

## Cypher One: Manual Tilt Interpretation vs. Cypher One Analysis

**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.

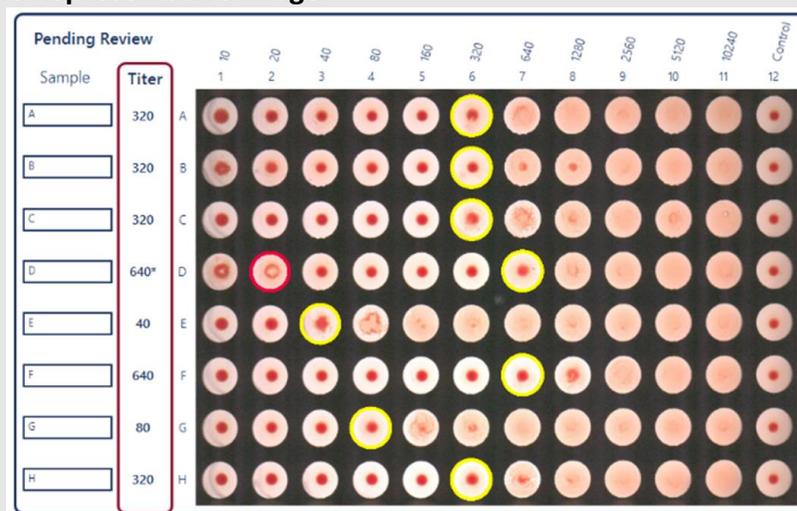
**Objective:** This objective of this Application Note is to compare the automated titer calls made by the Cypher One system to manual titer calls made by a single expert reader after tilting an HI plate. The question to be addressed is whether or not titling is required to assess titer in an HI assay.

During serological screening at a US Regulatory Agency clinical serum samples were evaluated for

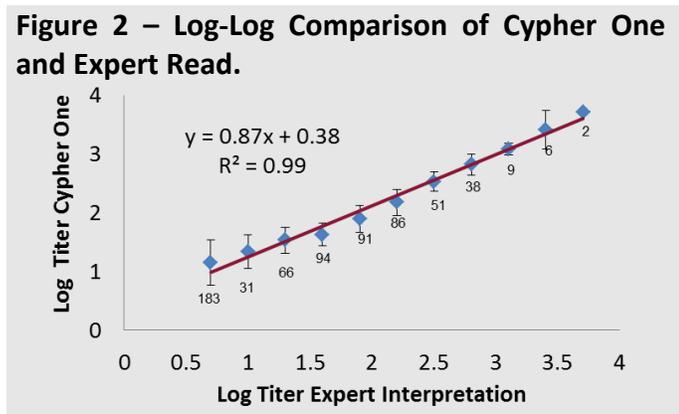
reactivity with influenza viruses in the presence of turkey red blood cells (RBC's). Microtiter plates were imaged using the Cypher One system first, then manually interpreted by a single expert who tilted the plate prior to reading. Tilting is conducted for samples that may contain non-specific inhibition as a means to distinguish true non-agglutinated wells from inhibited agglutination wells.

A representative image from Cypher One for this experiment is shown in **Figure 1**. The samples were diluted across each row (A through H) with column 12 containing negative controls. The dilution factor is marked 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.

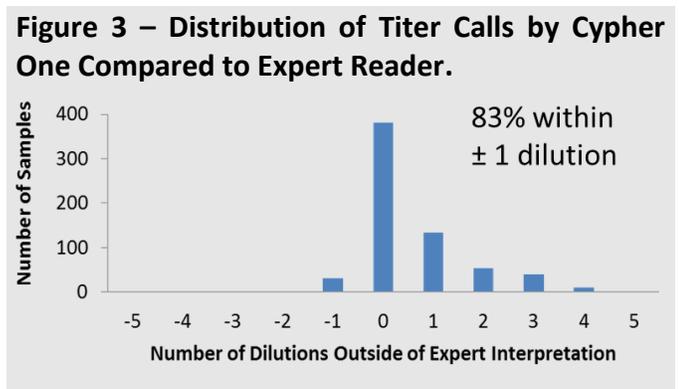
**Figure 1 – Cypher One Representative Image.**



**Figure 2** is a direct comparison of titer calls made by the Cypher One system to those made by the expert reader for 658 samples. The number of samples with a given titer is shown on the graph below each point. The data points are averages with  $\pm 1$  standard deviation representing the range. Based on this log-log analysis, it is clear that the data are highly correlated with an  $R^2$  value of 0.99.

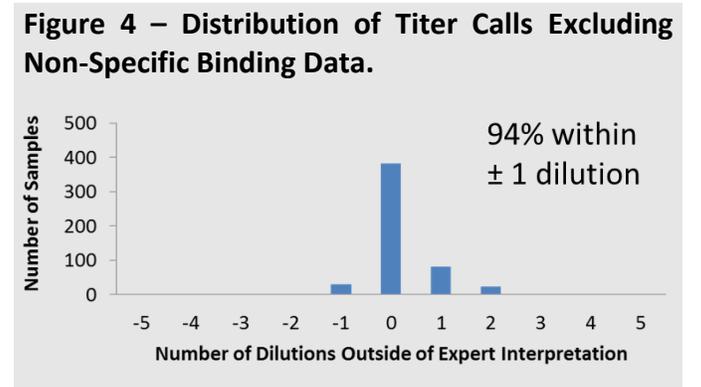


The histogram in **Figure 3** shows number of samples that were in perfect agreement (0 dilutions outside the expert interpretation) as well as the distribution in calls. For 658 samples analyzed, there was an 83% agreement with the expert reader within  $\pm 1$  dilution.



In order to establish a benchmark for performance, InDevR conducted an on-line survey of experienced HA/HI users. Each individual was shown images of rows from hemagglutination plates. Out of 390 unique “reads”, there was an 82% agreement within  $\pm 1$  dilution between the experienced users. Thus, the Cypher One reproducibility for HI assays **without tilting** the plate is equivalent to the performance of multiple human readers of the same plate.

**Non-Specific Inhibition:** Of the 658 samples in this data set, 79 were identified as exhibiting non-specific inhibition of agglutination. When these samples were excluded from the analysis, the agreement between Cypher One and the human expert was found to be 94% within  $\pm 1$  dilution (see **Figure 4**).



**Summary:** The Cypher One automated system exhibited good correlation and accuracy with respect to titer calls made by an expert reader, even with non-specific binding. Importantly, there was no need to tilt the plates, thereby eliminating a step from the process and further streamlining standardized analysis in the Cypher One system.

