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Protocol for primary cultures of HUVECs

I- Buffers and reagents

- **Fetal calf serum** (FCS) (Biosepra)
  - Unfreeze the FCS
  - Heat for 30 min. at +56°C (decomplementation; bottle closed)
  - Fractionate (25 ml) in sterile Falcon of 50 ml
  - Freeze the fractions at –20°C

- **Phosphate Buffer Saline** (PBS)
  - 100 ml of PBS 10X without Ca^{++} and Mg^{++} (Life Technologies)
  - 900 ml of distilled water
  - 2 g of glucose
  - Filtrate (0.22 µ), fractionate (50 ml) in sterile Falcon and freeze at –20°C

- **Collagenase** (0.2 % in PBS)
  - Dissolve 0.2 g of collagenase H from *Clostridium Histolyticum* (Roche) in 100 ml of PBS
  - Mix during 10 min. in the dark (aluminium foil)
  - Adjust the pH at 7.40 with NaOH 1N
  - Filtrate (0.22 µ)
  - Fractionate (10 ml) and freeze at –20°C

- **Buffer for conservation and transport of umbilical cords**
  - PBS 50 ml
  - Colimycine 1M 1 ml
  - Penicilline G 1M 1 ml
• **Culture medium**

**M199 "stock":**
- M199 Earle 10X 100 ml
- Distilled water 900 ml

**M199 “full”:**
- Glutamine 0.2 M 1 ml
- HEPES 1 M 1.5 ml
- NaHCO3 7.5 % (w/v) 1.8 ml
- Penicilline/streptomycine (10000/10000) 1 ml
- FCS (heated at 56°C and centrifuged) 20 ml
- M199 “stock” up to 100 ml

Conservation at +4°C up to 1 week.

**II- Cell culture**

• **The day before the manipulation:**
  - Coat the Petri dishes (35 mm; 7 dishes/cord) with 1 drop of fibronectin
  - Bring at the maternity 3 recipients containing 50 ml of conservation buffer for cords

• **The day of the manipulation:**
  - Fill up a recipient with ~ 500 ml of physiologic serum and maintain at 37 °C (“bain-marie”)
  - Under the hood, prepare (for 1 cord):
    - 2 syringes of 50 ml, 1 syringe of 30 ml and 1 syringe of 10 ml
    - 1 surgical clamping clip, 2 cannulas, string, sterile compresses, sterile scalpels and sterile gloves
  - Spread on the working area under the hood: 1 aluminium foil, 1 paper sheet and the “sterile envelope” of gloves (in this order)
• **Cord manipulation and culture:**
  - Tidily cut the 2 ends of the cord with scalpel
  - Introduce a cannula at each extremity of the vein (the widest vessel) and tightly maintain it with string
  - Wash the cord with PBS using the syringe of 50 ml
  - Inject the collagenase (0.2 %) at one end of the vein using the syringe of 30 ml
  - When leaking out the other end, tightly clamp it with the surgical clip
  - Maintain the syringe and protect the cord’s ends with “clean” aluminium foil
  - Incubate the cord 10 min in the physiologic serum at 37 °C
  - Upon the sterile envelope of gloves, gently squeeze the cord
  - Fill up a sterile falcon of 50 ml with 10 ml of “full” culture medium
  - Collect the cells in this falcon by washing the vein with 40 ml of PBS
  - Centrifuge at 900 rpm for 10 min
  - Carefully discard the supernatant and suspend cells in ~14 ml of “full” culture medium
  - Dissociate the cells (aspiration and repulsing X 3) with the syringe of 30 ml with needle
  - Add 2 ml of the cell suspension in each fibronectin-coated Petri dish

Cultures are placed in a humidified 95% air and 5% CO2 atmosphere at 37° C. The following day, non-adherent cells are removed by changing the culture medium. The culture medium is changed every two days and confluency is typically achieved in 6-8 days with “cobblestone appearance” in optical microscopy (~ 1.10^6 cells/dish).