

Protocol for the Preparation of Cells for Detection of *Mycoplasma* Species (August 2010)

I. Introduction

Serum and plasma samples are tested for the presence of neutralizing antibody responses by using a specific assay, as described in Protocol for 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 Protocol for the Preparation and Titration of HIV-1 Env-pseudotyped 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 Env-pseudotyped viruses.

II. Definitions

PCR: Polymerase Chain Reaction

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

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

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

Invitrogen

Sterile

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)

MycoAlert Mycoplasma Detection Kit

Lonza

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

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 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

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 the TZM-bl and 293T/17 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: 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 Protocol for Trypsin-EDTA Treatment for Disruption of Cell Monolayers.

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.

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.

