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## Unprocessed Human Serum Collection Protocol

### *A. Preparation Before Collection*

If possible, working environment, all consumables and equipment should be nuclease-free (PCR clean) during serum collection process.

### *B. Serum Collection:*

- a. For each individual, draw around 10 ml whole blood specimen into serum separator tubes (3-5 ml each tube), no anticoagulants should be added during collection. Approximately 4 ml serum could generally be harvested from 10 ml whole blood.
- b. Incubate the tubes in an upright position for 15-30 minutes under room temperature to allow the blood to clot.
- c. Centrifuge the serum separator tubes under 4°C at 1,000-2,000 × g for 10 minutes. Use minimal level of brake to stop centrifuge.
- d. For each individual, under the temperature of 2-8°C, immediately and carefully aspirate the liquid supernatant (serum) from the serum separator tubes and pool to one clean polypropylene tube with Pasteur pipettes. Do not disturb the cell layer or transfer any cells.
- e. Inspect turbidity of the serum, repeat c. to e. if turbid.
- f. Aliquot collected serum to PCR clean cryovials to avoid freeze-thaw cycles and store them at -80°C.
- g. The entire workflow should be finished as fast as possible and within the same day to avoid potential degradation of serum compositions.

### *C. Serum Storage:*

The collected human serum should be kept frozen at -80°C at all time except when being delivered to us. Avoid freeze-thaw cycle during storage to minimize RNA degradation.