

Analytical Development in Context of Viral Product Development

**ASGCT 2011 Translational Science Training Course:
Bench to Bedside Facilitating First in Human Trials**

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SAFC Carlsbad

Analytical Services

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Overview

-  General Overview on Analytical Applications in context with Viral Product Development
-  Focus on Viral Product Assays
 - TCID50 Case Study
-  Take Home Message

Analytical Services in Support of Production

From a CMO Perspective

SAFC Carlsbad Inc

CMO for Virus Manufacturing

Multi Product Facility

From Development to Phase 3
& Commercial

Analytical Services & Quality Control



Analytical Services & Quality Control

Support the Production Process

Method Transfer /
Development

→ Method Validation

In Process Testing

→ Product Release

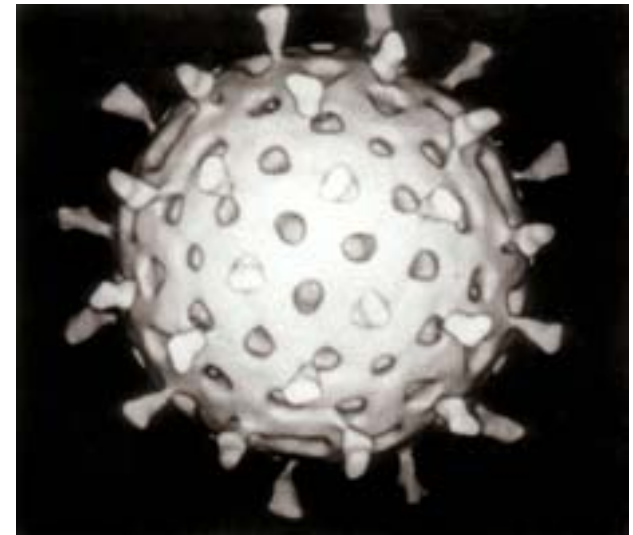
Raw Materials Testing → Stability Studies



Analytical Applications

Cover Drug Product & Impurities

Category	Product Parameter
Strength	Particle Concentration
Potency	Particle Activity
Purity	Host Cell DNA & Protein ¹
Identity	Virus Genome & Protein
Safety	Adventitious Agents ^{1,2}



1–Outsourced Testing. 2–May require Neutralizing Antibodies

Analytical Applications During Product Development

Early
Dev

Activity & Productivity

Titer, Total Particle, Potency

Identity

Genome or GOI Sequence & Protein

Safety

Sterility, Mycoplasma, Viruses, RCX

Characterization (CMC)

Genetic Stability, Size, Potency

Purity

Host Cell DNA & Protein

Stability

Titer, Total Particle, Potency

BLA

Regulatory Impact

Guidelines to regulate product requirements

Guidelines to regulate testing

- FDA
- ICH
- US, JP, EU Pharmacopeias

Method Validation



Focus on Methods for Strength & Activity

Infectious Titer & Total Particles

Pros and Cons for Selection of Dosing Units

- Total Particles vs Infectious Units

Value of Specific Activity

- Determine ratio of Total Particles vs Infectious Units

Assess Reliability of Methods

- Higher Variability with Cell Based Assay Methods

Methods for Infectious Titer & Total Particles

Titer Assays (cell based)

- TCID50
- Plaque
- CIU

Total Particles (physical)

- OD
- HPLC

Ratio Infectious Titer / Total Particle Titer

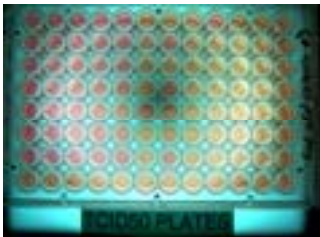
- Should be constant between lots
- May change during purification



Comparison of Titer Assays

TCID50 assay

- 96 well plates
- 7-14 day incubation
- Observe CPE
- Either positive or negative wells
- A single well has dramatic effect on overall titer number



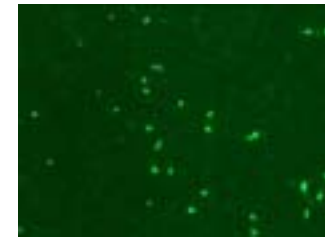
Plaque assay

- 6 well plates
- 7-14 day incubation
- Observe plaques of lysed cells
- Variable size
- Plaques can overlap
- Need to stay within narrow plaque number range (10 – 100)



CIU is independent of cytopathic effect

- 6 well plates
- Detect infected cells with virus specific antibodies and fluorescent readout
- 2-3 day incubation
- Count fluorescent cells



TCID50 Titer Assay Case Study

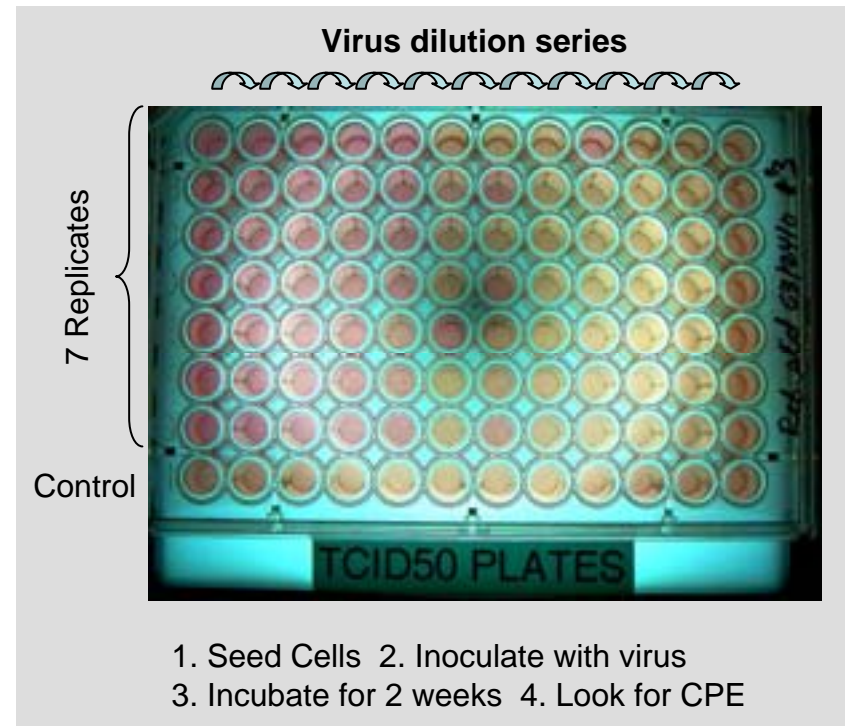
Assay Transfer & Validation

Titer Differences with client lab

Troubleshooting

New cell bank

Improved performance

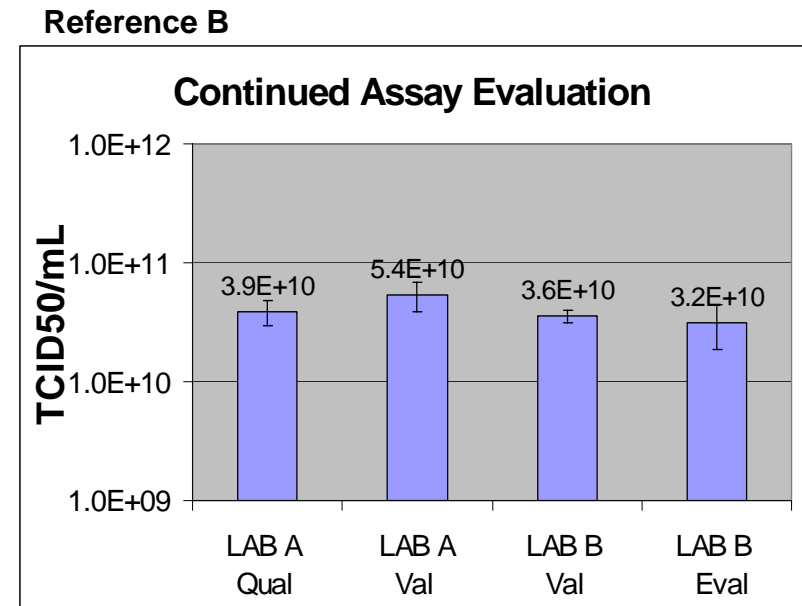
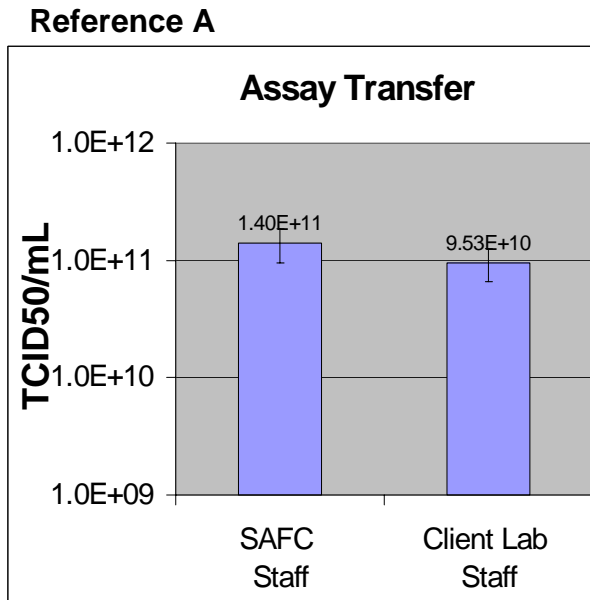


Assay Transfer

Side by side comparison with client lab scientist

Considerations:

- New cell bank
- Switch virus reference material
- Change lab location



TCID50 Assay Validation

Summary

Precision [Repeatability]

- 14% to 30% [8 assay runs]

Precision [Intermediate]

- 25% [10 assay runs]

Accuracy

- At 72% of historical data [10 assay runs]

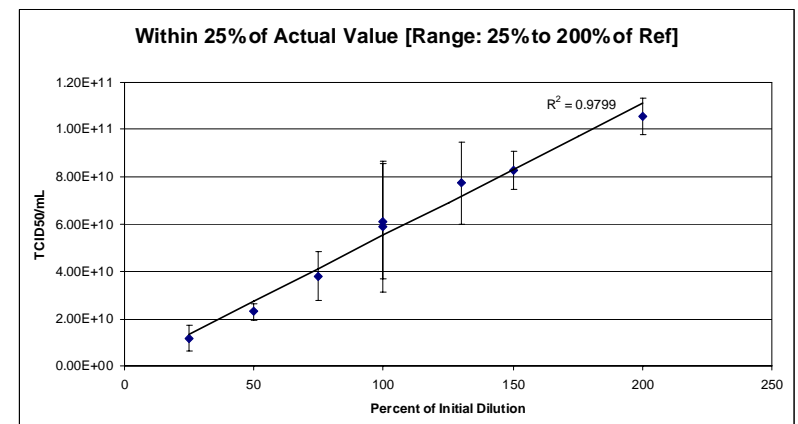
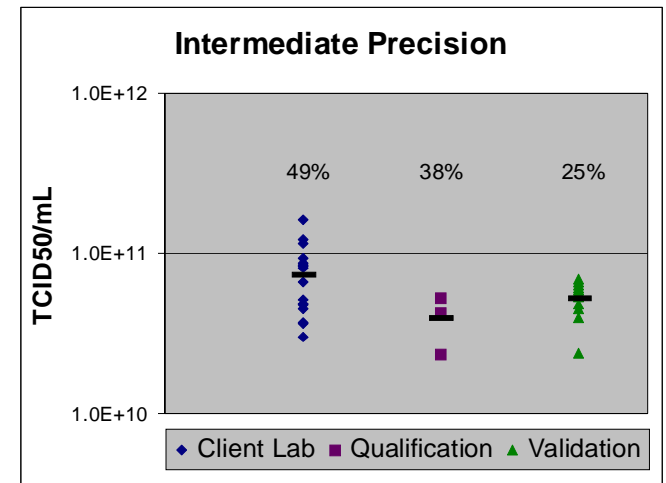
Linearity & Range

- Within 25% of actual concentration

Robustness

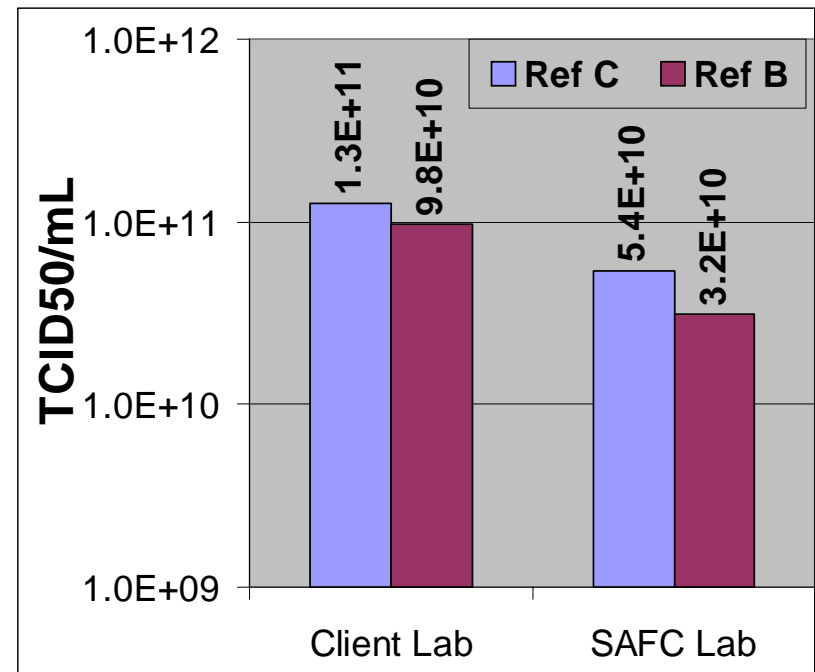
- Passage number, seeding time

Specificity



Lab Comparison

Lab to lab titer difference more than 2-fold



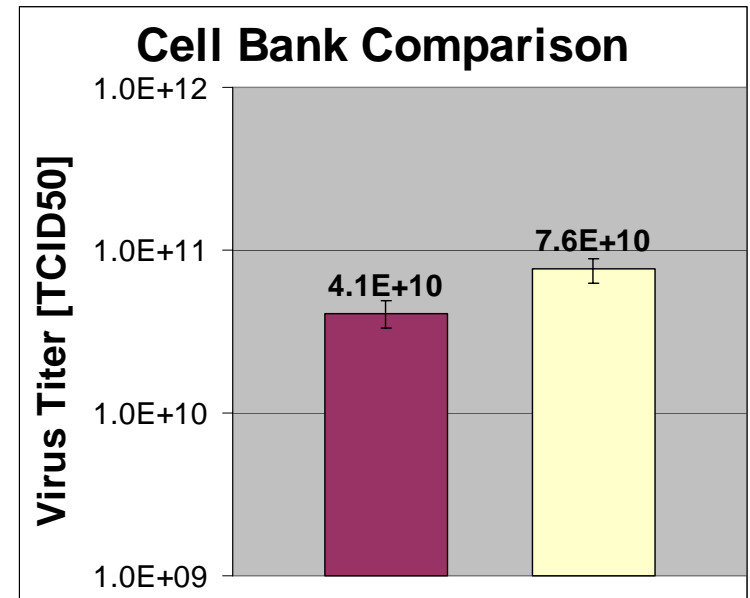
Troubleshooting

Cell Passaging

- Culture density
- FBS source
- Incubator
- **Analyst**
- Trypsin removal
- Trypsinization time
- Culture schedule
- Culture flask
- **Cell Bank**

TCID50 Assay

- Ref. Std. Stability
- Analyst
- Plating Density



Important Criteria for Cell Based Assays

Healthy cells

- Qualified QC cell bank
- Careful cell thaw
- Work in defined passage range

QC cell bank

- Prepare in virus free environment
- Monitor cell viability & recovery



Cell Based Titer Assays

Treat your cells well!

Cell thaw conditions

- Cells may be sensitive after the thaw

Cell passaging

- High or low cell densities may lead to cell sub selection
- Keep cells within a narrow cell density range
- Use low amounts of Trypsin or EDTA
- Wait with infection until cells have recovered from passaging (lag phase)
- Use cells when acclimated and stable
 - Do not use early passages (non-reliable cell conditions)
 - Do not use very late passages (cells could be aging)
 - For example: passages 5 to 15 may be good
 - Test for best passage range early on

Cell Based Titer Assays

Treat your cells well!

Long incubation times

- Maintain humidity
 - Loss of media
 - Cracked agarose plugs
- Maintain sterility in incubators
 - Frequent cleaning cycles

Comparison of Particle Assays

OD assay

- Particles must be disassembled to release viral genome
- Measure A260
- Can only be used with highly pure product

HPLC assay

- Separation on ion exchange column
- Requires reference standards
- Peaks may be asymmetric
- Up to 2 log assay range
- Can be used for in-process samples
- Limited sensitivity
 - A minimum of $1E+9$ to $1E+10$ particles required



Comparison of Assay Performance

TCID50 vs HPLC

Precision [Repeatability]

- 14% to 30% [8 assay runs] **[HPLC: < 5%]**

Precision [Intermediate]

- 25% [10 assay runs] **[HPLC: < 10%]**

Accuracy

- At 72% of historical data [10 assay runs]

Linearity & Range

- Within 25% of actual concentration

Robustness

- Passage number, seeding time

Specificity

Impact of TCID50 Assay Differences

Specific Activity

- Total Particle Concentration consistent
- Infectious titer differences affect specific activity

Dosing Based on TCID50 Values

- Effect on clinical dose
- Effect on cost per dose

Effect on Manufacturing Yields

- Cost per Production Run

Take Home Message

Analytical Development needs to be integrated early in product development projects

- Early efforts pay off later
- Is neutralizing antiserum required for safety testing?

Testing requires well organized sampling plans

- Testing labs need to be coordinated

Infectious Titer and Total Particle Assays required to determine specific activity

- Infectious & Particle Titers as well as their ratio should be consistent between production runs

Determine method for selection of clinical dose early.

- Use a precise and robust method, e.g. HPLC for total particle
- Choosing infectious titer method may require multiple testing to increase precision
- Robustness more important than sensitivity