

High Precision Cell Counting

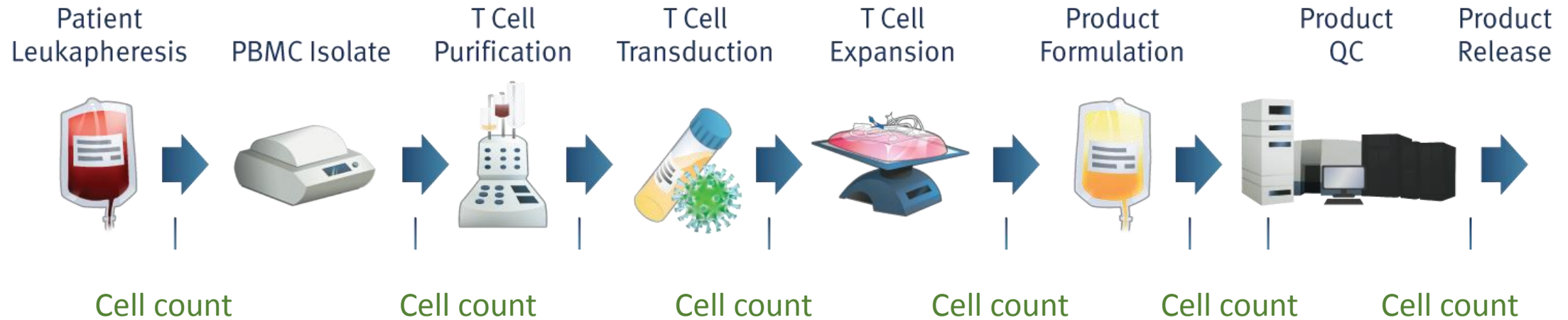
Variables to consider transitioning
from R&D to GMP to commercialization

Presented by: Matthew Riolo PhD

Conflict of Interest

I am the Lead Application Scientist for Chemometec Inc

Engineering T-cells



- One of the most important steps in production and manufacturing
- Used throughout entire process
- Determine whether to move forward

Upstream Considerations

- **Cell Type:** Immune cells, stem cells, CHO cells, HEK293T cells
- **Culture Method:** Culture flasks, bioreactors, media involved
- **Process Development:** Isolation, transfection, product formulation
Reagents involved - affects on cell health
- **Expansion into Manufacturing:** Multiple instrumentation, standardize protocol, ensure consistency
- **Storage:** Cryo-preserved reagents, affects on cell health and morphology

Cell Type

Limitations

- Aggregation
- Sample volume

Potential Issues

- Storage, transfection
- Debris

Cell Counting Approaches

- Total cell count vs Differential cell count
- Direct cell counting vs Indirect cell counting

Choosing the right cell counting instrument

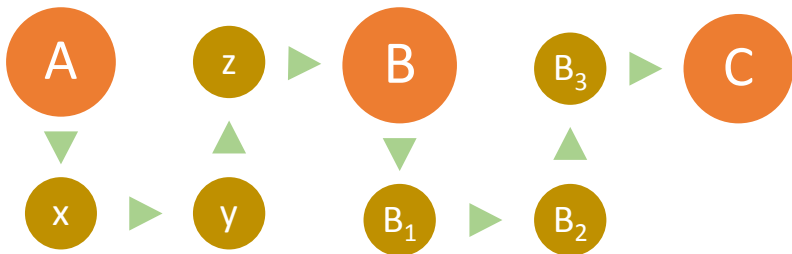
Consideration: Will this be going into GMP, commercialization?

- 21CFRpart11 software, documentation
- Anticipate expansion, regulation and challenges

Complex Protocol

vs

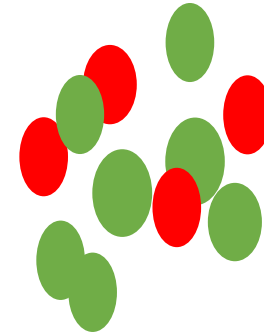
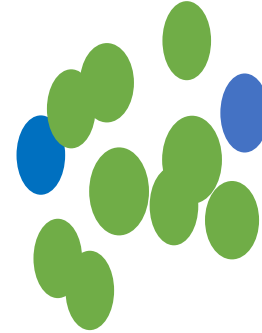
Simple Protocol



Why do different cell counters give different results

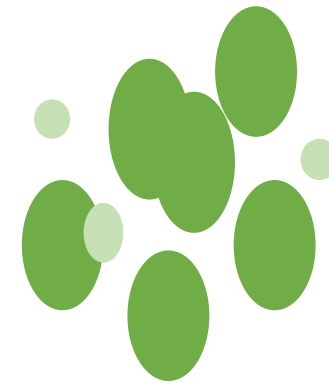
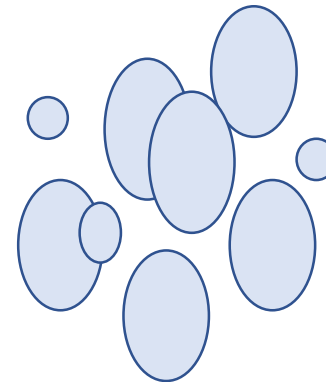
- **Staining reagents**

- Trypan Blue
- Acridine Orange
- DAPI
- Propidium Iodide



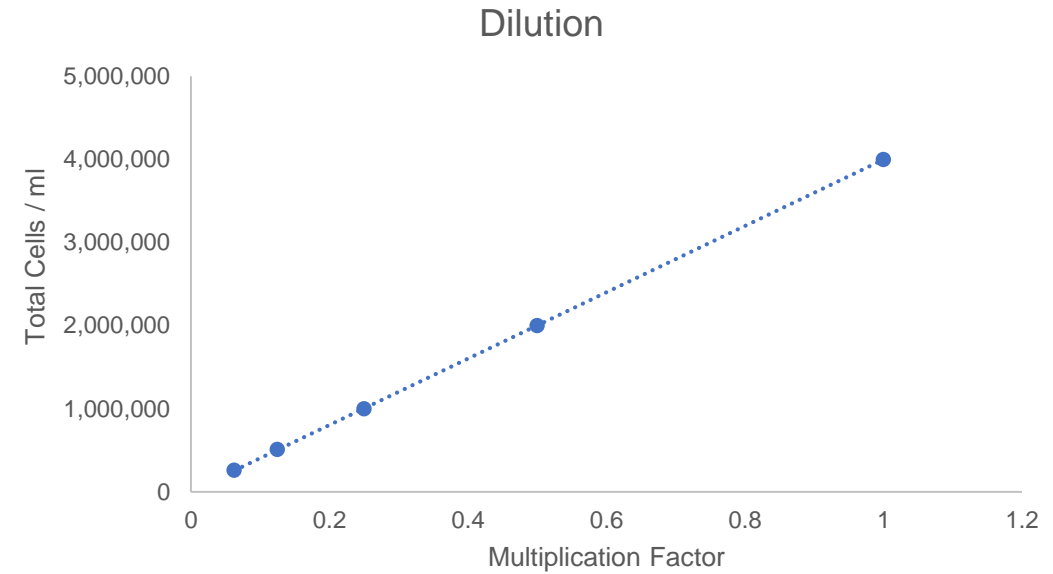
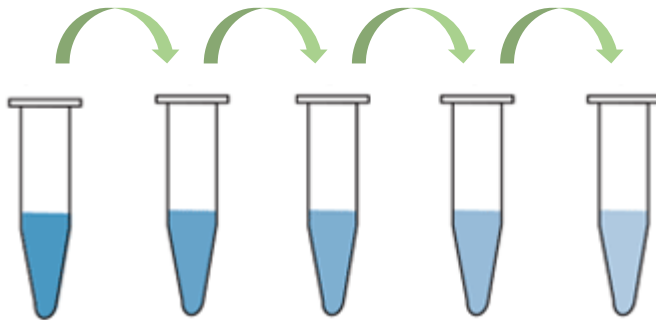
- **Parameters that define a cell**

- Brightfield image
 - Size and sharpness
- Fluorescence
 - Size and intensity

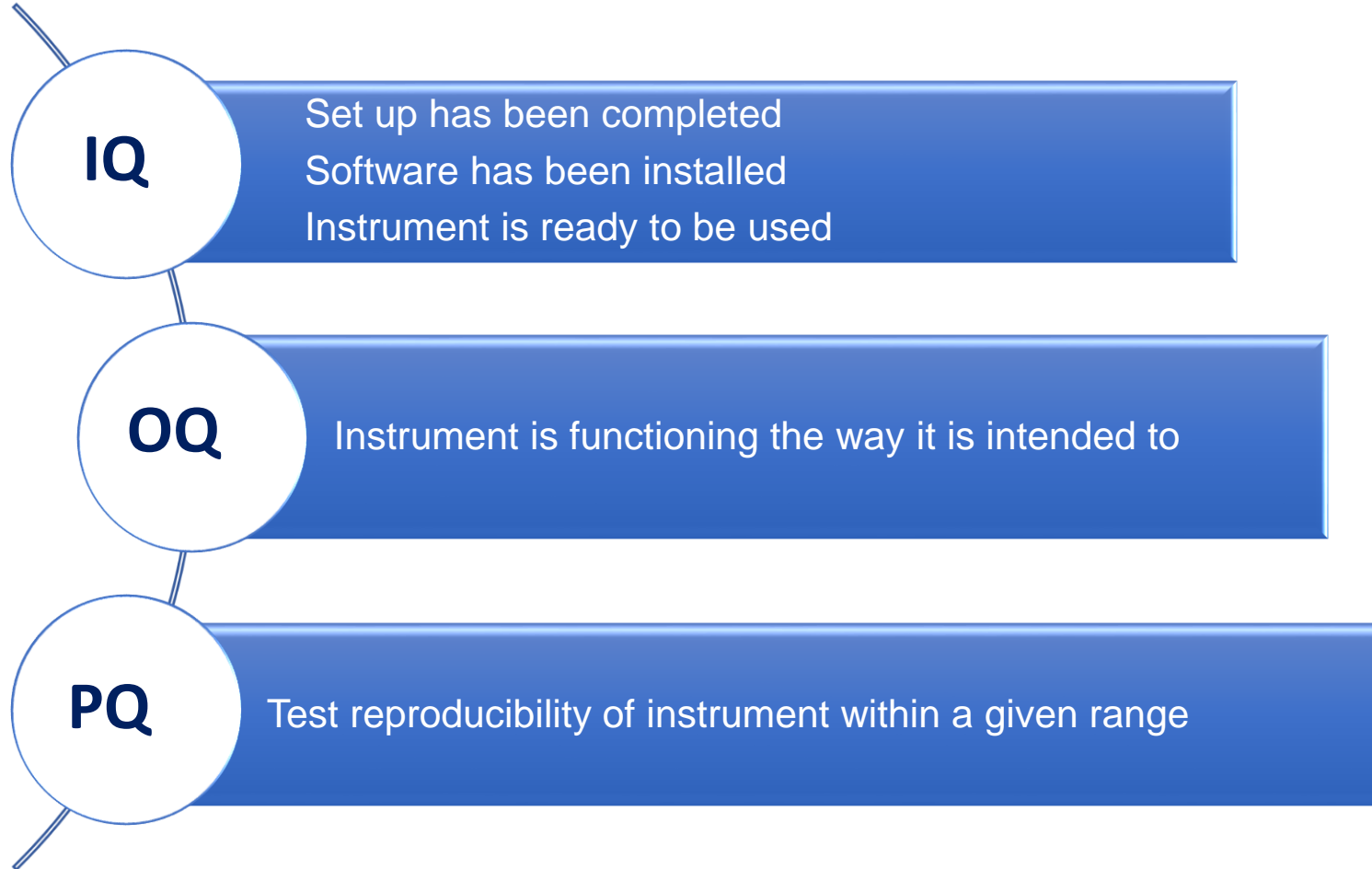


How to address different results

- Low user variability
- Low instrument variability
- Linear titration curve

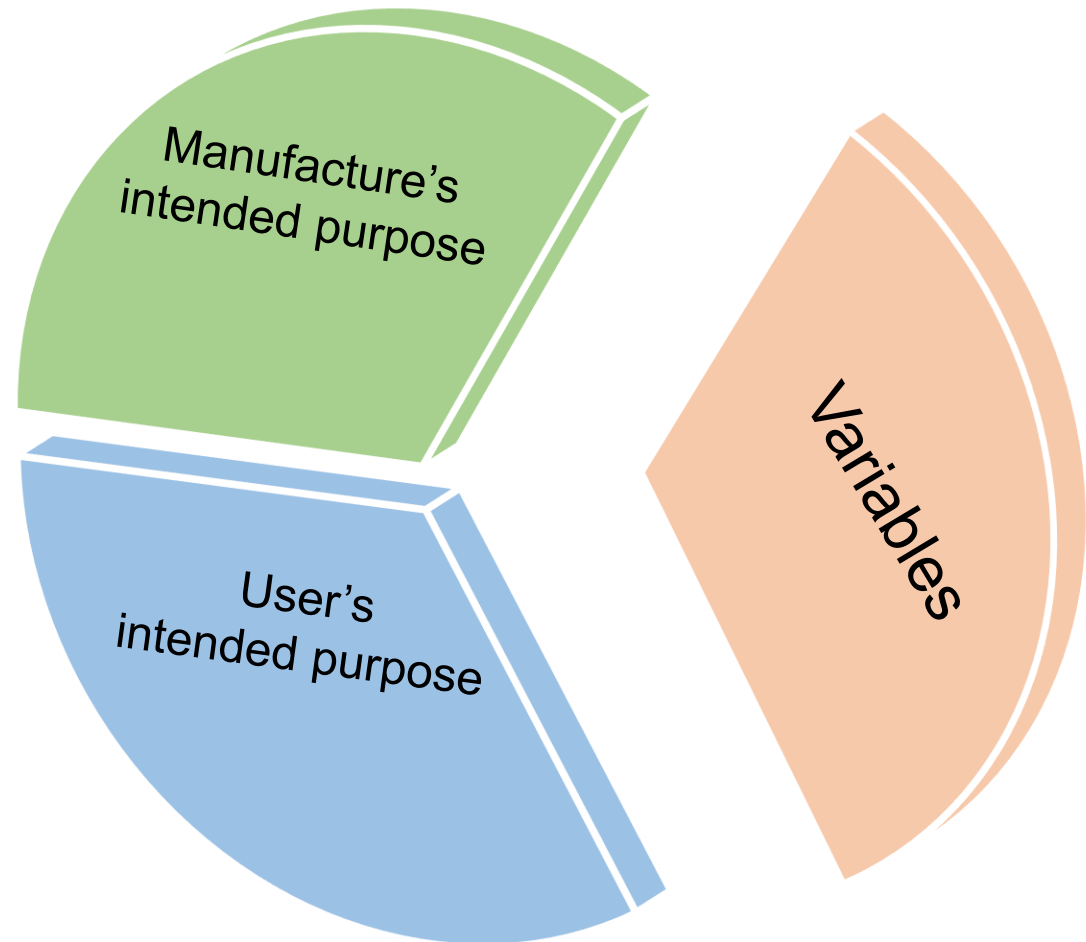


IQ, OQ, PQ



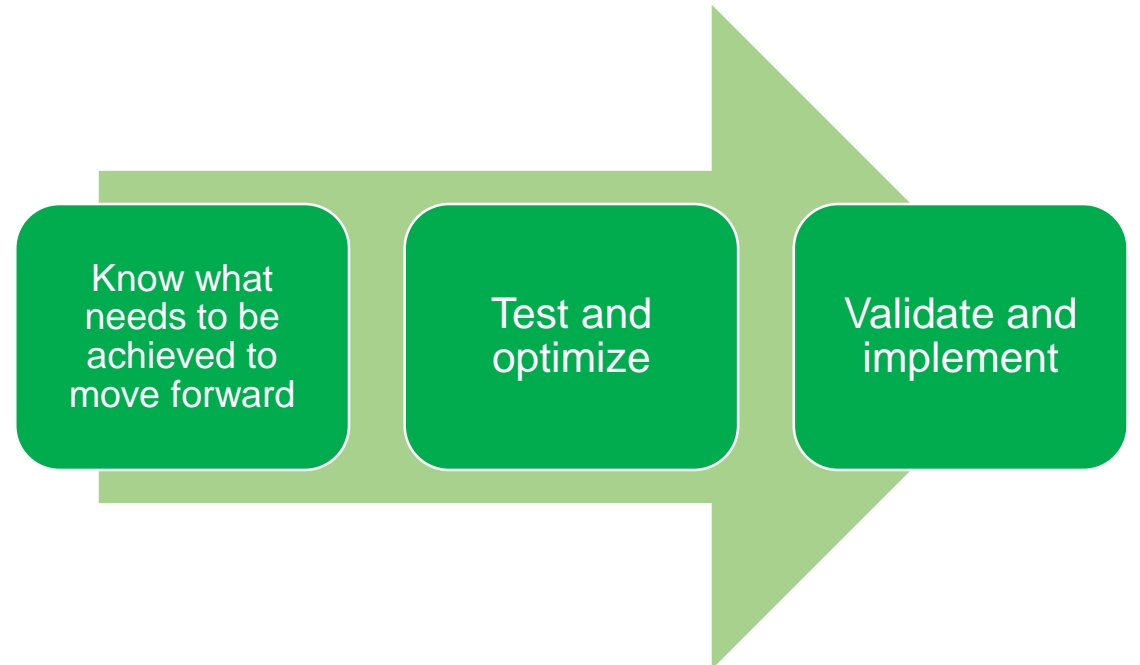
Intended purpose

- Manufacture's intended purpose
- User's Intended purpose
- Make sure these Align
- Variables to consider
 - Cell type
 - Process development
 - Media
 - Goals



Lessons learned in the field

- Objective
 - Clinical trials and commercialization
 - R&D to optimize process development / manufacturing
- Communication
 - Make sure everyone is on the same page
 - Work with vendor upstream
 - Keep in contact
- Knowledge of regulations
 - Project Manager
 - Senior Scientist



Challenges in cell counting

New variables effect validated procedures

- New steps in process development
- Reagents
- Cryo-preservation

New Technology

- Better precision
- Better definition of what a true cell is
- How to make that change

Staying updated with regulations

- Keeping up with 21CFRpart11
- Avoiding conflicts of interest