



Monitoring and **MoniQA** Quality Assurance

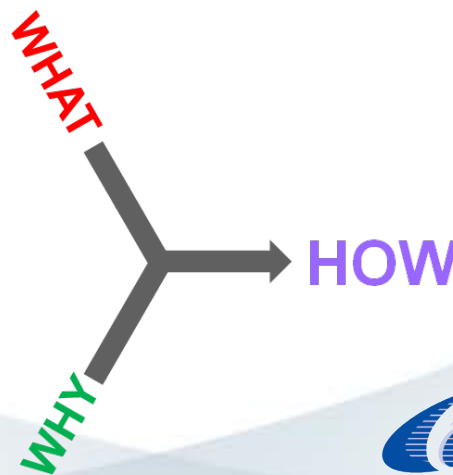
Validation of qualitative methods of analysis

Validation of qualitative methods

- The purpose of qualitative methods.
- The purpose of validation.
- Desiderata for a standard protocol for validation.
- MoniQA / IUPAC approach to achieving purposes and desiderata
- Examples
- Conclusions
- History and status

Validation of qualitative methods

- The purpose of qualitative methods. **WHAT**
- The purpose of validation. **WHY**
- Desiderata for a standard protocol for validation.
- MoniQA / IUPAC approach to achieving purposes and desiderata **HOW**
- Examples
- History and status



The purpose of a qualitative test

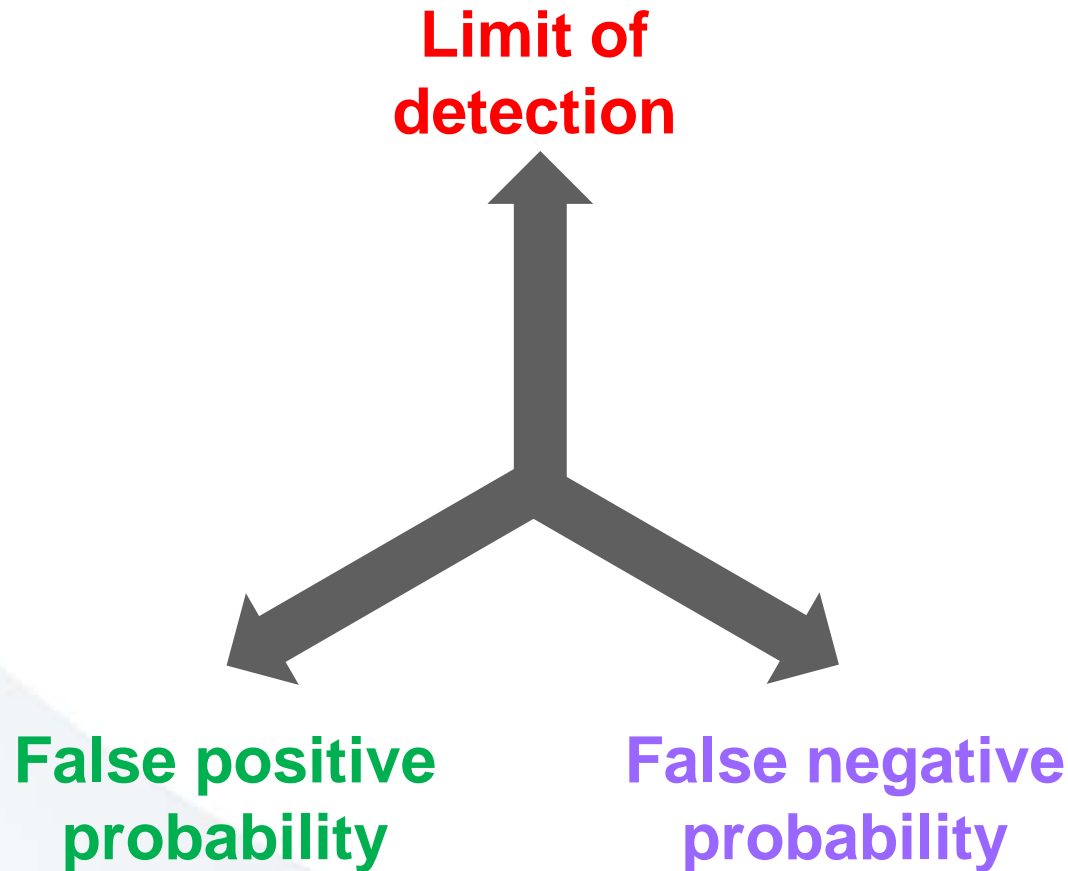
- **If we get a positive result:**
 - To be able to conclude with confidence that the target analyte is present in the sample

- **If we get a negative result:**
 - To be able to conclude with confidence that if the analyte is present at all then it must be below some low concentration.

The purpose of a qualitative test

- **If we get a positive result:**
 - To be able to conclude with confidence that the target analyte is present in the sample
 - Address by estimating the **probability of a false positive result for a negative sample**
- **If we get a negative result:**
 - To be able to conclude with confidence that if the analyte is present at all then it must be below some low concentration.
 - Address by estimating the **lowest concentration of analyte** for which the **false negative probability** is sufficiently low

Performance of a qualitative test: what we need to know



The purpose of validation: why validate

- To characterise method performance in such a way that new users can be confident that the method will provide fit for purpose results **when they use it in their laboratory**
- Provide a prediction about what method performance is likely to be when it is used after validation
 - Single laboratory: on a new day etc.
 - Inter-laboratory: in a new laboratory

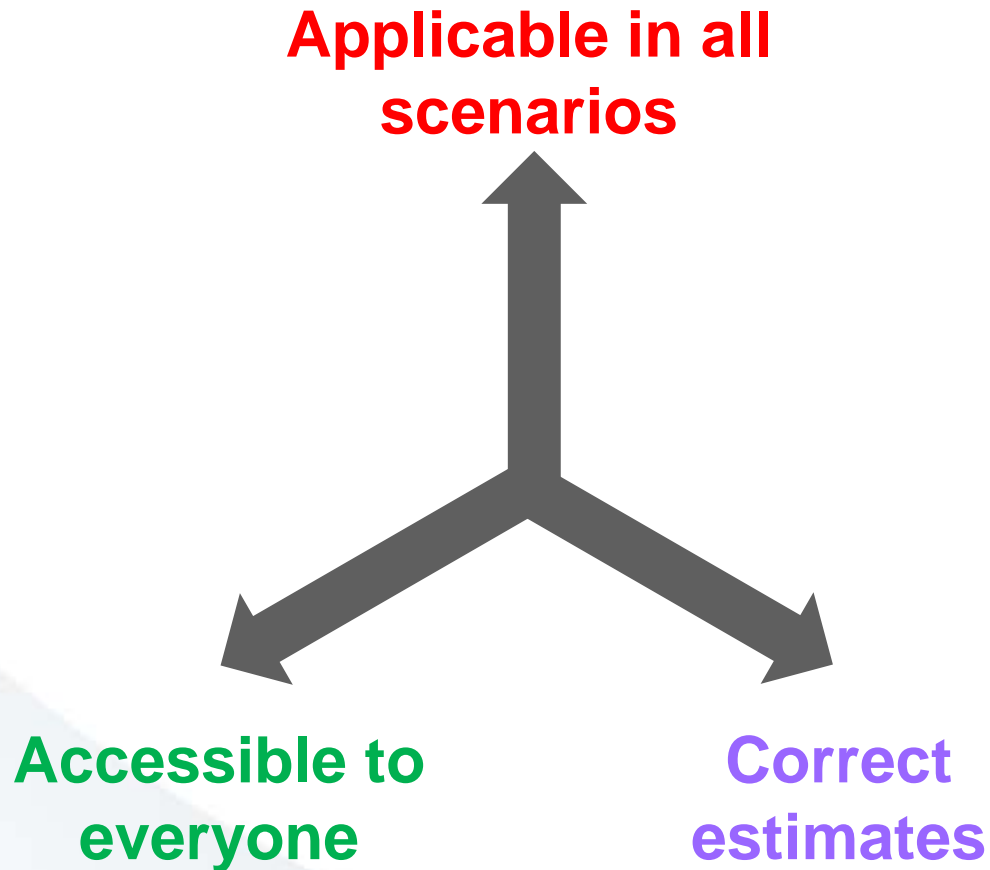
How to validate a qualitative method

- Inter-laboratory
 - A prediction of the interval within which we can expect the probability of a false positive result to lie when the method is applied in a new laboratory.
 - A prediction of the interval within which we can expect the false negative probability to lie at concentrations of interest. Hence, a prediction interval for limit of detection when the method is applied in a new laboratory.
- Single-laboratory
 -on a new day

Desirable features for a *standard* validation protocol

- Estimates of intervals for: false positive probability; false negative probability; and limit of detection should be **correct**
- The protocol should be **generally applicable** to all analytical sectors and technologies.
- **Simple.** The technology, knowledge and resources necessary to apply the protocol should not be a barrier to use.

Desirable features for a standard validation protocol



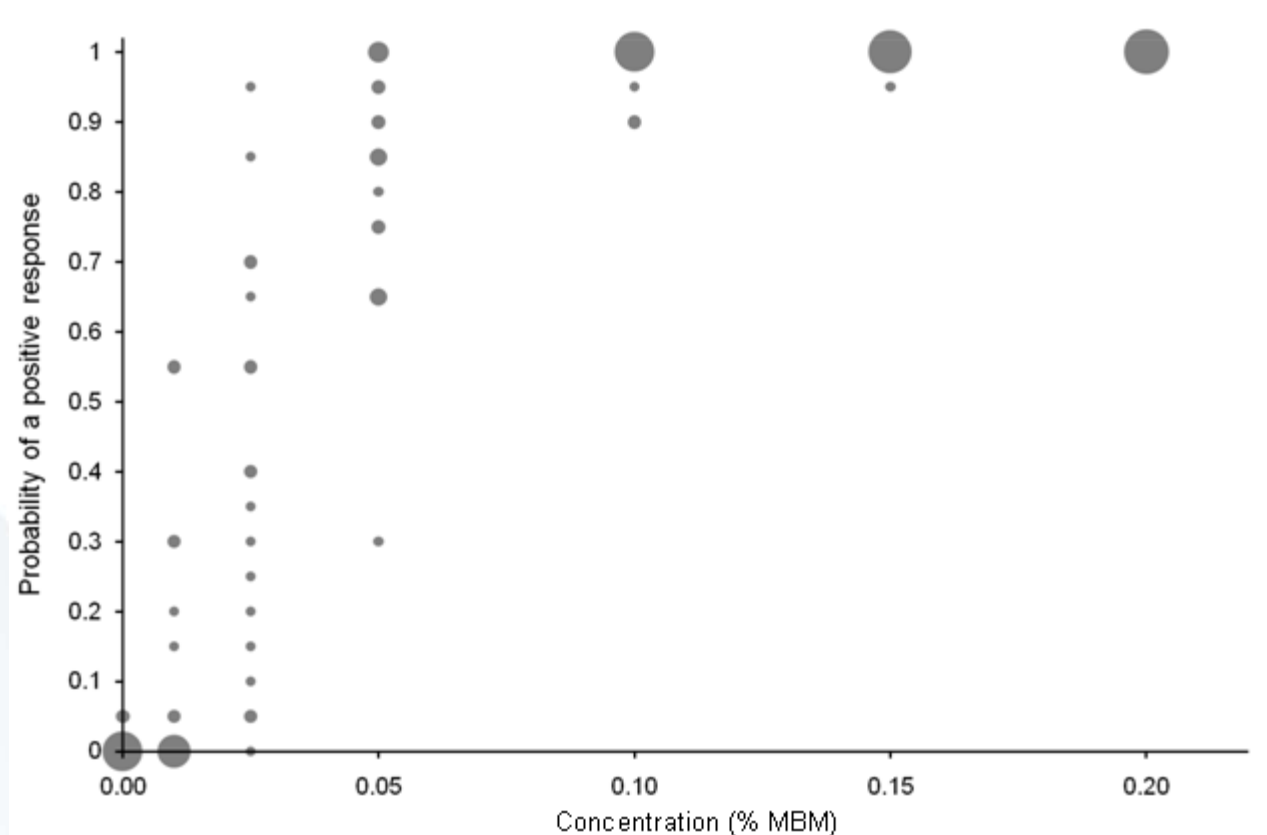
Our approach: inter-laboratory validation (1/2)

- Test at zero and at least one other level: the target LOD.
- Test replicate samples (at least 10) under repeatability conditions.
- Each laboratory reports the number of positives (x) out of n tests at each concentration.
- Estimate probability of detection for each laboratory at each level. $p_i = x_i/n$

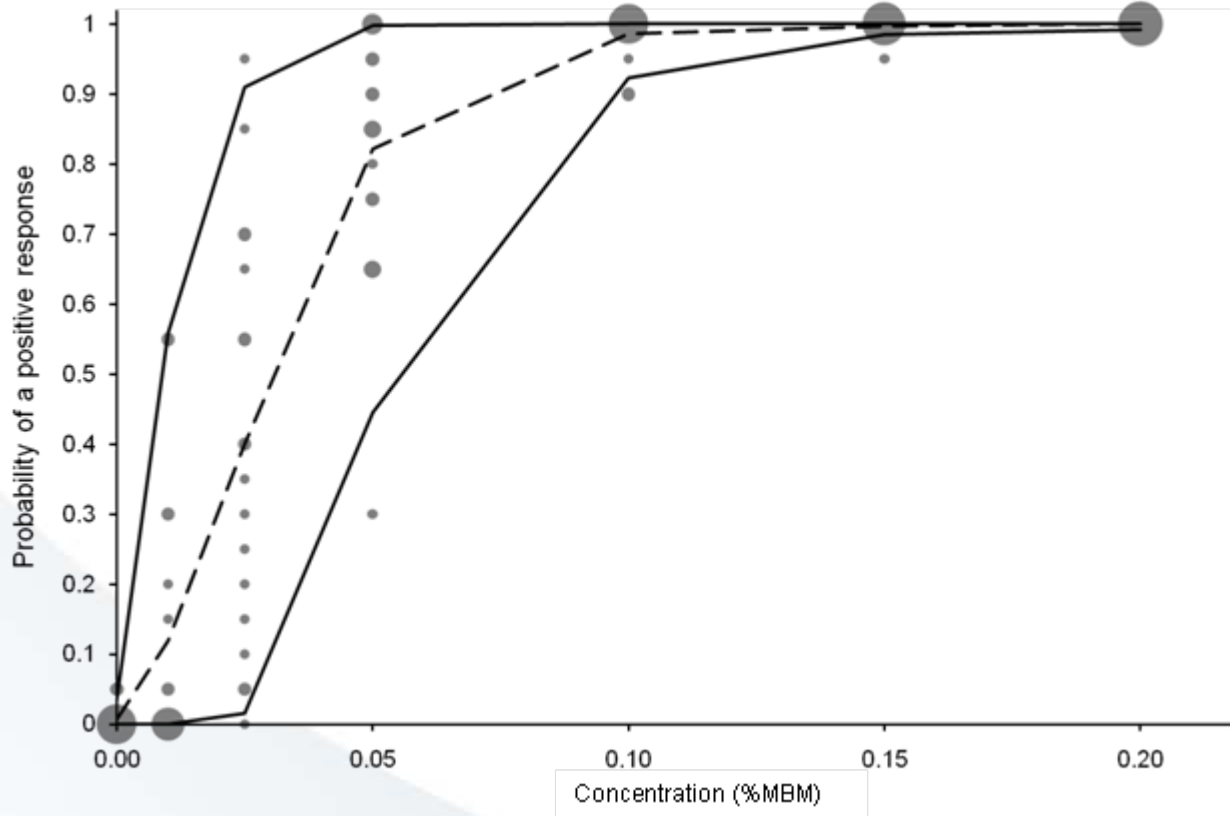
Our approach: inter-laboratory validation (2/2)

- Standard deviation of p_i between-laboratories gives estimate of reproducibility standard deviation (s_R).
- Moment matching of mean p and s_R gives prediction interval for probability of detection via a beta distribution.
- Interpret method performance (false positive probability, LOD) graphically.
- Assess between lab variation graphically via a beta-binomial distribution

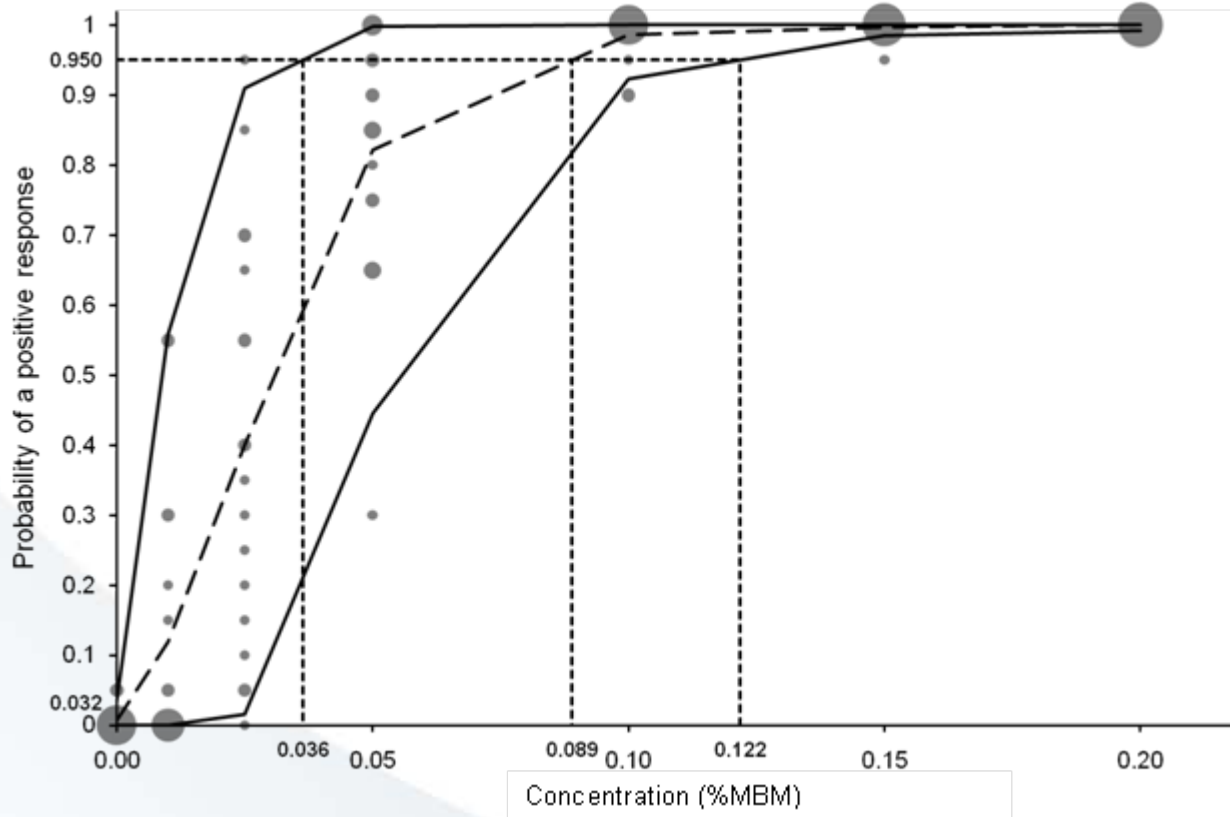
Example: MBM by PCR, 18 Labs, 7 levels, 20 replicates



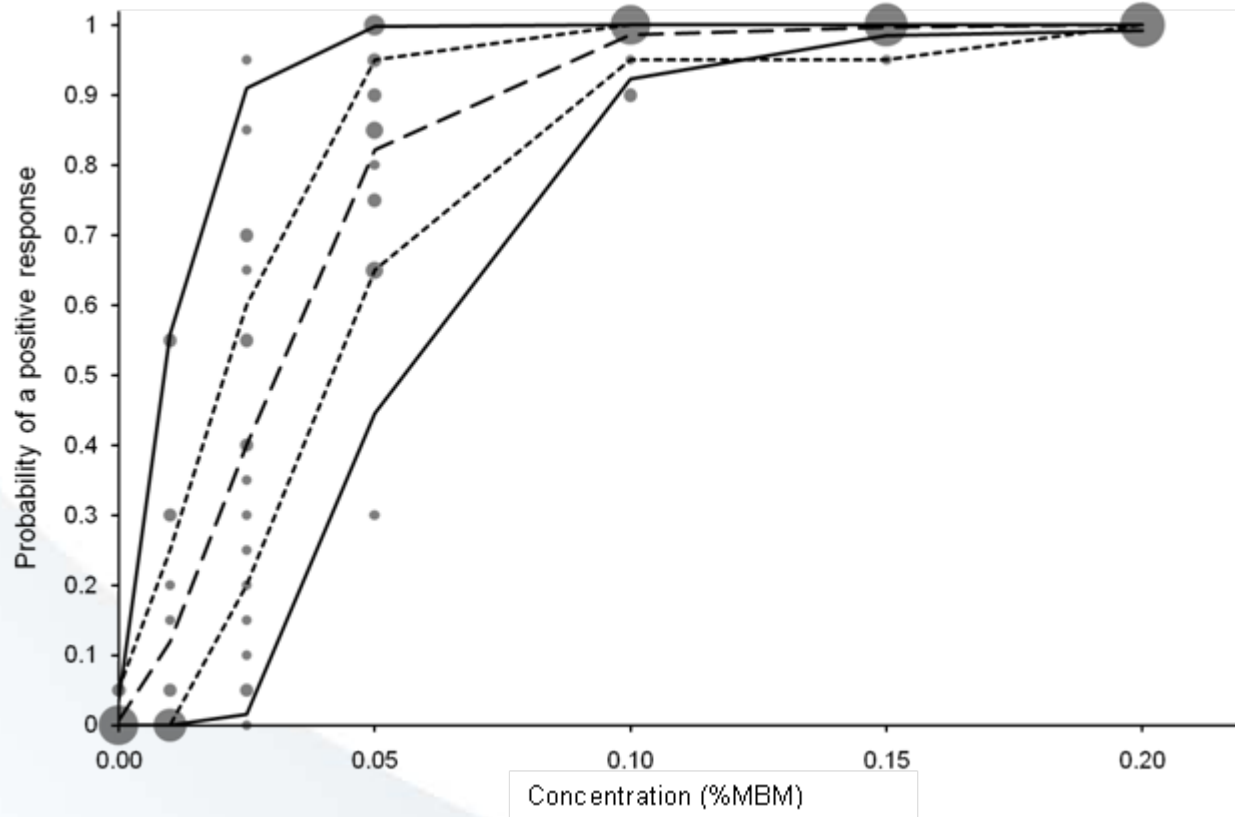
Interval for probability of detection in a lab



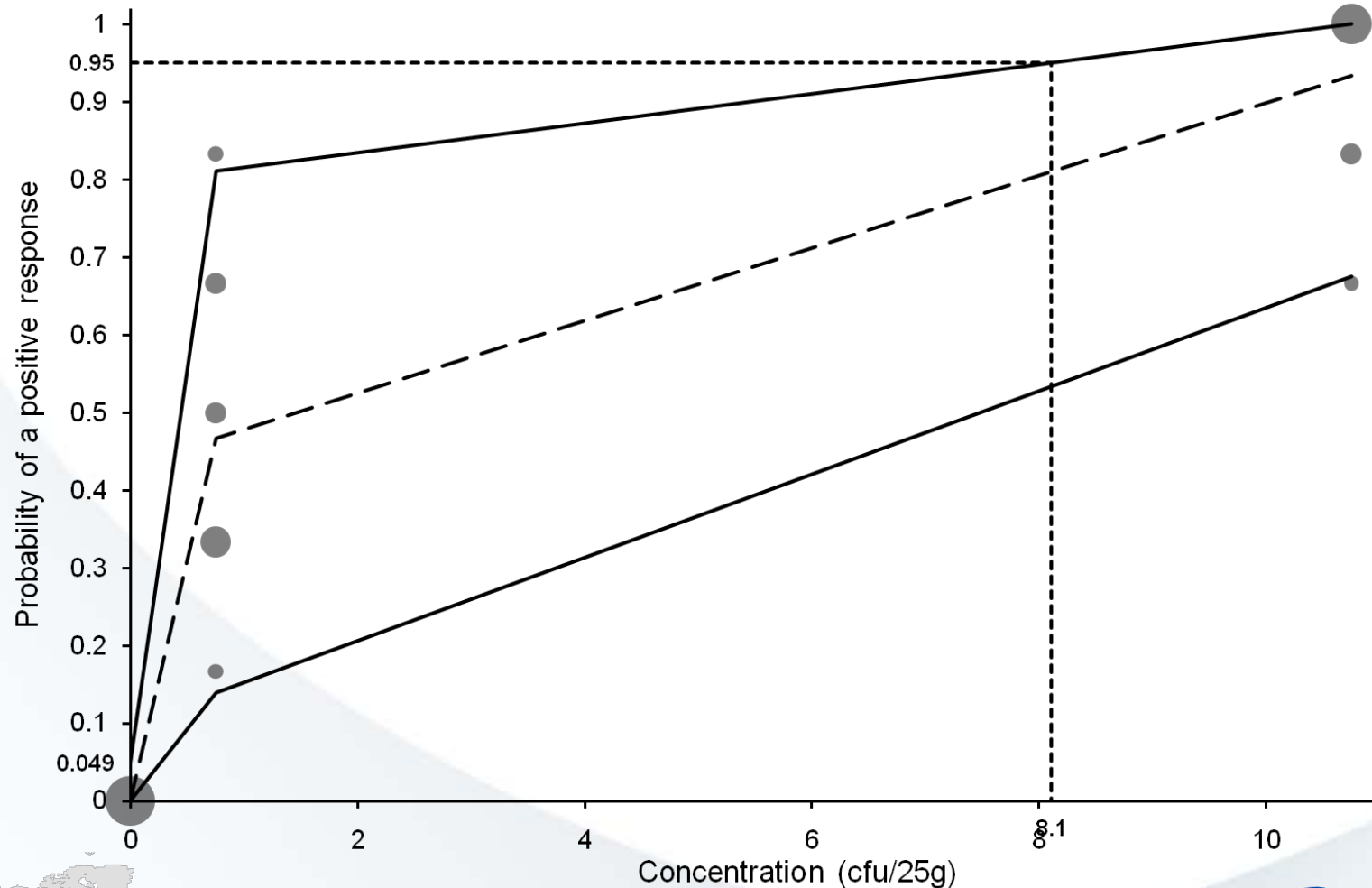
Interval for LOD and upper limit for false positive rate in a lab



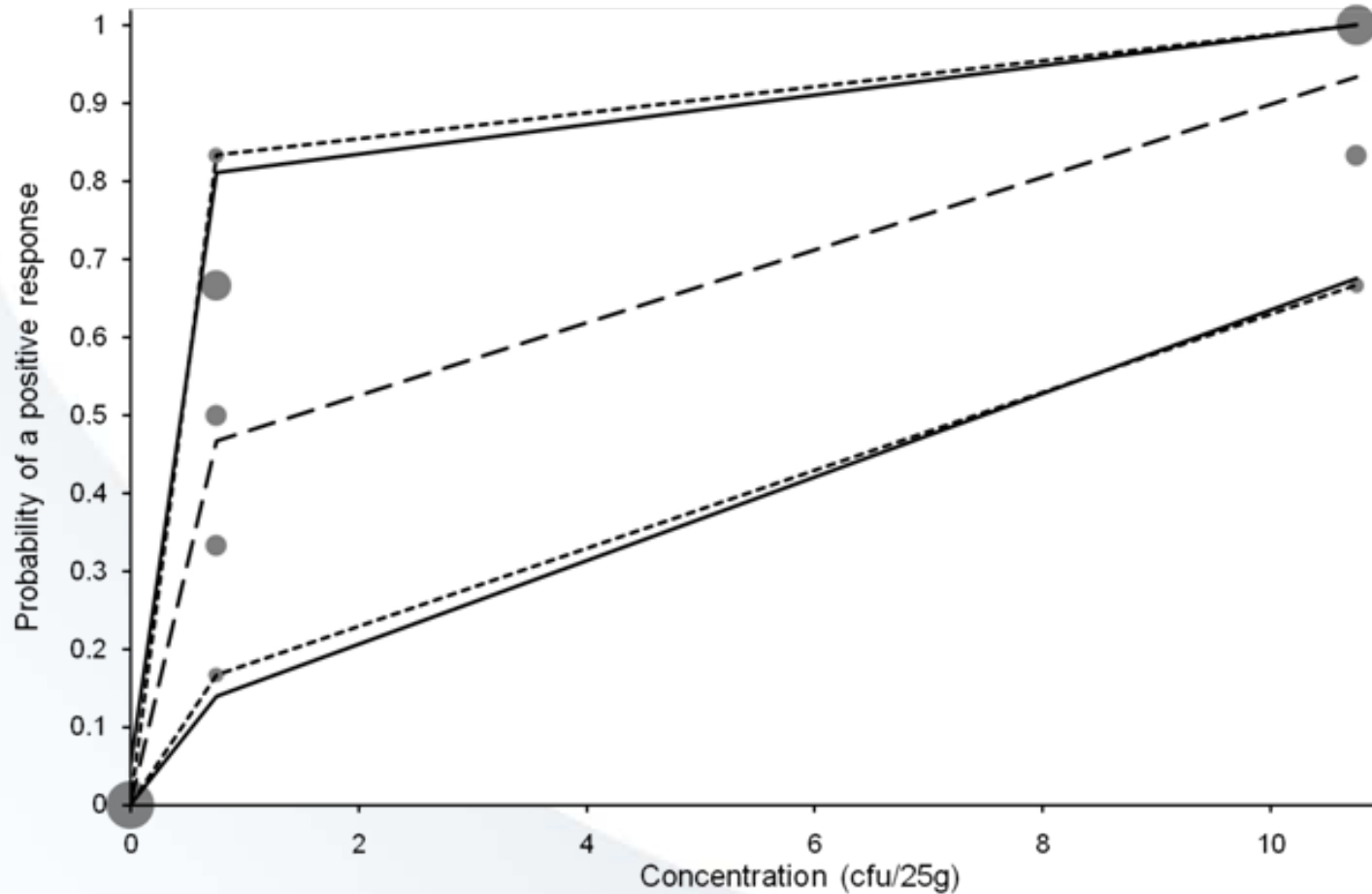
Graphical assessment of contribution made by between lab variation



Example: e.coli, 10 Labs, 3 levels, 6 replicates

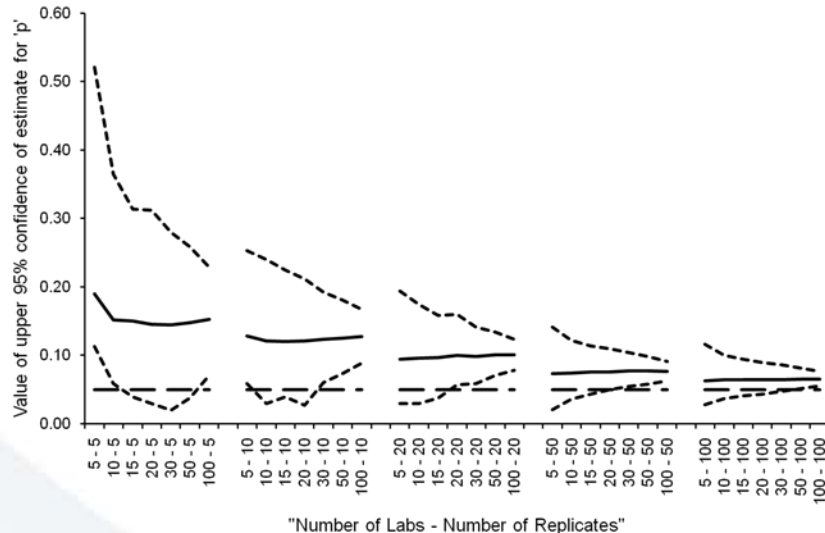


Example: e.coli, 10 Labs, 3 levels, 6 replicates



The performance of the draft protocol has been tested

- Simulation studies show the validation gives conservative performance assessments



- Favourable assessments based on small numbers of samples are reliable

Summary of validation protocol

- **Why:** provide confidence that method will give fit for purpose results when it is applied in a laboratory (or on a new day)
- **What:** False positive probability, limit of detection, false negative probability
- **How:** Linear interpolation of interval for probability of positive response in a laboratory based on S_R of p_i via a beta distribution.

Document status and history

- IUPAC project 2005-024-2-600 Establishment of guidelines for the validation of qualitative and semi quantitative (screening) methods by collaborative trial: a harmonized protocol
- MoniQA (Monitoring and Quality Assurance in the Food Supply Chain, Food-CT-2006-036337, European Commission, (<http://www.moniqa.org/>)
- Active since 2008
- This version submitted to JAOAC int.
- MoniQA protocol
- Developed version for submission to IUPAC