

**Protocol for Neutralizing Antibody Assay Reagent Bridging Studies
(Montefiori Lab)
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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. Serum and plasma samples are tested for the presence of neutralizing antibodies by using a specific assay that is described in “Protocol for Neutralizing antibody assay for HIV-1 in TZM-bl cells” and uses supporting procedures as described in “Protocol for Heat-inactivation of serum and plasma samples,” “Protocol for Preparation and titration of HIV-1 pseudoviruses,” and “Protocol for Preparation of Cell-Free Stocks of TCLA HIV-1 in Cell Lines.” To comply with GCLP regulations, parallel testing must be 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

III. REAGENTS AND MATERIALS

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

“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

ThermoLabsystem

Drummond

Light Microscope

Olympus

Specimens:

Reagents, cells and viruses listed in the following protocols:

“Protocol for Neutralizing antibody assay for HIV-1 in TZM-bl cells”

“Protocol for Heat-inactivation of serum and plasma samples”

“Protocol for Preparation and titration of HIV-1 pseudoviruses”

“Protocol for Preparation of Cell-Free Stocks of TCLA HIV-1 in Cell Lines”

IV. PROTOCOL

Criteria for Assessing When Bridging Studies Should be Performed

Control Reagents

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

NOTE 1: All receipt dates must be tested regardless if the reagents have the same lot number.

Virus Preparation

1. A bridging study should be performed each time a new stock of virus is prepared. If virus is harvested more than once from a single infection, bridging assays only need to be performed on one of the harvests.

TZM-bl Integrity

1. A bridging study should be performed each time a new aliquot of TZM-bl cells is thawed for use.

Fetal Bovine Serum (FBS)

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

NOTE 1: Bridging Studies may be indicated for additional reagents and will be included in this protocol as required.

Protocol for Performing Bridging Studies

Serologic Control Reagents

1. Perform a neutralizing antibody assay with the new batch of reagent at the same time that you assay a reference batch of the same reagent. The reference batch is one that has either been tested multiple times and has the expected properties or has been previously bridged to an acceptable reference standard.

2. It is recommended that serologic controls be bridged with a least two strains of virus that are sensitive to neutralization by the reagent.
3. All conditions of the bridging assays must be identical except for the serologic control reagents that are being bridged.

Virus Preparations

1. Perform a neutralizing antibody assay with the new batch of virus at the same time that you assay a reference batch of the same virus. The reference batch is one that has either been tested multiple times and has the expected properties or has been previously bridged to an acceptable reference standard.
2. It is recommended that viruses be bridged with 5 serologic reagents (e.g., sCD4, IgG1b12, 2F5, 4E10, and T).
3. All conditions of the bridging assays must be identical except for the viruses that are being bridged.

TZM-bl Integrity

1. Perform a set of neutralizing antibody assays using the current working culture of TZM-bl cells and the newly thawed cells.
2. It is recommended that TZM-bl cells be bridged with at least two strains of virus and 5 serologic reagents (e.g. sCD4, IgG1b12, 2F5, 4E10, and TriMAB).
3. All conditions of the bridging assays must be identical except for the cells that are being bridged.

Fetal Bovine Serum (FBS)

1. Perform the neutralization assay with the current and new lot numbers of FBS.
2. Assay the cells with a virus assigned by the Principal Investigator. Assay the virus against the following Control reagents (if available); sCD4, IgG1b12, 2F5, 4E10, and TriMAB. The starting concentration of controls will vary as indicated by the neutralizing antibody sensitivity of the virus.

NOTE 1: Cells must be passed at least once in the growth media containing the new lot of FBS before performing the neutralizing antibody assay.

3. All conditions of the bridging assays must be identical except for the FBS that is being bridged.

Establishing Pass/Fail Criteria

Pass: Test results for at least four assayed 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 background of the plate.

Fail: Test results for at least two reagents 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 background for the plate. Test will be repeated as necessary.

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 [†]	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 [†]	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	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallel Testing Passed [†]	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	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallel Testing Passed [†]	Date new culture in use

Signature: _____ Date: _____

Reviewed: _____ Date: _____