



Human Sera Collection Guidelines for Influenza Serology

Influenza Division
Centers for Disease Control and Prevention

Collection Details

1. For influenza serology using human sera, we suggest collecting the following volumes of whole blood:

| Category | Age of blood donor | Volume of blood to collect* |
|----------------------------|------------------------|-------------------------------|
| Pediatric | Less than 3 years old | 5.0 ml (≥ 1.5 ml) |
| Children | 3 through 11 years old | 5-10.0 ml (≥ 3 ml) |
| Teens, adults, and elderly | 12 years and older | ≥ 10.0 ml (≥ 3 ml) |

Sera may be tested in multiple assays and potentially with multiple viruses.

*In general, while the above volumes are preferred, smaller volumes can be accepted, if the situation does not allow for the collection of these amounts, e.g. for infants and young children. The minimum accepted volumes of whole blood to collect are 3ml from adults and older children and 1.5 ml from younger children.

2. Use tubes designated for the collection of serum, not plasma. We suggest the use of vacutainer tubes. The following vacutainer tubes are acceptable:
 - a. glass red top vacutainer tubes
 - b. plastic red top vacutainer tubes with clot activator
 - c. plastic gold top serum separator tubes (SST) with clot activator and gel
3. For testing of serum samples in influenza serology assays, it is very important to minimize hemolysis. Hemolyzed serum often has a negative impact on the cells in the influenza microneutralization assays. To minimize hemolysis, the use of a butterfly needle connected to a vacutainer tube is recommended for blood collection (Barnaby DP et al., 2016; Wollowitz A et al., 2013). The butterfly needle is a safe one-way system with blood being delivered into sealed vacutainer with a consistent pressure flow. The only source of possible hemolysis with this system is if the needle in the vein scrapes against vein wall and blood cells break up as they enter the needle bore. However, if butterfly needles are not available, the use of a needle that connects directly to the vacutainer tube is acceptable as an alternative.

Sera Collection and Storage Recommendations

1. Depends on the types of serum collection tubes used, follow manufacturer's instructions for the serum collection. In general, immediately after blood collection, gently invert the tubes several times to reach a proper mix. Do not shake the tubes. Vigorous mixing may cause foaming or hemolysis. Insufficient mixing or delayed mixing in serum tubes may result in delayed clotting. Collected blood should be stored at 4°C immediately. This can be done by placing the sample on ice, in a 4°C refrigerator, or in a cooler with cold packs.
2. Allow the blood to fully clot at room temperature 25°C (minimum 30 min -1 hour depending on the tubes used). It is recommended that clotted blood be centrifuged immediately to separate from serum. Depends on the types of tubes used, follow manufacture's recommendations for centrifugation speed. For example, BD vacutainer SST tubes can be centrifuged at 1100 to 1300g for 10 minutes in swing-head units or 15 minutes in fixed angle centrifugation units (balance the tubes in the centrifuge). After centrifugation, transfer the serum to a clean tube, the clotted blood may be discarded.

Note: after the blood fully clots, it is recommended that blood be centrifuged immediately (within 2 hours) to separate clotted blood from serum. If the centrifugation cannot be performed immediately, clotted blood should be stored at 4°C, and centrifuge as soon as possible. In rare scenarios if centrifugation is not immediately available at the site of the blood collection, blood may be stored at 4°C for up to 18 hours (though less ideal) prior to centrifugation.

3. Serum should be aliquoted into smaller volumes in labeled tubes to avoid repeated freeze-thaw. After aliquoting, serum samples should be immediately stored frozen at -20°C or colder.

Questions regarding the sera collection for influenza serology can be addressed to:

Dr. Min Levine
Influenza Division
Centers for Disease Control and Prevention
Email: MLevine@cdc.gov
Office: 404-639-3504

Shipping Guidelines

All shipments should be made via overnight carrier, to ensure delivery within 24 hours of shipment. All serum shipments should be packed with sufficient dry ice to ensure that samples remain frozen for a minimum of 48 hours after shipment.

Send serum samples to:

CDC - Influenza Serology
Attn: Dr. Min Levine / Ms. Lauren Horner
Bldg. 23, Room 8451
1600 Clifton Road, NE
Atlanta, GA 30329
Phone: 404-639-3504/404-639-4130

Send shipping questions and tracking info to: MLevine@cdc.gov/ lyz9@cdc.gov.

References:

Barnaby DP, Wollowitz A, White D, Pearlman S, Davitt M, Holihan L, Bijur P, Gallagher EJ. Generalizability and Effectiveness of Butterfly Phlebotomy in Reducing Hemolysis. Acad Emerg Med. 2016 Feb;23(2):204-7. 2016 Jan 14.

Wollowitz A, Bijur PE, Esses D, John Gallagher E. Use of butterfly needles to draw blood is independently associated with marked reduction in hemolysis compared to intravenous catheter. Acad Emerg Med. 2013 Nov;20(11):1151-5.