

**BAEC Isolation**

Juan Carlos Rodriguez

DMEM 10% FCS, Versene, Trypsin, Vitrogen.

1. Get fresh aortas, the more sterile the better. Keep the aorta at 4 °C.
2. Open aorta and expose the lumen and wash the luminal surface with media + antibiotics and warmed Versene several times.
3. Keep aorta with Trypsin/Versene (1:3) during 5'.
4. Use scrapple to rinse endothelial cells over a plate. Be cautious to avoid contamination with smooth muscle cells. After that, wash the aorta once with versene.
5. Mix with DMEM + serum, to inhibit trypsin activity.
6. Spin and wash several times.
7. Treat again with trypsin/Versene (1:5) during 10', 37 °C.
8. Spin again.
9. Finally, resuspend in DMEM+serum, and well 35 mm (6 well-plate) previously treated with Vitrogen.
10. Plate cells and allow to attach for 30 min. to 1h. Then remove non-attach cells.
11. Rinse the cells with DMEM, 1-3 h after plated. The rinses have to be strong (that means strong!) enough to remove smooth muscle but not endothelial cells.