# Derivatization for GC (August 31, 2019),

# MG-HeLa-samples, Fabiola Garcia

* generally keep blocks of samples of one randomization group together in one shaker/centrifuge, add chemicals in randomized fashion within one group *(do not go through numbers consecutively)*
* dry samples 15’ in the SpeedVac (manual mode, ***no cooling!***) to remove condensed water. Be sure that samples are completely dry, as water will disturb the derivatization. Work under the hood as all compounds are harmful!
* Prepare MeOX-Solution: Calculate the amount you’ll need and dissolve  
  40 mg of methoxyamine hydrochloride per mL of dry pyridine. (See also table below):
  + take ca 40 mg, dissolve in 1000 µL pyridine per 40 mg MeOX
* Add MeOX-Solution
  + 20 µL to each **Ident**, **Quant** or **sample**
  + **miscounted the samples. Had to prepare another MeOX-solution, which sample was treated how is noted in the sample list. I also lost the notes on how much MeOX I actually weighed, it was first about 30 mg, than another round with ca 25 mg**
* vortex, spin down
* Shake at 30 °C for 90’ (Eppendorf shaker), 1400 rpm
* At least 30’ before end take out MSTFA (N-methy-N-(trimethylsilyl) trifluoroacetamide) and Alkane-Mixture from fridge,  
  if alkanes do not redissolve completely briefly keep in hand to slightly warm the alkane stock
* Prepare MSTFA: combine 3 bottles (3x 1000 µL), add 30 µL alkane-mixture to combined bottles, if alkanes fall out heat both to 30 or 37 °C.
* Spin down in Centrifuge at 23 °C (room temperature, ***no cooling!***)
* Add MSTFA (with alkanes 10µL/1000 µL MSTFA):
  + 80 µL to each **sample, ident** or **quant**,
  + Rest 🡪 Wash
* vortex, spin down
* Shake at 37 °C for 60’, (Eppendorf shaker), 1400 rpm
* *label vials in the meantime,* 
  + *1 big each for idents*
  + *2 big for each quant*
  + *2 big for each sample*
  + *1 big for wash*
* Centrifuge samples for 10’ at maximum speed (Eppendorf table centrifuge, 18.000 rcf, at 23 °C, ***no cooling!***)
* Transfer supernatant into labeled glass vials
  + 2x 40 µL of each sample or quant,
  + 1x 60 µL of each Ident,
* Seal well, check if lid is tight (should not be turnable, but also septum should not be crinkled)
* Ready for GC!