Protocol for Neutralizing Antibody Assay Reagent Bridging Studies (May 2011)

I. INTRODUCTION

The Duke Neutralizing Antibody Assay Laboratory is responsible for assessing vaccine-elicited neutralizing antibody responses in clinical trials of candidate HIV-1 vaccines. Parallel testing is performed on reagents, when new lot numbers of reagents or preparations of cells or viruses are available, to ensure the integrity of the reagents and the validity of the assay. All current and new reagents for bridging studies will be evaluated using the neutralizing antibody assay in TZM-bl cells.

II. DEFINITIONS

FBS: Fetal Bovine Serum

TCID: Tissue Culture Infectious Dose

IMC: Infectious Molecular Clone

DPBS: Dulbecco's Phosphate Buffered Saline

III. REAGENTS AND MATERIALS

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality may be used when necessary.

Control Reagents
Polymun

Fetal Bovine Serum
Hyclone

TZM-bl Cells
NIH AIDS Research and Reference Reagent Program

A3R5 Cells
Colonel Jerome Kim & Dr. Robert McLinden, USMHRP

M7-Luc Cells
Dr. Nathaniel R. Landau, Salk Institute

293T/17 Cells
American Tissue Culture Collections

Env-pseudotyped viruses

IMC Viruses

Growth Medium

Invitrogen

DEAE-Dextran, hydrochloride, avg. Mol. Wt. 500,000
Sigma

Trypsin-EDTA (0.25% trypsin, 1mM EDTA)
Sigma

DPBS, Sterile
Invitrogen

Trypan Blue (0.4%)
Sigma

Britelite Plus Reporter Gene Assay System
PerkinElmer Life and Analytical Sciences

Viviren Live Cell Substrate
Promega

Microliter pipettor tips
Eppendorf, RAININ, Biohit

Disposable Pipettes, sterile, individually wrapped (1 ml, 5 ml, 10 ml, 25 ml, 50 ml)
Costar / VWR

Flat-bottom culture plates, 96-well, low evaporation, sterile
Fisher

Flat-bottom black solid plates, 96-well, Costar brand
Fisher

Flat-bottom white solid plates, 96-well
Costar / Fisher

Reagent reservoirs, 50 ml
Costar / VWR

Culture flasks with vented caps, sterile (T-75)
Fisher

“Control Reagents Parallel Testing Record” (Appendix A)

“Virus Preparation Parallel Testing Record” (Appendix B)

“TZM-bl Cell Integrity Post Thaw Parallel Testing Record” (Appendix C)

“Fetal Bovine Serum (FBS) Parallel Testing Record” (Appendix D)

Instrumentation:

Luminometer
Perkin Elmer Life Sciences

Biological Safety Cabinet
NuAIRE

Incubator
Forma Scientific

Pipettor
Biohit, RAININ, Eppendorf, Thermo Labsystem
Drummond

Light Microscope
Olympus

Centrifuge and Microcentrifuge
Jouan

Hemacytometer
INCYTO

Fluorescence Microscope
Olympus

Water Bath
Precision Scientific

Laboratory Refrigerator / -20°C Freezer
Sci-Cool

Low Temperature Freezer
Revco / Harris

Liquid Nitrogen Freezer Tank
MVE, Inc.

Specimens:

Control reagents, FBS, Env-pseudotyped viruses, Env.IMC viruses, cells (TZM-bl, A3R5.7, M7-Luc, 293T/17) listed in various protocols

IV. PROTOCOL

Control Reagents (e.g., sCD4, IgG1b12, 2F5, 4E10, TriMab)

1. A bridging study should be performed each time a new reagent is received from the manufacturer.

2. Run a parallel test of a current lot number with the new lot number or receipt date of the control antibody using the neutralizing antibody assay in TZM-bl cells.

3. Perform the assay with HIV-1 SF162.LS/293T/17 and HIV-1 QH0692.42/293T/17.

4. Use a 0.5 mg/ml stock solution of the assay control and start at a 1:20 dilution and do 3-fold dilutions (final starting concentration = 0.25 µg/ml). The starting concentration of controls will vary as indicated by the neutralizing antibody sensitivity of the virus.

NOTE 1: Pseudoviruses to be used in the evaluation may be reassigned by the PI.

Virus Preparation

1. A bridging study should be performed each time a new virus is prepared and after the TCID is completed.

2. Perform the neutralization assay with the current virus harvest date along with the first harvest date of the new virus at the dilution indicated by the TCID.

3. Assay the viruses against the following reagents (if applicable): sCD4, IgG1b12, 2F5, 4E10, and TriMab. The starting concentration of controls will vary as indicated by the neutralizing antibody sensitivity of the virus.

4. Consult the PI in order to proceed with the appropriate antibody concentration that will yield a full concentration curve at 50% neutralization.

5. Start the assay at 1:20 dilution and do 3-fold dilutions (final starting concentration will vary).

TZM-bl, A3R5, and M7-Luc Cell Integrity

1. A bridging study should be performed each time a new aliquot of cells is passed into a culture from liquid nitrogen storage.

2. Perform the neutralization assay with the current culture of TZM-bl, A3R5 and M7-Luc cells and the newly established culture of TZM-bl, A3R5 or M7-Luc cells.

3. Assay the cells with HIV- SF162.LS/293T/17 and HIV-1 QH0692.42/293T/17 when testing TZM-bl cells. Perform the assay with HIV-1 SF162.LucR.T2A.ecto/293T/17 and HIV-Bal.LucR.T2A.ecto/293T/17 when testing A3R5 and M7-Luc cells.

4. Assay the virus against the following control reagents (if applicable): sCD4, IgG1b12, 2F5, 4E10, and TriMab.

NOTE 2: The starting concentration of controls will vary as indicated by the neutralizing antibody sensitivity of the virus.

NOTE 3: Alternative viruses may be assigned by the PI.

5. Consult with the PI in order to proceed with the appropriate antibody concentration with that will yield a full concentration curve at 50% neutralization.

Fetal Bovine Serum

1. Perform a bridging study each time a new lot number is received from the manufacturer.

2. Perform the neutralization assay with the current and the new lot numbers of FBS.

NOTE 4: Growth medium should be prepared using the new lot number and a flask of cells kept in culture for at least two passages prior to the bridging test. Perform the neutralizing antibody assay in parallel with current cells kept in growth medium prepared with the old lot number.

3. Perform the parallel assay with the viruses used for bridging TZM-bl cells or with viruses assigned by the PI.

4. Assay with a selected virus against the following control reagents (if applicable): sCD4, IgG1b12, 2F5, 4E10, and TriMab. The starting concentration of controls will vary as indicated by the neutralizing antibody sensitivity of the virus.

5. Consult with the PI in order to proceed with the appropriate antibody concentration that will yield a full concentration curve at 50% neutralization.

293T/17 Integrity

1. Each time a new batch of cells is thawed, and before discarding the old cells, a virus should be grown in parallel using the old cells and the new cells. The yield of virus grown in the new batch of cells should not be lower than 3-fold compared to the virus grown in the old batch of cells.

Establishing Pass/Fail Criteria

Pass: Test results for at least four of the five assayed control reagents agree within 3-fold between the two sets of data. The mean RLU values of the virus control wells must be at least 10x the mean RLU values of the cell control wells of the plate.

Fail: Test results for at least two reagents (one reagent in Control Reagent Parallel testing are > 3-fold different between the two sets of data. The mean RLU values of the virus control wells are less than 10X than the mean RLU values of the cell control wells of the plate. The test will be repeated as necessary. If a failed reagent cannot pass the bridging test, the reagent should not be used.

Procedure for Recording and Reviewing Results

1. The technician should record the bridging results on the appropriate Parallel Testing sheet.

2. The technician should indicate whether the reagent used in the parallel testing has passed or failed the established criteria.

3. The technician performing the bridging assay should sign the Parallel Testing sheet(s).

4. The technician should submit the Parallel Testing sheet(s), along with the raw data, to the Principal Investigator (or designee) for review and signature.

5. The Parallel Testing sheet(s), along with the appropriate raw data and communication material, if applicable, should be filed within the Bridging Studies notebook.

V. REFERENCES

1. "Protocol for Neutralizing antibody assay for HIV-1 in TZM-bl cells"
2. "Protocol for Heat-inactivation of serum and plasma samples"
3. "Protocol for Preparation and titration of HIV-1 pseudoviruses"
4. "Protocol for Preparation of Cell-Free Stocks of TCLA HIV-1 in Cell Lines"

VI. APPENDICES

Appendix A: Control Reagent Parallel Testing Record

Appendix B: Pseudovirus Preparation Parallel Testing Record

Appendix C: TZM-bl Cell Integrity Post Thaw Parallel Testing Record

Appendix D: Fetal Bovine Serum (FBS) Parallel Testing Record

Appendix A: Control Reagent Parallel Testing Record

Neutralizing Antibody Assay **Control Reagent Parallel Testing**

Date:	Tech:	Virus:
Current Control:	New Control:	Virus Date:
Current Control Lot Number:	New Control Lot Number:	Virus ID:
Current Control Date Received:	New Control Date Received:	Experiment #:
Current Control Manufacturer:	New Control Manufacturer:	Parallel Testing Passed[1]:
ID50 in TZM-bl Cells (µg/ml)	ID50 in TZM-bl Cells (µg/ml)	Date in Use:

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Date:	Tech:	Virus:
Current Control:	New Control:	Virus Date:
Current Control Lot Number:	New Control Lot Number:	Virus ID:
Current Control Date Received:	New Control Date Received:	Experiment #:
Current Control Manufacturer:	New Control Manufacturer:	Parallel Testing Passed[1]:
ID50 in TZM-bl Cells (µg/ml)	ID50 in TZM-bl Cells (µg/ml)	Date In Use:

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Appendix B: Pseudovirus Preparation Testing Record

Neutralizing Antibody Assay Pseudovirus Preparation Parallel Testing

Date:					Tech:					
Virus:					Experiment #:					
Current Virus Preparation Date:					New Virus Preparation Date:					
Virus ID:					Virus ID:					
TCID:					TCID:					
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)					
sCD4	IgG1b12	2F5	4E10	2G12	sCD4	IgG1b12	2F5	4E10	2G12	Parallel Testing Passed[1]

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Date:					Tech:					
Virus:					Experiment #:					
Current Virus Preparation Date:					New Virus Preparation Date:					
Virus ID:					Virus ID:					
TCID:					TCID:					
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)					
sCD4	IgG1b12	2F5	4E10	TriMab	sCD4	IgG1b12	2F5	4E10	TriMab	Parallel Testing Passed[1]

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Appendix C: TZM-bl Cell Integrity Post Thaw Parallel Testing Record

Neutralizing Antibody Assay TZM-bl Cell Integrity Post Thaw Testing

Date:					Tech:						
Current Culture:					New Culture:					Virus:	
Passage Number:					Passage Number:					Virus Date:	
Thaw Date:					Thaw Date:					Virus ID:	
										Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallel Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Date:					Tech:						
Current Culture:					New Culture:					Virus:	
Passage Number:					Passage Number:					Virus Date:	
Thaw Date:					Thaw Date:					Virus ID:	
										Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallel Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Appendix D: Fetal Bovine Serum (FBS) Parallel Testing Record

Neutralizing Antibody Assay Fetal Bovine Serum (FBS) Lot to Lot Parallel Testing

Date:					Tech:						
Current Lot:					New Lot:					Virus:	
Lot Number:					Lot Number:					Virus Date:	
Expiration Date:					Expiration Date:					Virus ID:	
					Received Date:					Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E10	TriMab	sCD4	IgG1b12	2F5	4E10	TriMab	Parallell Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Date:					Tech:						
Current Lot:					New Lot:					Virus:	
Lot Number:					Lot Number:					Virus Date:	
Expiration Date:					Expiration Date:					Virus ID:	
					Received Date:					Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E10	TriMab	sCD4	IgG1b12	2F5	4E10	TriMab	Parallell Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____
Reviewed: _____ Date: _____