



TCID₅₀ Assay Protocol

The procedure is performed to determine the infectious titer of any virus which can cause cytopathic effects (CPE) in tissue culture over a reasonable period of 5 to 20 days while cells in culture remain viable. This procedure is performed to quantify how much infectious virus is in a preparation. Not all virus types cause CPE in tissue culture, and the cell line and virus must be carefully matched in order to see a cytopathic effect.

Virus	Cell Line	Days of culture until CPE complete
Adenovirus	293 for Recombinants A549 for Wild Type	7-10
Herpesvirus	Vero	3-5
Influenza	MDCK	3-5
Rhinovirus	H1 HeLa	5-7

The TCID₅₀ is determined in replicate cultures of serial dilutions of the virus sample. The titer of the virus stock is expressed as the TCID₅₀ which can be calculated using a statistical Excel program and is more accurate than a negative end-point.

VIRAPUR performs the TCID₅₀ assay using tenfold serial dilutions of the virus sample, and plates those samples in quadruplicate in 48-well tissue culture dishes.

This is a generic procedure, and the specific culture of the cell line such as the use of additives and the cell density and culture times may vary. Consult the managing virologist for appropriate cell lines, cell density, virus sample dilutions and incubation temperatures and times for the virus being tested.

Equipment and Materials Required

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips – 20 µl and 160 µl, 1000 µl
- Inverted microscope
- Tubes for dilution of virus - Falcon 2058 5 ml tubes or similar
- Hemacytometer with coverslip
- Cell media for infection (Infection Media - refer to Managing Virologist)
 - For adenoviruses, herpesviruses: use DMEM, high glucose, 2% fetal bovine serum plus antibiotics
 - For influenza viruses: use DMEM high glucose NO serum, TPCK trypsin at approximately 1 µg/ml and antibiotics.
- Growth media appropriate for cell line, ex DMEM plus 8% serum and antibiotics.
- 0.4% Trypan Blue Solution
- Lint-free wipes saturated with 70% isopropyl alcohol
- CO₂ incubator set at 37°C or 34°C or other temperature indicated

Procedure

1. One day previous to infection, prepare 48-well dishes by seeding each well with 7×10^4 cells in 0.5 ml DMEM plus 7.5% fetal bovine serum, 4 mM glutamine, antibiotics. Alternatively another cell density may be required, based on the cell line required for viral growth.
2. On the day of infection, make dilutions of virus sample in PBS. For influenza virus, use cell media plus TPCK trypsin for dilutions.
 - Tips used for virus transfers: Aerosol resistant 20 μ l, 160ul and 1000 μ l sterile tips.
3. Make a series of dilutions at 1:10 of the original virus sample. Fill first tube in series with 2.0 ml of PBS, fill the remaining 6 tubes in series with 1.8 ml of PBS.
4. Vortex virus sample, transfer 20 μ l of virus to first tube, vortex, discard tip.
5. With a new tip, transfer 200 μ l of first dilution to next tube. Vortex, discard tip.
6. Repeat series of dilutions through the last tube.

Additions of virus dilutions to cells:

1. Label lid of 48-well dish by drawing grid lines to delineate quadruplicates and number each grid to correspond to the virus sample and label the rows of the plate for the dilution which will be plated.
2. Include four negative wells on each plate which will not be infected.
3. Remove all but 0.1 ml of media from each well by vacuum aspiration. For influenza virus, monolayers should be rinsed once with serum free media before 0.1 ml infection media is added per well.
4. Starting from the most dilute sample, add 0.1 ml of virus dilution to each of the quadruplicate wells for that dilution.
5. Be careful not to track the pipette tip over the wells, and continue infecting the plate with 0.1 ml of virus dilution per well, infecting four wells per dilution, proceeding backwards through the dilutions.
6. Change the pipette tip when necessary.
7. Allow virus to adsorb to cells at 37° C for 2 hours or at a temperature indicated for the specific virus being titered (some viruses grow better at 34° C, including influenza).
8. After adsorption, remove virus inoculum with vacuum, beginning by sucking the most dilute wells and proceeding backwards to less dilute. Alternatively, leave the virus on the cells.
9. Add 0.5 ml Infection Medium (DMEM, 2% FBS, 4 mM Glutamine, 1X PSF) to each well. Do not touch the wells with the pipette. For influenza virus, add Infection Media without serum, with TPCK trypsin.
10. Place plates at 37° C or 34° C, depending upon instructions and monitor CPE using the inverted microscope over a period of one to four weeks, depending on the cultural characteristics of the virus in question.
11. Record the number of positive and negative wells.
12. Calculate the TCID50 titer using the Excel spreadsheet available for download from Yale School of Medicine at www.med.yale.edu/micropath/pdf/Infectivity%20calculator.xls.