

## HIV SYNCYTIUM-INDUCING (MT-2) ASSAY

### 1. BACKGROUND and CLINICAL SIGNIFICANCE

Host and viral factors may play a role in determining the way in which an individual responds to anti-retroviral therapy. Presence and maintenance of the non-syncytium inducing (NSI) HIV phenotype has been associated with reduced disease progression.

The assumption has been that a switch from syncytium-inducing (SI) back to NSI during anti-retroviral treatment would result in a positive response to therapy i.e. reduced chance of disease progression. However, this was not true in ACTG 175. There was no significant difference in disease progression between individuals who maintained the SI phenotype and those that switched from SI back to NSI [Fiscus et al, JID,1998]. Thus, it is possible that losing the SI phenotype does not necessarily indicate a switch back to a macrophage tropic phenotype. This was demonstrated in PACTG 138 which included 4 detectable conversions from SI to NSI between baseline and week 56-132 [Viani et al, JID,1998]. Of those 4 NSI isolates, only 2 demonstrated macrophage tropism. For the remaining 2 cases, neither the baseline SI nor the post treatment NSI isolates, were able to replicate in macrophages. Thus, a virus conversion back to NSI does not automatically indicate a change in tropism. In fact, dual tropic isolates have been isolated. The dual tropic activity of one such virus, 89.6, has been attributed to expanded co-receptor usage [Cell, 85:1149-1158]. Taken together, these data suggest more stringent criteria than SI/NSI alone may be necessary to define HIV tropism.

The MT-2 cell culture assay is used to detect SI variants of HIV. MT-2 cells, an HTLV-I immortalized T-cell line, are cultivated with cell-free supernatants from HIV-infected PBMC cultures. Inoculated MT-2 cell cultures are monitored every 2-3 days, up to two weeks, for development of typical cytopathic effect (CPE), i.e. large ballooning syncytia. The MT-2 assay is normally performed, in duplicate, in 96-well flat-bottom cell culture plates. Other plate, tube or flask formats are permissible if the concentrations and volumes are adjusted accordingly.

### 2. Specimen Requirements

Supernatants from HIV-infected PBMC cultures are suitable specimens. Optimally, fresh co-culture supernatants should be used,. Frozen supernatants may be acceptable, provided they have been stored at  $-70^{\circ}\text{C}$  or lower. Any previously frozen supernatant should be re-cultured in PBMCs concurrently with the MT-2 assay to confirm viability/infectibility.

### 3. Reagents

- 3.1 MT-2 cells: Human T-cells isolated from cord blood lymphocytes and co-cultured with cells from patients with adult T-cell leukemia. (NIH AIDS Reagent Repository catalog #237.)

- 3.2 MT-2 culture medium: 435 ml RPMI 1640 supplemented with 50 ml fetal bovine serum, 10mL penicillin (5000U/mL)/streptomycin (5000ug/mL) and 5mL of 200mM L-glutamine.
- 3.3 Positive control reference virus: NIH AIDS Reagent Repository virus strain A018 (catalog #629 )
- 3.4 MT-2 "thawing" medium: 385mL RPMI 1640 supplemented with 100mL fetal bovine serum, 10mL penicillin (5000 U/mL/streptomycin (5000ug/mL) and 5mL of 200 mM L-glutamine.

#### 4. Supplies and Equipment

Sterile 96-well flat-bottom cell culture plate (or sterile 24-well plate, using appropriate modifications in reagent volumes and cell number)

Micropipettors to deliver 50,150 and 200uL.

Sterile aerosol resistant disposable pipette tips.

Sterile centrifuge tubes.

25 and 75 cm<sup>3</sup> cell culture flasks

Gas permeable polypropylene (ziplock) bag

Sterile 2,10 and 25mL pipettes

Pipette-aid

Hemocytometer and cover slip

Inverted microscope

CO<sub>2</sub> incubator

Biological safety cabinet

Appropriate personal safety gear

#### 5. PROCEDURE

##### 5.1 Propagation of MT-2 cells:

- 5.1.1 Thaw 2 vials of frozen MT-2 stock cells rapidly in 37°C water bath.
- 5.1.2 Aseptically transfer cells to 15mL centrifuge tube with 10mL MT-2 media.
- 5.1.3 Centrifuge at 400 xg for 10 minutes at room temperature. Remove DMSO-containing freeze medium from the cells. Alternatively cells can be diluted 1:10 with MT-2 "thawing" medium containing extra FBS which is changed to fresh growth medium the following day.
- 5.1.4 Resuspend the cell pellet in 10mL MT-2 medium and transfer to a 25cm<sup>3</sup> cell culture flask.
- 5.1.5 Incubate at 37°C in 5% CO<sub>2</sub>.

- 5.1.6 Measure cell number and viability after 3 days incubation. Change half the culture medium and replace with fresh culture medium. Seed 75 cm<sup>3</sup> cell culture flasks with 30mLs at 5 x 10<sup>5</sup> viable cells/mL. Use 10mLs to seed 25 cm<sup>3</sup> cell culture flasks.
- 5.1.7 Incubate cultures at 37°C in 5% CO<sub>2</sub>.
- 5.1.8 Split cells 1:3 every 3 or 4 days or 1:10 once each week.

## 5.2 Freeze down procedure for MT-2 cell stock

- 5.2.1 Freeze down cells after verifying SI susceptibility of cells.
- 5.2.2 Use current consensus PBMC freezing method.

## 5.3 Propagation of Positive Control Virus Stock

- 5.3.1 Obtain stock positive control reference virus strain A018 from NIH AIDS Reagent Repository (catalog #629 ).
- 5.3.2 Store vial in -70°C until ready to propagate.
- 5.3.3 Thaw vial quickly .
- 5.3.4 Transfer virus suspension from vial to 75 cm<sup>3</sup> cell culture flasks containing 30mLs containing 5 x 10<sup>5</sup> viable MT2 cells/mL.
- 5.3.5 Incubate flask for 4 days at 37°C in 5% CO<sub>2</sub>.
- 5.3.6 Harvest culture supernatant in 100uL volumes in labeled cryovials.  
Store at -70°C.
- 5.3.7 Validate new positive control stock by setting up one aliquot in an MT2 assay in parallel with previous stock virus.

## 5.4 MT-2 phenotype assay

- 5.4.1 For 96 well layout. Add 200uL sterile PBS to each well designated "P" in the following diagram of a flat-bottomed 96 well cell culture plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	P	P	P	P	P	P	P	P	P	P	P
B	NEG	P	1	P	2	P	3	P	4	P	POS	P
C	NEG	P	1	P	2	P	3	P	4	P	POS	P
D	P	P	P	P	P	P	P	P	P	P	P	P
E	P	P	P	P	P	P	P	P	P	P	P	P
F	NEG	P	5	P	6	P	7	P	8	P	POS	P
G	NEG	P	5	P	6	P	7	P	8	P	POS	P
H	P	P	P	P	P	P	P	P	P	P	P	P

- 5.4.2 Harvest MT-2 cells which are growing vigorously, usually within several days. For the 96-well format, prepare a 4.0mL suspension of  $3.4 \times 10^5$  MT-2 cells/mL in MT-2 media.
- 5.4.3 Inoculate 150 uL of the cell suspension into each well designated with a specimen number or "NEG" or "POS".
- 5.4.4 Add 50uL of MT-2 culture media to the wells designated "NEG". Add 50uL of positive control virus to each of the wells designated "POS". Add 50uL of HIV-infected culture supernatant to duplicate specimen wells (1 through 8). The final volume in each well will be 200uL.
- 5.4.5 Incubate plate at 37°C in 5% CO<sub>2</sub>. (If the incubator is not humidified, seal the plate in a gas-permeable ziplock bag, then incubate.)
- 5.4.6 Visually examine each MT-2 well for syncytia formation on days 1, 3, 6, 9, 12 and 14, using an inverted microscope.
- 5.4.7 Read carefully against the negative and positive control wells. Positive syncytia formation is defined as 3 to 5 "balloons" or syncytia per well. (There should be 20 balloons or syncytia per well, if 24-well plates are being used.)
- 5.4.8 Document the first day of observed syncytia, e.g., "SI+d6" is an isolate which was first observed to produce syncytia on day 6.
- 5.4.9 After the wells have been examined, use a micropipettor and disposable tips to gently re-suspend the cells in each well, then remove and discard 130uL of suspension. Change tips between each well.
- 5.4.10 Feed each well with 150uL of MT-2 media. Return plate to ziplock bag, if needed, and continue incubation and observation.

5.4.11 Terminate the assay on day 14. If no syncytia are observed, score the isolate as nonsyncytia-inducing (NSI). If supernatants were previously frozen, assay new supernatants from PBMC parallel cultures for p24 to assure viral viability.

## **6 QUALITY CONTROL**

- 6.1 Positive virus control must produce observable syncytia within 7 days of incubation. First reading of the plate must be made by day 3 to avoid MT-2 cells dying and a positive reaction being missed.
- 6.2 Negative control wells must not develop syncytia. Note: minor ballooning may occur as the MT-2 cells divide, but these "balloons" are significantly smaller than in the positive wells and are not true syncytia.
- 6.3 If either control does not react as expected, the assay is suspect and should be repeated.
- 6.4 Cells should be passaged only up to 50 times. Monitor the reactions of the positive and negative controls at each passage. Discard the cell line if the positive control requires more time to develop syncytia or the negative control begins to develop significant ballooning that is difficult to distinguish from true syncytia. Older cells get non-specific ballooning, more difficult to maintain, and have a shorter life span.

## **7 Notes**

- 7.1 False negative SI results, interpreted as "NSI", can result from non-viable virus supernatant. In cases where culture supernatant viability is unknown, as in frozen stocks, a control PBMC culture should be performed.
- 7.2 False negative SI results can also occur if the MT-2 cells are compromised. Negative SI results should be confirmed by retesting if the positive SI control requires longer than 9 days to produce typical cytopathic effect, i.e. syncytia.

## **8 REPORTING**

- 8.1 Supernatants are scored as POSitive (SI), NEGative(NSI), or INDeterminant.
- 8.2 Record days to positivity. For example, POS+d6.
- 8.3 Record control results.
- 8.4 Record passage number of MT-2 cells.
- 8.5 Record results for ACTG specimens in the LDMS per current LDMS instructions.

## 9 REFERENCES

Japour AJ, Fiscus S, Mayers D, Reichelderfer P, Kuritzkes D. A microtiter MT-2 syncytia assay. IX International Conference on AIDS, 1993. PO-B41-2484; vol I, page 549. Berlin, Germany.

Harada S, Koyanagi Y and Yamamoto N. Infection of HTLV-III/LAV in HTLV-1 carrying cells MT-2 and MT-4 and application in a plaque assay. Science 229:563-566, 1985.

Tersmette M, Lange JM, deGoede RE, deWolf F, Eeftink-Schattenkerk JK, Schellenkens PT, Coutinho RA, Huisman JG, Goudsmi J and Miedema F. Association between Biological Properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet 1983-1985, 1989.

Koot M, Keet IP, Vos AH deGoede RE, Ross MT, Coutinho RA, Miedema F, Schellenkens PT and Tersmette M. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ Cell depletion and progression to AIDS. Ann Intern Med 118:681-688, 1993.

St. Clair MH, Hartigan PM, Andrews JC, Vavro CL, Simberkoff MS and Hamilton JD. Zidovudine resistance, syncytium-inducing phenotype, and HIV disease progression in a case-control study. The VA Cooperative Study Group. J Acquir Immune Deficiency Syndrome. 6:891-897, 1993.

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Procedure: HIV SYNCYTIUM-INDUCING (MT-2) ASSAY

Prepared by: ACTG Laboratory Technologist Committee

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Date Implemented into the Laboratory: \_\_\_\_\_

Updated on:

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Reviewed by:

Date:

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Supersedes Archived Protocol: DAIDS Virology Manual for HIV Laboratories,  
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