Influenza H1N1 HAI Protocol

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HAI Protocol

Treating Sera

Performing HAI

Supplies

1. **Receptor Destroying Enzyme (RDE) –** Accurate Chemical & Scientific Cat #370013
2. **Guinea Pig Red Blood Cells (GP RBCs) –** Lampire Biological Laboratories Cat #7243109
3. **Physiological Saline** – 0.85% NaCl
4. **PBS** – (pH 7.4)
5. **96 well round bottom plates –** Fisher Scientific Cat # 07-200-95
6. **Influenza A H1N1/California Strain in allantoic fluid**
7. **1.5 mL conical microcentrifuge tubes**
8. **CDC A/H1N1 California positive reference sera**
9. **Hopkins Vaccine Tube –** Kimble Cat # 45225-10

Treating Sera

1. Reconstitute RDE in Physiological Saline (add 20 mL of Physiological saline to one bottle of lyophilized RDE or follow manufactures' instructions)

2. Pipette 100 uL of subjects sera into a 1.5 mL conical microcentrifuge tube

3. Add 300 uL of reconstituted RDE (1:4 dilution) to the 1.5 mL conical microcentrifuge tube

4. Incubate (sera + RDE) overnight at 37ºC

5. The following day, inactivate Sera and RDE by incubating sample for 30 minutes in a 56ºC water bath

6. Remove GP RBC from cold storage (4ºC) and allow them to warm by incubating at room temperature for a 30 minutes

7. Pack GP RBC by centrifuging GP RBC at 1,200 rpm for 10 minutes BRAKE OFF at room temperature.

8. Add 20 uL of packed GP RBC to 400 uL of (sera + RDE) [1 part packed GP RBC to 20 parts (sera+RDE)]

9. Mix well then incubate sample at 4 ºC for 1 hour. Mix sample at 30 minutes.

10. Spin sample at 2,000 rpm for 1 minute to pelletize RBCs

11. Draw off 380 uL of treated sera and place in a clean 1.5 mL micro centrifuge tube

12. Add 570 uL of PBS to sample (1:2.5 dilution). Sera samples are now diluted 1:10

13. Label tubes with subject #, date, and "Treated and Hemabsorbed"

14. Store samples at 4 ºC till titered

Performing HAI

1. Remove vial of pre-washed 5.0% GP RBC from the cold room (4°C) and bring to room temperature by incubating at room temperature for 30 minutes

2. Ensure that GP RBC are ≤ 7 days old and mix well by inversion

3. Check the concentration of GP RBC by diluting 700 uL of GP RBC in 5 mL of PBS. Add 5 mL of diluted GP RBC Hopkins Vaccine Tube and spin at 1200 rpm **BRAKE OFF**

4. Read the percentage of GP RBCs in the 5 mL solution. Then use this percentage to make a 0.6% GP RBC solution.

5. One plate requires 5 mL of 0.6% GP RBC solution. **Cut the tip off of a p1000 pipette tip when pipetting RBCs to avoid RBC lysis.**

6. Remove one 120 uL aliquot, per plate, of H1N1 A/California virus from the -80°C freezer (or one 480uL aliquot per 4 plates)

7. Add 112.5 uL of virus to 2137.5 uL of PBS per plate
    a. Influenza virus should be added to the plate at a concentration of 4 HA units/25 uL. Each new stock of virus needs to be back titered to calculate the dilution necessary to achieve 4 HA units/25 uL. This can be done by following instructions for columns 1-2 of this protocol. The virus should be diluted such that complete agglutination occurs in the forth dilution (This means the virus concentration is at 4 HA units/25 uL).

8. Remove appropriate aliquots of **Treated** subject's sera and one aliquot of **Treated** control sera per plate from the cold room (4°C) and label plates and columns according to the template final page of protocol

9. Vortex each **Treated** subject's sera, then centrifuge samples at 3,000 rpm for 1 minute to pellet any aggregate which formed in the sera.

10. Add 25 uL of PBS to rows C-H

11. Vortex virus dilution then in **Back Titer** columns (columns 1-2) add 25 uL of diluted virus to rows A-C, pipette to mix in C.

12. Vortex **Treated** control sera then **dilute 5 uL ofcontrol sera in 95 uL of PBS** (1:20)

13. Add 25 uL of control sera to **Control** column (column 3) rows A-C, pipette to mix in C.

14. Add 25 uL of **Treated** subject sera to three columns rows A-C, pipette to mix in C according to template (**Do Not Vortex**).

15. Serially dilute 25 uL from row C to row H. Discard 25 uL from row H. Mix by pipetting and **Change tips after each dilution**

16. Add 25 uL of PBS to row A and **Back Titer** columns (columns 1&2) rows B-H

17. Add 25 uL of diluted virus to row B-H in **Control and Subject** Columns

18. Gently tap the plate then allow the plate to incubate for 15 minutes at room temperature, use a timer for each plate

19. After 15 minutes add 50 uL of 0.6% GP RBC to all wells, use 300ul tips and **change tips** with each row.

20. Allow plate to incubate for 1 hour, use a timer for each plate

21. Record HAI titer on the checklist by mark all wells which **completely** agglutinate.

22. Record HAI titer in the HAI workbook in the Influenza folder.

Plate #	**Back Titer**		**Con.**	**Subject _ _ _ _ _**			**Subject _ _ _ _ _**			**Subject _ _ _ _ _**		
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Reading HAI and QA/QC

1. HAI titer is quantified by the lowest dilution in which **Complete Agglutination** occurs.

2. Using the set up above, the Back Titer should agglutinate opposite of the sera wells and complete agglutination will occur in row E. **If complete agglutination does not occur in row E the assay failed and needs to be repeated.**

3. The CDC A/H1N1california anti-sera diluted 1:20 should agglutinate in row F. **If complete agglutination does not occur in row F the assay failed and needs to be repeated.**

4. **Every 50th sera sample should be repeated**

Influenza HAI Checklist
(Please refer to the protocol for more detailed directions)

- **Date Performed:_____ Initials: ___ ___ ___**

Check box after performing each step

☐ 23. Remove vial of pre-washed 5.0% Guinea Pig (GP) red blood cells (RBC) from the cold room (4°C) and bring to room temperature (approximately 20 minutes)

☐ 24. Ensure that GP RBC are ≤ 7 days old and mix well by inversion

☐ 25. Dilute GP RBC to 0.6%. **Cut the tip off of a p1000 pipette tip when pipetting RBCs to avoid RBC lysis.** Volume of RBCs should be based on concentration determined by Hopkins tube (spin 5ml dilution at 1200 rpm no brake for 7-10min)
   a. For four plates add _____uL of 5%RBCs to 20mL of PBS

☐ 26. Remove one 120 uL aliquot, per plate, of H1N1 A/California virus from the -80°C freezer (or one 480uL aliquot per 4 plates)

☐ 27. Add 112.5 uL of virus to 2137.5 uL of PBS per plate
   a. For four plates add 450 uL of virus to 8550uL of PBS

☐ 28. Remove appropriate aliquots of **Treated** subject's sera and one aliquot of **Treated** control sera per plate from the cold room (4°C) and label plates and columns according to the template final page of checklist

☐ 29. Vortex sera samples and then spin in microcentrifuge at 3000rpm for 1 minute to clear aggregate from serum

☐ 30. Add 25 uL of PBS to rows C-H

☐ 31. Vortex virus dilution then in **Back Titer** columns add 25 uL of diluted virus to rows A-C, pipette to mix in C.
   a. Columns 1 -2 on plate 1.

☐ 32. Vortex **Treated** control sera then in **Control** column add 25 uL of control sera to rows A-C, pipette to mix in C. Use Treated Control Sera which are at a **1:20 dilution** (beyond the 1:10 of all treated sera).
   a. Column 3 of plate 1.

☐ 33. Vortex **Treated** Subject sera before addition then in Columns according to Template add 25 uL of **Treated** subject sera to rows A-C, pipette to mix in C.

☐ 34. Serially dilute 25 uL from row C to row H. Discard 25 uL from row H. Mix by pipetting and **Change tips after each dilution**

☐ 35. Add 25 uL of PBS to row A and **Back Titer** columns rows B-H
   a. Column 1 and 2 of plate 1

☐ 36. Add 25 uL of diluted virus to row B-H in **Control and Subject** Columns

☐ 37. Gently tap the plate then allow the plate to incubate for 15 minutes at room temperature, use a timer for each plate

☐ 38. After 15 minutes add 50 uL of 0.6% GP RBC to all wells, use 300ul tips and **change tips** with each row.

☐ 39. Allow plate to incubate for 1 hour, use a timer for each plate

☐ 40. Record HAI titer on the checklist by mark all wells which **completely** agglutinate.

☐ 41. Record HAI titer in the HAI workbook in the Influenza folder.

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