Protocol for Preparation of Cells for Detection of *Mycoplasma* Species (Montefiori Lab) July 2008

I. INTRODUCTION

Serum and plasma samples are tested for the presence of neutralizing antibody responses by using a specific assay, as described in Protocol: Neutralizing Antibody Assay for HIV-1 in TZM-bl cells, that utilizes molecularly cloned Env-pseudotyped viruses generated in 293T/17 cells, as described in the Protocol: Preparation and Titration of HIV-1 Env-pseudotyped Viruses.

To comply with GCLP regulations, cell culture lines 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 [7.1]. Maintaining the integrity of these key cell lines is critical for ensuring the validity of the neutralizing antibody assay and the production of Env-pseudotyped viruses.

II. DEFINITIONS

GCLP: Good Clinical Laboratory Practice

DMEM: Dulbecco's Modified Eagle Medium

HEPES: N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid

III. REAGENTS AND MATERIALS

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

Complete Growth Media*

DMEM, with L-glutamine, sodium pyruvate, glucose and pyridoxine. Sterile, store refrigerated at 4°C.
Gibco BRL Life Technologies

Fetal Bovine Serum. Heat-inactivated at 56°C for 30 minutes, sterile. Store at -20°C. Once thawed, store at 4°C.
Hyclone

Gentamicin solution, 10 mg/ml. Sterile, store at 4°C.
Sigma

HEPES Buffer, 1 M. Sterile, store at 2°C – 8°C
Gibco

> *** Complete GM containing 10% heat-inactivated FBS, 50 µg gentamicin/ml, and 25 mM HEPES.** To make a 500 ml of complete GM, combine 435 ml DMEM, 50 ml FBS, 2.5 ml gentamicin and 12.5 ml HEPES in a sterile bottle, mix, store at 4°C for up to 2 months. Warm medium to 20-37°C prior to use.

Trypsin-EDTA (0.25% trypsin, 1 mM EDTA, sterile
Invitrogen

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

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

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

"*Mycoplasma* Testing Record" (Appendix A)

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
NuAire

Incubator
Forma Scientific

Pipettor
Drummond Scientific Co.

Light Microscope
Olympus

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

V. SPECIMENS

TZM-bl and 293T/17 cell lines listed in protocol: Protocol for Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells, and Protocol for Preparation and Titration of HIV-1 Env-pseudotyped Viruses.

VI. PROTOCOL

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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 both TZM-bl and 293T/17 cell lines. Both stocks must be tested for the presence f *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.

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.

1.3 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:*** Cell cultures must be discarded after either 60 passages or 5 months in culture, whichever comes first.

2. *Mycoplasma* Testing

2.1 In order to perform *Mycoplasma* testing, the technician trypsinizes a confluent flask of TZM-bl cells and/or 293T/17 cells in accordance to Protocols for Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells and Preparation and Titration of Env-pseudotyped Viruses.

2.2 Mycoplasma testing may be performed using a variety of commercially available kits or a third party laboratory. Refer to the manufacturer's instructions for the use of each individual kit and the pass/fail criteria.

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 2: Cell lines that test positive for *Mycoplasma* contamination must not be used for any assay in support of GCLP studies.

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.

VIII. Appendix

Appendix A: "*Mycoplasma* Testing Record"

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