Protocol for the Preparation of Cells for Detection of *Mycoplasma* Species
(December 2011)

I. Introduction

Serum and plasma samples are tested for the presence of neutralizing antibody responses by using assays as described in various Protocols (Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells; Neutarlizing Antibody Assay for HIV-1 in A3R5 Cells; Neutralizing Antibody Assay for HIV-1 in M7-Luc Cells; Preparation and Titration of HIV-1 Env-pseudotyped Viruses; and Preparation and Titration of IMC Viruses).

Cell line cultures must be screened for *Mycoplasma* contamination as *Mycoplasma* can cause alterations in cell growth rates, morphology, and cell viability as well as can spread to other cell cultures [1]. Maintaining the integrity of these key cell lines is critical for ensuring the validity and quality of the neutralizing antibody assay and the production of viruses.

II. Definitions

PCR: Polymerase Chain Reaction

Antibiotic-free GM: Growth Medium without the presence of antibiotics

IMC: Infectious Molecular Clone

GM: Growth Medium

FBS: Fetal Bovine Serum

DPBS: Dulbecco's Phosphate Buffered Saline

III. Reagents and Materials

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality than the recommended ones can be used whenever necessary.

Antibiotic-free Growth Media for TZM-bl Cells (see Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells)

Antibiotic-free Growth Media for M7-Luc Cells and A3R5 Cells (see Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in A3R5 Cells; Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in M7-Luc Cells)

Trypsin-EDTA (0.25% trypsin, 1 mM EDTA)
Vendor: Invitrogen
Sterile

DPBS
Vendor: Invitrogen
Sterile

Disposable pipettes, sterile, individually wrapped
Vendor: Falcon/VWR
1 ml pipettes
5 ml pipettes
10 ml pipettes
25 ml pipettes
50 ml pipettes

Culture flasks with vented caps, sterile
Vendor: Costar/VWR
T-75 flask

Conical tubes, sterile
Vendor: Costar/VWR
15 ml capacity
50 ml capacity

"*Mycoplasma* Testing Record" (Appendix A)

MycoAlert Mycoplasma Detection Kit
Vendor: Lonza

Cryogenic vials, 2.0 ml sterile screw cap, frosted label
Vendor: Starstedt Brand Products

IV. Instrumentation

Recommended manufacturers are listed. Unless otherwise specified, equipment of equal or better quality than the recommended ones can be used whenever necessary.

Biological Safety Cabinet
Manufacturer: Baker Co.

Incubator
Manufacturer: Forma Scientific

Pipettor
Manufacturer: Drummond

Light Microscope
Manufacturer: Olympus

Centrifuge
Manufacturer: Jouan
(low speed capable of up to 500 x g)
15 ml tube holder
50 ml tube holder

4°C Refrigerator
Manufacturer: Sci-Cool

-20°C Freezer
Manufacturer: Sci-Cool

Water Bath
Manufacturer: VWR

Hemacytometer
Manufacturer: INCYTO

V. Specimens

TZM-bl, M7-Luc, A3R5, CEM-NKR-CCR5-Luc, and 293T/17 cell lines listed in various Protocols.

VI. Protocol

1. Initial Qualification of Cell Lines

1.1 The Laboratory must maintain an archived inventory of frozen cells, designated as "Master Archive Stock" and "Working Archive Stock," for the TZM-bl, 293T/17, M7-Luc, A3R5 and CEM-NKR-CCR5-Luc (if applicable) cell lines. Both stocks must be tested for the presence of *Mycoplasma* in order to determine baseline purity. All cell lines in this archive should have tested negative for the presence of *Mycoplasma* species and be recorded in the appropriate laboratory log book.

1.2 During the initial qualification run, a vial of cells from the Working Archive Stock is thawed and cultured in vitro. Cells are tested for *Mycoplasma* contamination at Weeks 0, 2, 4, 8, 12, 18, and 24.

1.3 During each round of testing, the cells must be found negative for the presence of *Mycoplasma* species. If no positive results are obtained by the end of week 24, the routine testing schedule can be reduced to a period of time not to exceed every 3 months.

1.4 In the event that a cell culture tests positive for *Mycoplasma* during the qualification process, the culture must be discarded immediately, a new *Mycoplasma*-free cell line must be established, and another period of qualification testing performed as indicated above. This qualification run is also necessary if the laboratory begins culturing a new cell line.

NOTE 1: TZM-bl and 293T/17 cell cultures must be discarded after either 60 passages or 5 months in culture, whichever comes first. M7-Luc and A3R5 cell cultures must be discarded after 3 months.

2. Routine *Mycoplasma* Testing (TZM-bl, 293T/17, M7-Luc, A3R5, CEM-NKR-CCR5-Luc)

NOTE 2: *Mycoplasma* testing can be performed using a variety of commercially available kits or using a third-party laboratory. Refer to the manufacturer's instructions for the use of each individual kit.

NOTE 3: If sending the cells to a third-party laboratory for *Mycoplasma* testing, follow the instructions given by the testing laboratory.

2.1 Cells should be maintained according to the various Protocols for Cell Maintenance.

2.2 If required by the *Mycoplasma* testing kit/company, cells should be carried for at least 10 days or 3 passages in antibiotic-free GM.

2.3 A positive and negative control should be run in parallel with the testing of the cells. Positive and negative controls are commercially available.

2.4 All appropriate information pertaining to the cells that are being tested, as well as the "Pass" or "Fail" results, must be recorded on the *Mycoplasma* Testing Record (Appendix A).

2.5 In the event that a cell culture tests positive for *Mycoplasma*, the culture must be discarded immediately and a new *Mycoplasma*-free cell line must be established.

NOTE 4: Cell lines that test positive for *Mycoplasma* contamination must not be used for any assay.

3. Procedure for Recording and Reviewing Results

3.1 The *Mycoplasma* Testing Record (Appendix A) will be reviewed, initialed, and dated by a Lab Manager or appropriate personnel designated by the Principal Investigator.

3.2 The *Mycoplasma* Testing Record (Appendix A) must be filed in the laboratory.

VII. References

1. Kilani, A. "Mycoplasma Testing – An Overview." Clongen Laboratories, LLC. http://www.clongen.com/mycoplasma_testing_services2.htm.

2. MycoAlert User Manual

VIII. Appendix

A: "*Mycoplasma* Testing Record"

Technician	Test Date	Cell Type	Passage #	Results	Pass / Fail[1]	Designee Initials & Date