Breakout Report #3: Functional Cell Test (Potency Assay)

Overview:

- Potency Assay requires knowledge of “mechanism of action” (MOA) of cell therapy
- Functional test of cell therapy biological activity
- No standard measure for all cell therapy: individualized for each therapy

Take home message for measurement validation: Critical components & standards must be identified early for comparability & to limit assay variability

- Critical components include, but not limited to:
  - Serum and other media components
  - Cell banks to be used in assay (ex HUVECS)
  - Antibodies
  - Recombinant proteins
  - Plastic ware
  - Kits
  - Analysis equipment (microscopes, plate readers, etc)
- Standards include:
  - Positive control
  - Negative control
  - Materials to generate standard curves
  - Reference standards

Cell Functional Test (Potency Assay) Case Studies: 1) Tube Formation Assay & 2) Endothelial Cell Proliferation

Tube Formation Assay: Culture your cell therapy product, harvest the medium (conditioned medium), add to human umbilical vein endothelial cells (HUVECs) plated on matrigel, incubate, image and count formation of tubes

Endothelial Proliferation Assay: Culture your cell therapy product, harvest the medium (conditioned medium), add to human umbilical vein endothelial cells (HUVECs) plated in culture dishes, incubate, measure HUVEC proliferation

Biological Relevance:

- Use as an indicator for angiogenic activity for indications such as ischemia & myocardial infarction
- Endothelial cells are key part of vasculature & they typically must proliferate to form new vessels

Tube Formation Assay

Many drawbacks were identified including:

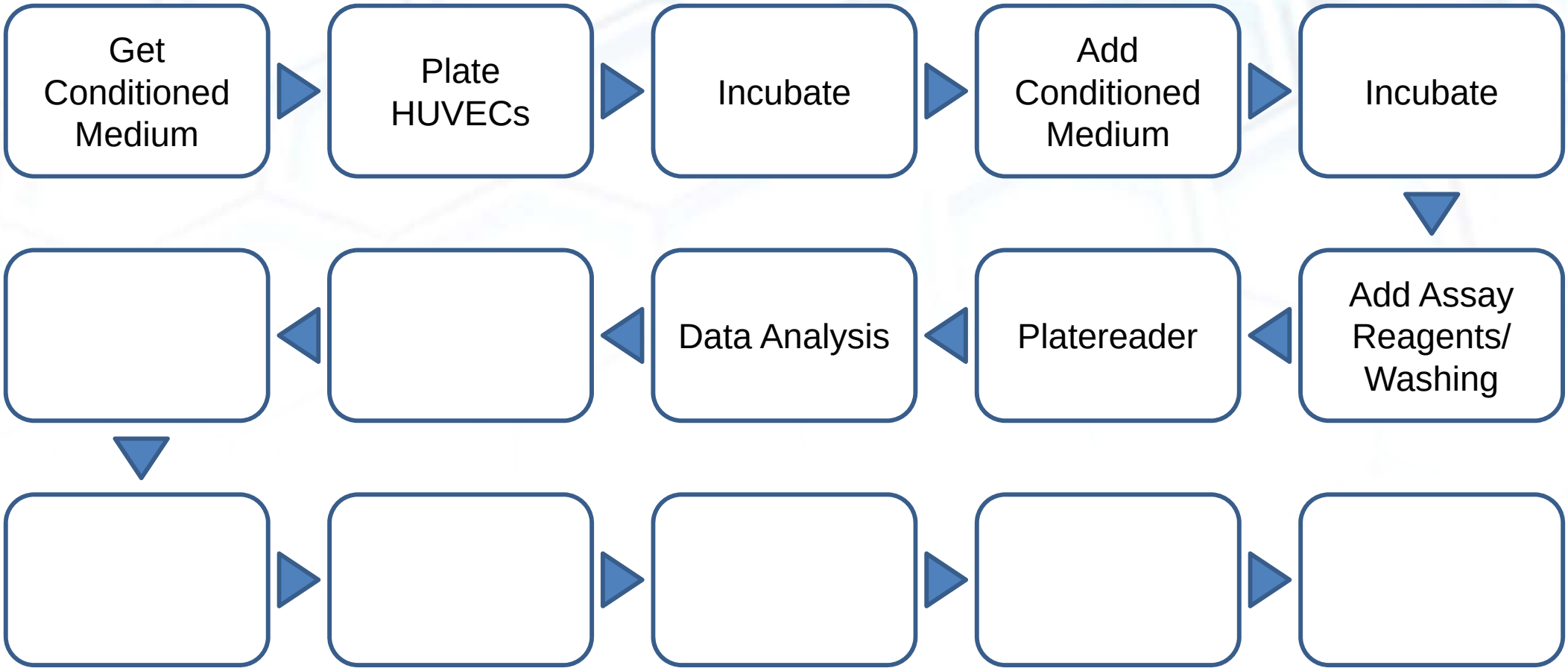
- Inconsistent results with HUVECS
- Inconsistent Matrigel lots
- Lack of a standard cell line to induce tube formation
- No standardized counting methodology
- Need for a standardized controls such as recombinant proteins with quantified tube formation activity linked to concentration

Controls that are needed:

- Negative control: Basal media
- Positive Controls:
  - Basal media + serum
  - recombinant protein (VEGF) curve
  - Reference standard (cell therapy shown to work in preclincial or clinical models)

Final discussion: Without improvements to these standards and critical materials, it will be difficult to develop a standardized tube formation assay to use for potency measurements in a GMP/manufacturing setting

Endothelial Cell Proliferation Measurement Process Flow Chart:



Endothelial Cell Proliferation

- Positive Control:
  - FBS: weak stimulator of EC proliferation
  - FBS + VEGF (or other GFs): Works best, but variable due to FBS, & you run out of lots of FBS, requiring continuous verification of new lots of FBS
  - Must retain old batches of conditioned medium (CM) that worked (for future troubleshooting)
  - NEED: Chemically-defined positive control
  - NEED: A reference material cell that reliably produces conditioned medium that stimulates EC proliferation
- Negative Control:
  - Medium without FBS (doesn't account for effects of FBS)
  - Medium with FBS (variable FBS composition by lots)
  - Conditioned medium is depleted of nutrients from culture with the CTP
  - Immuno-depleted conditioned medium (but you have to know MOA & which growth factors to deplete)
    - Catch 22: But if you know the growth factors, then you could deliver the GFs & eliminate the CTP

Endothelial Cell Proliferation

- Reference Material Cells (fully characterized via hyperlink to “cell counting” and cell viability” Breakout Sessions)
  - HUVECs are suboptimal: primary cells, vary by donor, limited supply
  - NEED POSITIVE CONTROL CELLS: Cells that reliably respond to angiogenic factors by proliferating
    - This case is amenable to a reference material cell since the cell is not the CTP and is instead part of the measurement system; resulting in potentially greater consensus in the community on how to standardize
  - NEED NEGATIVE CONTROL CELLS: Cells that do not respond to angiogenic factors to establish that the response is specific
- Training/SOP
  - Need a rigorous way to train people and establish the SOP
  - Requires the mindset that the measurement will need to be performed after you leave the company