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Protocol for Islet Media Change

1. PURPOSE:

This protocol describes how to change the media of a flask containing islets.

2. SCOPE:

The main reason for carrying out a media change on the islets is to remove the used media and replace it with fresh media.

3. MATERIALS:

1. Flask(s) containing islets.
2. PIM(S) Media (Prodo labs – Cat no. -).
3. PIM(R) Media (Prodo labs-Cat no-)
4. Pipettes and Pipettor.
5. Empty sterile container (to collect used media).

4. Procedure

To avoid or minimize the chance of contamination, the steps below are performed in a laminar flow hood with good sterile technique.

1. Warm up Media at room temperature until it comes to room temperature.
2. Take out the flask(s) from the incubator and place the flask(s) in a laminar flow hood. Keep the flasks vertically positioned (with the cap facing upward). Loosen the caps of the flask(s).
3. Position flask(s) at an angle, tilting it towards the longer edge, so all islets can congregate to the lower corner of the flask due to gravity. This is achieved by aligning a 250ml and 50ml conical rack side by side and resting the T150 flasks at an angle on the 50ml conical rack.
4. Leave all flask(s) positioned in this manner for 30 minutes to let all islets settle down.
5. Go to the middle point of the media in one flask and take a 1ml sample, place in a petri dish and examine under the microscope.
6. If no islets more than 50 micron in size are visible proceed to the next step. If islets are visible allow more settling time until the media sample has no islets present.
7. Take the media at room temperature into the laminar flow hood aseptically.
8. While maintaining the position of the flask (as in step 4.3) aspirate out 50% of the used media from the surface, without disturbing the islets that are settled on the bottom corner.
9. Pour the aspirated used media into the empty sterile container to ensure that no islets were aspirated out.
10. Pour the same amount of appropriate media (PIM(R) for overnight media change and PIM(S) for long term culture) that has been aspirated out.
11. Repeat steps 8 – 10 for all remaining flask(s).
12. Once all flask(s) are complete, tighten caps on all flask(s) and return to incubator set to 37°C.
13. If there are islets in the sterile container, centrifuge the media at 180g for 2 min, aspirate the supernatant and re suspend the islets in appropriate volume of fresh media and re-culture

in appropriate sized flask.