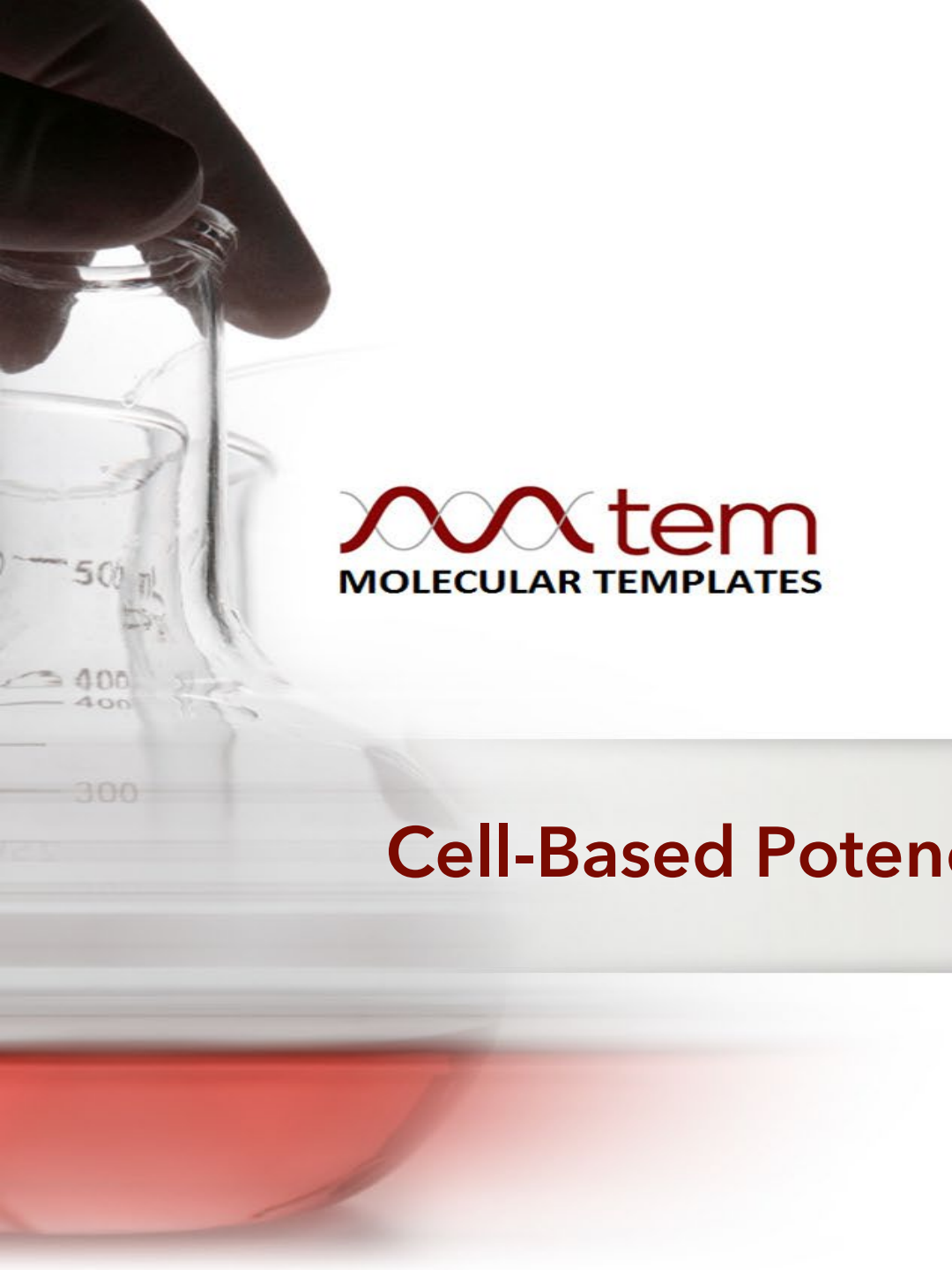

MOLECULAR TEMPLATES

Cell-Based Potency Assay : Designs and Considerations

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CASSS CMC Strategy Forum 2022

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Acknowledgment

Molecular Templates Product Attribute Sciences group

Talk Outline

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- Introduction to Potency
- Developing Cell based Potency Assay
- Characterization of the assay
- Conclusion

What is Potency?

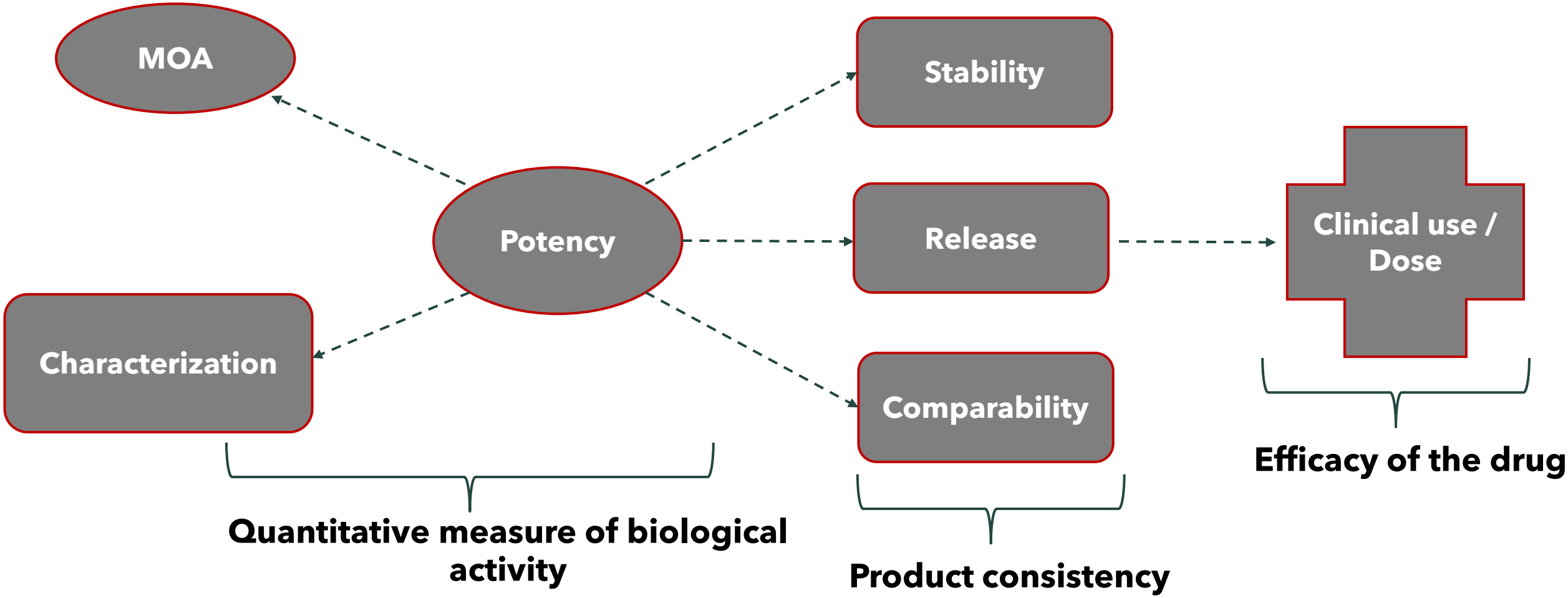
- Quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties. (21 CFR 600.3)
- ICHQ6B defines a valid bioassay :
    - Animal Based : Organism's Biological response
    - Cell-Based : Biochemical/ Physiological response at cellular level
    - Biochemical Assay : Enzymatic reaction rate
    - Other Procedures : Ligand and receptor binding

- Reflect the Mode of Action (MOA) of the product

Why a Potency Assay?

- Mechanism of action (MOA) can be evaluated using well-designed potency assay
- Quantitatively measure the biological activity of a compound
- A well-designed potency assay is correlative of product quality
- An appropriate potency assay reliably predicts clinical efficacy

Uses of Potency Assay

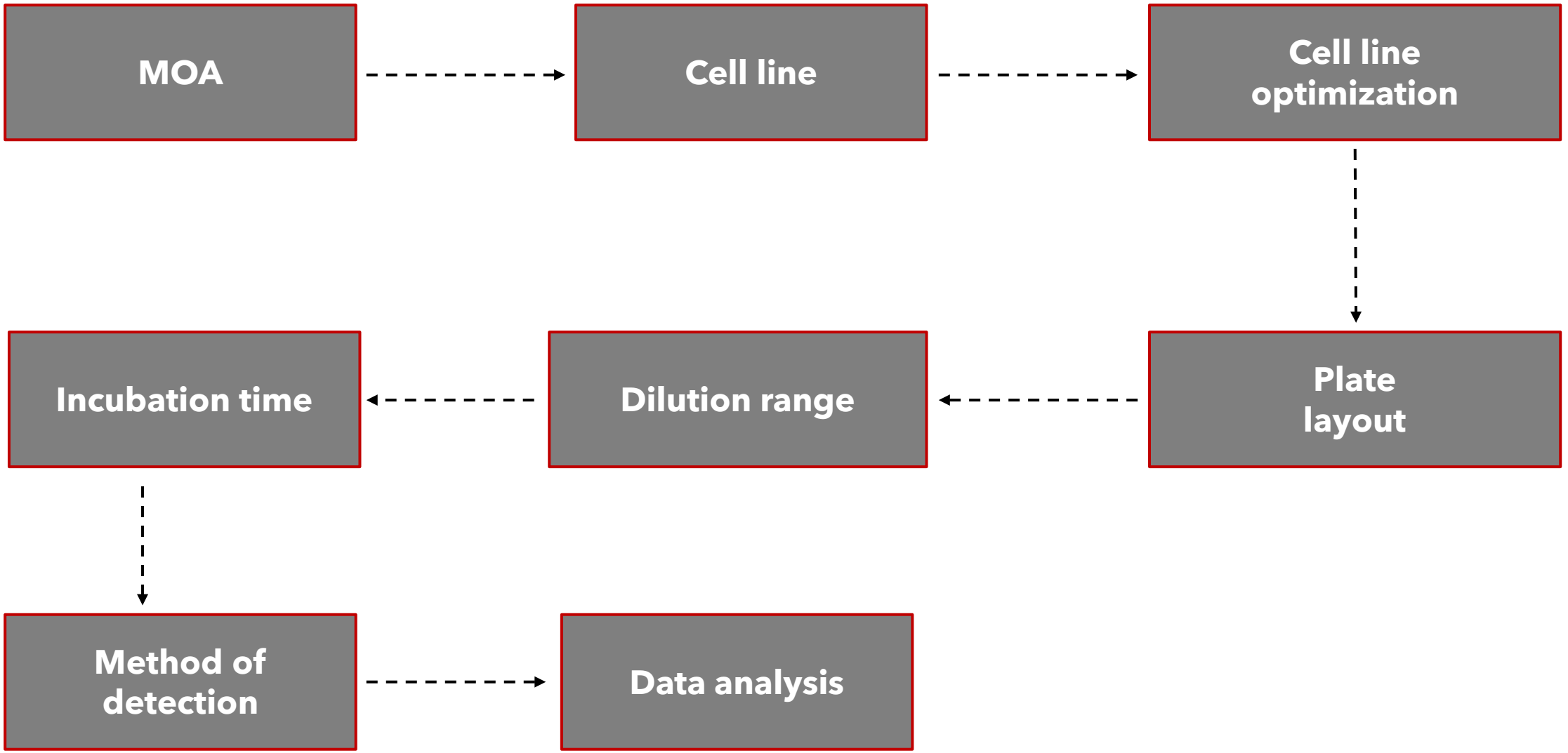


Why cell-based potency assay

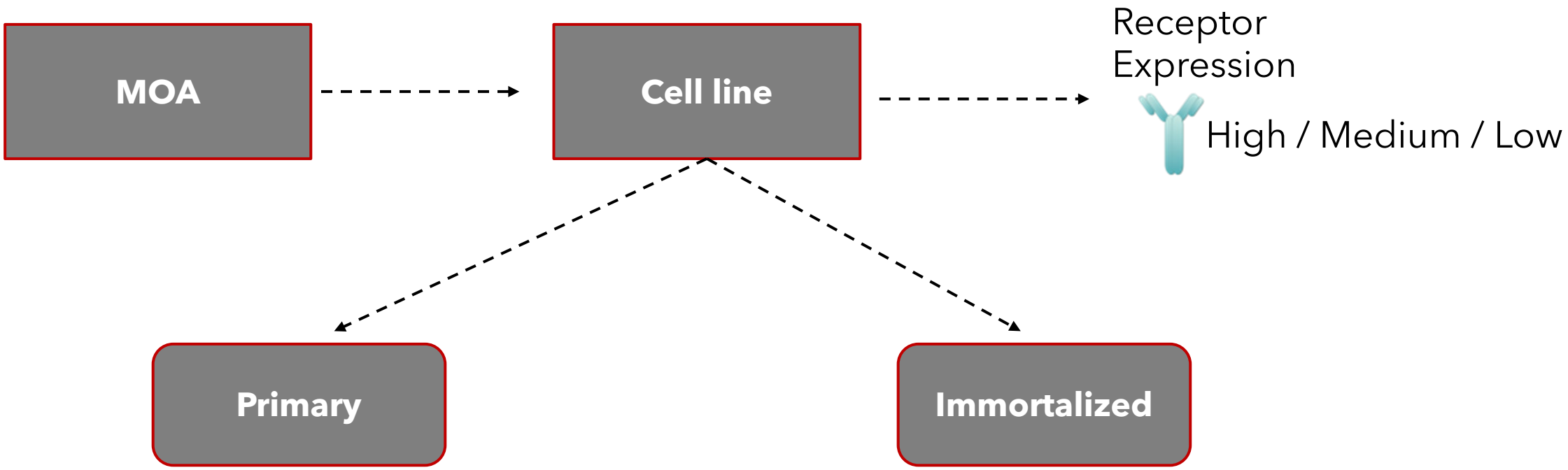
Cell based potency :

- More efficient, faster, and less expensive than animal studies
- Straight-forward route to establish an analytical assay with biological relevance

Cell-based Potency Assay Development



Cell line



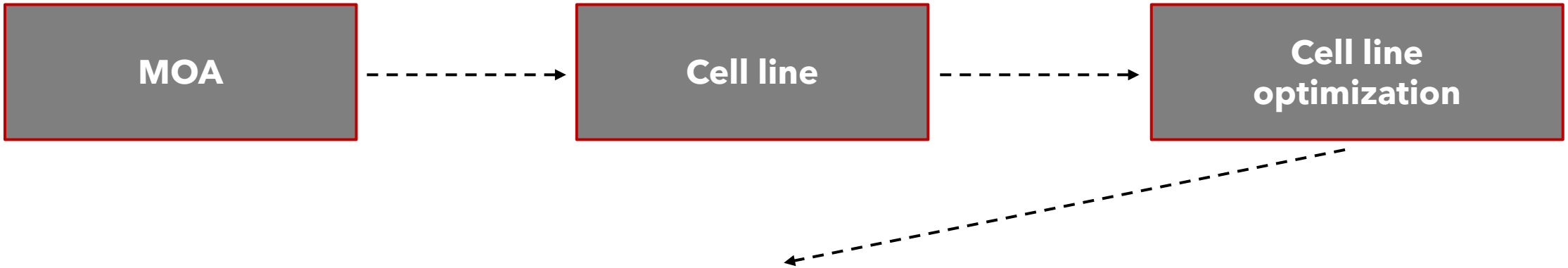
- PBMCs to study drugs like mosunetuzumab that target CD20 on b-cells.

- mAb like pembrolizumab can be tested on cells having the correct receptor are an excellent choice due to the availability

Cell line

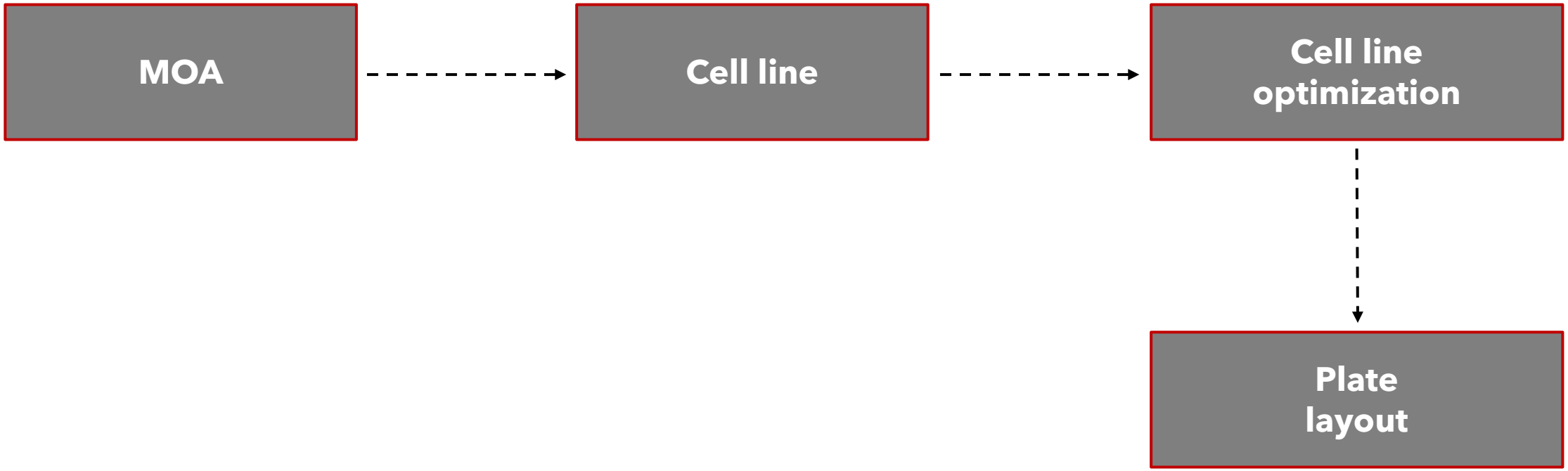
	Pros	Cons
Primary Cell line	• Biologically relevant system • Retains genetic integrity and physiology • Bypass animal use	• Variable between batches • Short cell culture lives ~ 2 weeks • Cannot be stored long-term • Cell line heterogenicity
Immortalized Cell line	• Cost effective • Not variable between batches • Longer cell culture lives • Can be stored for years.	• Not have the relevant attributes or functions of normal cells.

Cell line optimization



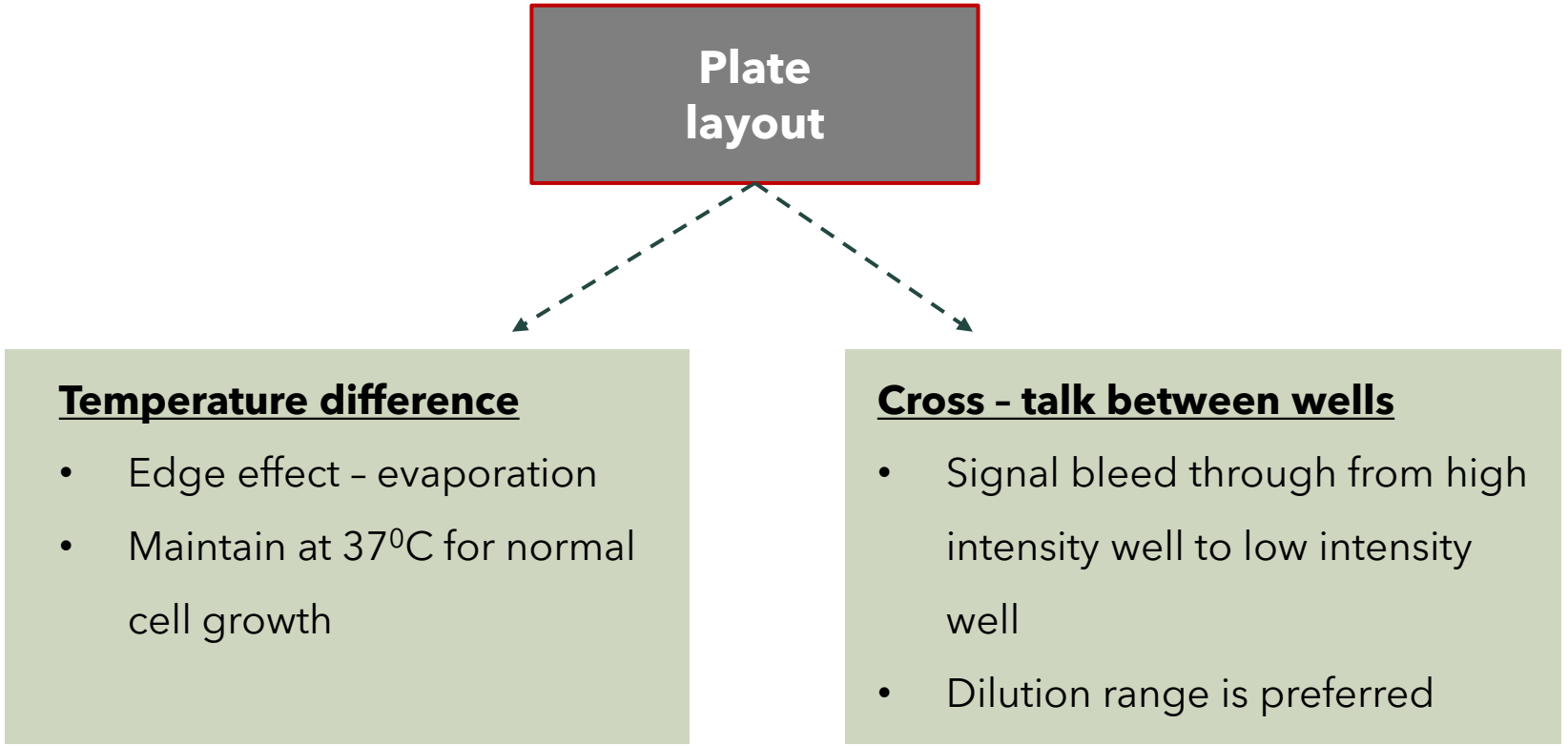
- Check for mycoplasma monthly to prevent contaminations leading to false positive results
- Passage number for immortalized cell lines up to 40
  - >40 passages cells might not grow at the same phase as younger cells leading to variability in the cells' response to intoxication
- Enough cell stocks are made and stored in liquid N_2 for long-term storage
- Plates prepared with lowest number to highest number of cells per well (500 – 5000 cells per well) to determine number of cells per well

Cell-based Potency Assay Development

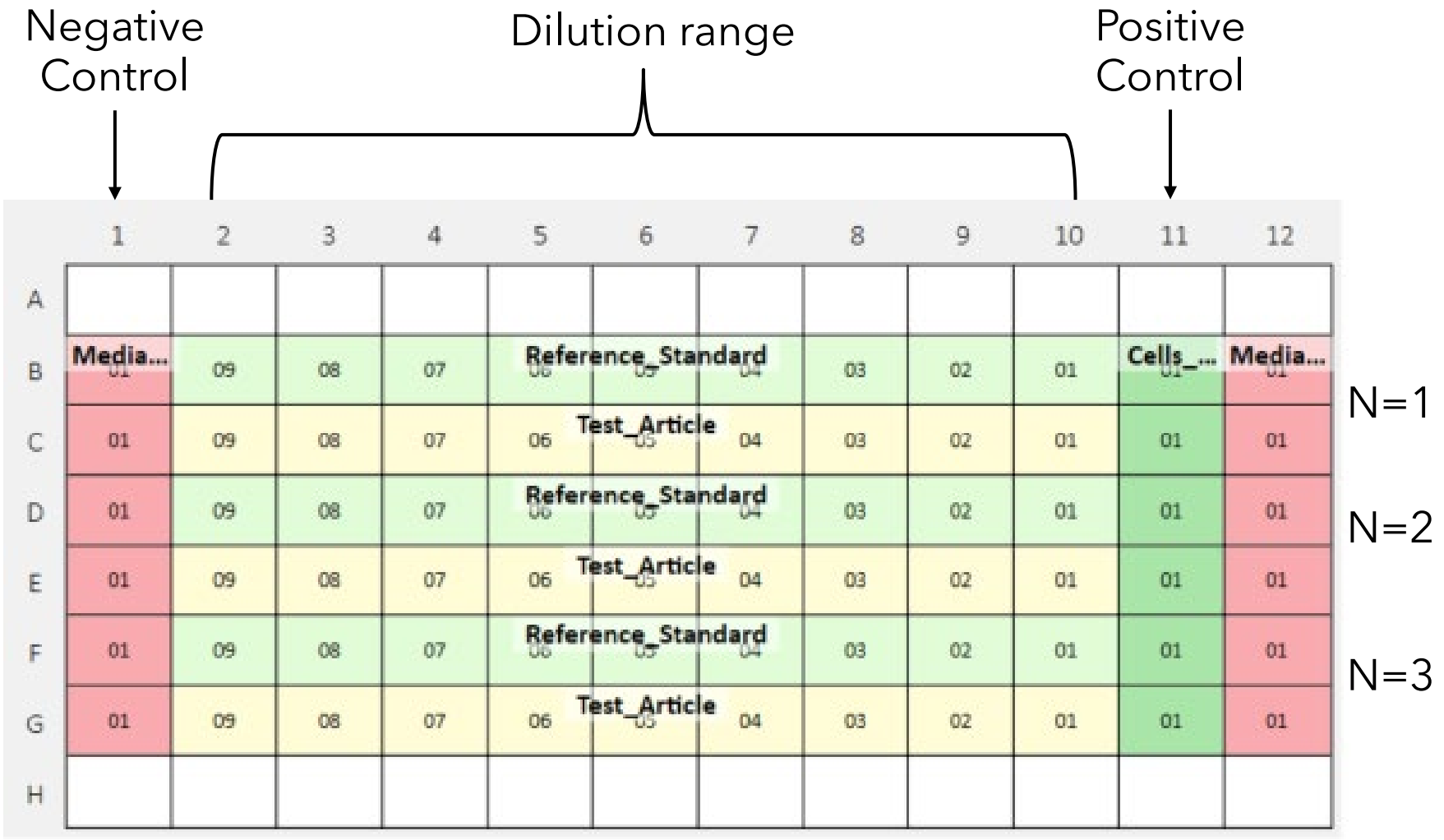


Considerations for Plate Layout

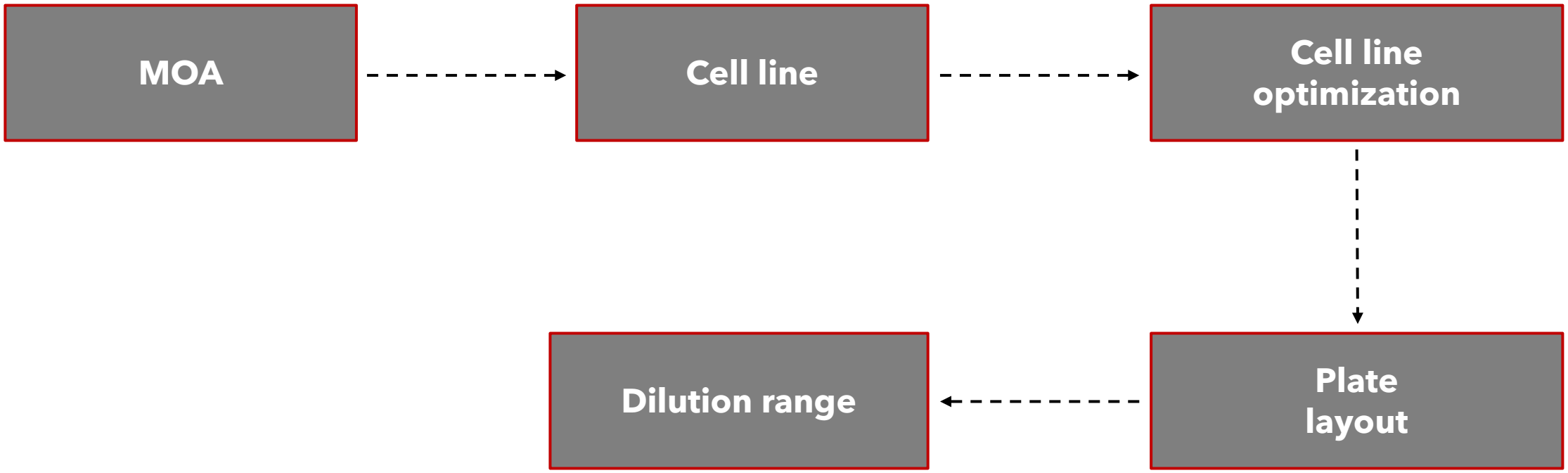
- The area of plate wells and the growth rate of the cell line are factors in the number of cells seeded per well.
  - 96 well preferred over 384 well due to reduced variability



Sample Plate layout



Cell-based Potency Assay Development



Dilution range

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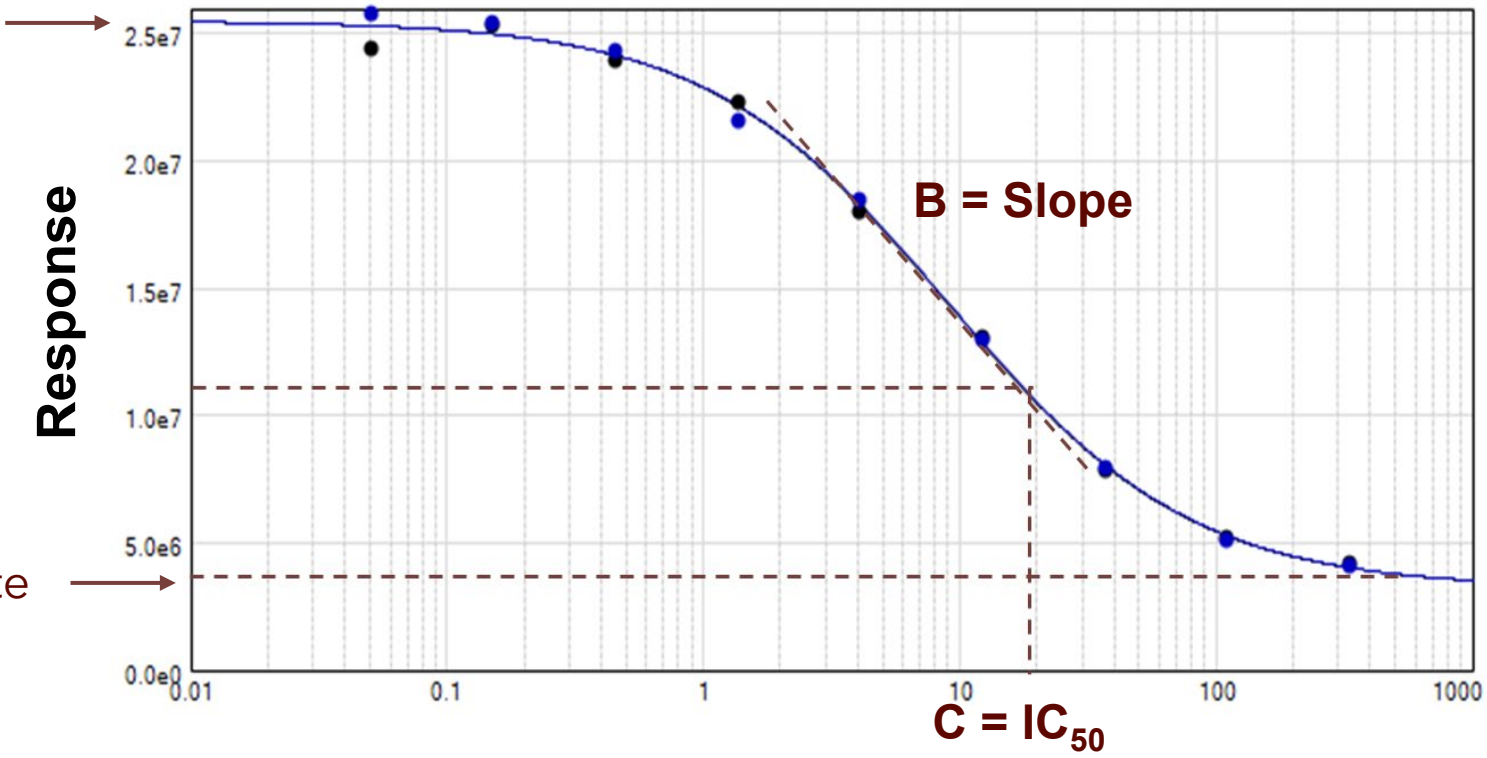
USP 1032-1034 addresses bioassays

- Focus should be on Relative Potency due to inherent variation of biological material
- Response vs log Concentration
- Guidance says that parallelism is to be used to evaluate the suitability of bioassays
- 4 parameter Parallel Line Analysis (4-PLA) is used in most cases, as the curve is sigmoidal
- A minimum of 9 points are to be used to determine the dose/response curve

4 parameter Parallel Line Analysis

A = Upper asymptote

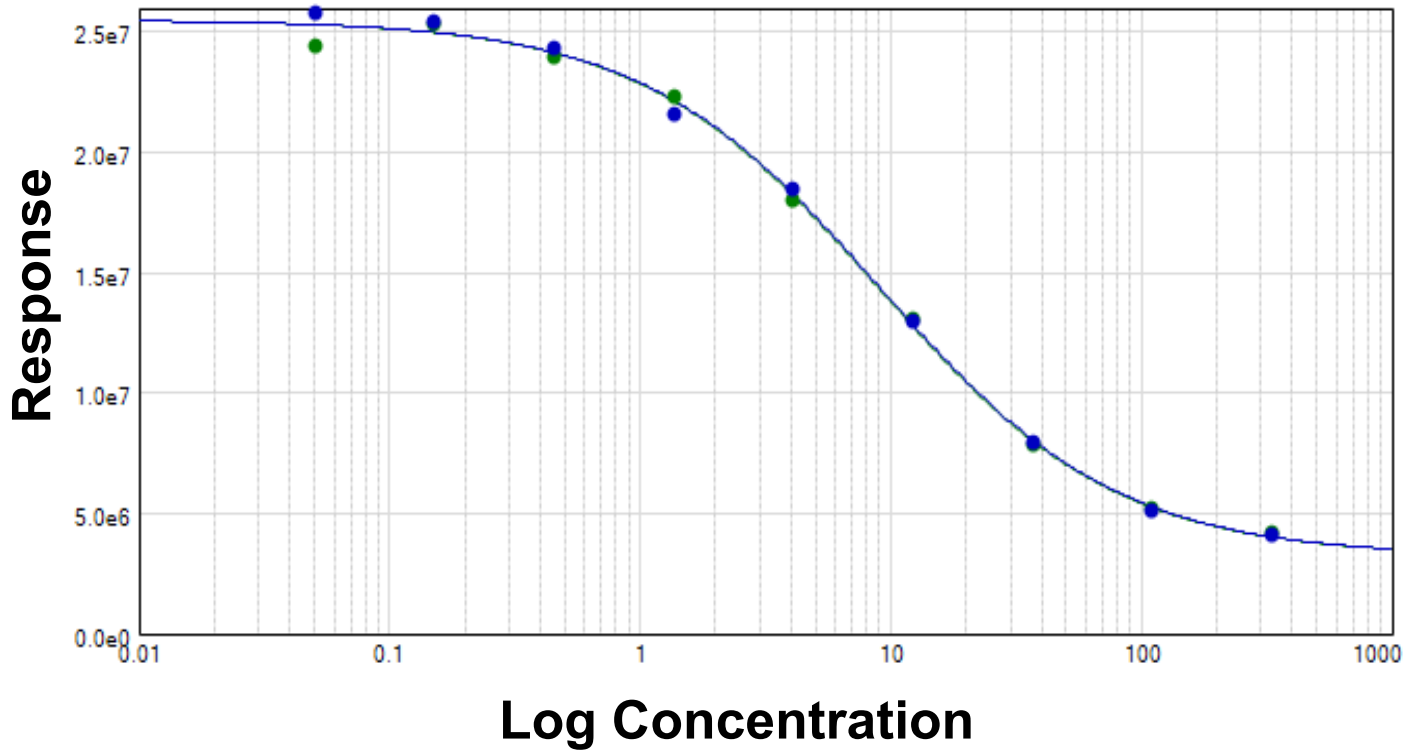
D = Lower asymptote



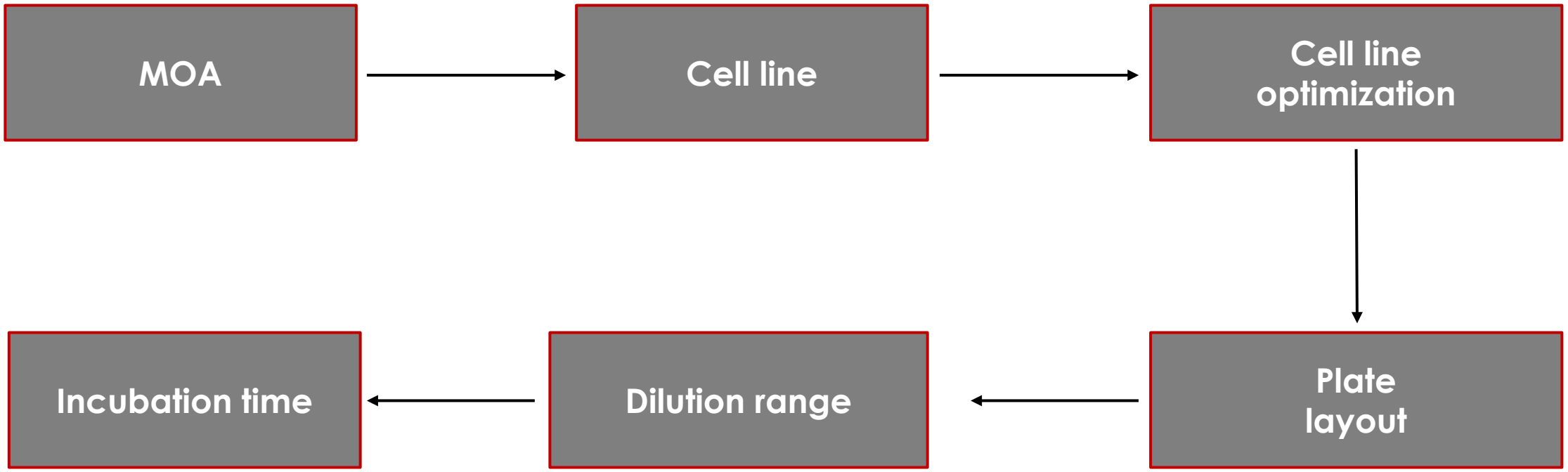
$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}}$$

Dilution range

- Dilution amount affects range of points as well as spacing
- Initial concentration determines where on the curve the points lie.



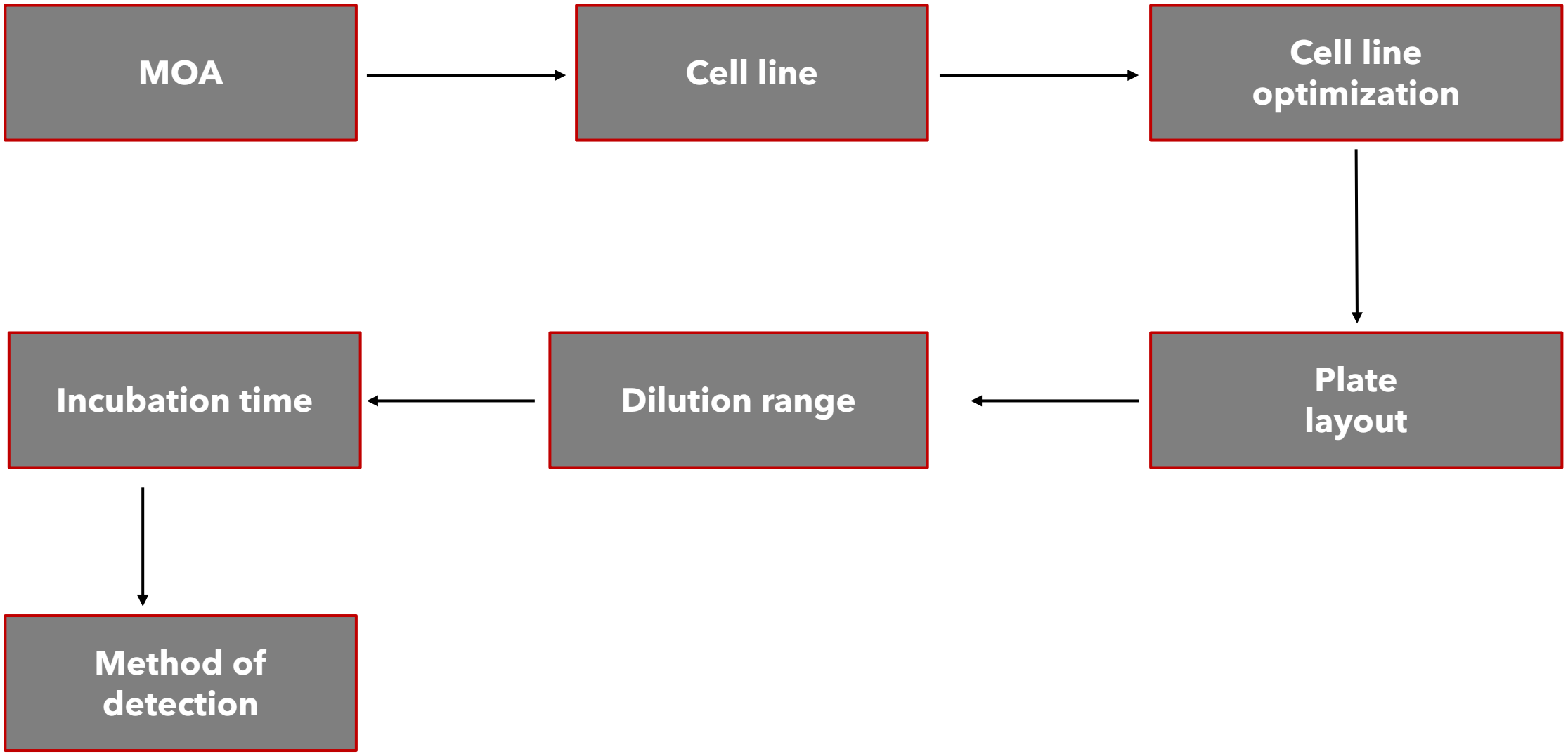
Cell-based Potency Assay Development



Incubation time / Readout

- Incubation after treating with drug (24-72h) at 37^{o}C having 5% CO_2 supply
- How long after adding the reagent the incubation time is critical usually up to 4h for Fluorescence reagent
    - Longer incubation leads to auto fluorescence and bleaching
    - Lesser incubation will result in underdeveloped reaction (color)
    - Optimizing the right time is critical for the assay to be successful
- At shorter timepoints, such as 24h the drug may not have an effect cell growth
- At longer timepoints, such as 96h the drug effect has been obscured by cell growth
- An optimal time is established where there is well resolved activity such as 72h

Cell-based Potency Assay Development



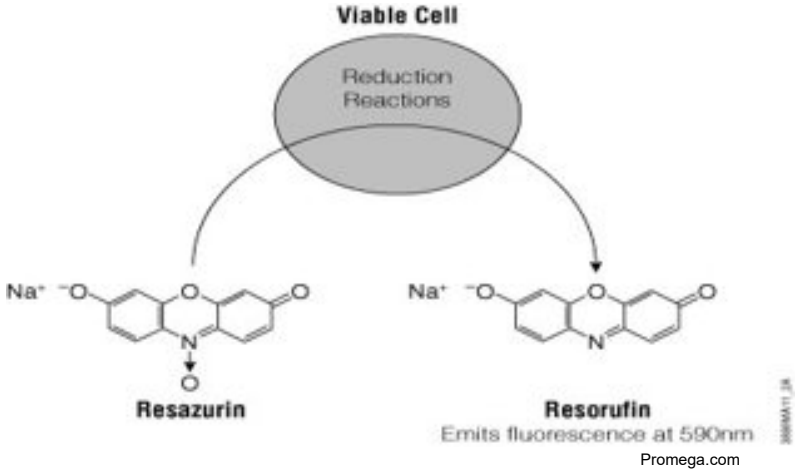
Method of detection

Method of detection

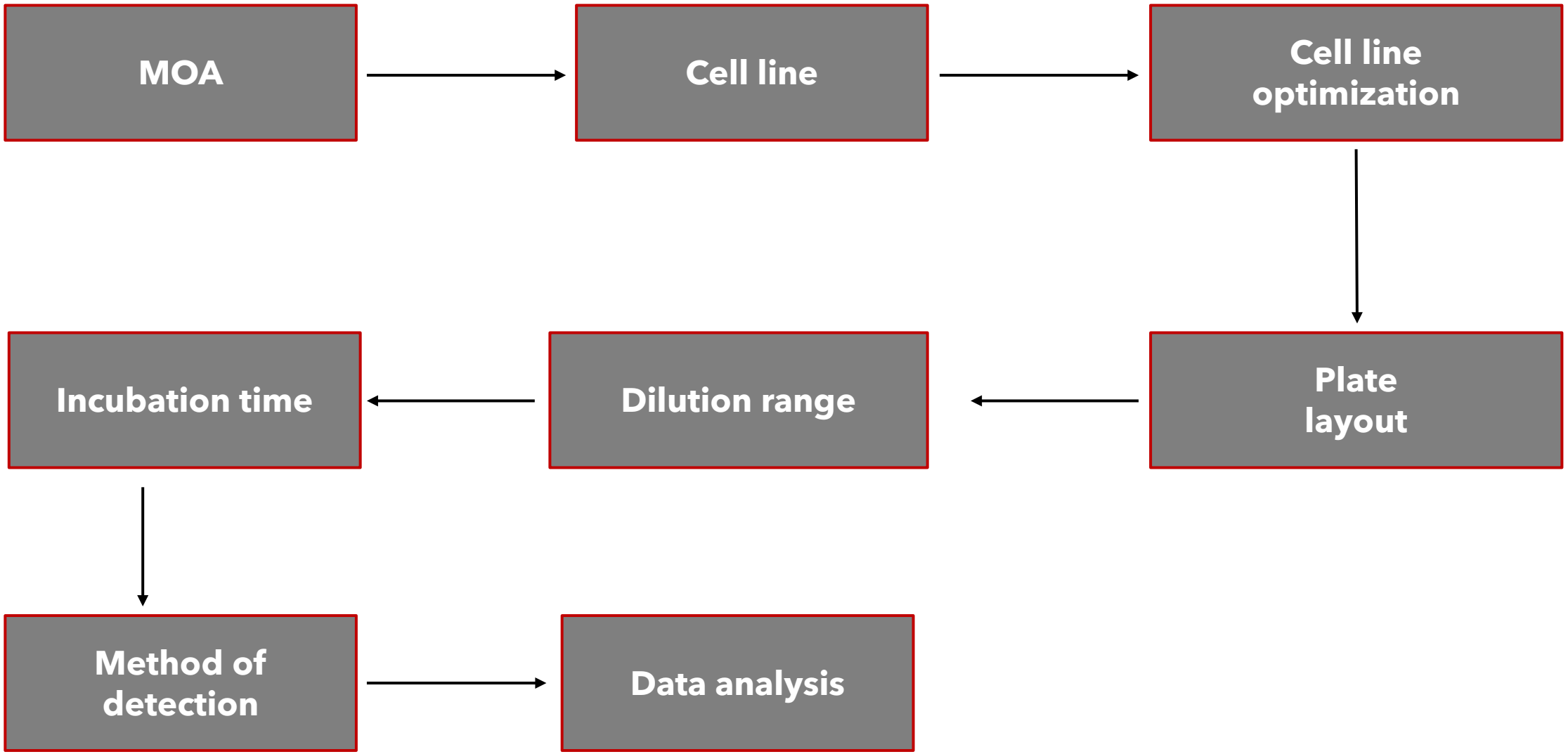
Luminescence is the most sensitive with the shortest incubation time (10 min), but also most expensive (plate, reagents, reader)

Absorbance is the least sensitive

Fluorescence requires a 4-hour incubation period but is more sensitive than absorbance and less costly than luminescence



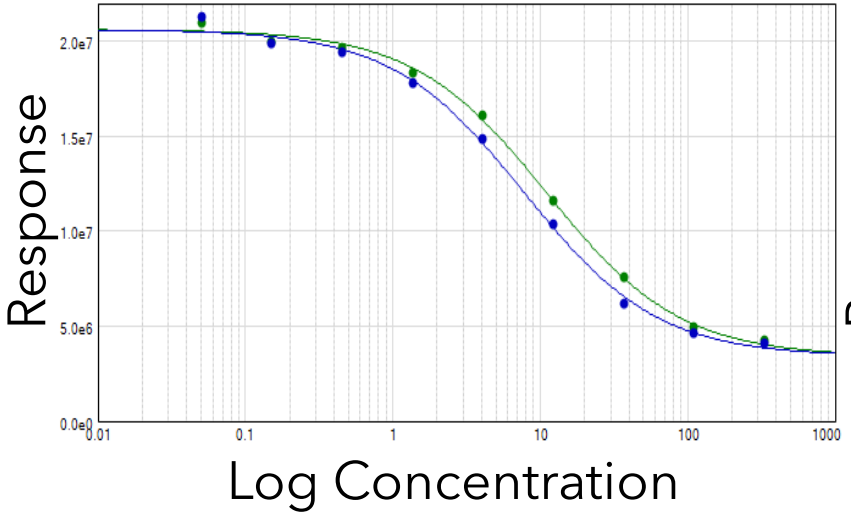
Cell-based Potency Assay Development



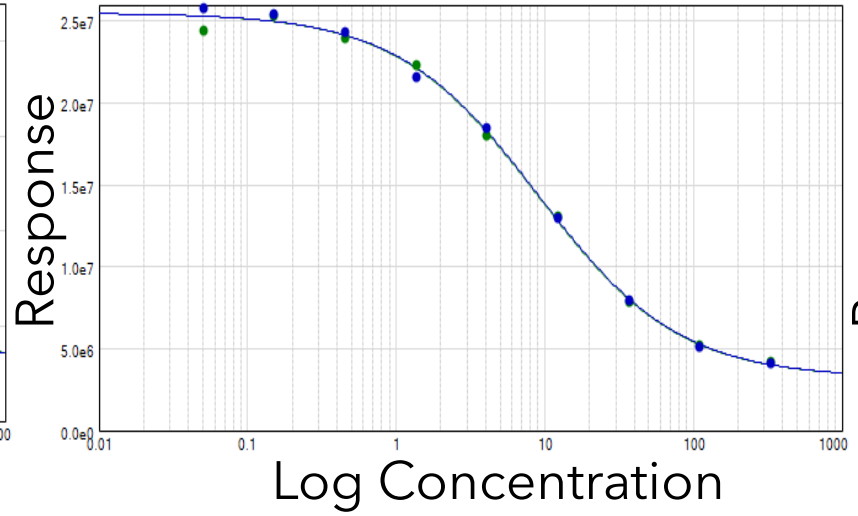
Data analysis

- Due to inherent variability in the assay, potency assay is reported as relative to the reference standard.
- 2- full dose response curves are generated for each plate.
- Potency = IC50 of Reference / IC50 of Test

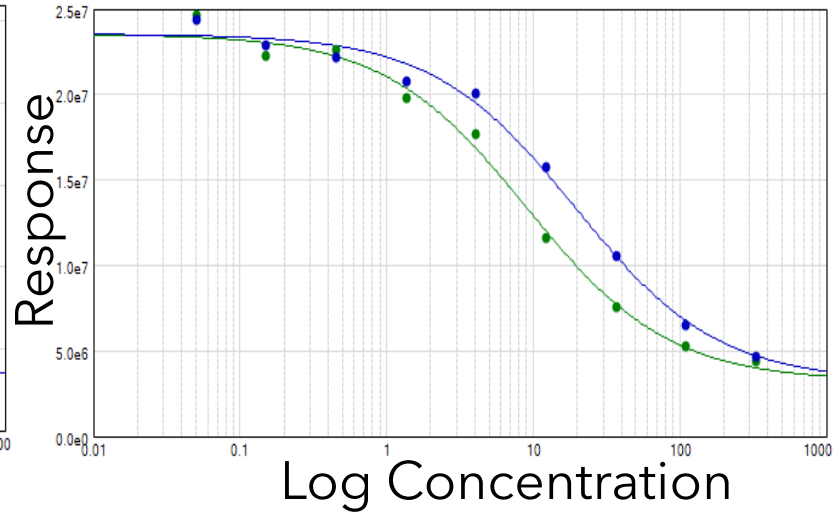
More Potent



Same as Reference



Less Potent



= Reference Standard

= Test Article

Characterize a cell-based Potency Assay

Define Assay Specific Criteria

- Goodness of fit to confirm precision of measurements usually R-squared value >=0.98
- Z- factor Signal to Noise Ratio >=1.5

Evaluate reference similarities

- Run the same reference vs reference sample to achieve close to 1.0 estimated relative potency.
- Closer the value to 1.0 the more similar the samples are. Values outside (0.8-1.2) would fail similarity criteria, leading to re-evaluating the assay development.

Evaluate product related impurity samples

- Acidic variants, Basic variants, HMW species etc., to distinguish the potency compared to reference material

Evaluate performance parameters

- Accuracy, Linearity, Specificity, Precision and Range

Conclusion

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- Develop an assay to focus on minimum variability
- Choice of cell line and the assay reflects the MOA
- Assay is designed with appropriate positive and negative controls
- Assay performed relative to a reference standard on every plate
- Triplicate samples are used in every plate
- Assay designed for amenable QC environment