ISLET CULTURE

Abbreviations

Rpm (rotation per minute)

Safety routines, environmental, and work risks

Dithizone is classified as a poison.

All work with human material carries the risk of transmitting infectious diseases. See Skyddsföreskrift laboratoriearbete KITM AL4731 and Hygienregler för Akademiska sjukhuset AL9112

Procedure

Preparations

Start with islets that are washed according to Bestämning av antalet ö-ekvivalenter Estimation of total islet equivalents AL5173.Keep the islets cold in culture medium until everything is ready for culturing in bags.

Procedure

1. Prepare the culture medium according to Lösningsprotokoll och odlingsmedium AL6662 and add 50 ml of ABO-compatible human serum per culture medium bottle (about 500 ml).
2. About 200 ml of completed culture medium is used per bag. The islet volume should not exceed 150 µl per bag.
3. Put a spike in the islet culture bag and attach an islet culture label on top of the Baxter label. The islet culture label is filled in after receiving information necessary for proper identification.
4. Hook up the custom made bag holder to the horizontal metal bar spanning the top of the culture hood. Put a 50 ml syringe without plunger into the bag holder. Remove the male luer (with membrane) from the spike in the islet culture bag and place the luer in a sterile Petri dish for later use.
5. Connect the syringe to the culture bag.
6. Transfer about 50 ml of culture medium to the bag via the syringe.
7. Start with the islet suspension that has been prepared according to Bestämning av antalet ö-ekvivalenter Estimation of total islet equivalents AL5173. Resuspend the tissue up to 50 ml culture medium / 150 µl. The pellet volume for each culture bag may not exceed 150 µl. E.g 300 µl pellet is brought up to 100 ml and divided into 2 culture bags, 400 µl pellet is brought up to 150 ml and divided to 3 culture bags etc.
8. Gently but thoroughly mix the tissue and transfer 50 ml to the bag via the syringe.

9. Add 100 ml culture medium into the culture bag so that final volume will 200 ml. Repeat for additional bag and rinse the 250 ml conicals twice with 50 ml culture medium.
10. Remove the bag from the syringe and close with the sterile male luer (previously removed in step 3).
11. Sign and date the islet culture label and make sure that pancreas number, donor bloodgroup and fraction number is recorded there.
12. Put the bag onto the culture racks and place them into the 37 °C incubator. Never put culture bags from different donors on the same shelf in the incubator.
13. Fill in the NICS database.

Protocol

Protocol in NICS database.

Archiving

Protocol information is archived for a minimum of 10 years.

Equipment

Apparatus

Biological Safety Hood	MTA 075970
Incubator	MTA 075570, 075571, 074130, 074131

Material

Islet culture bags	746200
Syringe, 50ml	746080
Sampling site coupler, “spike”	746094
Labels for islet culture	746286
Petri dish, Sterilin 50 mm	746201

Reagents

Reagents

Culture medium	767594
Human serum, ABO-compatible	758220

Overview

The islets are cultured for a 24-hour period after the isolation and before deciding whether or not to transplant.

"Good to know"

After adding serum to the culture medium it expires in one day. Without serum, but with supplements, the culture medium expires in one week if kept sterile and cold.

References

Related documents

Skyddsföreskrift KITM

Bestämning av antalet ö-ekvivalenter Estimation of total islet equivalents AL5173 .

Lösningsprotokoll och odlingsmedium AL6662

Hygienregler för Akademiska sjukhuset AL9112

Bilaga 2. /*Appendix 2.*

Odlingsmedium ö-cellslab

Tillsatser Kylda+Frysta datum:
Signatur:

Serum Blodgrupp:
Datum + Sign serum: