Troubleshooting guide (Biomedicine T5, MolMed HT11)

Problem	Possible causes	Probable solutions
Very few cells in the culture, thus no RNA & poor FACS data	Wrong cell count due to the difficulty in distinguishing RBC from leukocytes Cells were washed away and lost	Add RBC lysis buffer step Culture more cells Use 14mL tubes, which gives firmer and “protected” pellets
No or very low CD93 mRNA expression	Failed reverse transcription (No cDNA) Failed amplification on CD93 (bad assay) High apoptosis and low neutrophils population (time frame) Mixed cell population and different cell types have different CD93 mRNA expression levels	Check reference gene Check known positive controls Count live and dead cells when we harvest them and compare Cell-type-specific culture Grow more cells
One of the SNP assay did not work	Bad assay No DNA	Run the products on an agarose gel Positive control from known samples Check the other assay, it worked.
Bacterial contamination	Not working in sterile lab	Count the cells to see Work in sterile area (hood or similar) Put the lid on as soon as possible Increase antibiotics concentration in the media

		Some indicators (eg pH) to monitor
	Working with a bacteria incubator	Don’t do that.
	Colleagues’ extensive use of bacteria culture than past years	Clean it well... or well...
No or degraded RNA	Presence of RNase	Use RNA stablilizing agent or RNase inhibitor at all times
	Not low enough temperature	
	Long processing time	
Low intensity for CD93 in ELISA	Removal of “jelly” may have removed some cells and soluable CD93	Work sterile
	Soluble CD93 stuck on bacteria	Culture more cells
	24h lifespan of neutrophils	Decrease grow time, faster handling time