

Program of External Proficiency Testing for the TZM-bl Neutralizing Antibody Assay

□ Initial rounds of testing

- Assess inter-laboratory variation under conditions of relaxed standardization

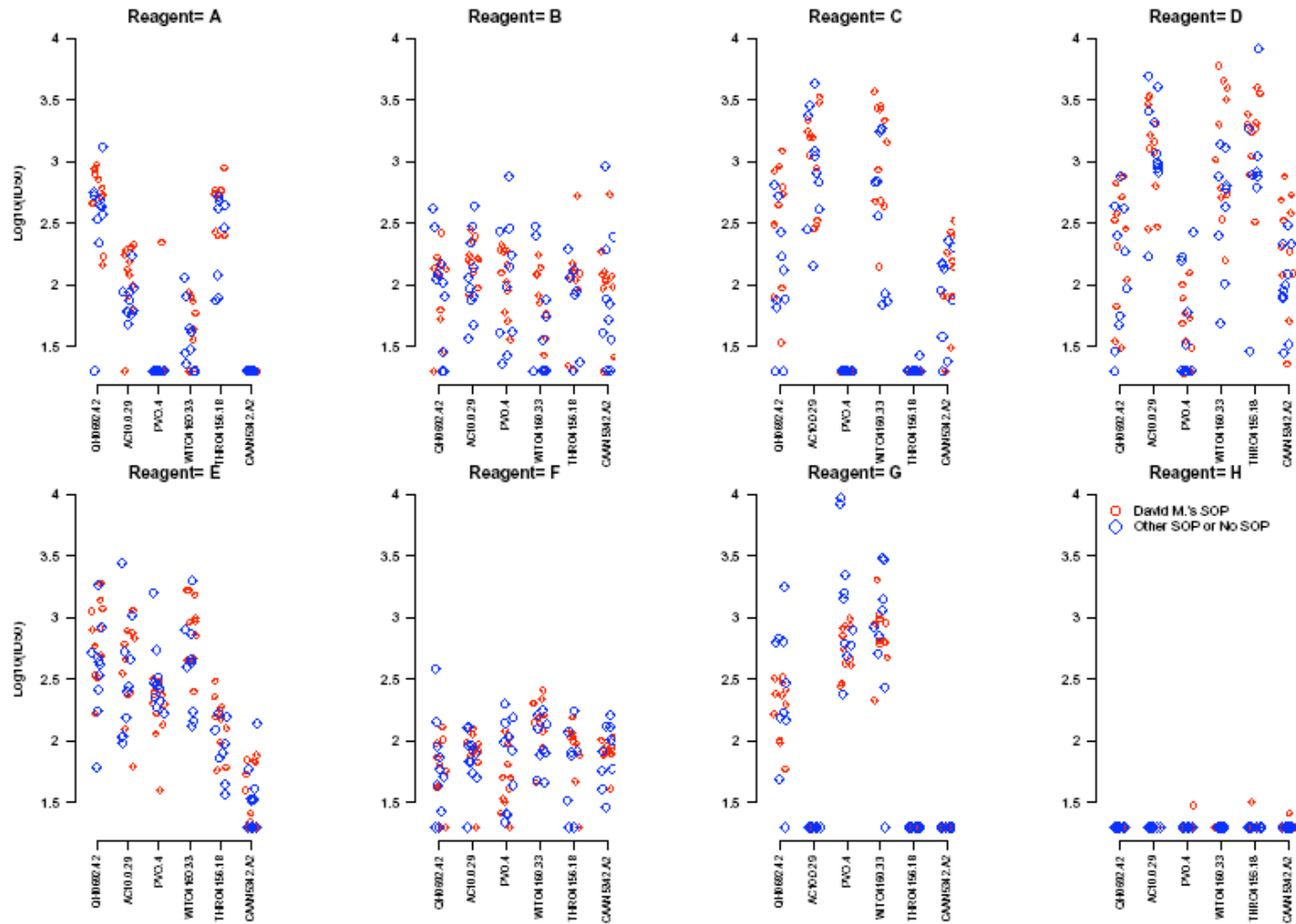
□ Subsequent rounds of testing

- 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 Round of Proficiency Testing Aimed at Developing a Standardized and Validated Program for the TZM-bl NAb Assay

- Prospective study plan (study goals and design, reagents, instructions, assay checklist, statistical analysis plan, QAU oversight)
- 17 laboratories participated
- Assays performed with blinded serologic reagents:
 - ✓ Panel included:
 - 6 clade B reference strains as Env-pseudotyped viruses
 - sCD4, IgG1b12, 2G12, 2F5, 4E10, TriMab, 2 HIV-1-positive serum pools, 1 HIV-1-negative serum.
- Participating labs supplied a copy of their SOP
- Each laboratory made their own stocks of pseudoviruses
- Statistical analysis performed by SCHARP (Seattle)

Outcome of First Round Proficiency Testing



SOURCE: SCHARP MaggieWang /trials/vaccines/David_Montellor/TZMAssayvalidation/codes/Intertab_validation.R

R version 2.1.0, 2005-04-18 (2006-04-27 1

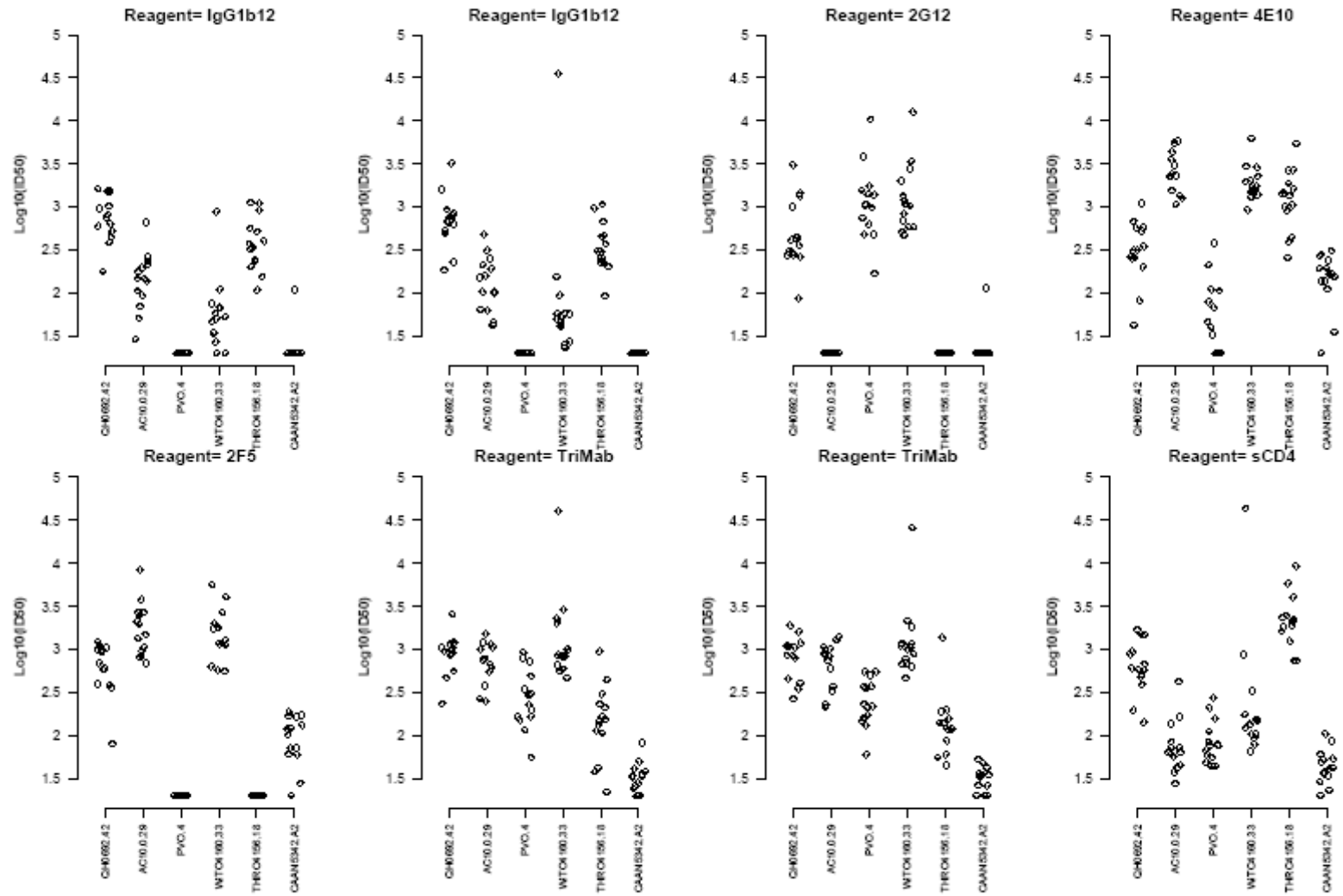
Outcome of First Round Proficiency Testing

- 5 laboratories considered to be highly concordant – based on 3-fold variation from mean of all laboratories**
- Variability of the other labs could not be easily explained by differences in assay SOP's**
- Current evidence strongly implicates the pseudovirus stocks generated in each laboratory to be the major source of variation**

Second Round of Proficiency Testing Aimed at Developing a Standardized and Validated Program for the TZM-bl NAb Assay

- ❑ Prospective study plan
- ❑ 14 laboratories participated
- ❑ Assays performed with blinded serologic reagents:
 - ✓ Panel included:
 - 6 clade B reference strains as Env-pseudotyped viruses
 - sCD4, IgG1b12, 2G12, 2F5, 4E10, TriMab
 - 2 MAbs were shipped as blinded duplicates
- ❑ Participating labs supplied copy of their SOP
- ❑ Each laboratory using the same stock of pre-prepared pseudoviruses
- ❑ Statistical analysis to be performed by SCHARP (Seattle)

Unblinded Scatter Plot for Proficiency Data 2nd round -- ID50 (14 labs)



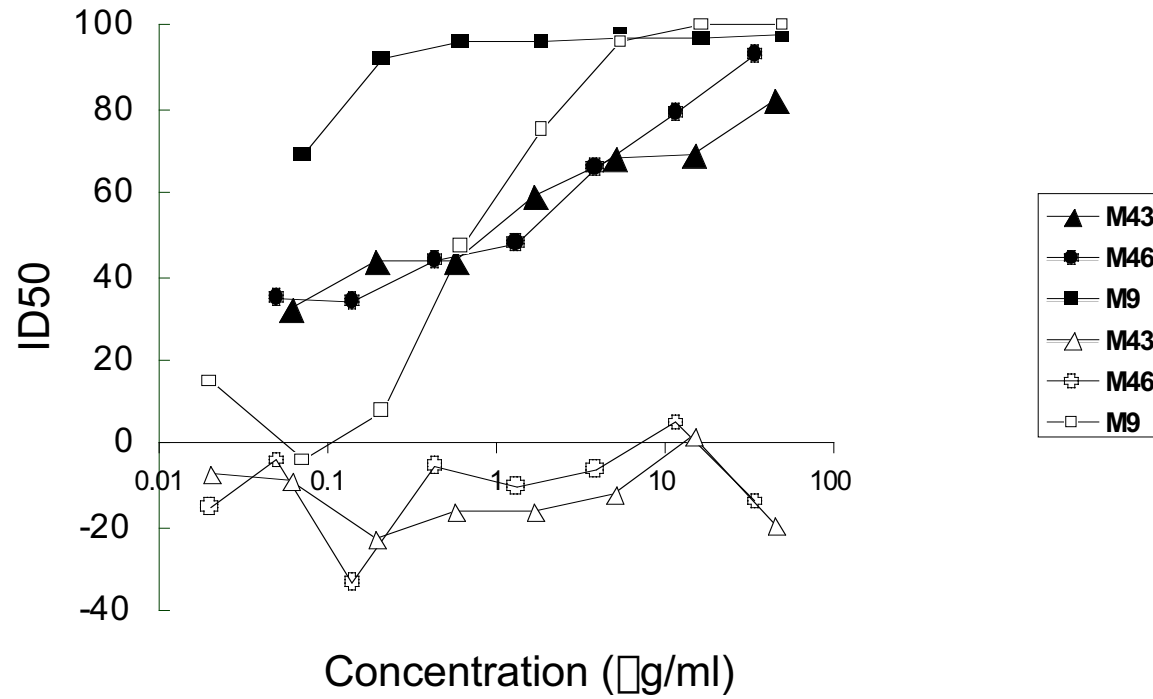
SOURCE: SCHARP MaggieWang /trials/vaccine/David_Montefiori/TZMAssayvalidation/code/Interlab_validation_2round_March2007.R

Version 2.3.0 (2006-04-24) | 2007-03-13 10:13

Key Limitation of the New Assay Technology

**Not all neutralizing antibodies are detected in
genetically engineered cell lines...**

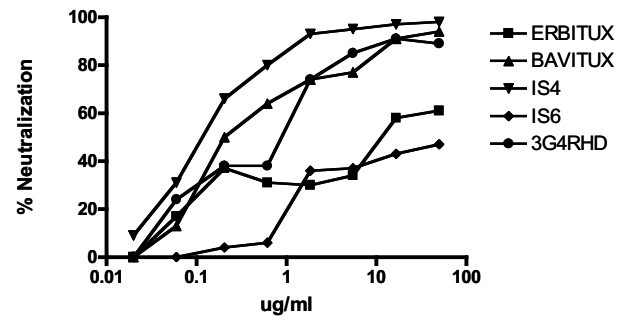
Neutralization Curves with Du123



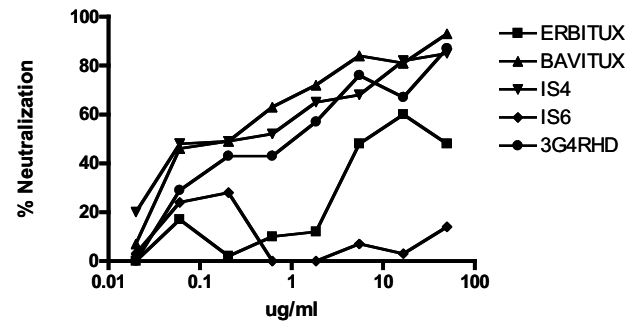
Solid symbols: PBMC virus assayed in PBMC
Open symbols: 293-T pseudovirus assayed in TZM-bl cells

Title

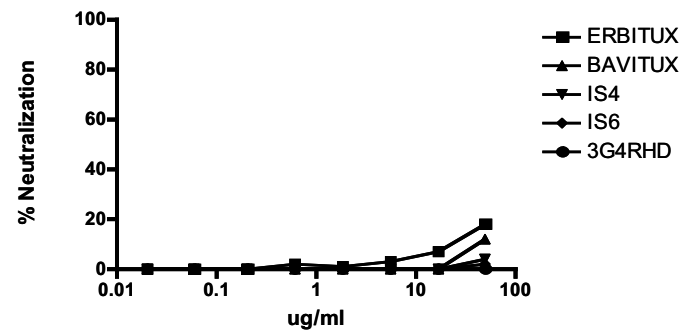
QH0692 PBMC-grown/PBMC Assay



QH0692.42 +Luc/PBMC Assay



QH0692.42-Luc/TZM-bi Assay



What Explains the Phenomenon?

- Is it the cells in which the virus is grown? Unlikely- 293T-grown virus is neutralized in PBMC; PBMC virus is not neutralized in TZM-bl.
- Is it an FcR-mediated activity? No- Fab neutralizes in the PBMC assay but not in the TZM-bl assay.
- Is it DEAE-dextran? No- Antibodies fail to neutralize in the TZM-bl assay when DEAE-dextran is excluded.
- Is it the level of CCR5 expression? Unlikely- Antibodies fail to neutralize in CEMx174-CCR5 cells (very low CCR5).
- Is it the target cells? Maybe- Antibodies fail to neutralize in TZM-bl, U87.CD4.CCR5 and CEMx174.CCR5 cells.

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PARTICIPANTS IN THE PROFICIENCY TESTING PROGRAM:

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