Standard operation procedure

Title: Virus coated magnetic microparticles

PURPOSE

To outline the steps required to safely handle magnetic microparticles coated with the Contaminant Level 2 whole Rubella virus (strain HPV 77) on digital microfluidic devices; and to describe the process for safe disposal of waste generated by working with these agents. All microparticle suspensions should be considered potentially infectious.

PRIOR TO BEGINNING WORK

All staff working with Containment Level 2 biological receive research-specific training by the Principal Investigator (PI), and complete Biosafety training in addition to all other EHS safety training.

PERSONAL PROTECTIVE EQUIPMENT REQUIREMENTS

1. Wear appropriate personal protective equipment (PPE) when handling microparticle suspension and/or performing sections of this protocol:

    a. Nitrile gloves
    b. Lab coat or gown
    c. Safety glasses

2. All off-device fluidic operations and handling (using microcentrigufe tubes) must be performed inside a biosafety cabinet;

3. All device fluidic operations and handling must be performed inside a biosafety cabinet.

MATERIALS AND EQUIPMENT

- PPE (lab coat, nitrile gloves, safety glasses);
- Yellow biowaste bin;
- Autoclave/biohazard bags;
- Pipettes and pipette tips (as needed for work);
- 1% sodium hypochlorite solution;
- Becker (for pipette tip disposal);
- Biosafety cabinet;
- Digital microfluidics (DMF) instrument;
- Digital microfluidic devices.

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.

REAGENTS

- Virus coated microparticle suspension (Abbott Architect Rubella IgG and IgM commercial kits);

- Resuspension solution – Superblock™ blocking buffer + 0.1% Ethylenediamine tetrakis(ethoxylate-block-propoxylate) tetrol (Tetronic 90R4);
- Wash buffer solution - Phosphate buffer saline (PBS) + 0.1% Ethylenediamine tetrakis(ethoxylate-block-propoxylate) tetrol (Tetronic 90R4);

PROCEDURE

All pipette tips used is this procedure should be discarded into a Becker containing 1% sodium hypochlorite solution and let it sit for 30 min.

A. Decontamination of BSC

Refer to BSC standard operation protocol.

B. Bead washing and solution preparation

1. Transport microcentrifuge, microparticle suspension, resuspension solution, pipettes, pipette tips, magnet and 1.6 µL microcentrifuge tubes to a BSC;
2. Mix microparticle suspension by flicking its container;
3. Label microcentrifuge tube;
4. Aliquot 1000 µL of bead suspension into microcentrifuge tube;
5. Place tube on magnet for 2 minutes, and remove supernatant;
6. Wash microparticles with 1000 µL of resuspension solution (mix by flicking tube);
7. Centrifuge tube for 5-10 s;
8. Repeat steps 4-6 twice;
9. Place tube on magnet for 2 minutes, and remove supernatant;
10. Add 100 µL of resuspension solution (mix by flicking tube) to obtain bead suspension at working concentration.
11. Store microcentrifuge tube labelled and tightly closed at 4 ℃.
12. Discard gloves into yellow biowaste bin.
13. Wash hands with soap and water.

C. Digital microfluidic device assays

1. Transport DMF instrument, devices and reagents to a BSC;
2. Load device into DMF instrument and perform initial droplet movements test protocols using a PBS + 0.1% 90R4 wash buffer solution;
3. Load microparticles and reagents according to assay protocol to be performed.
4. After using, place device in 5% sodium hypochlorite solution for 30 min;
5. Remove bleach by aspersions and discard solids (device containing microparticles and paper wicks) into yellow biowaste bin.
6. Discard non-biohazardous liquid waste (chemical reagent solutions) in proper waste containers;
7. Discard gloves into yellow biowaste bin.
8. Wash hands with soap and water.

D. Disposal of liquid waste containing biological agents

1. Treat liquid waste with a 1% sodium hypochlorite solution. Allow the solution to stand for at least 30 minutes.
2. Rinse solution down the drain.

WARNINGS AND PRECAUTIONS

A. Microparticle suspension (as supplied by Abbott Architect Rubella IgG and IgM kit)

Contains methylsothiazolones and sodium azide. Methylsothiazolones may cause allergic skin reaction. Sodium azide liberate toxic gas when in contact with acids.

1. Do not use reagent kits beyond expiration date.
2. Keep container tightly closed at 4 8C.
3. Avoid breathing mist / vapors / spray.
4. Contaminated work clothing should not be allowed out of the workplace.
5. Wear protective gloves, protective clothing and eye protection.
6. If on skin: wash with plenty of water.
7. If skin irritation or rash occurs: Get medical attention.
8. Take off contaminated clothing and wash before reuse.

Safety data sheet available at abbottdiagnostics.com.

B. Ethylenediamine tetrakis(ethoxylate-block-propoxylate) tetrol (Tetronic 90R4)

May cause allergic skin reaction. Causes serious eye irritation.

1. Avoid contact with skin and eyes. Avoid inhalation of vapor or mist.
2. Keep container tightly closed in a dry and well-ventilated place.
3. Wear protective gloves, protective clothing and eye protection.
4. If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
5. In case of skin contact, wash off with soap and plenty of water. Consult a physician.
6. In case of eye contact, rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
7. If swallowed, rinse mouth with water. Consult a physician.

Safety data sheet available at https://www.sigmaaldrich.com.