Hemagglutination Inhibition Assay

protocol type: assay [@](http://bioportal.bioontology.org/ontologies/1058)

**Project**: Flu

**Laboratory**: Human Immune Monitoring Core

# Document history

Add a row when creating a new doc or making meaningful modifications:

|  |  |  |
| --- | --- | --- |
| **Date** | **What** | **Who** |
| 2/13/2013 | V1.0: doc created | Cindy Huynh |
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# Variables

Please list the crucial conditional (aka, “independent) and response (aka, “dependent”) variables:

**Conditional variable**: Factor that modifies the effect of the putative causal factor(s) being investigated

**Response variable**:  The variable that you measure, namely, the instrument readout

|  |  |
| --- | --- |
| **Variable Type** | **Variable Name** |
| Conditional [@](http://bioportal.bioontology.org/ontologies/1058) | Serum from flu vaccinated individualsand influenza strains serially diluted, and chicken red blood cells |
| Response [@](http://bioportal.bioontology.org/ontologies/1058) | Level of aggluination of chicken red blood cells in the presence of influenza strains and vaccinated human serum |

# Reagents

List all reagents used in this protocol. You MUST include the catalog number if it exists.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Source** | **Catalog Number** | **Lot Number** |
| Phosphate Buffered Saline | Life Technologies | 14190144 | 1227361 |
| 5M Sodium Chloride | AccuGene | 51202 | 000020005 |
| Receptor Destroying Enzyme | Accurate Chemical & Scientific Corporation | 370013 | 412061 |
| Chicken Blood | Colorado Serum Company | 31151 | 999230 |

# Major equipment and software

Example major equipment: analytical instrument, e.g., FACS machine

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Source** | **Catalog Number** | **Software and Version** |
| Not Applicable |  |  |  |

**PROTOCOL STEPS**

# Experimental groups and controls

Examples: - Naïve201: sample#201; PBMCs in media only, no cytokine stimulation

* Stim201: sample #201; PBMCs + IL-12

|  |  |  |
| --- | --- | --- |
| **Name** | **Description** | **Control (Y/N)** |
| Sample | RDE treated serum sample serially diluted in PBS | N |
| Virus Control | Viral strain is titered for 8HA | Y |
| CONS2 | HIMC human control serum | Y |
| PBS Control | PBS is tested for viral contamination | Y |

Below are the actual protocol steps:

# Sample Preparation (Day 1)

# Thaw serum samples and Restriction Destroying Engzyme (RDE) in 37°C water bath

# Transfer 50uL of each sample into a labeled 1.5mL microcentrifuge tube

# Add 150uL of RDE to each tube, mix well

# Place samples into the CO2 incubator at 37°C for overnight incubation

# Plating and Running the Assay (Day 2)

# Place samples into 56°C water bath for a 45 minute incubation, chill on ice, then add 300uL of 0.9% NaCl to each samples

# Allow samples to rest at room temperature for the remainder of the assay

# Prepare 0.5% chicken red blood cells (cRBC) working stock solution from 10% stock solution and keep on ice

# Dilute each viral strains to 8HA and keep on ice

# Add 25uL of PBS to each well from columns 1-10, with row A empty

# Add 50uL of PBS to all of column 12

# Aliquot 50uL of serum as in figure below in row A and in column 11 respectively

# Add 75uL of virus working dilution to well A10

# Serially dilute with 25uL from columns 1 to 10, discard the last 25uL

# Add 25uL of virus working dilutions to every well of columns 1-9

# Incubate for 15 minutes at room temperature

# Add 50uL 0.5% cRBC solution to entire plate

# Incubate for 1 hour at room temperature then read plates

### NOTES

How to read a plate:

* “+” hemagglutination is present, the well is hazy with no cRBC button
* “-“ hemagglutination is absent, the well is relatively clear with cRBC button
* HAI titer is the reciprocal of dilution of last well that inhibits hemagglutination

# References

Please include citations when you refer to papers within the protocol