PRECAUTIONS TO AVOID ENDOTOXIN CONTAMINATION OF LDL AND ACETYL-LDL

Preparation of Endotoxin-Free Equipment

1. Soak glassware and stir bars (for dialysis) overnight in a 1% solution of alkaline detergent (E-TOXA-CLEAN, Sigma E9029).
2. Rinse all glassware 8 to 10 times with warm running tap water, 5 times with distilled water, and finally twice with endotoxin-free water (Hyclone, SH30529.03).
3. Soak centrifuge tubes (including screw tops, rubber inserts, and rings), acetyl-LDL stirring vial, stir bar, and dialysis tubing clips overnight,. Then rinse them as above, and air dry under the hood.
4. Autoclave all glassware and stir bars but DO NOT AUTOCLAVE CENTRIFUGE TUBES!!

LP buffer

1. Make LP buffer with endotoxin-free water, and autoclave before use.
2. Clean the pH meter probe by rinsing it with endotoxin-free water.
3. Use a fresh bottle of HCl to adjust the pH.

Preparation of LDL:

1. Wipe each rotor with 70% ethanol before use. Because the centrifuge tubes used in the last spin have an open top, make sure to clean the rotor and rotor top thoroughly right before use, and do not apply excess grease around the rotor top.
2. Work under the sterile hood as much as possible, and change gloves frequently.

Acetylation of LDL:

1. Use fresh sodium acetate hydrate solution made with endotoxin-free water.
2. Work under the sterile hood as much as possible, and keep the vial closed during stirring. Change gloves frequently.
3. Instead of stirring the solution in the cold room, which can be a source of contamination, place the vial in a small ice-water mix beaker on a stir plate in the lab. Make sure ice is always in the beaker during stirring.
4. Use thick wall dialysis tubing (Spectrum Cat# 08-667A) for dialysis.

Verification: Test all preps using the Limulus amebocyte lysate (LAL) assay. Here is a typical result for acetyl-LDL: a 100 µg/ml solution gave a result of 0.08 EU, so a working solution of 50 µg/ml would contain < 0.05 EU/ml (< ~5 pg/ml), which is considered clean for experiments. If further certainty is needed, one can show that as much as 10 pg/ml endotoxin does not mimic whatever endpoint is being assayed with 50 µg/ml acetyl-LDL.