Q&A ATCC® Excellence in Research Webinar “ATCC® Influenza Research Materials”

General Questions

1. Will we be able to download the presentation?
   This presentation will be available to watch on demand on the ATCC website, or click here.

2. I purchased a strain of Influenza A virus that is H1N1 and was isolated from swine, but I am not getting a positive PCR result using primers for detecting the pandemic strain. What could be wrong?
   Not all influenza A H1N1 strains from swine are from the recent pandemic. If the H1N1 strain is not from the recent pandemic, it will not be detected using the diagnostic PCR tests currently available.

   The only influenza A pandemic strains we have confirmed by PCR are ATCC® VR-1736™ and VR-1737™. RNA is available for these strains also, as catalog numbers ATCC® VR-1736D and VR-1737D.

3. What is the difference between a Plaque Assay and TCID50?
   Both assays are used to calculate viral titer. The plaque assay was initially developed to count and measure the infectivity level of bacteriophages. This technique has since been modified for use in animal virology, and has been a reliable determination of titer for a number of tissue-culture adapted viruses. The basis of this assay is to measure the ability of a single infectious virus to form a plaque on a cell culture monolayer. A plaque is developed as a part of the viral infection cycle, where following viral replication, the host cell dies.

   Viral titer can also be determined in vitro by calculating the infectious dose. For tissue culture adapted strains, this calculation is ascertained through an endpoint dilution assay in cell culture. The most reproducible endpoint of the dilution assay is the dilution of the virus that will produce a pathological change in 50% of the cell cultures inoculated. This number is expressed as 50% the infectious dose, or TCID50. The accuracy of this method is related to the number of replicates at each dilution.

   Overall, the plaque assay is much more sensitive than TCID50. For all newly acquisitioned tissue culture-adapted viruses, ATCC calculates titer using the plaque assay.

4. Can we calculate titer in PFU/mL from the TCID50? How are they related?
   Assuming that the same cell system is used, that the virus forms plaques on those cells, and that no procedures are added which would inhibit plaque formation, 1 mL of virus stock would be expected to have about half of the number of plaque forming units (PFUs) as TCID50. This is
only an estimate but is based on the rationale that the limiting dilution which would infect 50% of the cell layers challenged would often be expected to initially produce a single plaque in the cell layers which become infected. In some instances, two or more plaques might by chance form, and thus the actual number of PFUs should be determined experimentally. Mathematically, the expected PFUs would be somewhat greater than one-half the TCID\textsubscript{50}, since the negative tubes in the TCID\textsubscript{50} represent zero plaque forming units and the positive tubes each represent one or more plaque forming units. A more precise estimate is obtained by applying the Poisson distribution.

5. For frozen viral preparations, how should we thaw the vial? Should we quick thaw? ATCC recommends thawing in a 37°C water bath, until just melted.

6. Is the viral RNA you provide a pure stock? It is possible that RNA from the host cell line for tissue-culture adapted strains may also be present.