



Adenovirus potency assays

Novel approaches to current challenges

G. Marzio, PhD

Head Molecular Virology

Product R&D

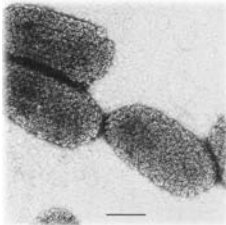


Overview

- Introduction
- Single-virus potency assay
- Improved potency assay

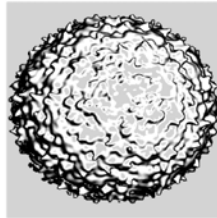
Crucell's products

Antibody



Rabies

Classical

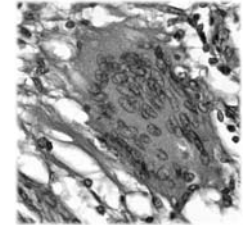


West Nile

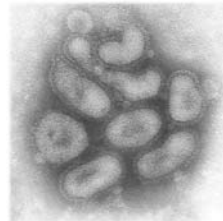
Adenovector



Ebola



Tuberculosis



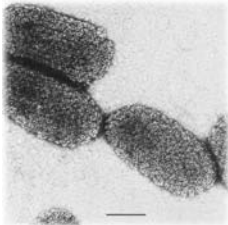
Influenza



Malaria

Crucell's products

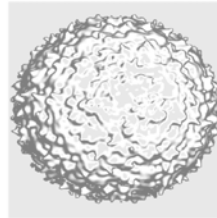
Antibody



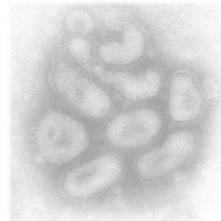
Rabies

RIFFT assay

Classical

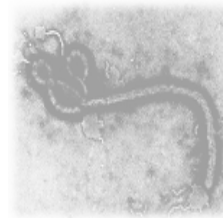


West Nile

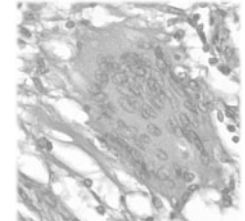


Influenza

Adenovector



Ebola



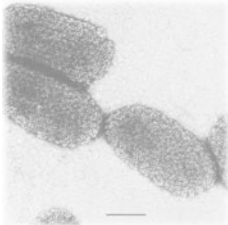
Tuberculosis



Malaria

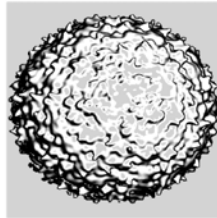
Crucell's products

Antibody



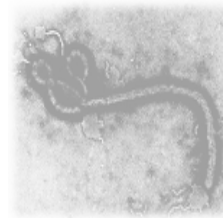
Rabies

Classical

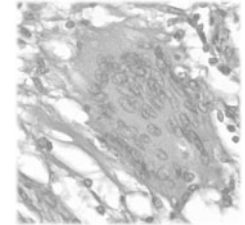


West Nile

Adenovector

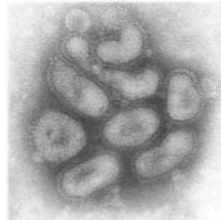


Ebola



Tuberculosis

RIFFT assay



Influenza

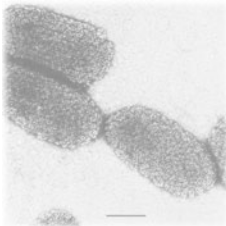
SRID assay



Malaria

Crucell's products

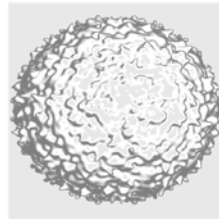
Antibody



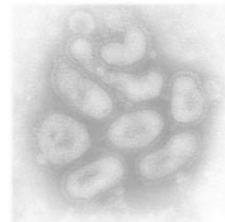
Rabies

RIFFT assay

Classical



West Nile



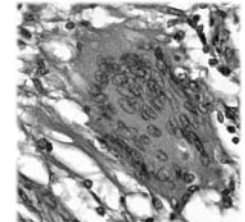
Influenza

SRID assay

Adenovector



Ebola



Tuberculosis



Malaria

??? assay

Potency guidelines

Measures the biological function of the product

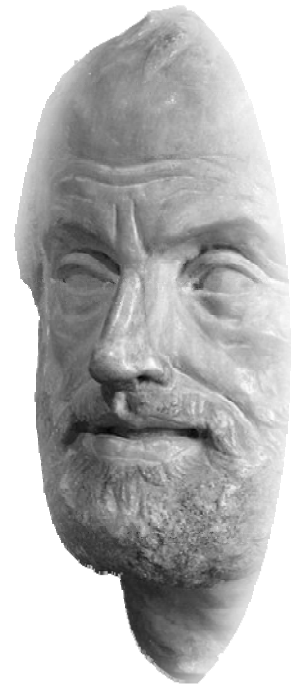
- **Licensure**
Validated product-specific quantitative assay
- **Phase III**
Qualified product-specific quantitative assay
- **Phase I/II**
Quantitative infectivity assay
Qualitative biological assay

Potency syllogism

TRANSGENE EXPRESSION FOLLOWS INFECTIVITY

POTENCY *CAN BE* TRANSGENE EXPRESSION

INFECTIVITY *CAN BE* POTENCY



Assumption

?

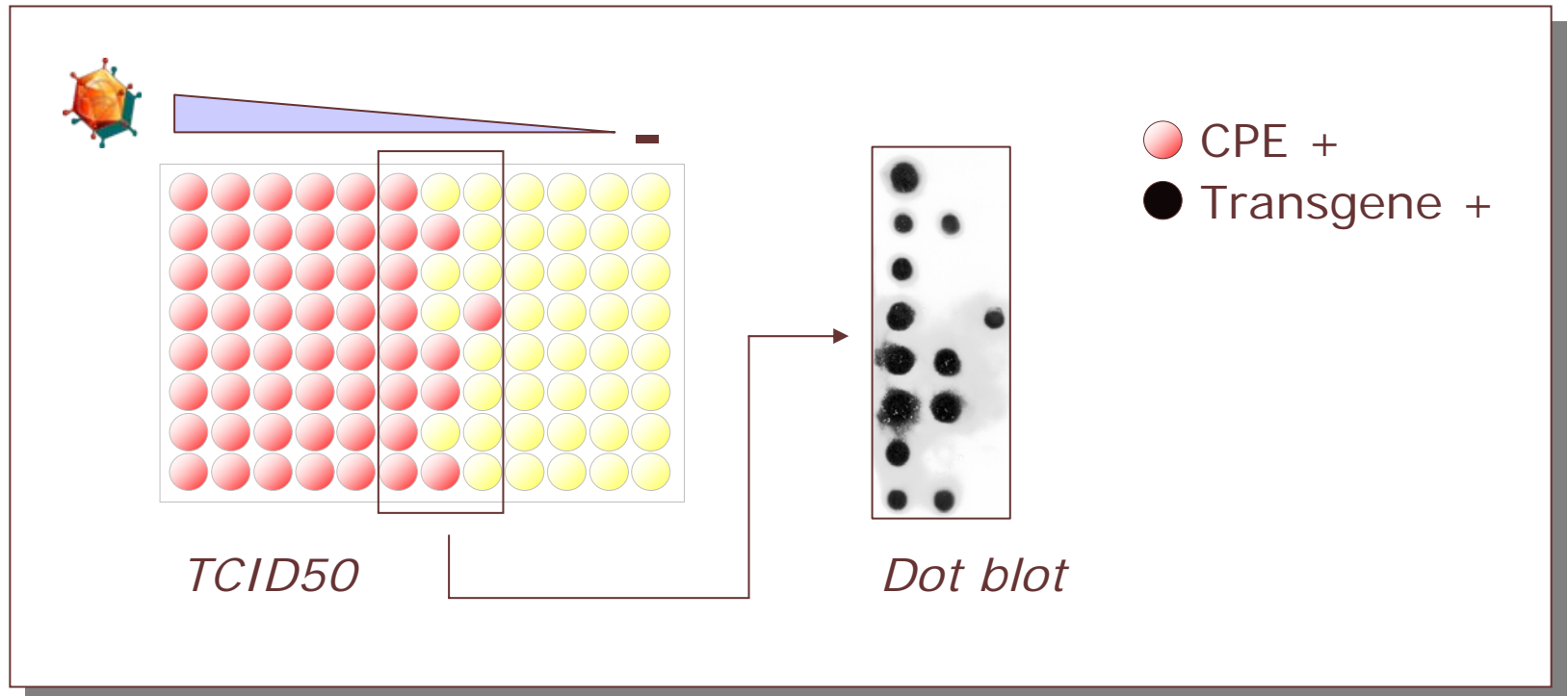
1 infectious unit = 1 transgene unit

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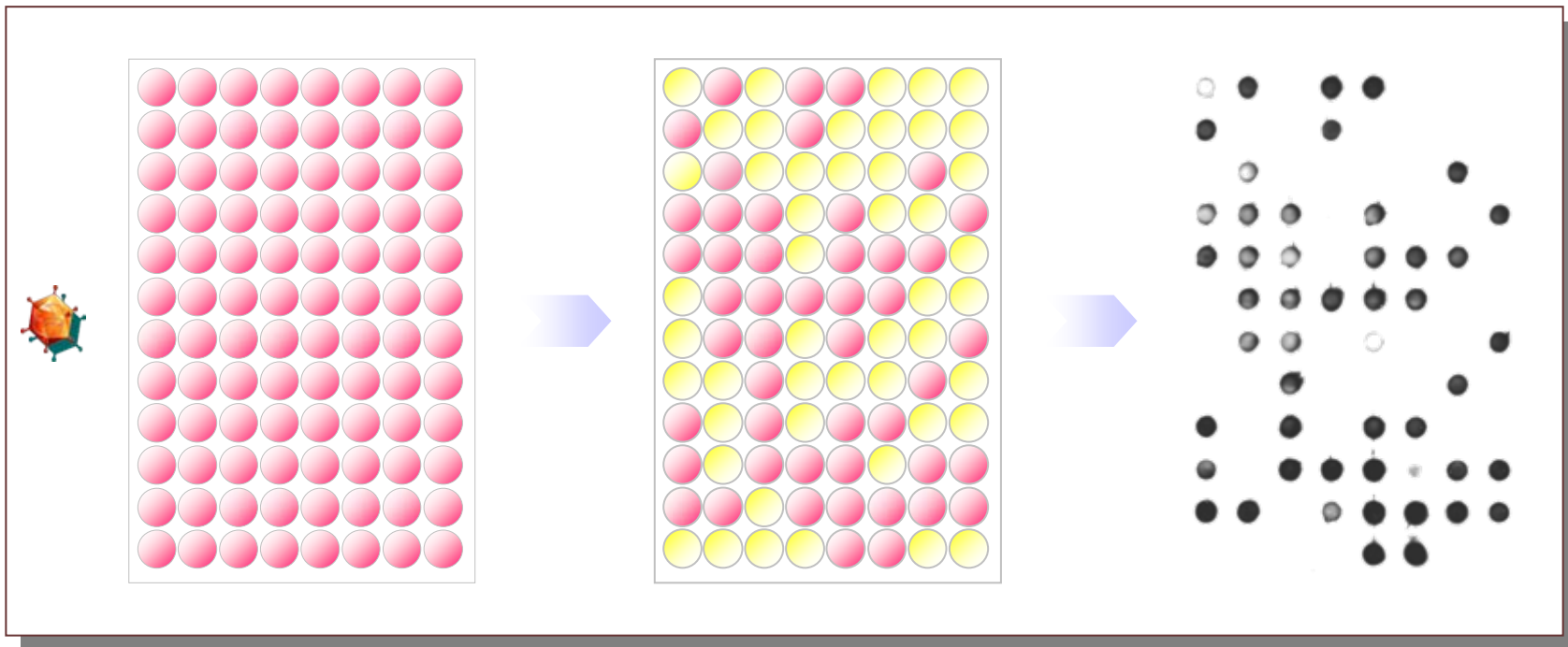
Proof of concept

Infectious vs. Transgene Units



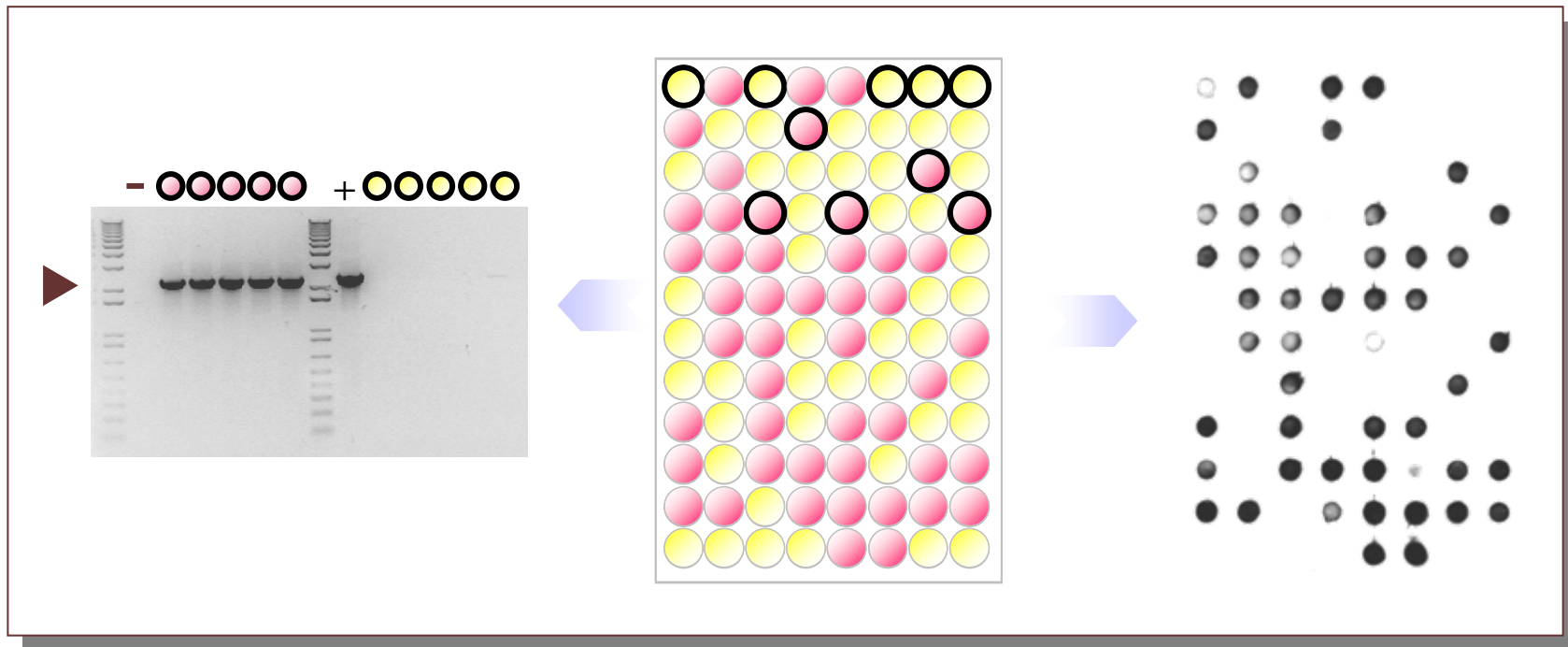
Single virus potency assay

- Dilute to 1 IU/100 ul
- Inoculate 96 well plate, 100 ul/well
- Score CPE



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Future

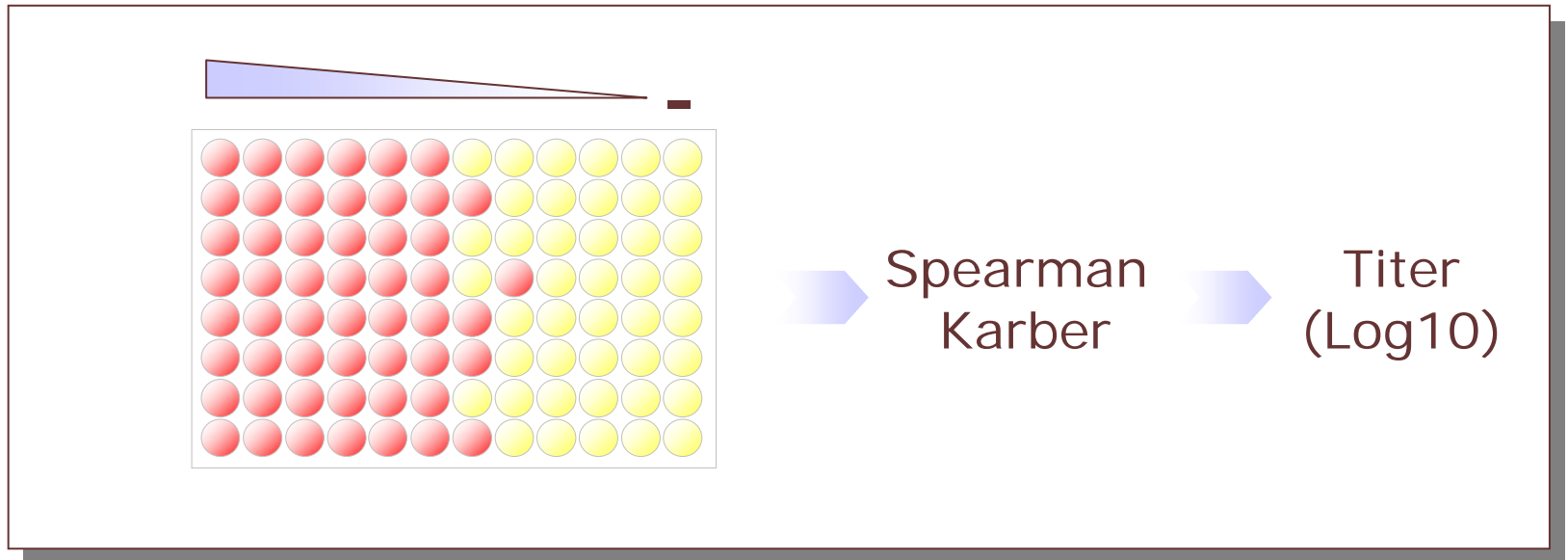
- Compare batch to batch variation
- Correlate with potency in vivo

Overview

- Potency vs. infectivity
- Single-virus potency assay
- Improved potency assay

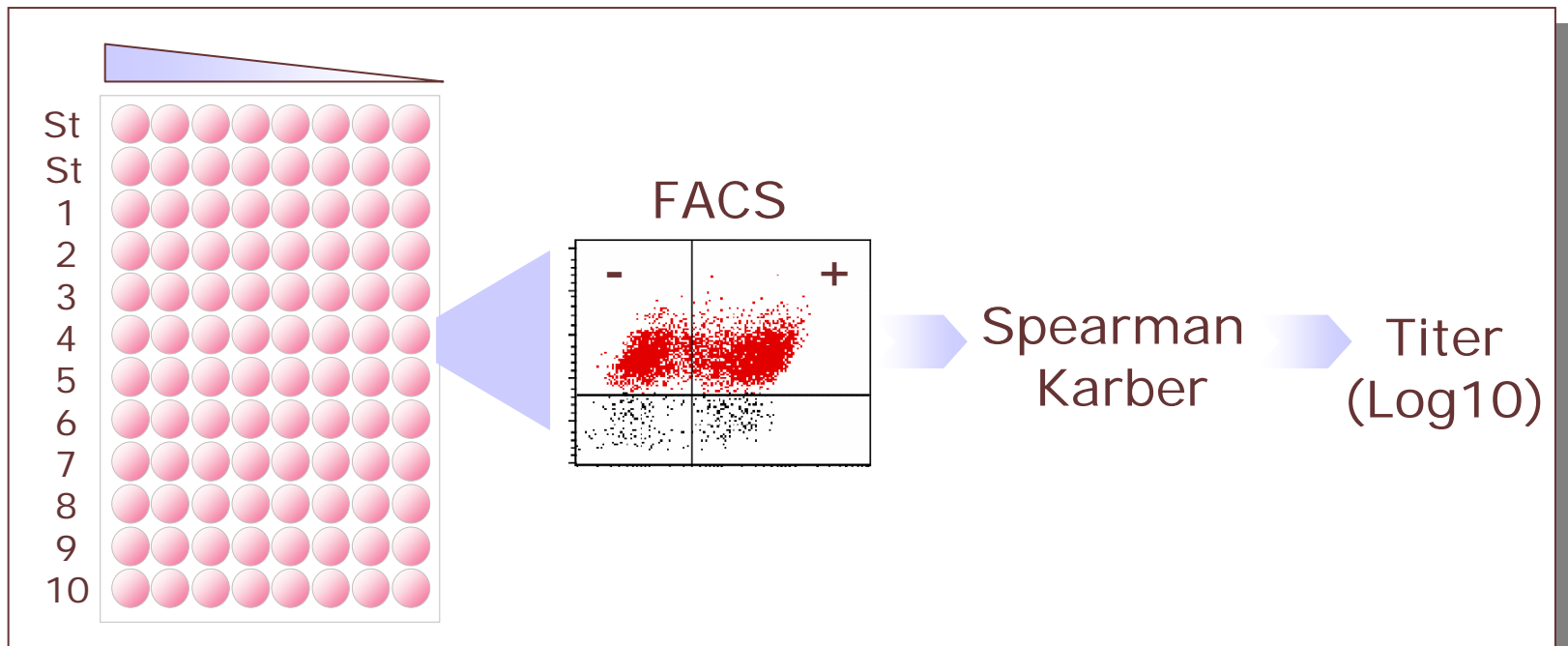
TCID50

- 8 replica wells/dilution
- 1:5 dilutions
- 1 sample/plate
- 2 weeks



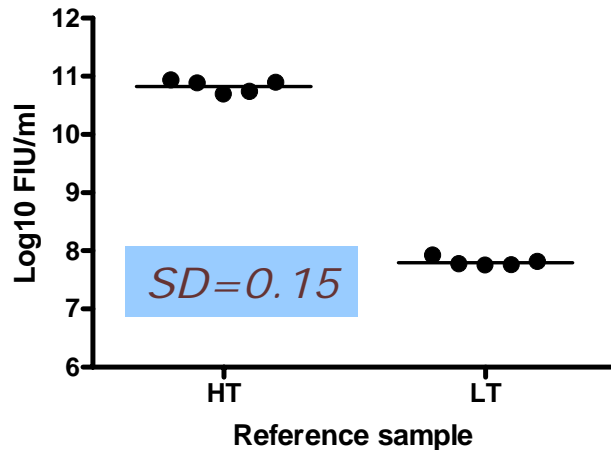
FACS Titration - method

- 5000 events/dilution
- 1:6 dilutions
- 10 samples/plate
- 2 days

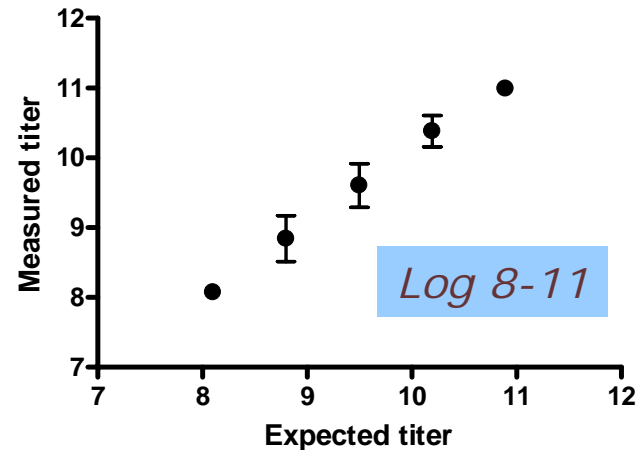


FACS Titration - qualification

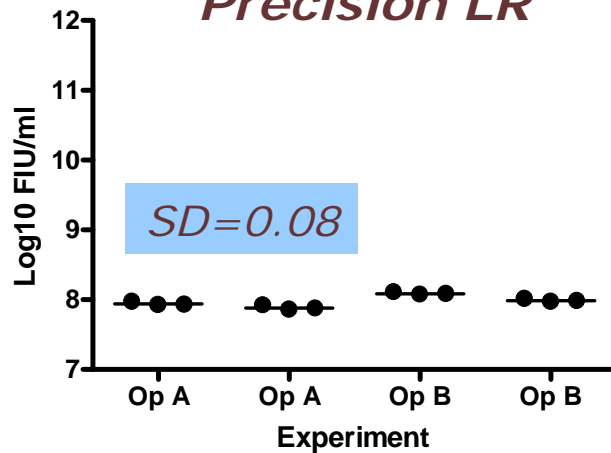
Repeatability



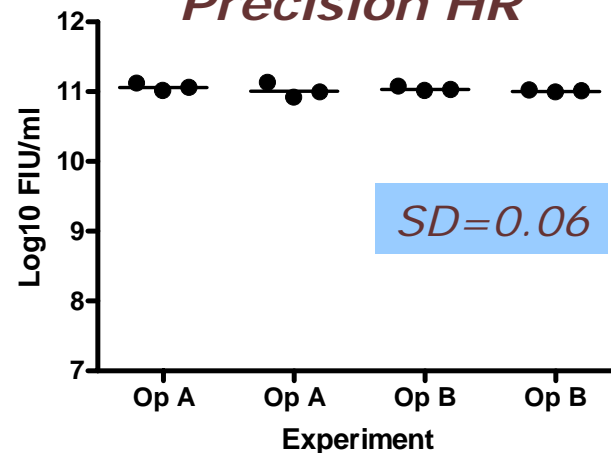
Linearity



Precision LR



Precision HR



TCID50 vs. FACS

	TCID50 (8 wells / 1:5)	FACS (1wells / 1:6)
Variation (2xSD, log10)	+/- 0.5	+/- 0.3
Throughput (samples/plate)	1	10
Time to results (days)	14	2
Accuracy	N.A.	97-100%

Acknowledgements



Acknowledgements

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