Assay Requirements

- Sensitive, quantitative, reproducible, high throughput and have correlative value
- Optimized and validated to meet GCLP requirements for human clinical trials
- Reagents need to be standardized and traceable

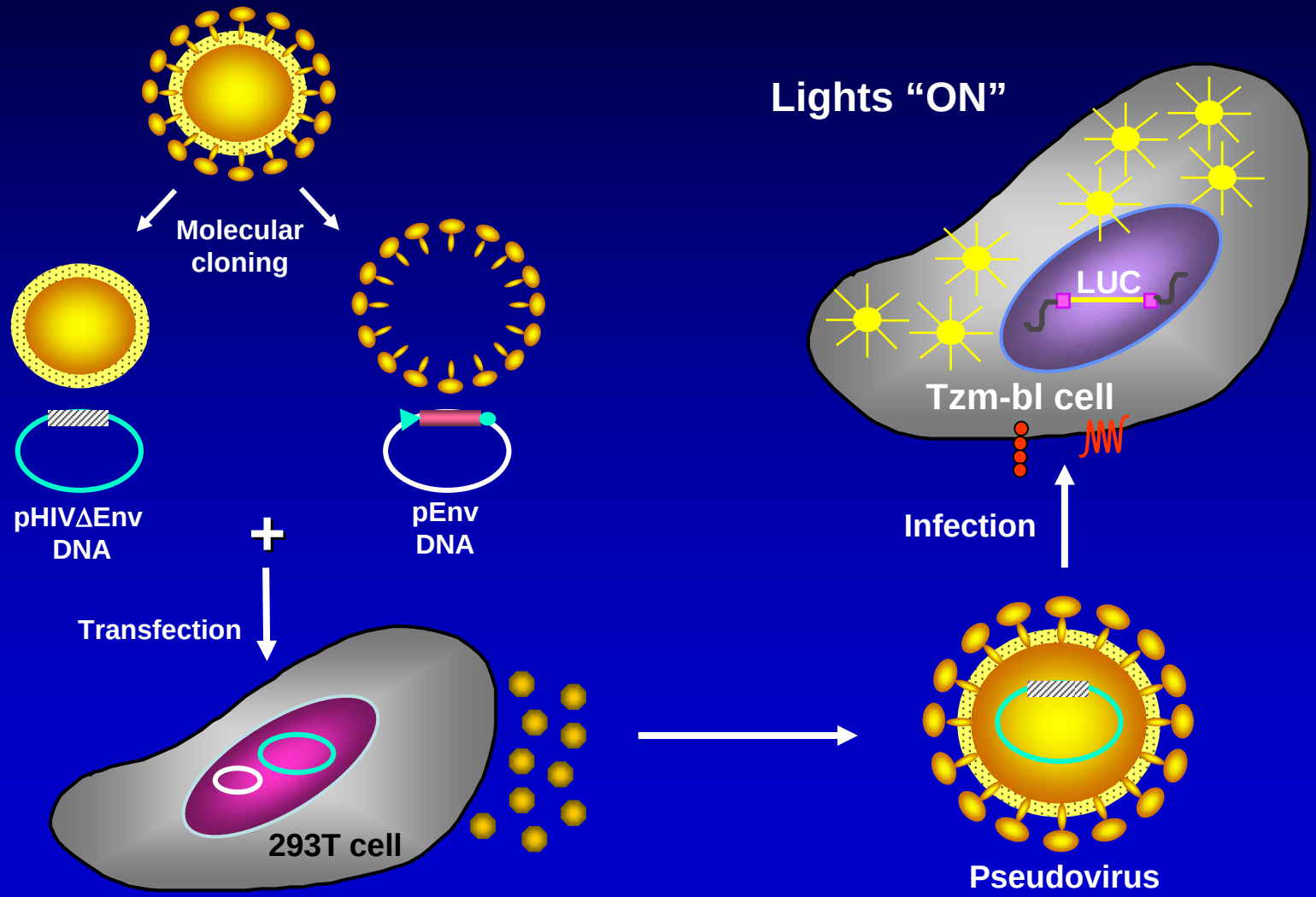
Neutralizing Ab Assays

New technologies have allowed dramatic improvements in assay performance and validation

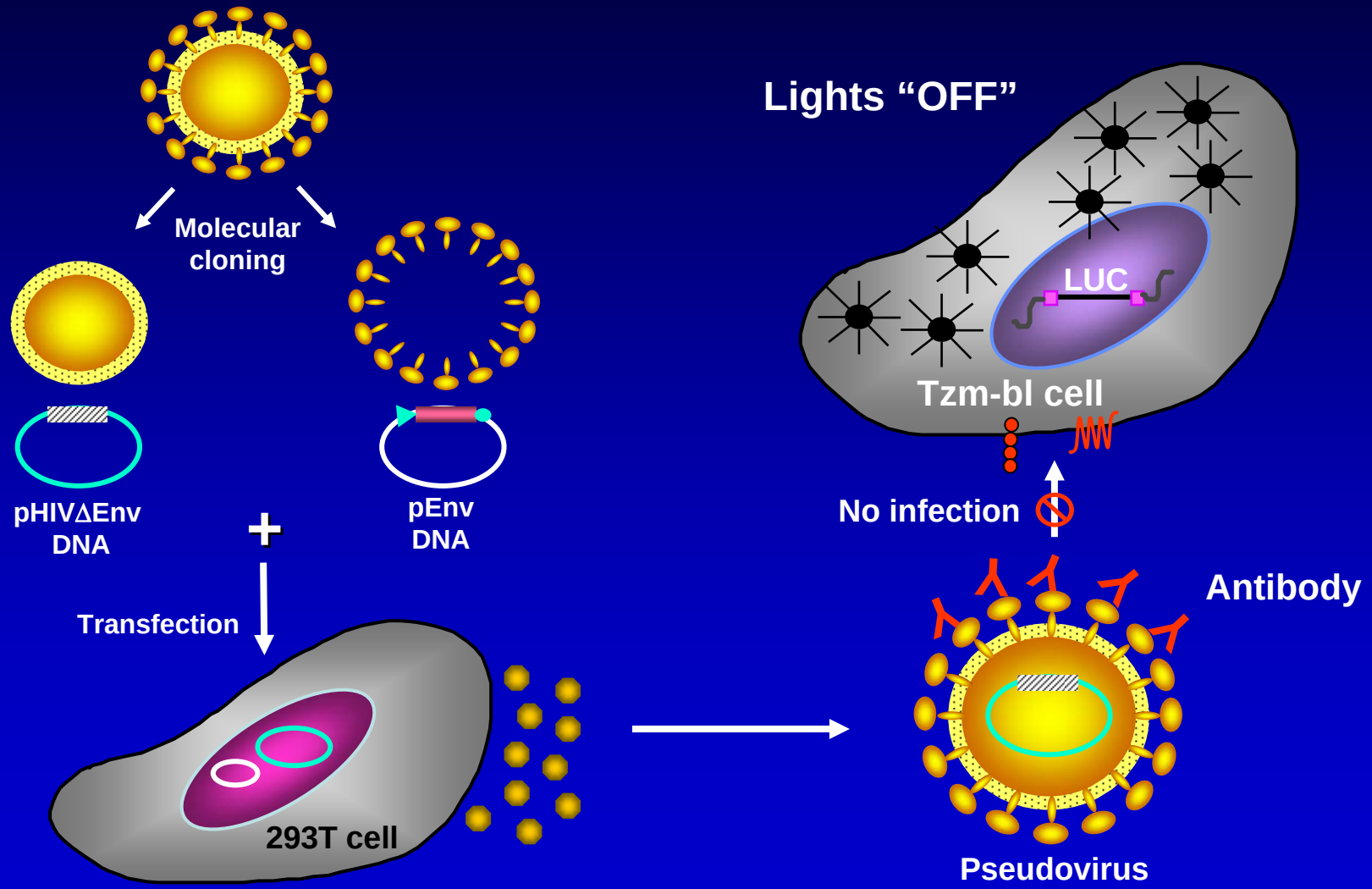
Luciferase Reporter Gene Assay in TZM-bl Cells Based on Single-Round Infection with Molecularly Cloned Env-Pseudotyped Viruses

- TZM-bl (JC53-bl) is a genetically engineered HeLa cell line that expresses CD4, CXCR4 and CCR5 and contains Tat-inducible Luc and β-Gal reporter genes:
  - High success rate in single-round infections
  - Enhanced throughput capacity (2-day assay)
  - Increased precision (can measure 50% neutralization)
  - Improved level of standardization (e.g., stable cell line, clonal viruses)
  - Recently optimized and validated

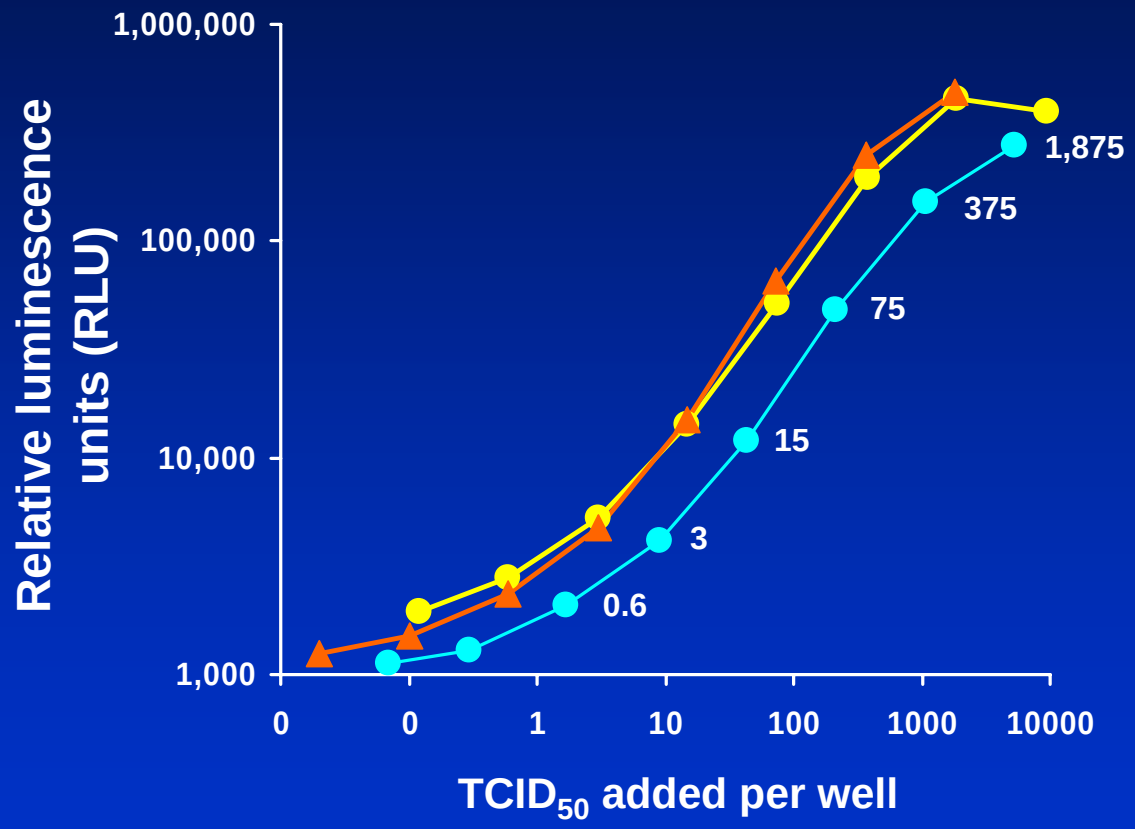
SEQUENTIAL EVENTS IN DETECTING NEUTRALIZATION OF ENV-PSEUDOVIRUSES IN TZM-BL CELLS



SEQUENTIAL EVENTS IN DETECTING NEUTRALIZATION OF ENV-PSEUDOVIRUSES IN TZM-BL CELLS



LINEAR RANGE OF INFECTION IN TZM-BL CELLS



Molecularly cloned Env pseudoviruses:

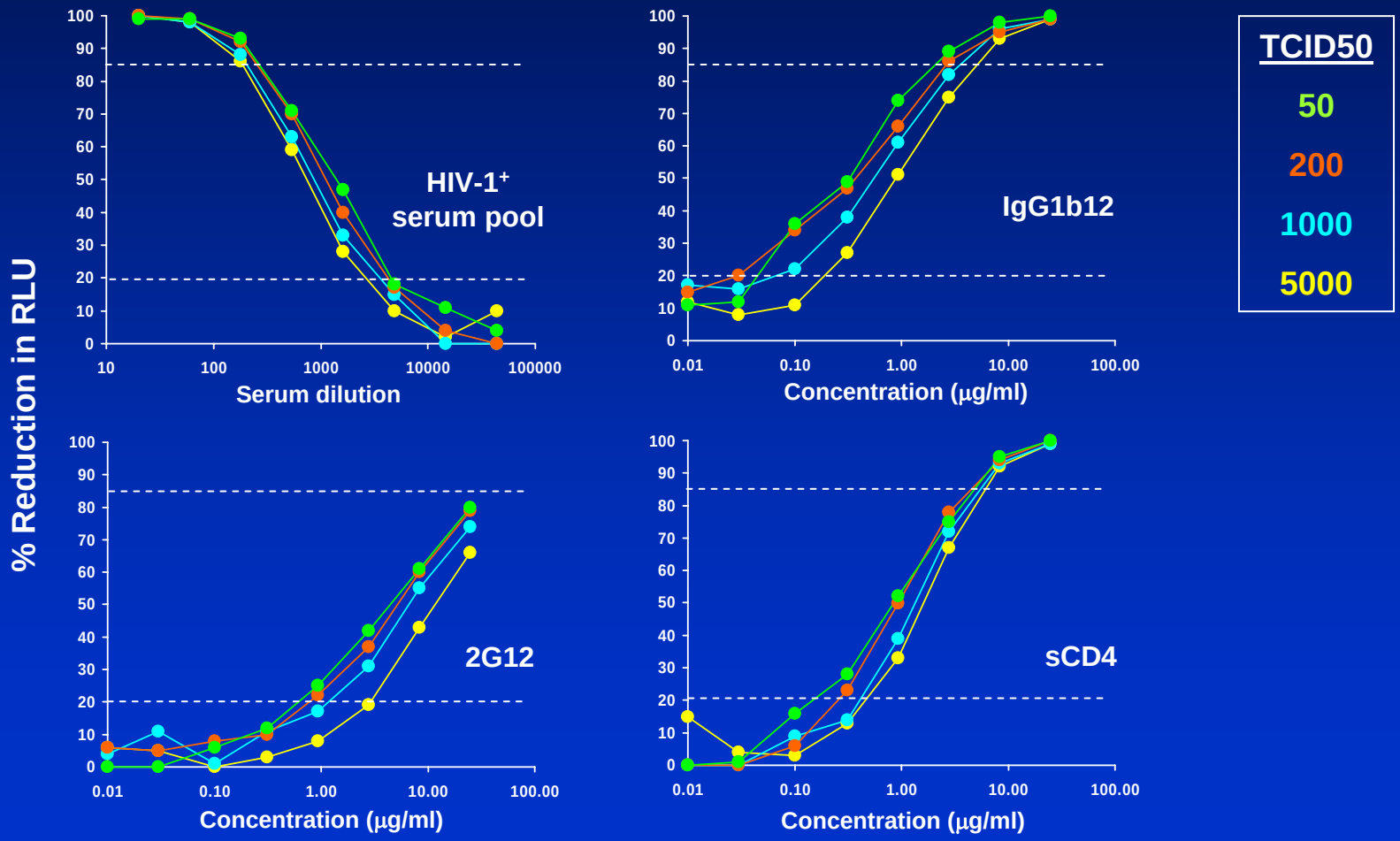
SS1196.1

QH0692.42

6101.10

LINEAR PORTION OF THE NEUTRALIZATION CURVE: 20% - 85%

Molecularly cloned pseudotype virus 1196.01, 48 hr incubation



OPTIMIZATION OF THE TZM-BL ASSAY

- Cell culture conditions
- Range of isolates that infect adequately
- Cell number
- Virus dose
- Incubation time
- Choice of 96-well plates for luminescence
- Luminescence readings
- DEAE-dextran
- Indinavir
- Uncloned vs cloned virus

VALIDATION OF THE TZM-BL ASSAY

Specificity:

- **Background activity of normal human serum and plasma**

Accuracy:

- **Comparisons have been made to other in-house assays and assays performed in other labs**

Precision:

- **Well-to-well variability in cell control, virus control and test wells**
- **Intra- and inter-asssay variability**
- **Intra- and inter-operator variability**

Limits of Quantitation:

- **Upper and lower limits established**

Linearity & Range:

- **Neutralization curves generated with positive serum samples and mAbs show a consistent pattern of linearity over a range of 20-85% reductions in RLU. Values in this range are directly proportional to the concentration of neutralizing antibodies in the sample.**

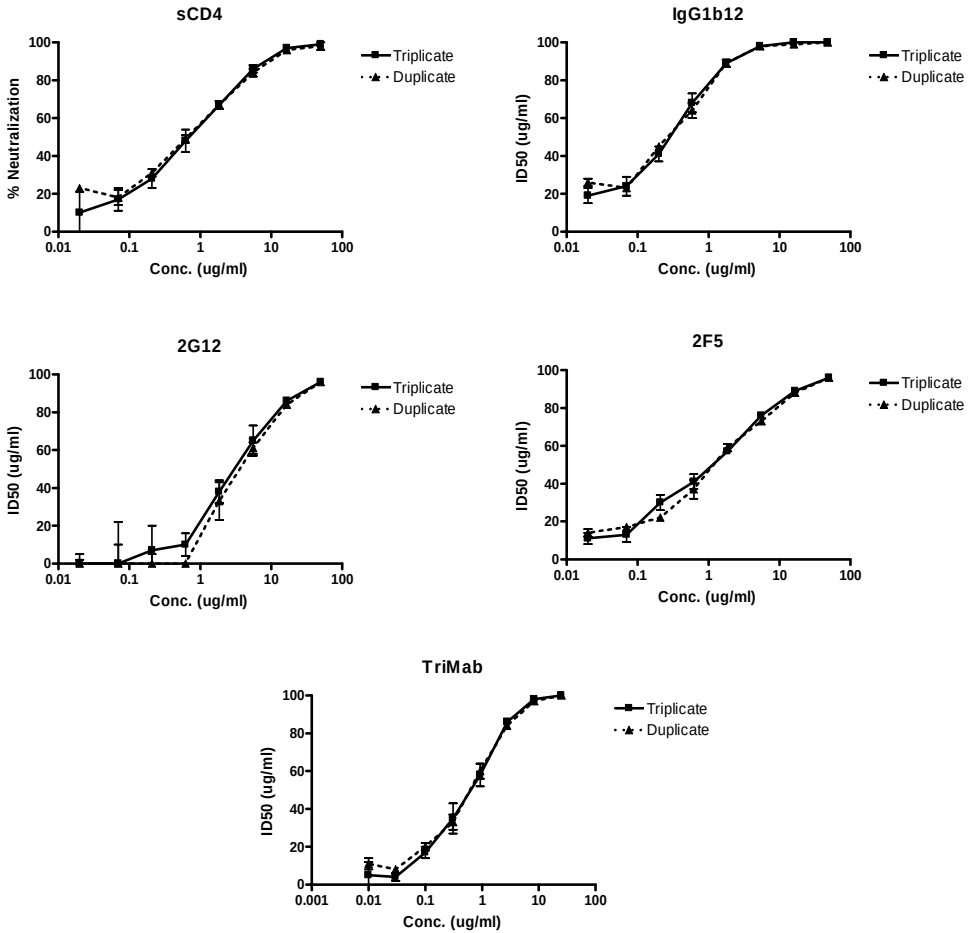
Ruggedness & Robustness:

- **Stability of CD4, CCR5 and CXCR4 expression**
- **Stability of TZM-bl infectivity after multiple passages**
- **Effect of DEAE-dextran on neutralizing antibody activity**
- **Effect of heat-inactivation on neutralizing antibody activity**
- **Serum vs plasma**
- **Uniformity of multiple luminometers**

Example of Intra-Assay Variation:

Validation of Duplicate Assay Well Format

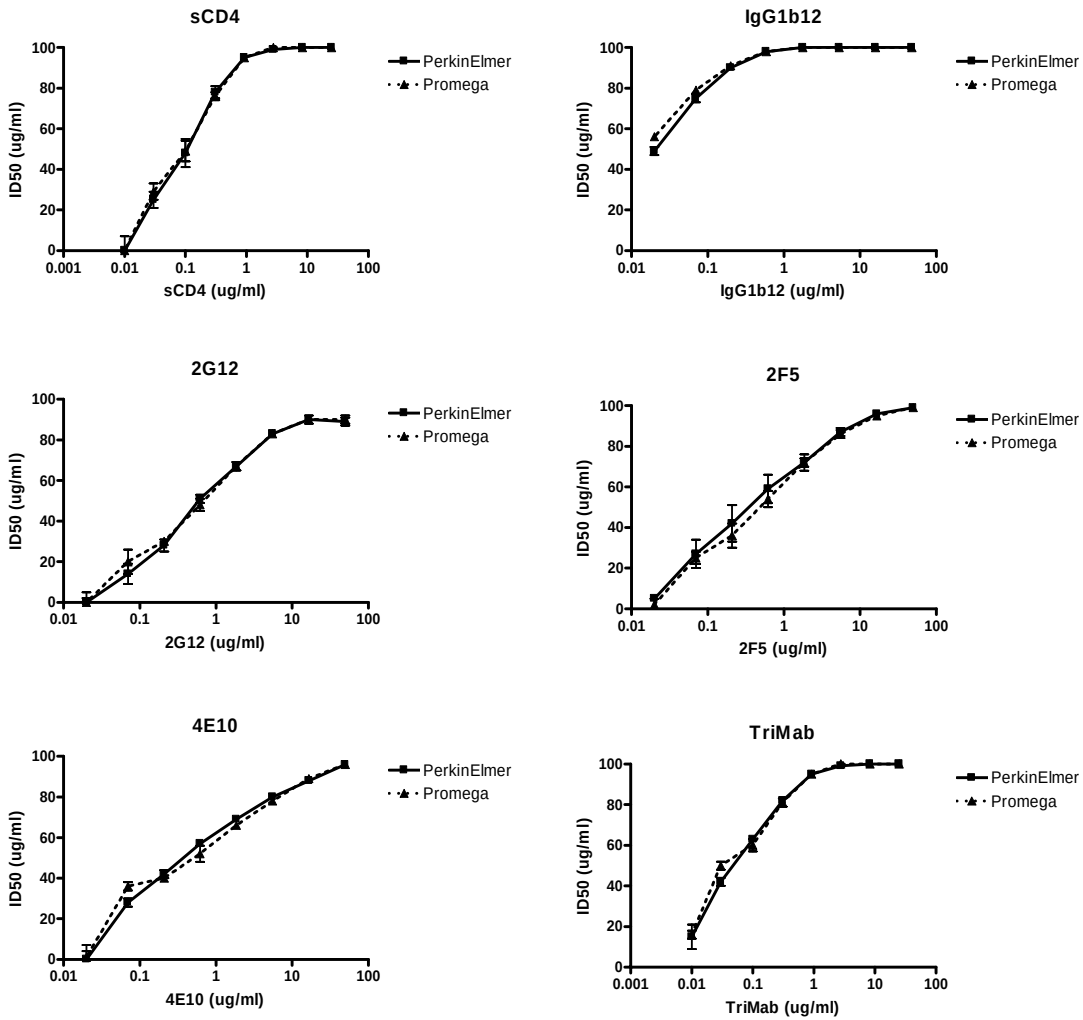
Assays with QH0692.42



Examples of Intra-Assay Variation:

Comparison of Two Luciferase Kits (PerkinElmer vs Promega)

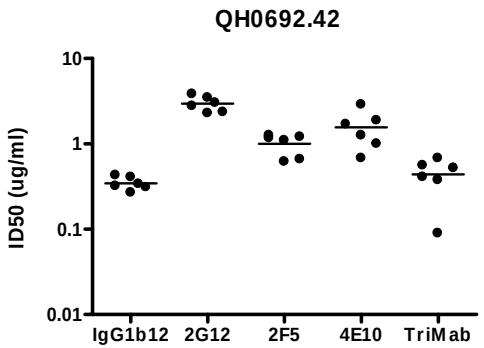
SF162.LS

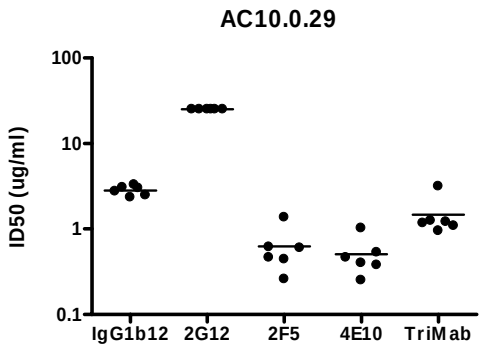


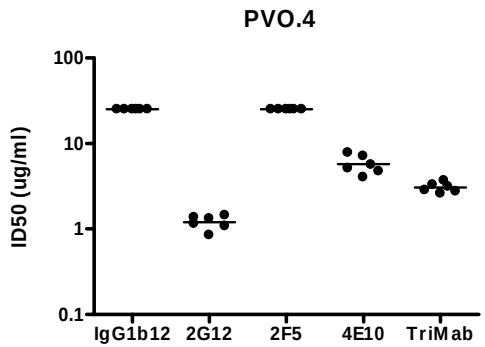
Inter-Operator Variation: Internal Proficiency Testing

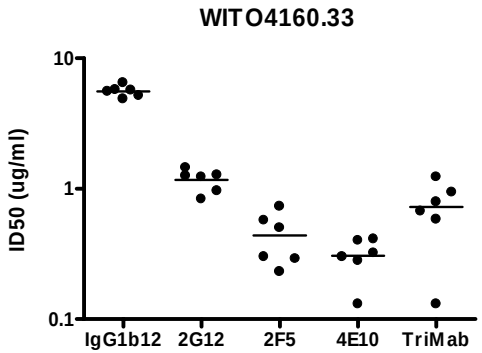
Six operators assayed 7 positive serologic reagents against 6 reference strains of Env-pseudotyped HIV-1 in TZM-bl cells (SOP HVTN02-A0009).

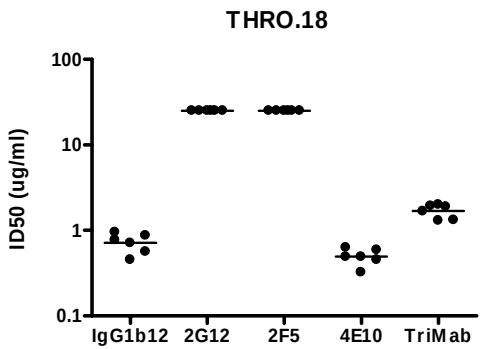
Internal Proficiency Test Results with MAbs

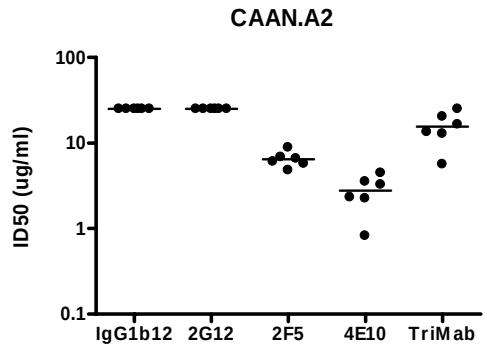




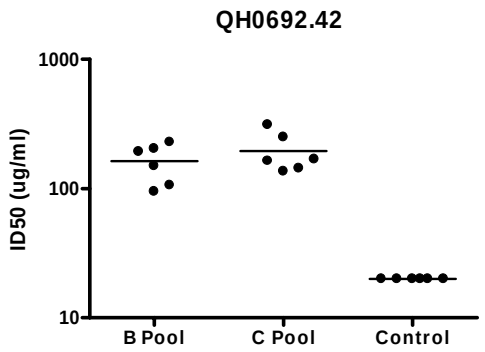


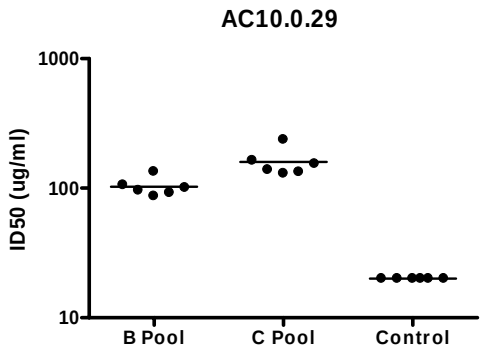


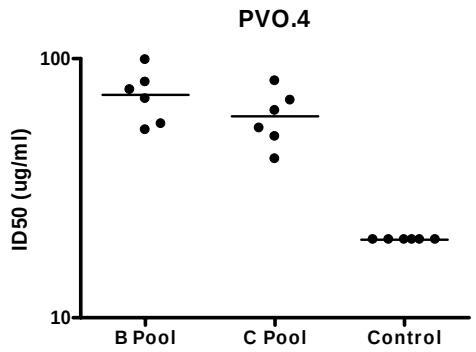


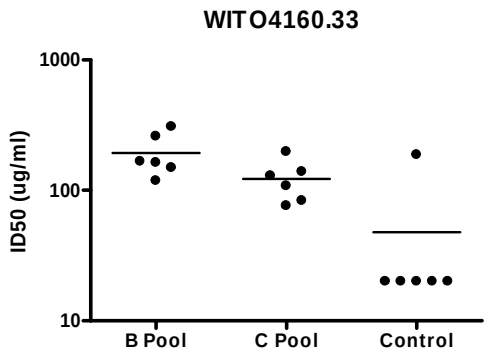


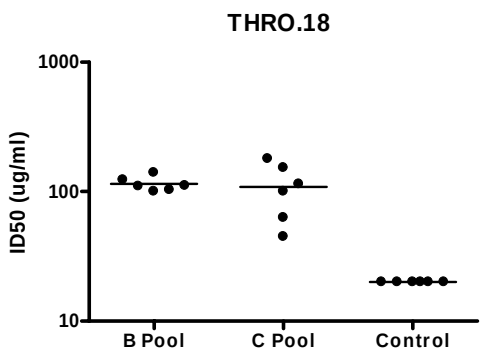
Internal Proficiency Test Results with Serum Samples

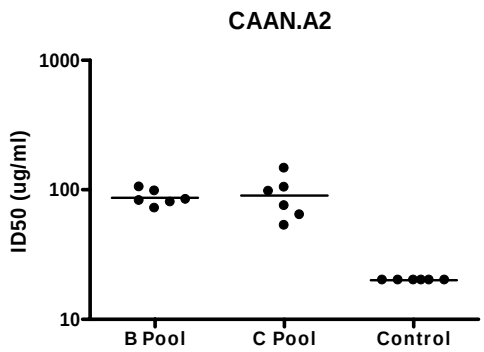




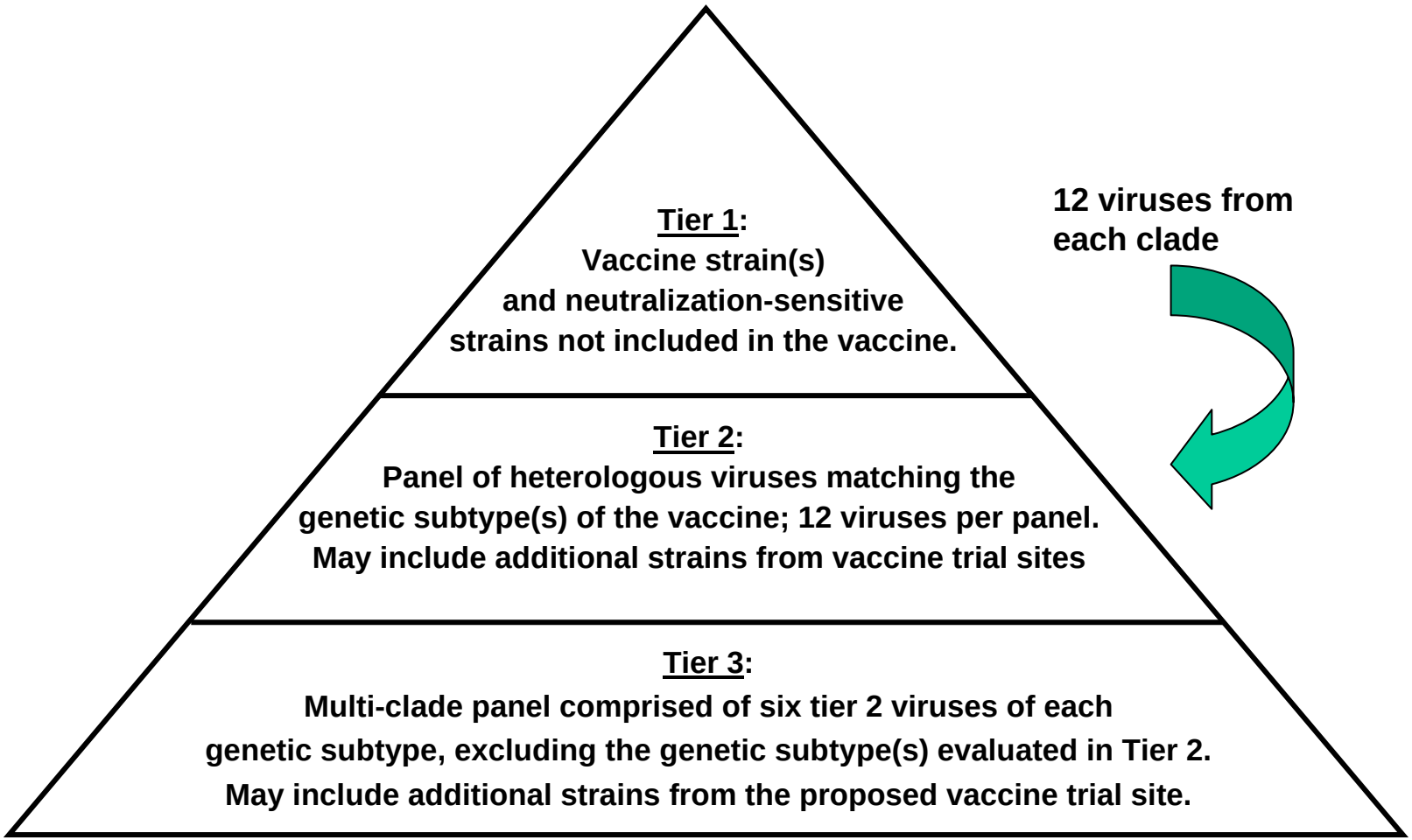








Tiered Approach to Assessing Vaccine-Elicited Neutralizing Responses



J. Virology, 79:10103-10107 (2005)

Criteria for Selection: panel composition

- Weighted in favor of recently collected viruses
  - Avoid potential genetic/antigenic drift over time
- Sexually transmitted viruses from acute/early infection
- Grouping by 6 major clades - 90% circulating HIV-1
  - Virus panels for A, B, C, D, E, A/G
  - Additional panels corresponding to vaccine trial sites
- Viruses with a representative distribution of neutralization phenotypes
  - HIV-1^{+} sera, mAbs, vaccine sera
- Use molecular clones for stability, reproducibility and epitope analysis
  - Non-replicating pseudoviruses
  - PBMC-grown viruses could also be made available

How many viruses in each panel?

Statistical considerations - Peter Gilbert (SCHARP)

Estimate the # of viruses and vaccine recipients that would allow adequate power to differentiate immunogens.

- **Two vaccines in phase I - 20 subjects per arm**
- **Panel of 12 clade B viruses**

90% power to see a difference between vaccine that neutralized 10% of viruses vs. 35%

Advantage of Clonal Viruses

- Reagent Characterization – precisely known Env sequence
- Stability – on regeneration of virus stock, same virus each time
- Easily transferable as plasmids
- Precision and reproducibility – clonal virus is the same in each assay
- Facilitate GCLP assay validation for clinical studies
- Facilitate the mapping of antibody specificities – relation to known Env sequence.

RECENT PROGRESS:

Standard Panel of 12 Clade B HIV-1 Reference Strains

- **Full-length functional Env plasmids – recently donated to NIAID AIDS repository**
- **Clinically, demographically well characterized**
- **Acute/early sexually-acquired infection, R5 biologic phenotype**
- **Neutralization phenotype - representative primary isolates**
- **Genetically distinct and well-characterized, e.g. N-glycans, V-regions, MAb epitopes – sequences deposited in GenBank**
- **Gender diversity - most M-M, several F-M and one M-F transmissions**

J. Virol. 79:10108-10125 (2005)

Ongoing and Future Plans

- Compose global panels for clades A, C, D, E, A/G
- Compose panels from international vaccine trial sites
- International networks - identify and donate reagents for non-B virus panels
- Address scientific questions identified by workshop
- Evaluate AIDSVAX phase III sera against the clade B panel (minimum bar)
- Design and implement an external proficiency testing program

Plans for an External Proficiency Testing Program: TZM-bl Neutralizing Antibody Assay

- **Initial round of testing**
  - **Assess inter-laboratory variation under conditions of relaxed standardization**

- **Subsequent rounds of testing**
  - **Confirm the key parameters that affect assay performance**
  - **Revise and validate the assay SOP**
  - **Develop an SOP for proficiency testing**
  - **Validate the proficiency testing SOP**

First iteration of testing should begin in October 2005

Standard Neutralizing Antibody Potency Test for HIV-1 Vaccines?

- **Magnitude: Geometric mean titer of neutralizing Abs against one or more Tier 1 viruses.**
- **Breadth: Frequency of positive neutralization against a standard panel of reference strains.**
- **Consideration: Protein boosting in the case of genetic vector vaccines.**

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