Standard Operating Procedure
Title: Handling of human samples for diagnostic assays on digital microfluidics

Related SOPs

Collection of blood samples from healthy participants
Storage and handling of human samples suspected of measles or rubella infection
Virus coated magnetic microparticles

Purpose

To handle and store human samples for developing digital microfluidic (DMF) immunoassays for measles and rubella IgG and IgM. Since the assays rely on virus-functionalized magnetic particles and human samples that may have active infection (e.g., IgM containing samples), biosafety precautions need to be taken.

Scope

Potentially hazardous and infectious materials used in this procedure include rubella-functionalized magnetic particles, human serum samples from suspected measles cases, blood/serum from healthy donors recruited at the University of Toronto, and commercially available serological panels.

General laboratory safety guidelines are to be followed during this work.

Materials and equipment

- Digital microfluidic chips
- Digital microfluidic control system (e.g. MR Box)
- P20, P200 and P1000 pipettes
- Spray bottle of 70% ethanol
- 1% sodium hypochlorite solution
- Sample (either human serum from suspected measles cases, blood/serum from healthy donors recruited at the University of Toronto, or commercially available serological panels)
- Sample diluent (PBS 4% BSA 0.1% Tetronic 90R4)
- Measles antigen- or rubella virus-functionalized magnetic particles
- Superblock buffer with 0.1% 90R4
- DMF wash buffer (PBS with 0.1% 90R4)
- Anti-human IgG conjugated to horseradish peroxidase (HRP) **or** biotinylated anti-human IgG and streptavidin-conjugated HRP
- Thermo SuperSignal™ ELISA Femto Substrate (luminol and peroxide), supplemented to 0.05% 90R4
- Kim wipes
- Liquid waste container (beaker or conical flask)
  Rubella virus-coated magnetic particles and all human derived samples should be treated as potentially infectious. General biosafety level 2 procedures must be followed.

Personal protective equipment (PPE) and containment

PPE such as lab coats and gloves should be worn at all times in the laboratory. All work with potentially infectious material and human samples must be performed in a biosafety containment level 2 cabinet. All centrifuging and vortexing must be performed within the BSCL2 cabinet. Wash hands after removing gloves.

Any breach of the skin (scratch, cut, wound) needs to be protected from contact with biological agents. Cover open wounds, cuts, scratches, and grazes with waterproof dressings and gloves. If you exhibit any open wounds (broken skin) in areas that cannot be covered by dressings or clothing, re-evaluate the work in process. Suggestions for mitigating the exposure in the case of broken skin that cannot be covered include, for example where the wound is on the face, work with a full-face shield; work in the BSC, or have someone else do the work.

Sample preparation

1.1 Stored samples (either human serum from suspected measles cases or commercially available serological panels) should are stored at -20 °C.
1.2 Frozen samples must be completely thawed before opening.
1.3 Wipe down the outside of the sample vial with 70% ethanol.
1.4 Pipette sample into a fresh microcentrifuge tube and dilute 1:10 – 1:20, as per assay development needs, with sample diluent. Do not prepare more than 50 μL of sample per assay.

Bead preparation

2.1 Measles antigen-coated or rubella virus-coated magnetic particles are stored at 4 °C.
2.2 Vortex the particles to resuspend them prior to pipetting.
2.3 Remove particles from the container by pipette, bearing in mind that you will concentrate them 10-fold. A single assay requires ~2 μL of particles. Do not prepare more than 100 μL (final volume) of particles at a time. Pipette into a new 1.5 mL microcentrifuge tube.
2.4 Centrifuge on table-top centrifuge for 10 sec. and pellet particles with a magnet for at least 15 seconds.
2.5 Remove supernantant and discard in waste container.
2.6 Wash beads 3X with DMF wash buffer, centrifuging and pelleting each time. All supernatant is discarded into the liquid waste container.
2.7 Resuspend in Superblock buffer with 0.1% 90R4 at 1/10th the initial volume (e.g. if you removed 1 mL of beads, resuspend in 100 μL).

DMF instrument setup

3.1 The DMF instrument (MR Box) should be setup in the BSCL2 cabinet. The laptop can remain outside of the BSCL2 cabinet.
3.2 Load a DMF chip into the MR Box and ensure all pogopin and ground connections are made.
3.3 Start MicroDrop and load the pre-saved immunoassay protocol. If developing/modifying the existing protocol, create a new protocol and save it accordingly.
3.4 Load a waste Kim wipe in between the top and bottom plates to absorb liquid waste from the device.

DMF ELISA general procedure

4.1 Vortex beads 2-5 sec. before loading. Pipette beads onto the DMF chip and load into the chip by turning on the reservoir electrode.
4.2 Dispense a droplet of magnetic particles
4.3 Pellet the particles by engaging the magnet in the MR Box

4.4 Remove the supernatant droplet and drive the droplet to the Kim wipe waste reservoir
4.5 Pipette sample onto the DMF chip and load into the chip by turning on the reservoir electrode.
4.6 Dispense a droplet of sample and deliver to the magnetic particles.
4.7 Incubate the sample with the magnetic particles as needed, mixing all the time.
4.8 Remove the supernatant by engaging the magnet on the MR Box and removing the supernatant to the waste Kim wipe.
4.9 Pipette wash buffer onto the DMF chip and load into the reservoir
4.10 Dispense wash buffer and deliver to the magnetic particles
4.11 Remove the pelleting magnet and wash the particles in a circular motion on the chip
4.12 Pellet the particles by engaging the magnet in the MR Box and remove the supernatant to waste
4.13 Repeat washing (4.10-4.12) as needed.
4.14 Pipette anti-human IgG (either conjugated to HRP or biotinylated) onto the DMF chip and load it into the chip by turning on the reservoir electrode.
4.15 Dispense a droplet of anti-human IgG and deliver to the magnetic particles.
4.16 Remove the magnet and incubate the particles with the anti-human IgG as needed, mixing all the time.
4.17 Remove the supernatant by engaging the magnet on the MR Box and removing the supernatant to the waste Kim wipe.
4.18 Wash the particles, as needed, per 4.10-4.12
4.19 **If using HRP-conjugated anti-human IgG, skip this step and go to 4.22** Pipette streptavidin-conjugated HRP onto the DMF chip and load into the chip by turning on the reservoir electrode.
4.20 Dispense a droplet of strepatividin-conjugated HRP and deliver to the magnetic particles.
4.21 Incubate the strepatividin-conjugated HRP with the magnetic particles as needed, mixing all the time.
4.22 Remove the supernatant by engaging the magnet on the MR Box and removing the supernatant to the waste Kim wipe.
4.23 Wash the particles, as needed, per 4.10-4.12
4.24 Pipette SuperSignal substrate onto the DMF chip and load into the chip by turning on the reservoir electrode.
4.25 Dispense a droplet of SuperSignal substrate and deliver to the magnetic particles.
4.26 Incubate the SuperSignal substrate with the magnetic particles as needed, mixing all the time.
4.27 Deliver the droplet to the PMT detection zone for measurement.
4.28 Move all droplets to Kim wipe waste reservoir.

Disposal and decontamination

5.1 Remove waste Kim wipe from DMF device and spray with 70% ethanol before disposing in biohazardous waste.
5.2 Remove the DMF chip from the MR Box. Do not remove the top plate, as this may aerosolize any remaining droplets on the chip. Dispose of the whole chip in the biohazardous waste.
5.3 Any remaining sample, liquid waste, and unused magnetic particles should be added to 70% ethanol or 1% sodium hypochlorite for 20 minutes. Dispose of as chemical waste.
5.4 All surfaces must be cleaned with 70% ethanol. Be sure to wipe down the pipettes.