Cypher One: Influenza HA with Guinea Pig RBC's using V-Bottom Plates

Background: Cypher One™ is a new tool for rapid automated analysis of hemagglutination (HA) and hemagglutination inhibition (HI) assays. Until now interpretation of HA and HI assays required specialized expertise to accurately read and manually record the data, with experienced users often differing in their interpretation of the titer value. The Cypher One system will not only standardize analysis but also provide a digital, traceable record to enhance data quality and reliability.

Objective: This objective of this Application Note is to demonstrate the analysis capability of the Cypher One system for influenza HA assays performed using guinea pig red blood cells (RBC's) in conical (V-bottom) plates.

Forty influenza virus samples in clarified allantoic fluid were evaluated by hemagglutination in the presence of guinea pig red blood cells (RBC's) using conical (V-bottom) plates. In order to eliminate most of the error associated with manually reading the plates, virus samples were prepared at known concentrations relative to each other. A representative image from Cypher One for this experiment is shown in **Figure**

1. Virus samples are shown in rows A-H and column 12 contains the negative control. Note that the software displays the dilution factor above each column. Wells that are highlighted with a yellow circle are the titer calls made by Cypher One using analysis settings that were conserved for the entire dataset. These values are also listed in the "Titer" column to the left of the plate image.

As is standard practice, the titer of a given sample was determined in each row based on serial dilution across the row. In this experiment, each virus sample (rows A-H) was also diluted by a factor of two relative to the previous sample. Thus, the titer value was expected to vary by a factor of 2 between each sample/row. A total of 5 plates were analyzed and the sample placement was randomized to evaluate imaging effects.

Figure 1 – Cypher One Representative Image.
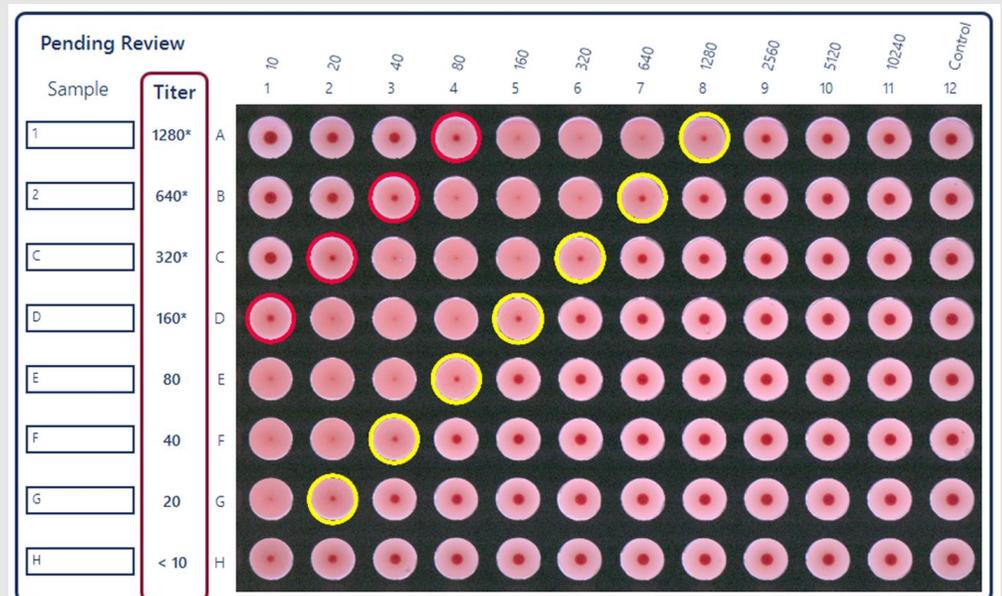
The data were evaluated for both correlation and accuracy. **Figure 2** is a direct comparison of titer calls made by the Cypher One system relative to the expected titer for all 40 samples. Based on a log-log analysis, it is clear that the data are highly correlated, as expected.

Figure 2 – Log-Log Comparison of Cypher One with Expected Titer.
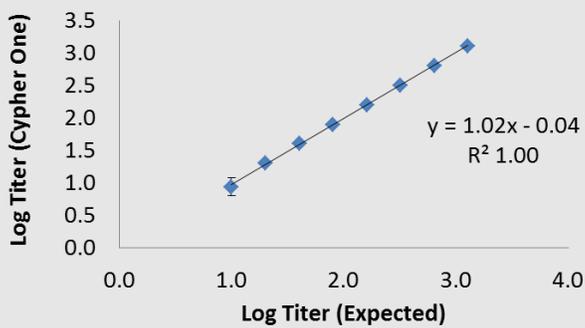
The histogram in **Figure 3** shows the number of samples that were in perfect agreement (0 dilutions outside the expected titer) as well as the distribution in calls relative to the expected value. For 40 samples analyzed, there was 100% agreement with expected within ± 1 dilution.

Figure 3 – Distribution of Titer Calls by Cypher One Relative to Expected Titer.
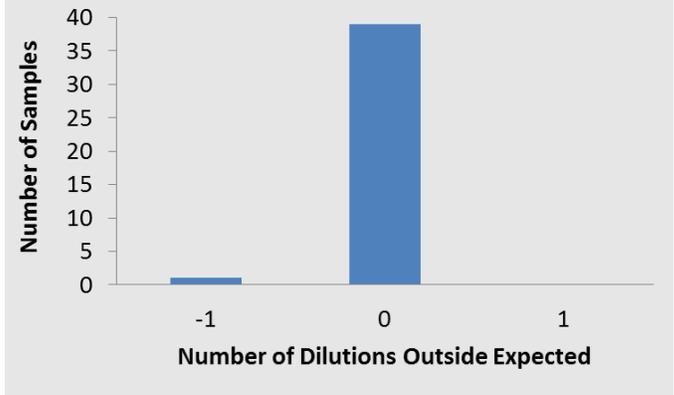
Summary: The Cypher One system performed well with a standardized sample set composed of influenza viruses in clarified allantoic fluid tested with guinea pig RBCs.