

# 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:

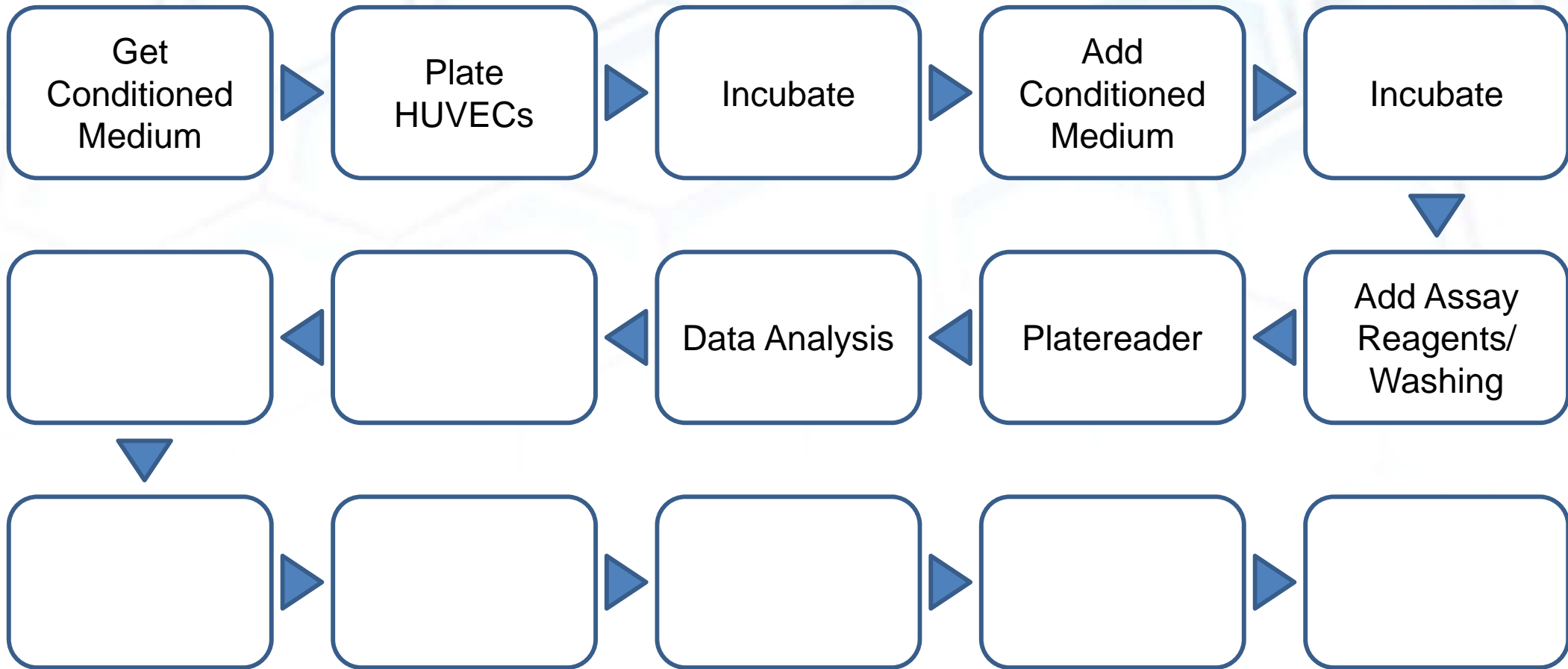
- Inconsistent results with HUVECS
- Inconsistent Matrigel lots
- Lack of a standard cell line to induce tube formation
- No standardized counting methodology
- Need for a standardized controls such as recombinant proteins with quantified tube formation activity linked to concentration

## 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 preclinical 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