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Hemagglutination Inhibition Assay (HAI) using TiterSafe Hemagglutinating Particles

Safety note: TiterSafe is created using a lentiviral system and should be used under laboratory conditions. Always wear gloves, eye protection, and a lab coat when handling TiterSafe.

Required Reagents

- TiterSafe (Integral Cat# HAP-XXX)
  - Store at -80°C until use
- 10% solution Guinea Pig Red Blood Cells (Rockland, Cat# R402-0050 or equivalent)
- Clear, U-bottom, 96-well plates (VWR, Cat# 82050-622 or equivalent)
- PBS-/- (HyClone, Cat# SH30256.01 or equivalent)
- Relevant hyperimmune serum

Note: Catalog numbers correspond to our recommended products. They can be substituted as desired.
Note: Do not use RBCs past the expiration date.
Note: Human sera is recommended to receive receptor destroying enzyme (RDE) treatment such as cholera filtrate prior to use. The necessity of RDE treatment for sera from other species should be determined.

A. Hemagglutination Inhibition Assay (HAI)

Note: The recommended standard dilution of HA particles to use in an HAI assay is the dilution at 4 HA units. The dilution at 4HA units can be calculated by running an HA assay and determining the dilution at 1HA unit (1HA unit= lowest dilution that fully hemagglutinates red blood cells).

a. Prepare serum samples by diluting 1:4 in PBS-/- (Antibodies or other inhibitors must be tested by user)
b. Dilute TiterSafe in PBS-/- to a dilution at 4HA units. Calculate enough diluted TiterSafe for 25µL/well of the 96-well plate.
c. Obtain a 96-well U-bottom plate and add 25µl of PBS-/- to all appropriate rows.
   - Be sure to include a PBS-/- only, negative control row (**see Note 1**).
   - As an optional step, include an additional row to perform a back titration of TiterSafe (**see Note 2).**
d. Add 25µl of the 1:4 diluted serum to column 1 of the plate and gently triturate 5x to mix.
e. Perform a 2-fold serial dilution across the plate.
   - Transfer 25µl from one column 1 to column 2.
   - Gently triturate 5x to mix and change tips.
   - Repeat steps for each column.
   - Discard excess 25µl of diluted material from column 12.
f. Add 25µl of the diluted TiterSafe to all sample wells
g. Gently tap the plate ~10 times on all 4 sides to mix the TiterSafe and serum together.
h. Cover the plate with tape and incubate for 30 minutes at room temperature.

i. Use PBS-/- to dilute the Guinea Pig RBCs to a 1% solution. Calculate enough of the 1% RBC solution for 50µl/well of the 96-well plate (**see Note 3**).

j. Add 50µl of the diluted RBCs to each sample well of the 96-well plate.

k. Gently tap the plate ~10 times on all 4 sides to mix the TiterSafe and RBCs together.

l. Cover the plate with tape and incubate for 1 hour at room temperature.

m. Image or otherwise record results after 1 hour.

B. Interpreting the results (see Note 4)

a. Without disturbing the plate, visually inspect wells and note the lowest serum dilution that shows complete non-agglutination. This is the HAI titer.

Notes:

1. The PBS-only control wells provide a visual confirmation of the absence of hemagglutination. Hemagglutination within these wells could indicate an issue with the RBCs. Obtain a new, fresh stock of RBCs if this occurs.
2. A back titration ensures that the dilution of TiterSafe being added to the plate correlates to 4 HA units. To perform a back titration, serially titrate TiterSafe 2-fold with PBS-/-, starting at the dilution at 4HA units. A dilution of TiterSafe at 4 HA units will hemagglutinate the RBCs in the first and second well of the back titration. *Note: due to assay variability, partial or full hemagglutination may be noted in the third well.*
3. Other species of RBCs can be used for certain strains of Influenza. The TiterSafe particles have been validated with Guinea Pig RBCs. Refer to the following paper to optimize/test other species of RBCs: *Kaufmann, L., Syedbasha, M., Vogt, D., Hollenstein, Y., Hartmann, J., Linnik, J.E., Egli, A. An Optimized Hemagglutination Inhibition (HI) Assay to Quantify Influenza-specific Antibody Titers. J. Vis. Exp. (130), e55833, doi:10.3791/55833 (2017).*
4. Recording HAI assay results can be done after visual assessment by the user, but it is recommended to image the plate as a record of the raw data. An imaging device used to capture colorimetric gels, such as Coomassie blue, is recommended. Interpretation can be subjective and may differ slightly between operators and/or the method used.

An example plate lay-out can found below:

	Serum Dilution (1:X)											
	8	16	32	64	128	256	512	1024	2048	4096	8192	16384
Serum 1												
Serum 2				Titer Safe dilution at 4 HA units								
Serum 3												
PBS Only (No TiterSafe)