CELL COUNTING BREAKOUT SESSION OVERVIEW (2 H 45 MIN)

Scope:

- Focus is on measurement validation and not biological relevance
- Pretend we have unlimited resources (time, personnel, money)

Brief introduction by Sumona Sarkar (NIST) and Janet Davis (Janssen R&D)

Cell counting measurement process flow chart

Sources of variability/Ishikawa diagrams

Consider use/needs for reference materials/process controls/inter-lab studies

SCOPE

Total vs Differential Counting

Total count – object counting

Differential count – count of sub-population, based on biological distinction

- Viable cell count (i.e. trypan blue dye exclusion)
- Specific cell type count (i.e. biomarker labeling, or cell shape distinction)

*Our focus for this session will be on analytical methods for **direct counts** and may be applicable to both total and differential counts*

Direct vs Indirect Counting

Direct methods – data output is count or concentration data

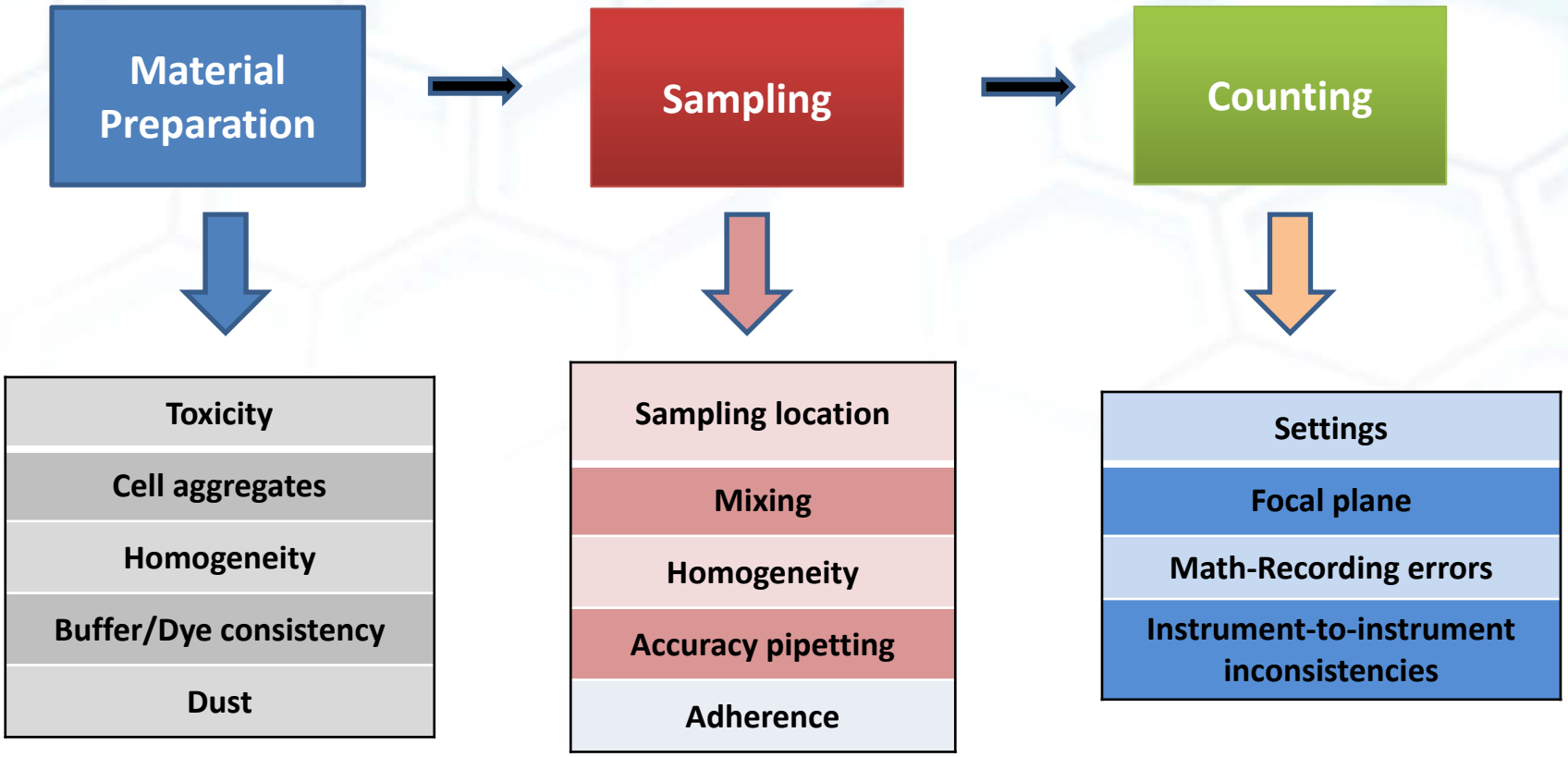
- Coulter counters
- Microscopy counters
- Flow cytometers

Indirect methods – data output is compared to a calibration curve to obtain concentration data

- Packed cell volume
- Glucose/lactate assays
- Total DNA

Troubleshooting

Cause and Effect Examples

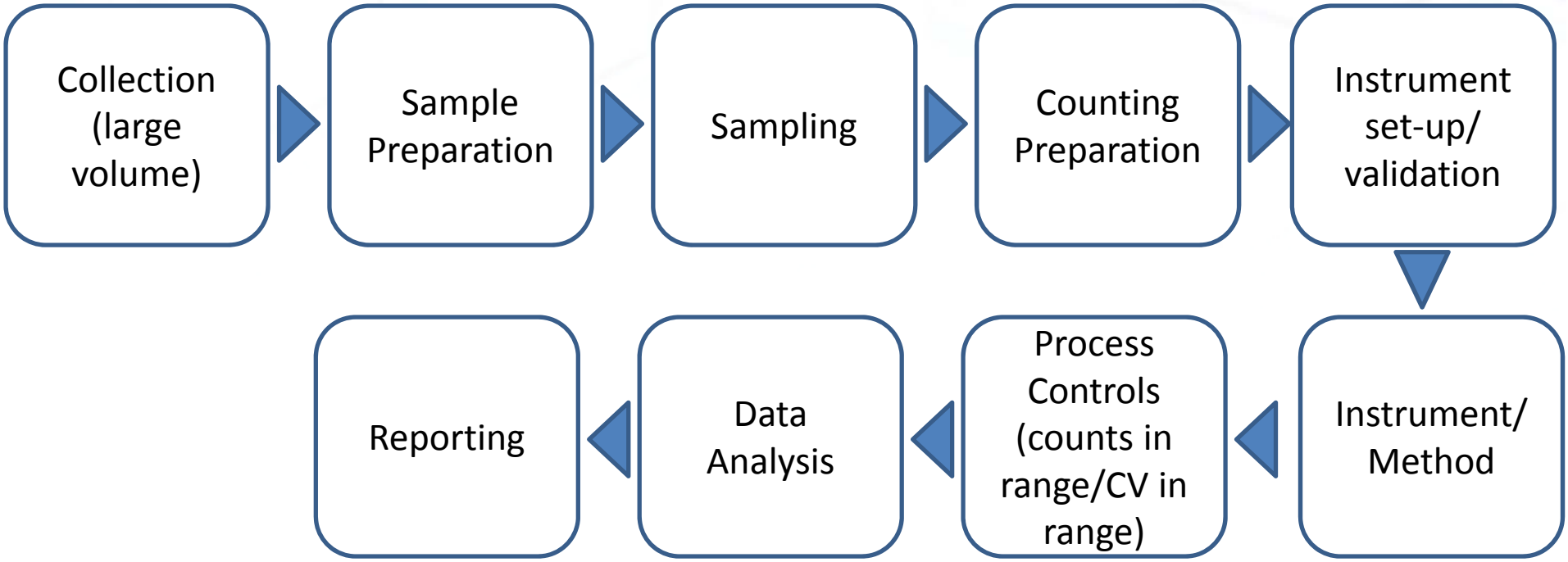


Look for Patterns Contributing to Data Artifacts

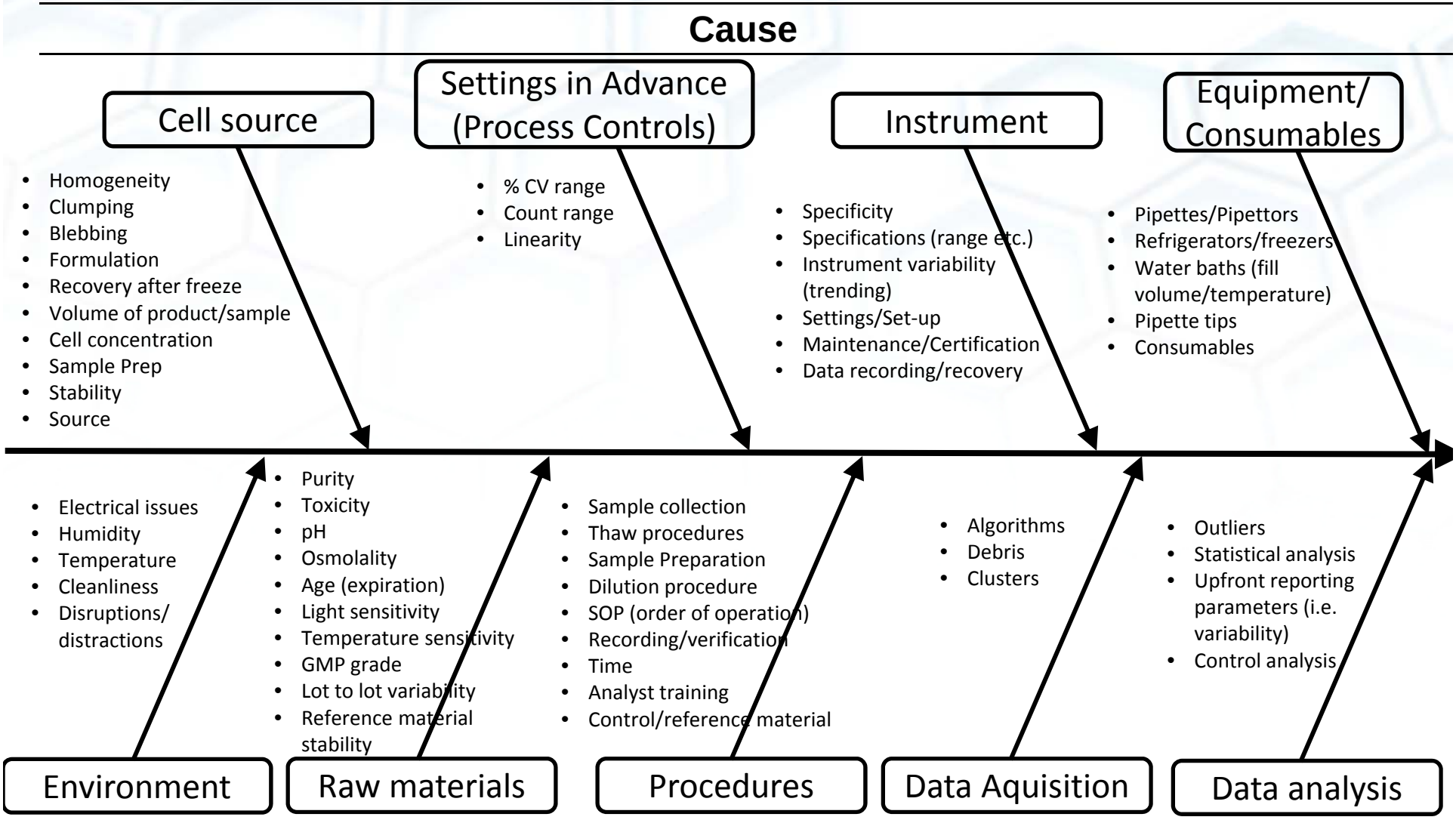
Cell Counting Measurement Process Flow Chart

Upstream Considerations for Assay Development

- Intended Use
- Material Choice (Population Definition)
- Choice of Instrument (IQ/OQ/PQ)



Ishikawa Diagram (TEMPLATE)



REFERENCE/CALIBRATION MATERIALS

- Beads
  - Instrument calibration/qualification

Alternative to Reference Materials

Goal: Provide confidence in measurements and facilitate comparison when reference materials are not easily envisioned

Due to lack of sample:

Stability

Traceability

Homogeneity

Strategy: Develop experimental and statistical techniques to quantify cell count measurement performance, independent of a reference material or a reference method

- Cell controls/calibrants/references
  - Challenges
    - Banking
    - Stability
    - Preparation consistency
    - Formulation
    - Lot-to lot variability/passage stability
    - Cell line vs primary cell source
    - Labeling
  - Spike-in controls
    - Dilution
    - Viability
    - Sampling
    - Concerns
      - Bias
      - Interference
      - Characterization

Collection and Sample Preparation: Measurement Controls

Variables/Factors	Why an Issue?	Control Experiment /Approach	Reference Material?
Variability in starting material		Test different lots	
Concentration effects		Test at different concentrations	
Volume effects		Test at different volumes	
Formulation effects		Test different dilutents	
Stability/Timing		Run order effects/loss of cells over time	
Thaw process			
Dilution integrity			

- SOP's for sample preparation recommended

Process Controls

- Formulation effects
- Raw materials
- Interference (dust/debris/cells)
- Time/temperature
- Volume of sample taken
- Lot to lot variation
- Range Linearity
- Variability
    - Day to day
    - Operator to operator
    - Instrument to instrument
    - dilution