Measurement science activities under ICCVAM

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NIST Practices

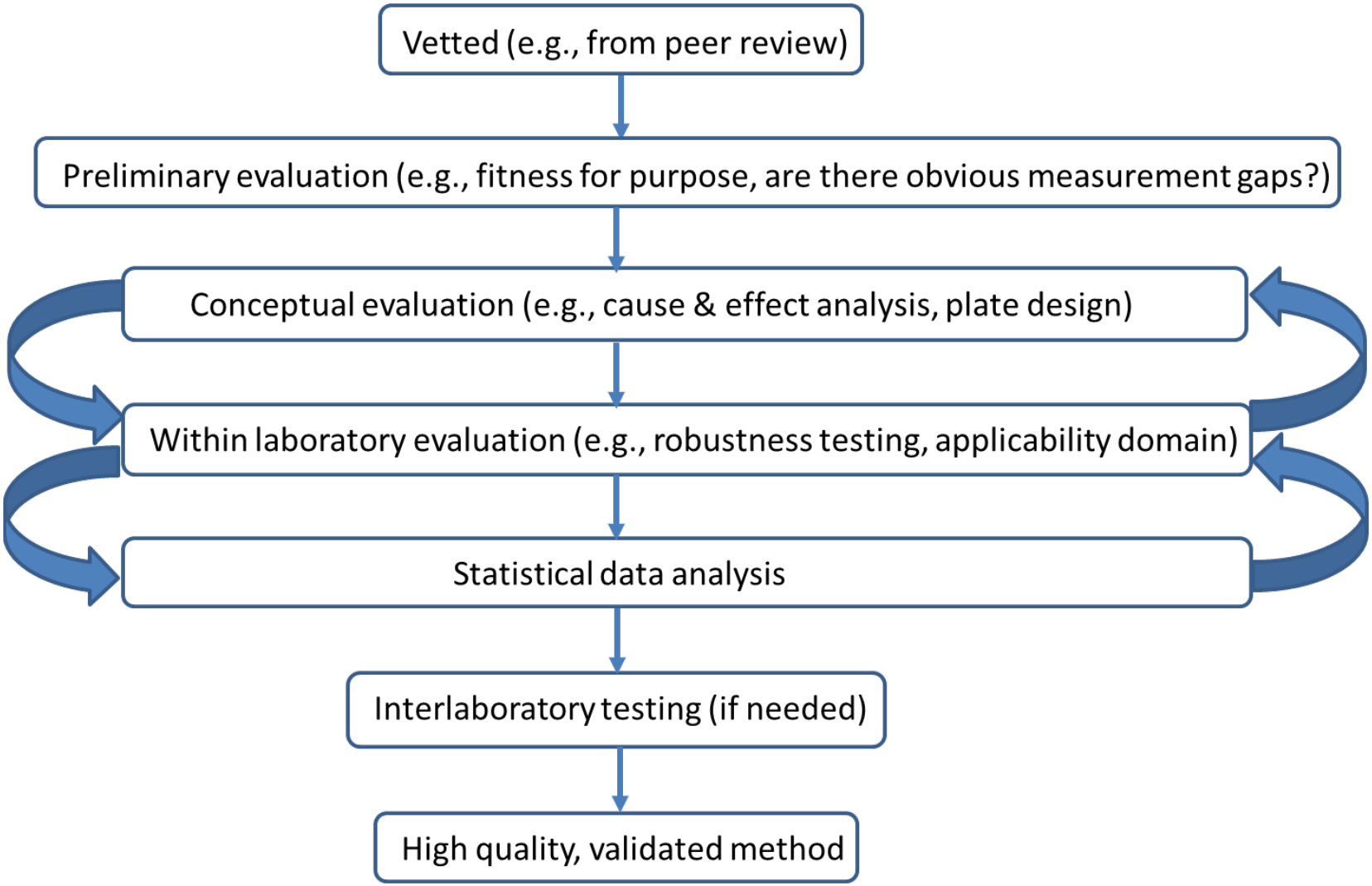
- **Measurements**
  - Develop new measurement methods
  - Improve accuracy/precision of measurements
- **Reference Materials**
  - Well-defined materials for use as a reference when making measurements
  - Enables inter-lab comparability
  - Physical artifacts for calibrating instruments
- **Standards**
  - Documentary standards, ASTM, ISO
  - Reference data (chemical spectra)
- **Assay development within ICCVAM**
  - No regulatory responsibilities but supports other agencies with improving the quality of assays potentially useful for regulatory purposes
  - Interlab comparison with EASA method with NIOSH, FDA, and CPSC/NIST coordinated by NIEHS started in 2017 using cuvette based method

Food-matrix reference materials to facilitate nutritional labeling

NIST Synthetic RNA controls (ERCCs) used in sequencing of Ebola virus genomes to characterize patterns of viral transmission

Is an assay ready for measurement assurance?

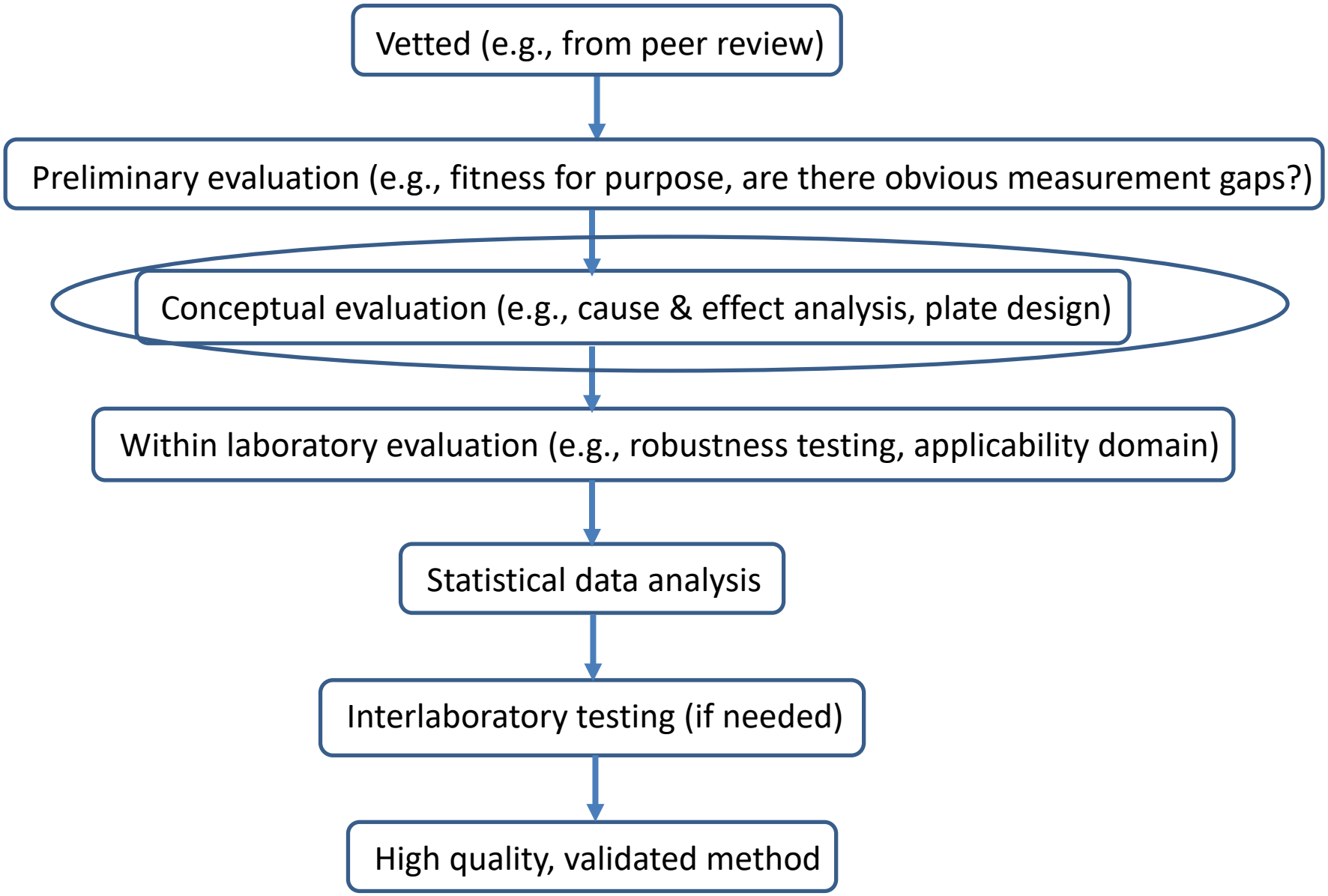
Is there a need for increased confidence in an assay measurement?



Decision tree analysis of the the electrophilic allergen screening assay (EASA): A collaboration with CPSC

- Adverse outcome pathway event measurement for skin sensitization and vetted by ICCVAM, OECD, others
- Technical measurement gaps in initial method- Instrumentation limitations, lack of sufficient controls, challenges in data analysis
- Comprehensive evaluation of sources of uncertainty
- New plate design to include multiple process control measurements- 96-well plate, plate reader ready, in-process controls, dose-response for performance evaluation
- Preliminary qualification rounds within laboratory
- Statistical analysis and interpretation based on error propagation
- Full interlab study underway

Sources of uncertainty in the EASA



Flow chart

1. Add solvent system (50 % Phosphate buffer: 50 % acetonitrile) to wells

↓

2. Add positive chemical control or test chemicals to relevant wells

↓

3. Add the probe molecule (NBT or PDA) to relevant wells, and cover plate with plate seal

↓

4. Place the plate in the plate reader, and take kinetic measurements for 50 min.

Cause and Effect Diagram

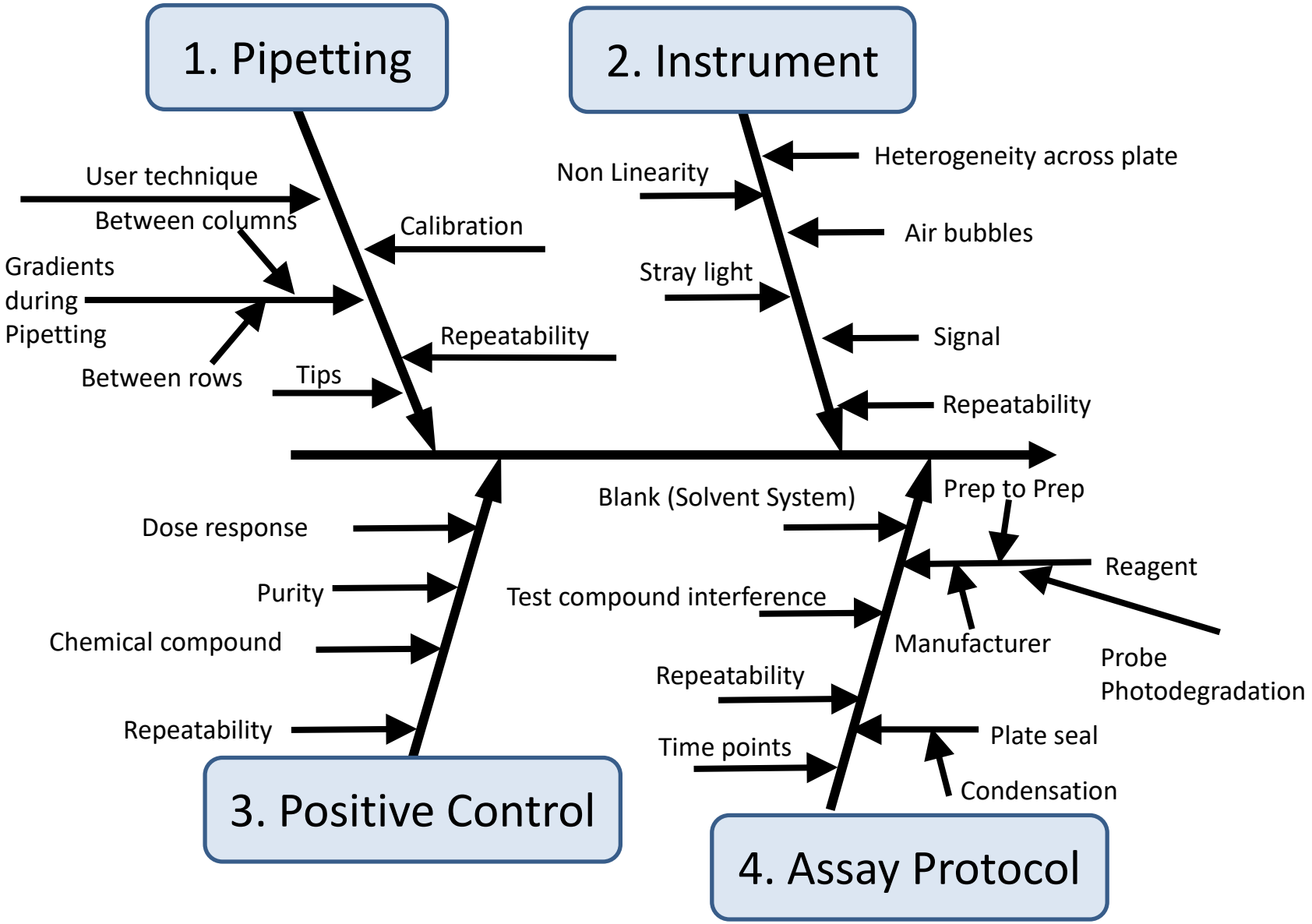
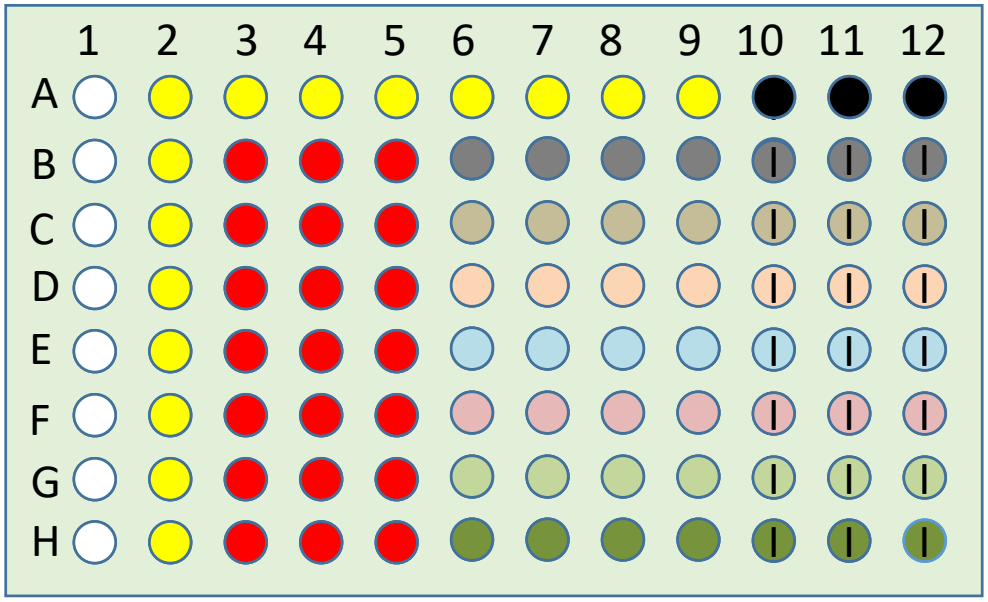


Plate Design for EASA assay



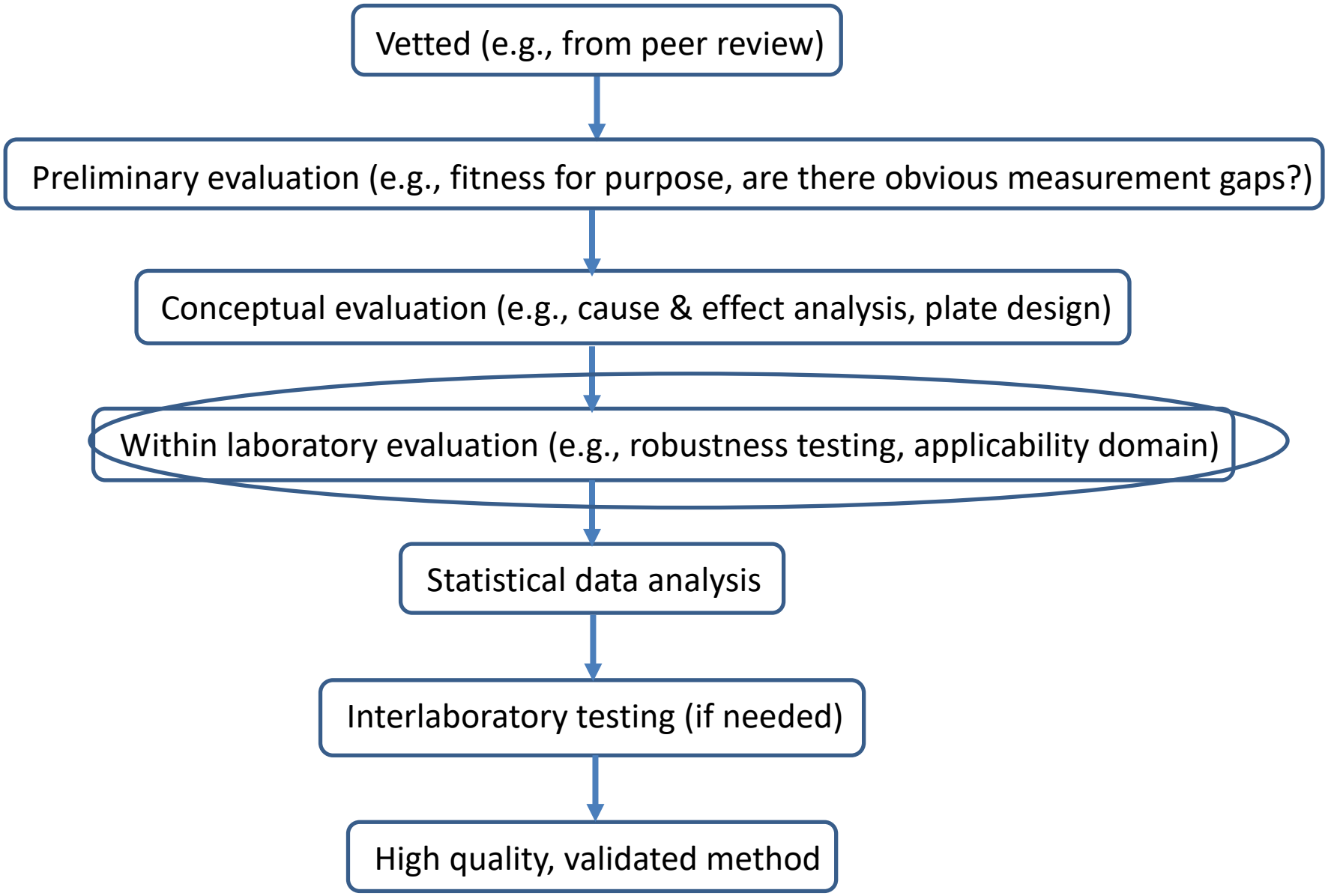
- Blank (Solvent System)
- Negative Control
- Positive Control (serial dilution)
- Test chemicals
- Test chemical interference wells

Process control measurements:

1. Within pipette step variability
2. Between pipette step variability
3. Solvent system (blanks)
4. Serial dilution of positive chemical control
5. Instrument performance/bubbles (680 nm)
6. Test chemical interference

- **Process control measurements encode quality onto the plate.**

Steps to add measurement assurance for *in vitro* assays



Evaluating system parameters for EASA

- Photodegradation of probe molecules
- Plate reader homogeneity and impact of pipetting direction
- Assay duration
- Potential for bias from bubbles in wells
- How to handle bias from test chemicals which absorb or fluoresce similarly to probe molecules
- Usage of polar and semipolar solvents
- Select positive controls based on ease of handling, low toxicity
- Initial test chemical concentration
- Performance of different types of plates and plate seals

- **A main goal was to select measurement parameters in the protocol that were scientifically defensible and based on data instead of expert judgement.**
- **Robustness testing and plate design revealed biases undetected during the original cuvette assay**

Preliminary tests results from prototype testing

- 64 chemicals have been evaluated including 50 sent from NTP and 10 from the original cuvette assay
- Comparison to *in vitro* direct peptide reactivity assay (DPRA) data yielded 100 % agreement (18 compounds)
- Comparison to *in vivo* local lymph node assay (LLNA) data yielded 89 % agreement (36 compounds)

Is assay protocol and format fit-for-purpose with respect to analytical performance? Yes

Are the assay results fit-for-purpose with respect to biological relevance? Yes

Statistical evaluation

A T-score is calculated by taking the “Effect” and dividing by the standard error. In order to take all uncertainty into account, all sources of variability must be included in the calculation. In this case, we took into account the variability of: the Negative Control, the NC/PC Blank, the TC and the TC Blank.

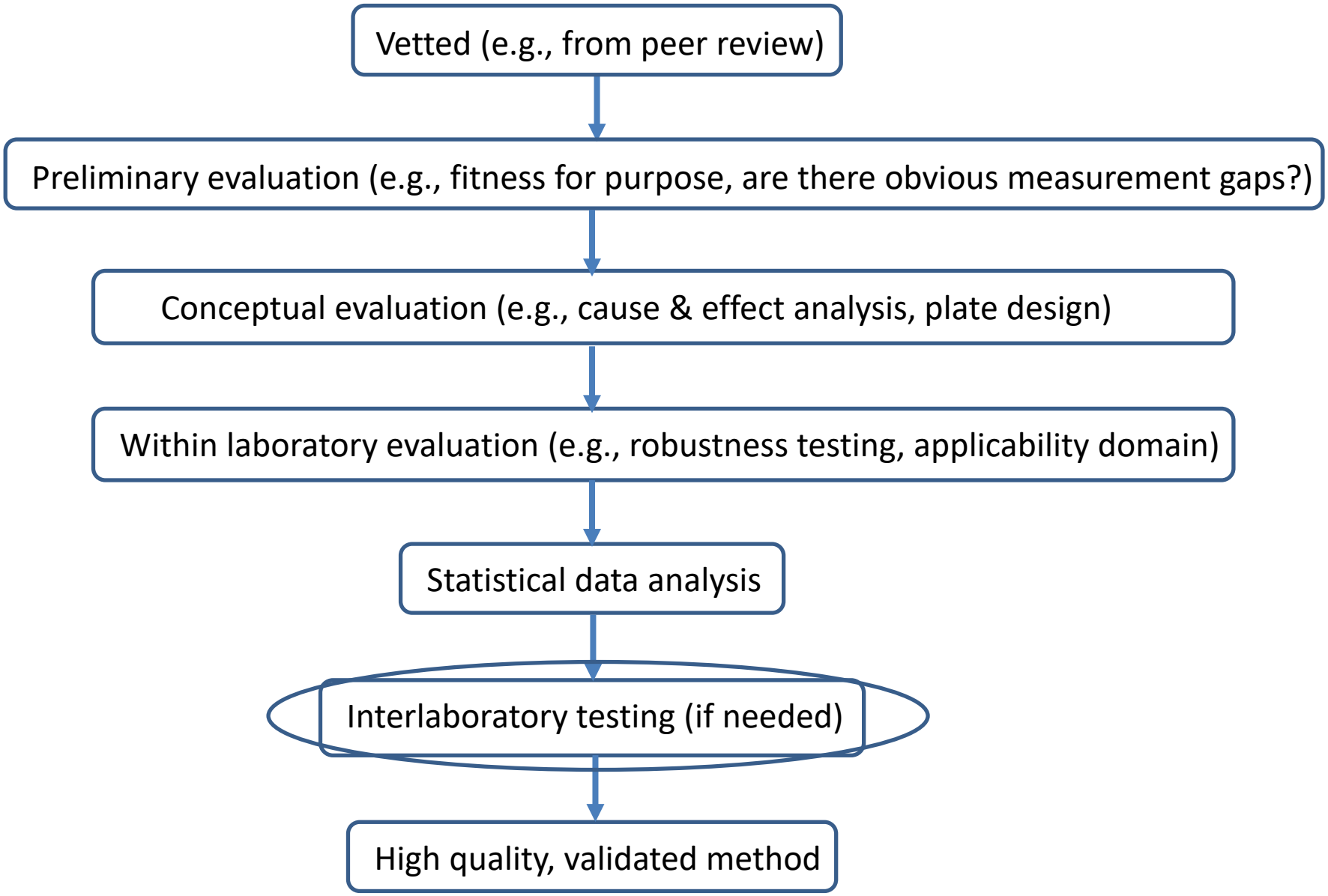
NC – Negative Control
S – NC/PC Blank
TC – Test Compound
TCB – Test Compound Blank
sd – standard deviation
n – number of replicates

$$T = \frac{(\overline{NC} - \overline{S}) - (\overline{TC} - \overline{TC_B})}{\sqrt{\frac{sd_{NC}^2}{n_{NC}} + \frac{sd_S^2}{n_S} + \frac{sd_{TC}^2}{n_{TC}} + \frac{sd_{TC_B}^2}{n_{TC_B}}}}$$

Effect (or in our case Depletion)

Cumulative Uncertainty

Steps to add measurement assurance for *in vitro* assays



Interlaboratory comparison using performance standards

OECD
Organisation for Economic Co-operation and Development

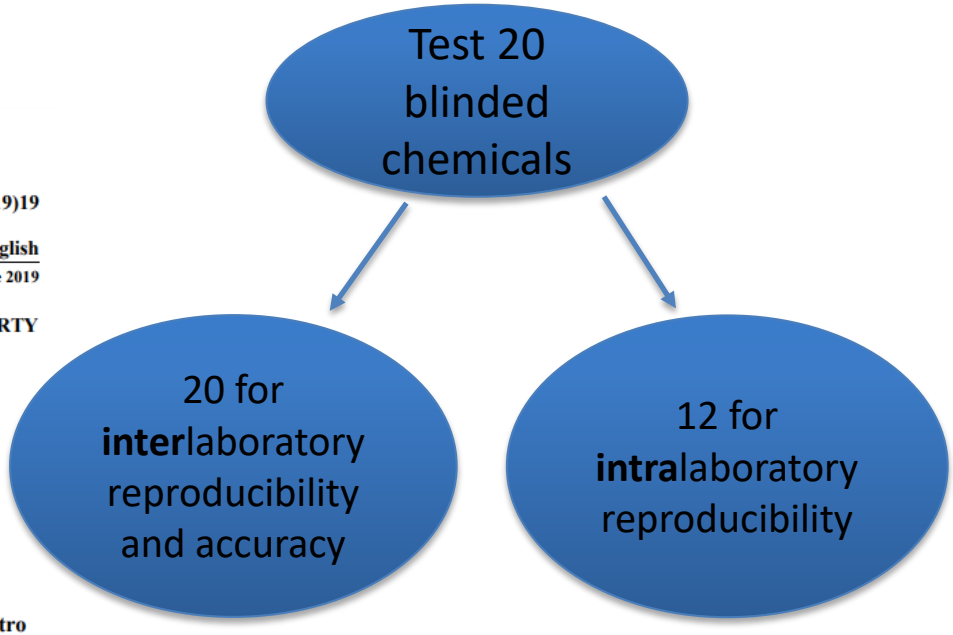
ENV/JM/MONO(2019)19

Unclassified | English - Or. English

28 June 2019

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Performance Standards for the assessment of proposed similar or modified in vitro skin sensitisation DPRA and ADRA test methods



Status

- Positive and negative control testing completed
- Blinded chemicals will be tested when labs reopen

CPSC

DoD

NIST National Institute of Standards and Technology U.S. Department of Commerce

FDA U.S. FOOD & DRUG ADMINISTRATION

NIH National Institute of Environmental Health Sciences

Collaborators at NIST and CPSC for assay development and interlaboratory testing

NIST
Elijah Petersen
John Elliott
Blaza Toman

CPSC
John Gordon
Rick Uhl

FDA
Diego Rua

DOD
Emily Reinke

NICEATM/ILS
Judy Strickland
Jim Truax

Meeting at NIST on March 8, 2019

NRC postdoc opportunity at NIST

Improving Measurement Assurance of *In Vitro* Toxicity Assays

Applications can be submitted in August 1 or February 1

2-year appointment

~ 72k stipend

Contact Elijah Petersen (elijah.Petersen@nist.gov) for more information