Cypher One Plate Flexibility and Custom Analysis

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SUMMARY

Labs performing hemagglutination assays utilize a variety of plate layouts and control types. In order to accommodate the vast array of configurations, the Cypher One analysis can be paired with an Excel workbook to customize analysis and enable enhanced QC analysis to further advance a more standardized workflow.

Introduction

Hemagglutination assays are well established methods (1) with broad applications throughout the influenza vaccine, diagnostic, and surveillance communities to measure virus and antibody titers. Hemagglutination inhibition assays are recommended by the Global Influenza Surveillance Network (GISN) of the World Health Organization (WHO) for serological diagnosis and surveillance of influenza (2), and many laboratories use hemagglutination inhibition assays to assess immunogenicity and during analysis of which virus strains to including in seasonal flu vaccines. There are additional applications for hemagglutination assays beyond influenza including veterinary diagnostic testing for mycoplasma, parvovirus, and other diseases.

This wide range of applications results in a variety of assay configurations to meet the specific needs of the laboratory and method. This includes dilution of samples either across a row or down a column, multiple samples per row or column, inclusion of a variety of control types and replicate testing to enable geometric mean titer (GMT) calculations.

Variability in Plate Setup

In surveying publications and published hemagglutination assay protocols (2-5) as well as discussions with laboratory personnel, more than 15 unique plate setup configurations have been identified. Two layouts were identified as the most commonly used formats and incorporated as templates into the Cypher One software (**Figure 1**). First, the standard row format with samples diluted across each row starting in column 1 with a red blood cell (or negative) control well in column 12. Second, the standard column format with samples diluted down each column starting in row A with a red blood cell (or negative) control well in row H.

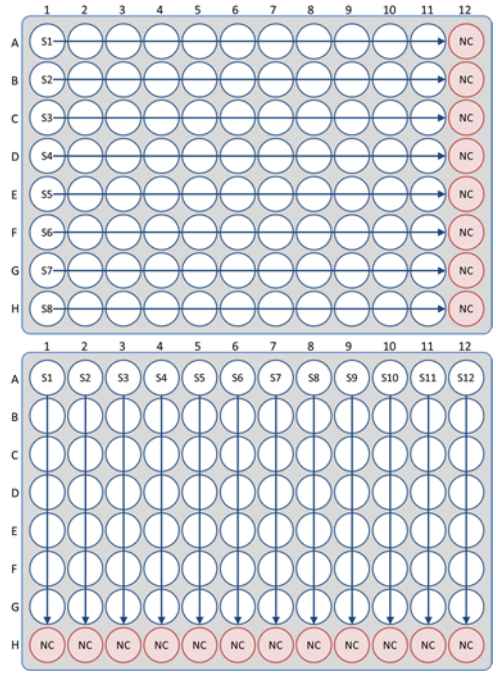

Figure 1: Standard Row and Column Formats.

Alternate layout configurations may incorporate alternate placement of controls, additional controls including those listed in the next section, multiple samples per row, or multiple replicates of each sample.

Variability in Control Usage

A total of 6 unique control types were identified for use in hemagglutination assays in a survey of publications, protocols and laboratories (**Table 1**). The Red Blood Cell (RBC) or Negative control is the most commonly used type and inclusion can aid in automated analysis by providing a standard button or pellet for reference. Other controls can be used to assess reagent performance, appropriate virus or antigen concentrations and determine the presence or absence of non-specific inhibition (NSI).

Control Name	Components	Acceptance Criteria
RBC or Negative	RBC + PBS	Well-formed pellets or buttons of consistent size
Virus or Antigen	RBC + Virus or Antigen + PBS	Full agglutination
Backtiter*	RBC + Titered Virus or Antigen + PBS	Last fully agglutinated well equal to virus titer in assay
Test Sera*	RBC + Test Sera + PBS	Well-formed pellets or buttons with no NSI
Positive Sera*	RBC + Positive Serum + Virus or Antigen + PBS	Well-formed pellets or buttons
Negative Sera*	RBC + Negative Serum + Virus or Antigen + PBS	Full agglutination

*Typically used only in HI assays.

Table 1: Control Types in Hemagglutination Assays.

For HA and HI assays, extensive testing has demonstrated that inclusion of RBC controls on each plate provides more robust results with greater confidence. Therefore, these controls are included in the native plate configurations provided in the Cypher One software.

Variability in Analysis Needs

Analysis standardization of hemagglutination assays is a well-documented need (4). Outside of the inherent variability in titer calls, there is variability in need and desire for other analysis. This can include a calculation of GMTs or tracking and trending of controls. For example, the long-term or lot-to-lot tracking and trending of controls is not a measurement that has been available through traditional, manual analysis of hemagglutination assays. By capturing an image of the plate and performing a mathematical analysis to assign a value to each well, this analysis can be enabled to provide greater confidence in the assay performance and results generated.

Enabling Flexibility and Customization

While Cypher One will accommodate the two most prevalent row and column formats (Figure 1), the system also provides an opportunity to leverage the data collected for analysis of alternate layouts or quality control assessments. During image analysis, Cypher One assigns a value to each well of the plate, which can be exported to Excel for offline analysis.

Briefly, these exported values have been successfully used to:

1. Analyze plate configurations with 2 samples per row.
2. Analyze samples utilizing sera controls instead of RBC controls to flag for possible NSI.
3. Alert users to invalid controls or possible NSI.

Conclusions

There is a range of plate configurations, control types and analysis needs for hemagglutination assays. Cypher One can accommodate most

protocols for hemagglutination assays either natively or in conjunction with offline Excel analysis.

References

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