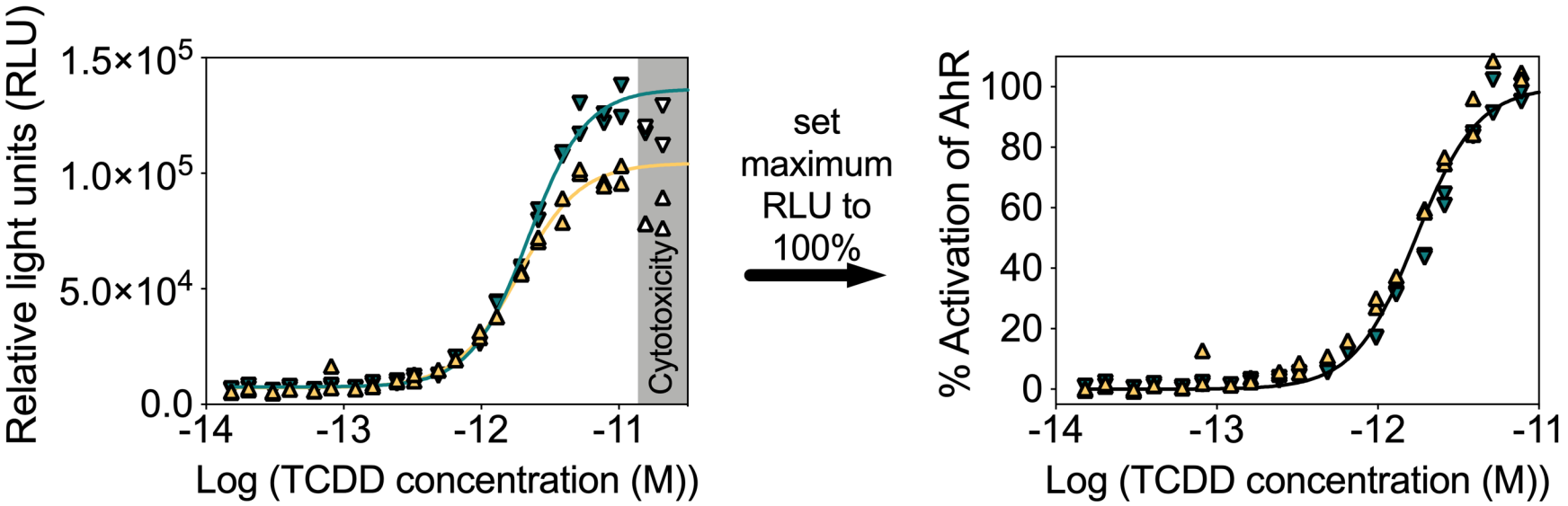
Inter-assay

- verifies that there is no assay drift or bias over time
- Samples should always be analysed on at least two independent runs performed on different days
- variability of the results at this stage is an indication of variability of the bioanalytical method (assuming there is no sample degradation)
- although inter-assay variability can be reported as an indication of the confidence in the analytical result, it is not a measure of the true variability of the water sample



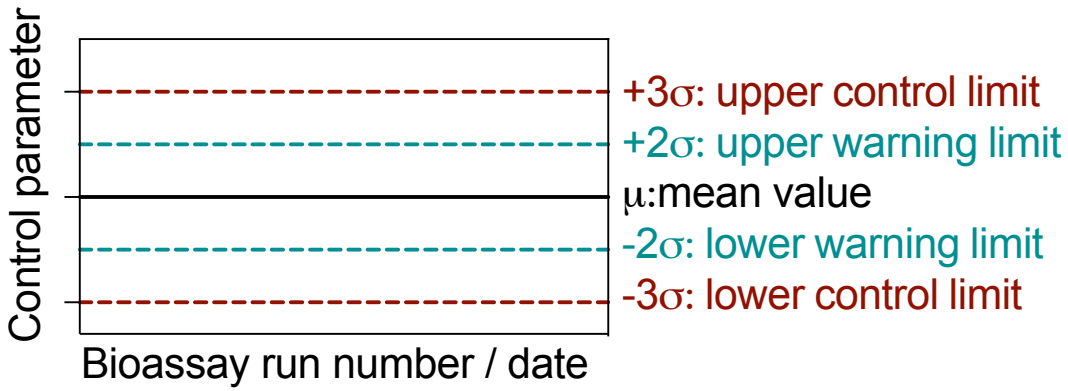
Standard curve

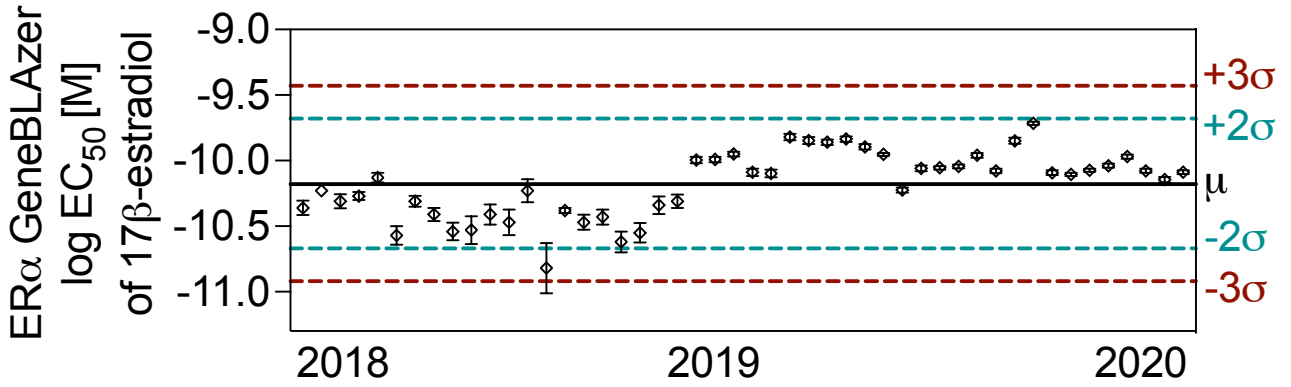
- One standard on every plate for QC and to determine the % effect
- Colour change (absorbance), relative light (RLU) or relative fluorescence units (RFU) may be variable but if normalised to the maximum of the positive control independent repeats fall on each other
- Reference compound (standard): natural hormone 17β-estradiol ; positive control 4-nonylphenol
- Often used to derive the BEQ

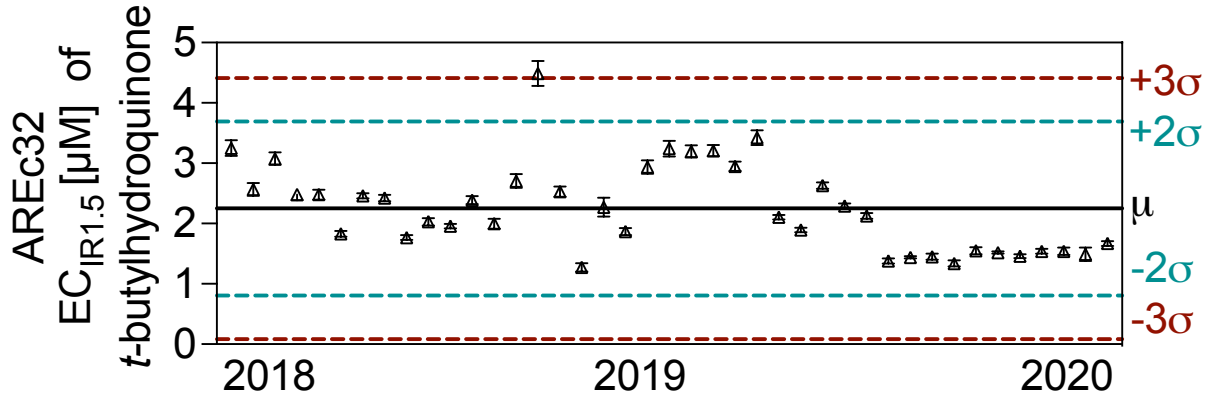


Control charts (Shewhart or X-charts)

- Mean value as well as warning and control limits are based on mean value and standard deviations of all previous runs
- Any value that falls outside these control limits indicates abnormal bioassay behaviour in which case the entire bioassay data set should be discarded and the samples re-tested







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