In clinical trials, time is often one of your worse opponents, among others including variability and money concerns. Besides these considerations, subjects/patients well being must be considered. In clinical trials involving molecular biology, this work package is often one of the most time consuming and the most expensive.

Based on these facts, we developed in our lab a new method focused on immune system based on the combination of an inflammatory model with gene expression monitoring. This method is MIQE compliant.

In this experiment, the control group is blood from healthy subjects without in vitro LPS stimulation. The test group’s blood have received LPS and has been incubated at 37°C for different times. Samples have not been thaw more than once and always on ice. Storage temperature was -80°C. DNase treatment is done after extraction, right before RT.

cDNA synthesis is done using QuantiTect RT kit from Qiagen, without modification (100 ng). Purity is assessed with OD 260/280 and 130. The ratios 260/280 are between 1.76 and 1.84, 260/230 between 2.11 and 2.29. cDNA are stored on ice before qPCR (a few hours).

RESULTS: As expected, LPS stimulation induces an inflammatory response. This is show by increase in immune markers. Due to low number of samples (only three), the statistical power is not very high, but thanks to MIQE, the biological relevance is clear.

Solaris predesigned assay: The pre designed assay helps a lot in performing analysis.
~ No time required for designing primers and probes
~ All in the same tube: very convenient, reduces pipeting concerns

Superbases and MGB: The amplicons are highly optimized, thanks to reduced size of primers and probes.
~ Incorporation of superbases™, which increases the melting temperature for smaller primers and minor groove binder (MGB)
~ Increases stability of the probes
~ Smaller primers and probes allows wider coverage of the genome, so over 98% of the genome is covered
~ Fluorescence relies on structural change rather than hydrolysis

References: Bustin et al, The MIQE guidelines; Minimum information for publication of quantitative real-time PCR experiments, 2009; Pouillart et al, NUTRIOSE®, a prebiotic low-digestible carbohydrate stimulates gut mucosal immunity and prevents TNBS-induced colitis in piglets, 2009