ENHANCING THE SPEED OF VACCINE DEVELOPMENT BY UTILIZING FULLY AUTOMATED HIGH THROUGHPUT CELL POTENCY ASSAYS

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High-throughput Automation

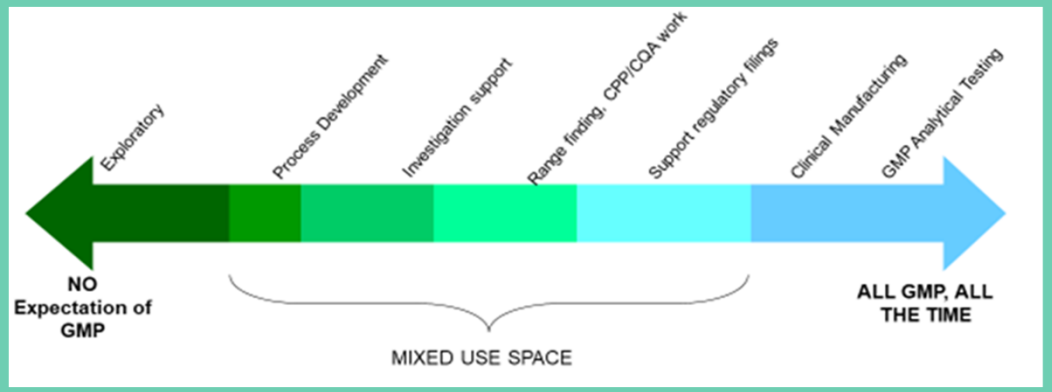
ANALYTICAL RESEARCH & DEVELOPMENT

High-throughput Automation is a popular solution to accelerating process development by increasing sample numbers and data quality while decreasing data turnaround time, and assay variability. A wide range of analytical techniques and specialized equipment can be used for high throughput automation.

Who are our partners:

- Process development teams
- Formulation
- Continually evaluating partnerships

Phases we support:



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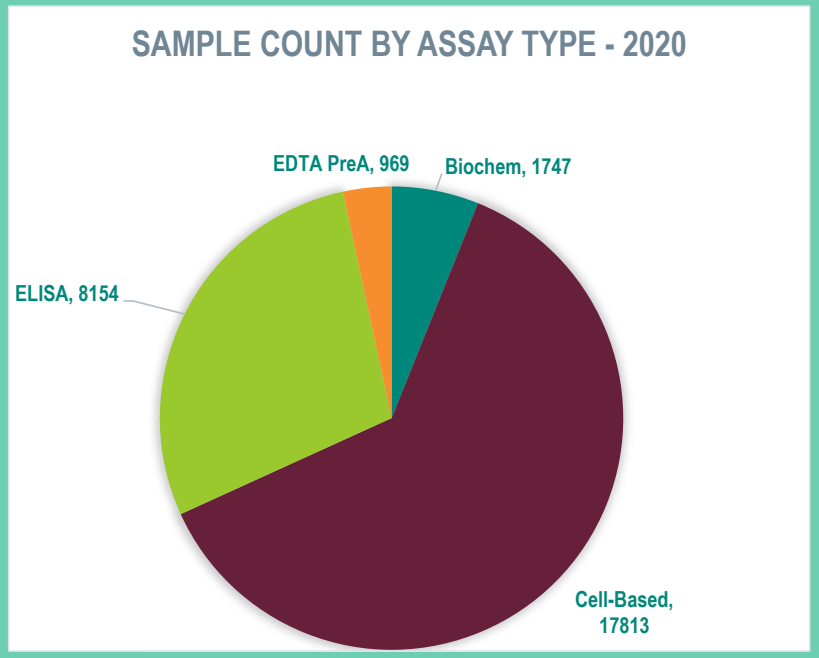
Automation Strategy

ANALYTICAL RESEARCH & DEVELOPMENT

- Focus
  - Labor intensive assays (ELISAs, Cell Based Assays…)
  - High sample volume assays
  - In use for long periods of time
- "Walk-away" automation
  - Maximize resource liberation
  - 1 day ELISA assays
- Automation of data reduction and reporting
- 10 assays
- 8 vaccine candidates



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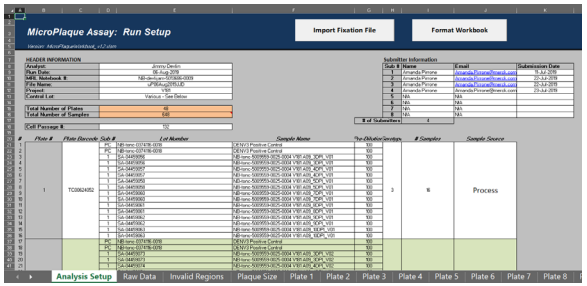
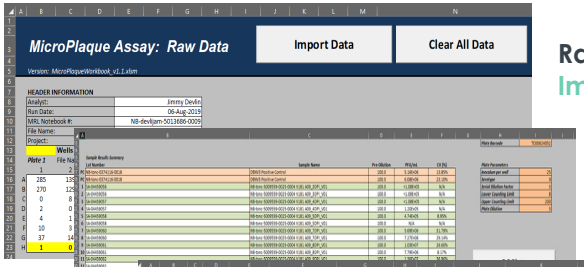
Assay Data Workbook Overview

ANALYTICAL RESEARCH & DEVELOPMENT

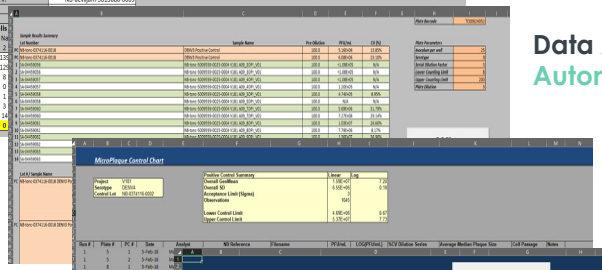


Sample Entry Worksheet
Dilution/replication
Sample order
Plate Sero*type*

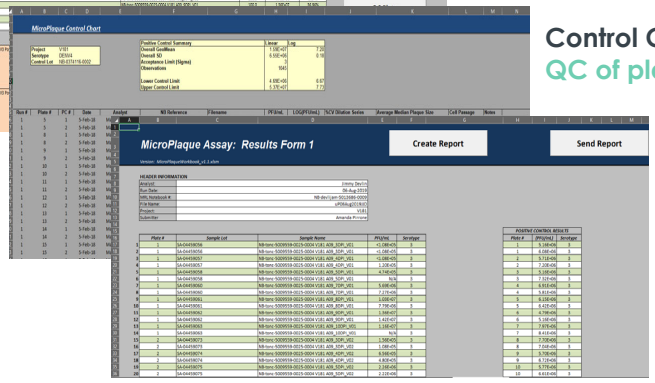


Raw Data Worksheet
Imports data from reader

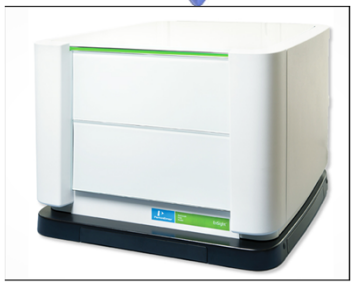
Data Analysis Worksheet
Automates calculations



Control Charting
QC of plate data against historical



Results Report Worksheet
Generates & sends report



Generates raw data file

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CASE STUDY

Background on Covid Vaccine candidates

ANALYTICAL RESEARCH & DEVELOPMENT

Utilize a recombinant Vesicular Stomatitis Virus (VSV) platform by replacing the VSV-G protein with the SARS-CoV-2 S Protein

The CoV Spike gene is large - VSV genome size is increased by ~ 3kb

The VSV and CoV replication cycles differ

- Replication occurs in different cellular compartments
- CoV Spikes concentrate in different regions of the cell compared to VSV G
- CoV Spikes tend to be fusogenic

Assay Concept / Comparison

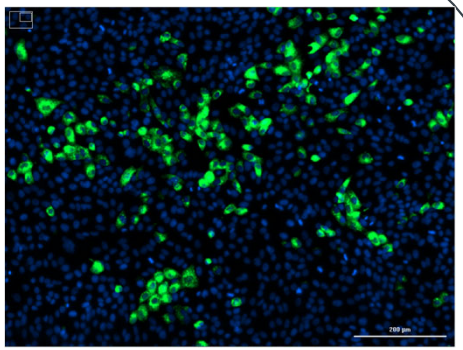
- Plaque Assays – to quantify infectious viral units that are capable of infection and cell-to-cell transmission (i.e. viral replication)
  - Goal is to allow multiple infection rounds per infectious virion to form plaque colonies
  - Generally direct quantitation assay (pfu/mL read out)
  - Typically used as a dose assay for viral vaccines

- Infectivity Assays – to quantify viral units capable of infecting a cell and expressing viral protein(s) on the surface of a cell
  - Goal is to measure only primary infection events per viral unit
  - Generally a relative potency read-out (%RP); can also be direct quantitation (FFU/mL or IU/mL)
  - Typically used as a measure of potency for process and formulation development; can also be used as dose assay

HTA Cell Based Assays Platforms

ANALYTICAL RESEARCH & DEVELOPMENT

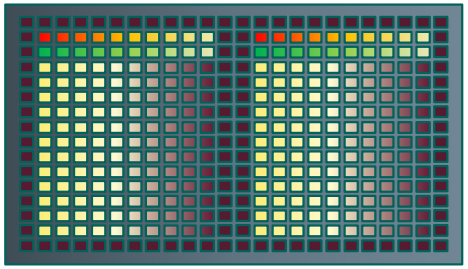
HighRes Biosolutions System "Selene"

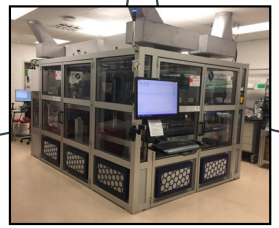


V590 Infectivity using BioTek Cytation5

HT Infectivity Assay Platform

- Fully automated (48-60) 384 well plate run capacity – 12 samples/plate (in duplicate)
- 576 - 720 samples per run
- 2-3 runs/week
- 96-capacity built-in
- BioTek Cytation5



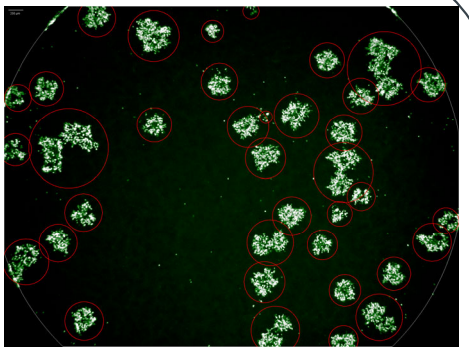


Our first HighRes Biosolutions System "Phoenix" →
Set up for both Assay Platforms in lower capacity and throughput

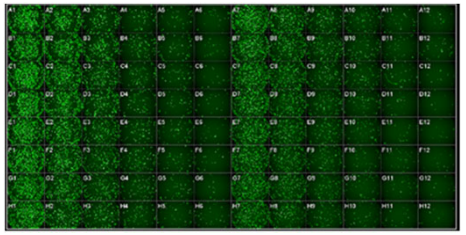
PAA System "Helios"

HT µPlaque Assay Platform

- Fully automated (44-48) 96 well plate run capacity – 14 samples/plate (in singleton)
- ~600-700 samples per run
- 1-2 runs/week
- 384 well capability built-in
- PE EnSight & BioTek Cytation3



V590 Immuno-plaque
Image captured using PE EnSight (Algorithm Counted: 44)



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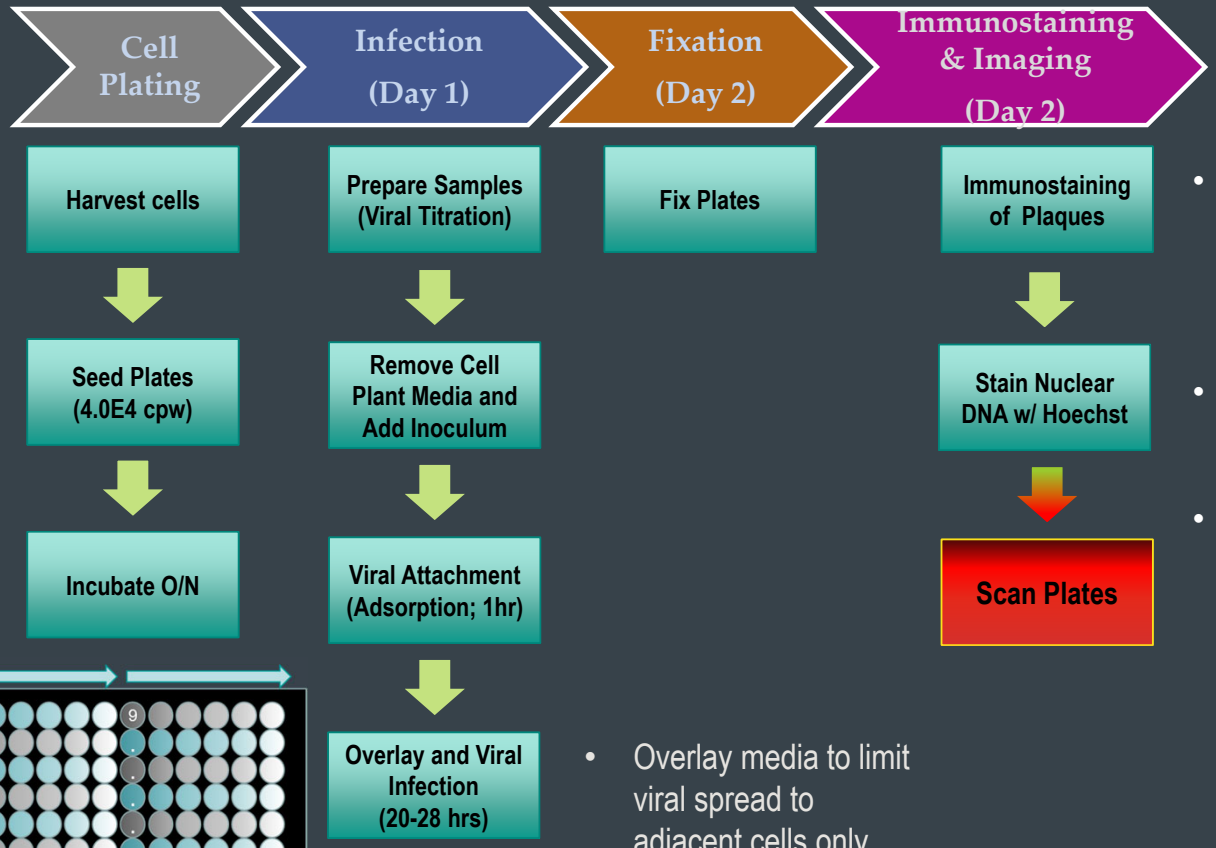
96-well Plaque Assay Workflow



High Throughput Format – Across Plate
- 14 sample slots

- Overlay media to limit viral spread to adjacent cells only

- Immunostaining of plaques with viral specific *mAb*
  - AlexaFluor secondary
- Stain nuclei with Hoechst
- Read plates on PE Ensight
  - Brightfield (% Confl.)
  - GFP (Plaques)
  - Nuclear Count

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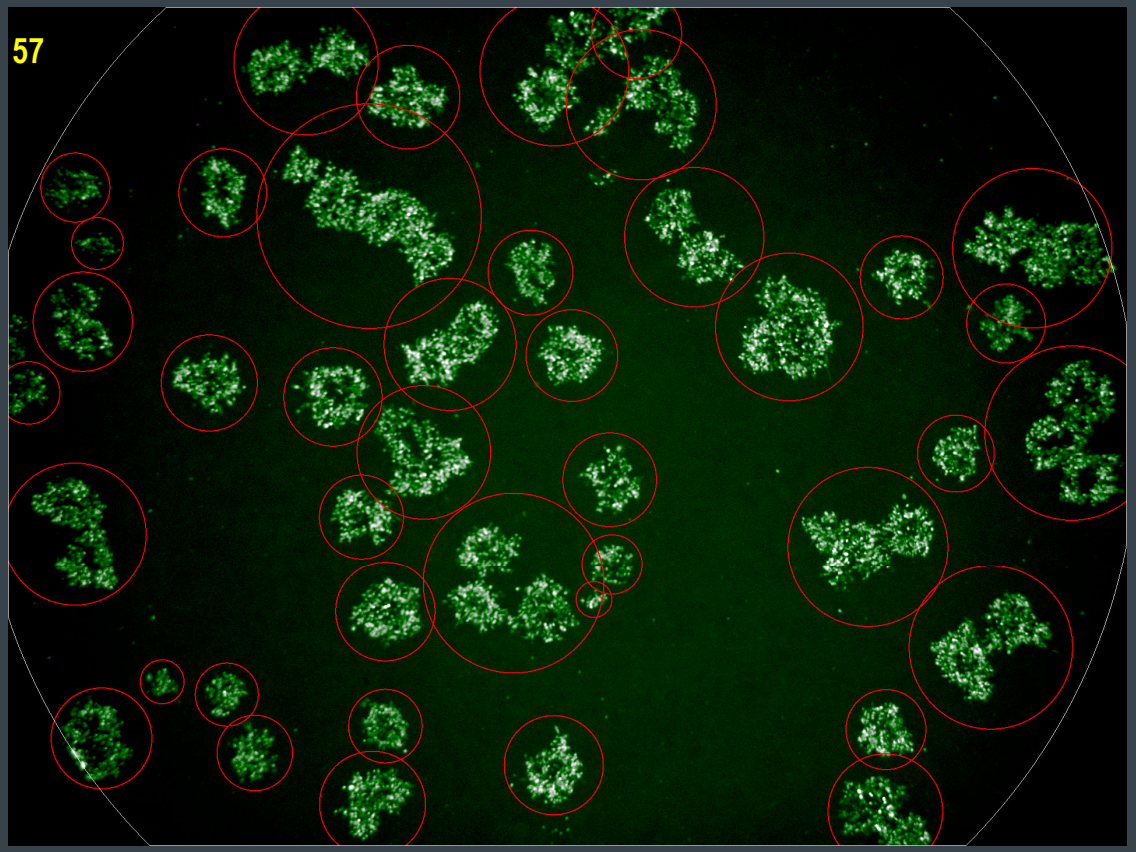
Plaque Counting Algorithm on PE EnSight / Kaleido Software



1. Count single, continuous fluorescent objects
2. Depending on sizing parameters, partition objects, by area alone, into estimated plaques
   - Doesn't leverage gaussian or watershed algorithms; these are computationally intensive

Experience with this for about 4-5 years → Algorithm has evolved over time and is well defined

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Multi-Factored DOE for µPlaque

ANALYTICAL RESEARCH & DEVELOPMENT

Assay Variables Adjustments

1. Cell substrate, plate seeding density, and passaging concentrations
2. FBS concentration in assay media and overlay media
3. Trypsin concentration in assay media and overlay media; Impact of Trypsin on potency
4. Pen/Strep in assay media and overlay media
5. Primary and secondary antibody candidates for immuno-staining
6. Basal media used for cell growth, planting, and assay dilutions
7. Viral adsorption and infection kinetics
8. Sample stability through freeze thaw cycles, time on deck (diluted) and time at stock conc.
9. Sample dilutions for various stages of process and formulation development
10. Imaging parameters including plaque size, brightness, and overall plaque quality

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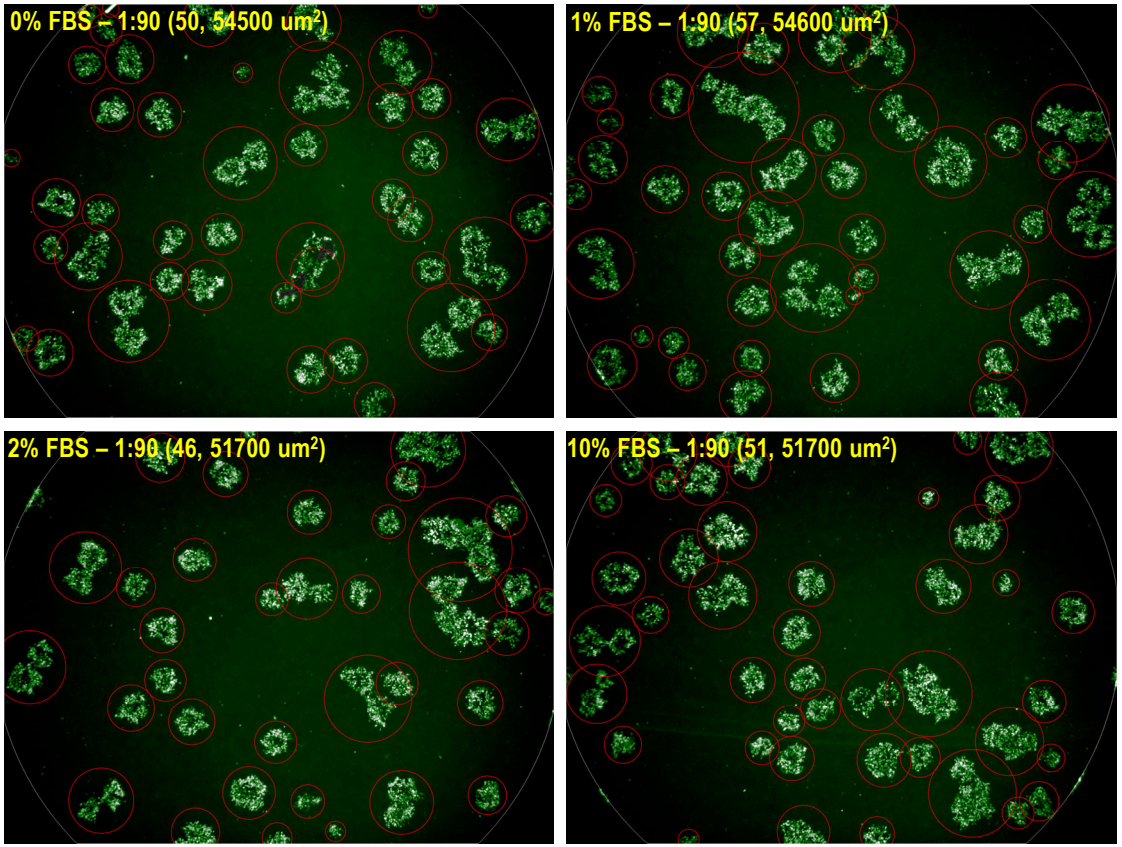
Serum Content – Viral Attachment (10% FBS in Overlay)

ANALYTICAL RESEARCH & DEVELOPMENT



	PFU/mL	%CV
0% FBS during Infection	2.01E+05	6%
1% FBS during Infection	2.83E+05	12%
2% FBS during Infection	2.46E+05	10%
10% FBS during Infection	1.61E+05	24%

Constant Conditions:

Final Bulk Drug Substance

10% FBS in Overlay Media

Conclusions:

Serum is critical for cell health but can be inhibitory on viral uptake

Not a significant difference in titers between serum content; decrease 10% FBS

- Cell health improves with increasing FBS conc.
- 1% and 2% FBS optimal for sensitivity and cell health

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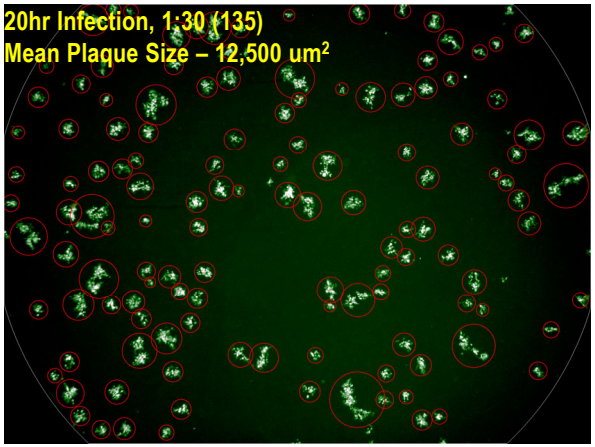
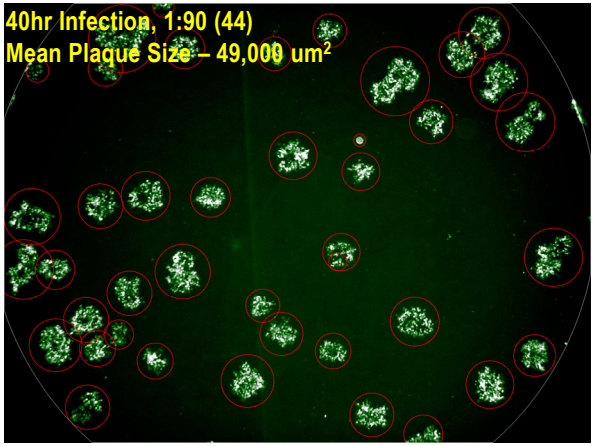
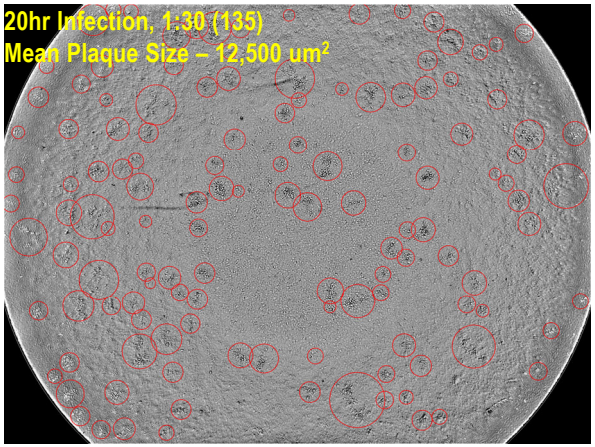
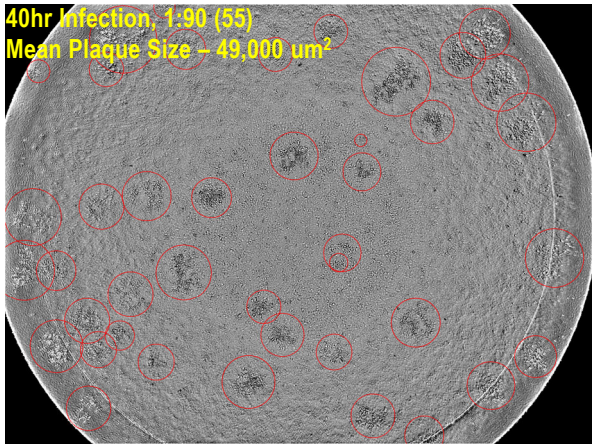
20 hour vs 40 hour infection

ANALYTICAL RESEARCH & DEVELOPMENT









Sample Used:

Final Bulk Drug Substance

Conclusions:

20 hour incubation time has smaller but quantifiable plaques

- Allows earlier detection
- Wider quantifiable range per well (i.e. more plaques per surface area)

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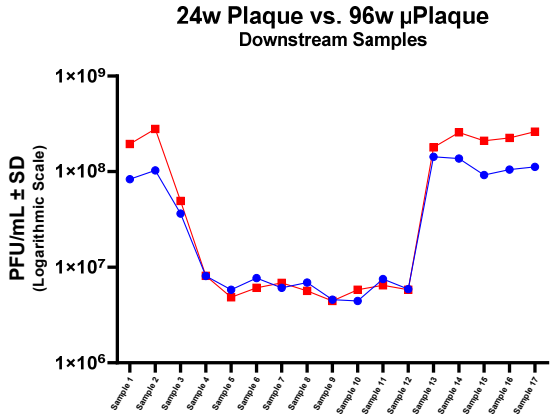
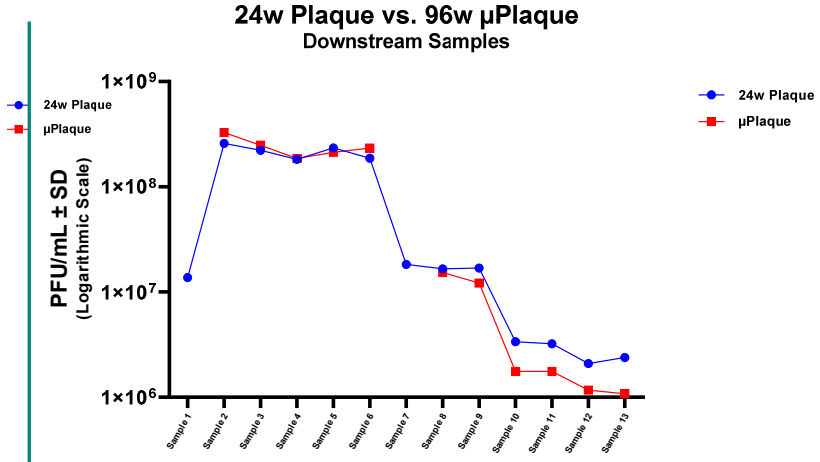
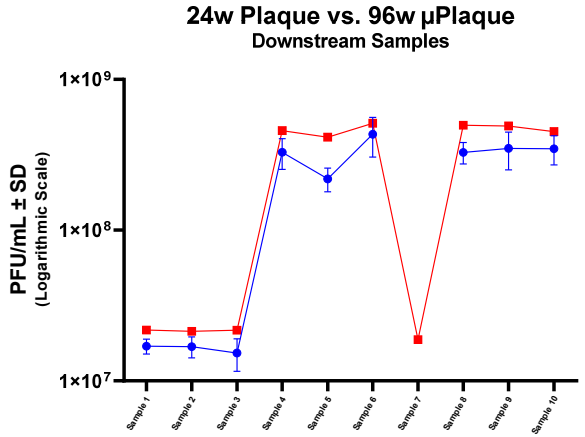
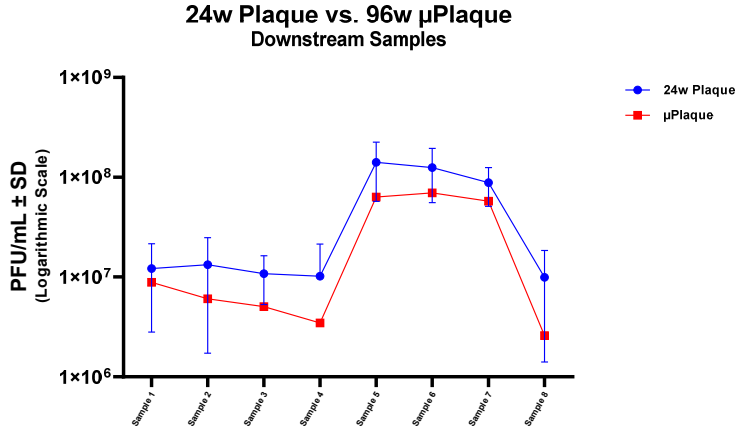
Process Development Downstream Samples Correlation

ANALYTICAL RESEARCH & DEVELOPMENT









Notes

Unit differences between assays

- 24w → Geomean titers with %RSD variance
- 96w → Mean titers with %CV variance

Correlation studies not controlled for cell passage, same vials, etc.

Summary

Good trending between the assays pointing towards a strong correlation

Better alignment in peak response observed between the assays

- Variance in trends likely due to cell passage → known issue with Vero cells and has been observed with other projects as well

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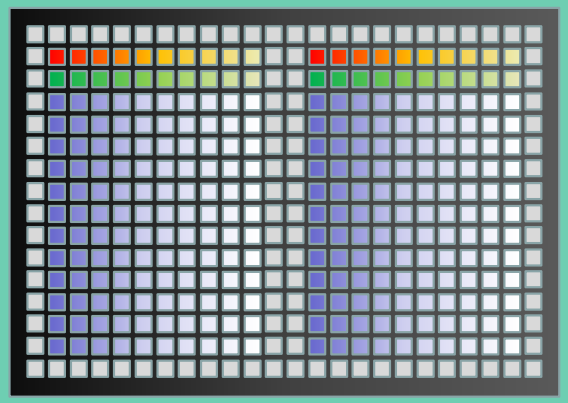
Infectivity Assay Steps

ANALYTICAL RESEARCH & DEVELOPMENT

Cell Plating → Infection (Day 1) → Fixation (Day 2) → Immunostaining (Day 2) → Imaging of Plates → Data Analysis

Serial Dilution of Virus

Incubate at 37°C, 5%CO_2 for 8-20 Hours



- 14 rows of standard / samples *in duplicate*
  - High Throughput: 12 samples / plate
- Outer edges not infected – Assay Blanks

Permeabilize

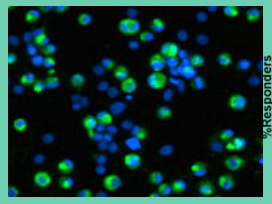
Block & Hoechst Stain

1° Ab

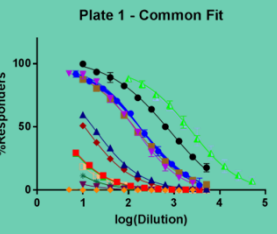
2° Ab (AlexaFluor)

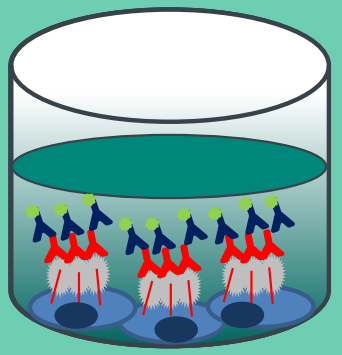
Add PBS and Transfer to reader

Cytation 3



Relative Potency





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Multi-Factored DOE for Infectivity

ANALYTICAL RESEARCH & DEVELOPMENT

Assay Variables Adjustments

1. Cell substrate, plate seeding density, and passaging concentrations
2. Basal media used for cell growth, planting, and assay dilutions
3. Direct vs. Indirect infection kinetics and incubation periods
4. Assay media FBS source
5. Primary and secondary antibody candidates for immuno-staining
6. Sample stability through freeze thaw cycles, time on deck (diluted) and time at stock conc.
7. Sample dilutions for various stages of process and formulation development
8. Plate washer height, cycle count, and aspirate/dispense rate
9. Mitigation of "hook effect" through cell seeding density, media refeed/refresh, and addition of ApoE and rTrypsin
10. Concordance with µPlaque data

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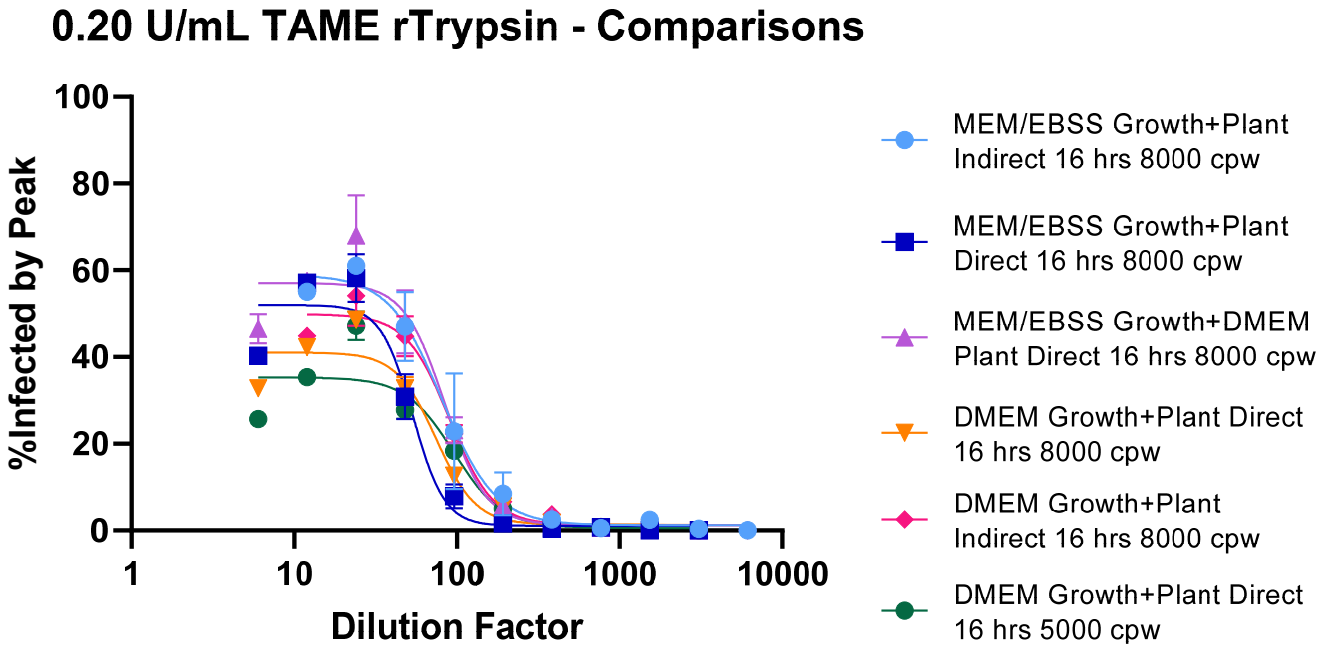
Infectivity Data – Hook Effect

ANALYTICAL RESEARCH & DEVELOPMENT



Sample Used:

Final Bulk Drug Substance

Conditions Varied

- cell seeding density,
- Media
- media refeed/refresh,

Conclusions:

Unfortunately, Hook Effect still present

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Conclusions

ANALYTICAL RESEARCH & DEVELOPMENT

- The 96-well µPlaque assay was successfully developed, optimized, and deployed for COVID-19 program support within 6 weeks

- Since then, it has provided key data for more than 7300 samples at an average turn around time of 8 days and is being run, at minimum, once a week.

- While the Infectivity assay did not successfully complete development, due to hook effects we gained additional understanding on platform support.

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THANK YOU!

Acknowledgements

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QUESTIONS?

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