CYPHER ONE: AUTOMATED INTERPRETATION OF HEMAGGLUTINATION INHIBITION (HAI) ASSAYS

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Abstract

Background and novelty: Hemagglutination (HA) and hemagglutination inhibition (HAI) assays have been utilized for 70+ years and play a critical role in flu vaccine development. In particular, HAI is critical in antigenic characterization of flu viruses and in evaluating immunogenicity of cell-based and traditional egg-based vaccines. HAI assays are prone to poor lab-to-lab consistency due to subjectivity in interpretation between human "readers" where a difference of ±1 dilution is often considered an 'equivalent' result. In addition, the presence of non-specific inhibition (NSI) can further complicate analysis. The Cypher One system both images plates for HA/HAI assays and automates the analysis in an effort to standardize interpretation and create a permanent record of results generated. Here we address whether or not the common practice of "tilting" HAI plates is necessary to obtain accurate results.

Experimental approach: We compared performance of Cypher One automated interpretation to visual interpretation of a human expert reader for a HAI dataset of 1,238 samples analyzed during serology testing by a US government agency. Cypher One titer was obtained without tilting and compared to that obtained by the human expert reader employing plate tilting for interpretation.

Results and discussion: Percent agreement between manual interpretation with tilt and Cypher One automated interpretation without tilt was 94.3% ± 1 dilution. Importantly, ~25% of clinical samples screened exhibited NSI, which is known to complicate manual interpretation and often cited as a cause for the need to tilt the plate for accurate interpretation. Even in the presence of NSI Cypher One showed high agreement for HAI without the need to tilt the plate. The Cypher One system thus achieved high accuracy and consistency while providing a digital record of plate images and associated results to meet data integrity requirements.

HAI Assay: Background

- **Hemagglutination:** HA proteins of influenza viruses cross-link red blood cells (RBCs) of avian or mammalian origin through binding to sialic acids on the cell surface, resulting in sustained suspension of RBCs in solution.
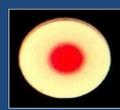
- **Hemagglutination Inhibition (HAI) Assay:** titrated antibodies are added along with a fixed antigen concentration to determine the endpoint where hemagglutination is inhibited by the presence of the antibodies. This inhibition is visually identified as the red blood cells (RBCs) in solution precipitate, resulting in a solid red "button" at the bottom of the well.

- **Standard HAI Interpretation:** visual examination by a human reader while the plate is tilted at a 45⁰ angle to assign a titer value (inverse of the dilution factor) based on how each sample "runs" relative to a representative negative control well.

- **Problem:** Lack of standardized reagents and consistent technique combined with unpredictable presence of <u>non-specific inhibitors</u> that challenge the interpretation translate to high inter-lab and inter-reader variation.

Fully Agglutinated

Agglutination Inhibited (Non-Agglutinated)

Presence of Non-Specific Inhibition Creates an Interpretation Challenge

Decreasing Ab concentration → Control   Decreasing Ab concentration → Control

No Non-Specific Inhibition

Examples Highlighting Non-specific Inhibition

Method

- 1,238 blinded human serum samples analyzed by HAI against 7 different antigens

- All samples underwent a standard receptor destroying enzyme (RDE) treatment

- Serum serially diluted (2-fold) across the row (i.e. A1-A11) in U-shaped 96-well plates

- 0.5% Turkey RBCs were added to all wells after the initial incubation period

- Column 12 used as a non-agglutinated negative control (no serum, no virus)

- <u>Cypher Imaging</u>: Each plate was first imaged in the flat orientation (not tilted) using Cypher One and immediately transferred to the experienced human reader for analysis

- <u>Human Interpretation</u>: The experienced human reader placed each plate at a 45⁰ angle for approximately 1 minute prior to making an interpretation

  - The final titer value (inverse of the dilution factor) was assigned as the last non-agglutinated dilution within the series.

  - In cases where all wells within the row were fully agglutinated (+) or when non-specific inhibition was detected, a HAI titer of 5 was arbitrarily assigned because the initial serum dilution was 1:10

Conclusions

- Despite prevalent non-specific inhibition, Cypher One automated interpretation without plate tilting demonstrated high (94.3%) overall agreement with the interpretation of a well-experienced human reader using plate tilting.

- Titer calls were consistent between the methods and exhibited a strong linear correlation.

- Cypher One can provide a number of advantages over standard manual interpretation:

  - Elimination of the need to tilt prior to interpretation of images

  - Standardization of assay interpretation by eliminating user-to-user subjectivity

  - Generation of a permanent digital record for each plate and audit trail, eliminating risk of transcription errors and ensuring data integrity

  - Reduction of time to result

  - Consistent imaging conditions across all plates

Cypher One: Automated Solution

- High fidelity imaging of standard HA and HAI assay configurations

- Standardizes assay interpretation with an innovative analysis algorithm

- Rapid analysis provides consistent and reliable results

- Enhanced data integrity with a traceable digital record

- User-friendly and intuitive software interface

- ~2x image resolution achievable with the human eye
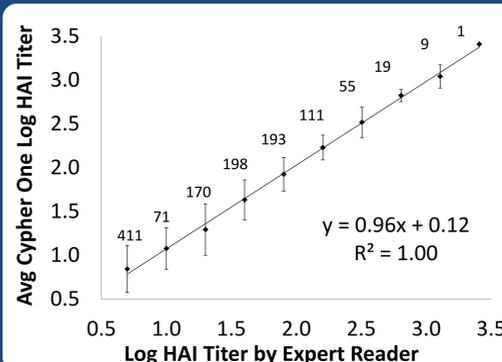
Results and Discussion

- The Cypher One image analysis algorithm evaluates the extent of agglutination to determine and display the titer

- Cypher One titer values obtained prior to plate tilting were compared to the human reader interpretation with plate tilting

Cypher One vs. Expert User Interpretation

Antigen	N	% Agreement (+/- 1 Dilution)
H1N1 A/Michigan/45/20	193	99.5%
B/Florida/78/2015	94	90.4%
B/Brisbane/60/2008	193	94.3%
B/Florida/78/2015	193	90.7%
B/Arizona/10/2015	190	92.6%
H1N1 A/CA/07/2009	193	94.8%
B/Phuket/3073/2013	182	96.2%
Complete Data Set	**1238**	**94.3%**

- All titer values were normalized to the expert reader interpretation, and the difference in number of dilutions at the transition point between the two methods was recorded.
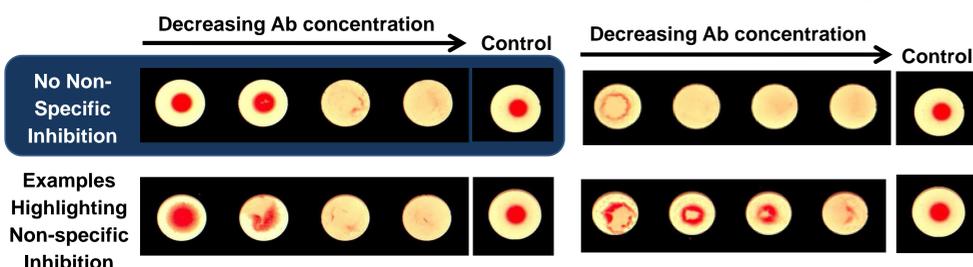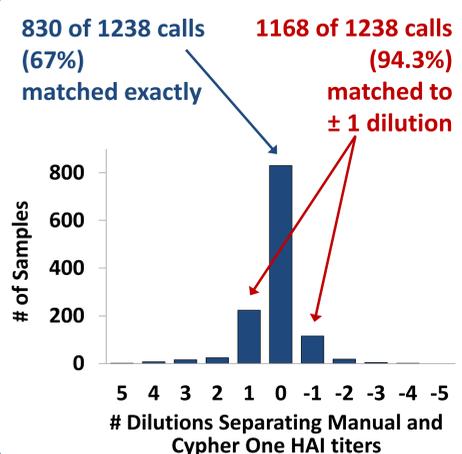
An agreement within ± 1 dilution or a 2-fold difference from the reference titer is considered an equivalent result due to the variability in the assay and interpretation expected between users.

- 2 adjustable algorithm parameters can be optimized for a variety of sample types (different RBCs such as guinea pig, turkey, and equine, and different RBC concentrations)

830 of 1238 calls (67%) matched exactly

1168 of 1238 calls (94.3%) matched to ± 1 dilution

# of Samples

5 4 3 2 1 0 -1 -2 -3 -4 -5
 # Dilutions Separating Manual and Cypher One HAI titers

- A linear regression of the data has a slope of 0.96 and a Pearson's correlation coefficient of 1, indicating a strong linear correlation between the two interpretation methods.

- The number above each data point in the figure is the total number of samples with a specific titer call as defined by the experienced reader titer value.

Avg Cypher One Log HAI Titer

$y = 0.96x + 0.12$
$R^2 = 1.00$

Log HAI Titer by Expert Reader

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