Titration of MC99 Virus on Clone 1A2 Cells

a. Make replicate 1:2 dilutions of MC99 virus prep in 96-well plate in RPMI-1640 media containing 10% FBS, gentamicin and no phenol red. We prefer to use media without phenol red because it interferes with spectrophotometric Absorbance reading at 450λ.

b. In separate 96-well plate place 5000 Clone 1A2 cells/well/150µl.

c. Transfer 50µl/well of virus dilutions onto plate with cells (be sure to leave one row of uninfected cell controls). Incubate for 6 days in 37°C humidified incubator.

d. After incubation, add 50ul/well of XTT-PMS or 20µg/ml WST-1 solution (Boehringer-Mannheim, follow manufacturer instructions) and incubate for 4hr at 37°C.

e. Read plate on 96-well spectrophotometer ($A_{450-650}$) and determine virus dilution which gives approximately 80% killing of cells as compared to uninfected controls.

Testing Potential Anti-HIV Compounds

Please refer to attached plate format for suggested orientation for testing two compounds/plate. Drug diluted in outer wells receives no cells or virus and $A_{450-650}$ from these wells is subtracted from test $A_{450-650}$ to eliminate effects of colored or microbially contaminated compounds. Drug is tested with cells in the absence of virus to determine toxic effects on the cells. We also recommend testing plates in duplicate.

Preparation of Drug Dilution Plate

a. Make replicate dilutions of compound in 96-well plate in RPMI-1640 with 10% FBS, 10µg/ml gentamicin and no phenol red. We recommend 1:2 dilutions for crude extracts and half-log or log dilutions for synthetic compounds. We typically start at 2000µg/ml for crude extracts and $2X10^{-4}M$ for synthetics. Prepare sufficient volume for testing on two plates (i.e. at least 400ul of each drug dilution.

Preparation of Test Plate

b. Using the attached plate format the following media additions are needed to ensure a final volume of 200ul/well: into a round bottom 96-well plate, place 50ul media in wells 3-10, B-E, these are the cells+virus+drug test wells. Place 100ul media in columns 2,B-G and 11, B-G, these are the cells alone and cells+virus control wells. Place 200ul media in column 1, A-H, these are the media blank wells and 100ul media in rows A, 1-11 and H, 1-11, these are the drug alone wells. Also place 100ul in columns 3-10, F-G, these are the drug+uninfected cell and uninfected cells alone wells. Place 200 ul of PBS in column 12, A-H, this column is not used. Transfer 50ul of each drug dilution to the appropriate wells of a round bottom 96-well plate.

c. Add clone 1A2 cells (5000/50ul/well) to inner 60 wells. (Split cells in flask so that they will be in the log-phase of growth, between $2X10^5$ and $1x10^6$/ml, when used for this assay).

d. Add 50ul/well of virus to appropriate wells (columns 2-11, B-E).

e. Incubate six days in humidified, 5% CO_2 incubator.

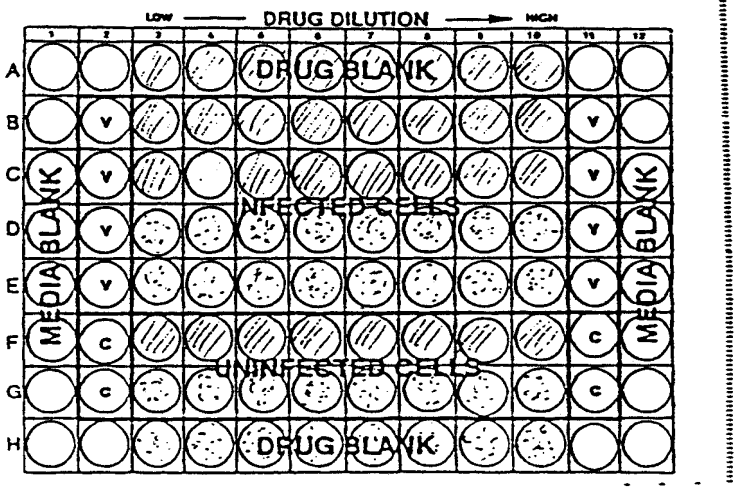
f. Add 50ul/well XTT-PMS or 20ul/well WST-1 (Boehringer-Mannheim, follow manufacturer instructions) to all wells. Reincubate for four hours.

g. Make visual observations of wells to confirm that wells with high formazan production (high O.D.) corresponds to a healthy cell pellet.

h. Read O.D.'s $A_{450-650}$ with a 96-well spectrophotometer (*NOTE: A shareware program for analysis is available for this plate format that is compatible with Exel, Lotus 123 and Quatro Pro.)

i. Compare test O.D. to uninfected cell control O.D. Plot concentration of drug vs % viability (test O.D.-drug alone O.D./uninfected control O.D.) to determine EC_{50}. IC_{50} can be similarly determined by plotting the drug+uninfected cell O.D.-drug alone O.D./uninfected control O.D.

Standard Plate Configuration



V = Virus control
C = cell control
/// = Drug 1
∴ = Drug 2