The diagnostic use of in vitro molecular assays can be limited by the lack of guidelines for collection, handling, stabilization and storage of biosamples. One of the goal of the EC funded project SPIDIA (Standardisation and Improvement of pre-analytical Procedures for in vitro diagnostics, www.spidia.eu) was the development of evidence-based guidelines through implementation of two [1] pan-European External Quality Assessment (EQA). Here we reported the results of the Second EQA.

119 laboratories were recruited from 21 European countries by the announcement published on European Federation of Laboratory Medicine (EFLM) website. At deadline, 109 laboratories (92 %) returned extracted RNA to the SPIDIA facility (Clinical Biochemistry Laboratory, University of Florence).

Because blood from a single donor was not of sufficient volume to provide proficiency specimens to all study participants, two blood donors were enrolled, blood from each donor was aliquoted into TO control and proficiency specimens. The participating laboratories were therefore randomized into two groups, each group receiving proficiency specimens associated with one donor. They received K2EDTA blood samples (Tube C & Tube D) with or without stabilizer (PAXgene Blood RNA tube® or K2EDTA, according to their request). They performed RNA extraction following their own procedure and filled a questionnaire and result form to collect the data. RNA C had to be extracted from Tube C immediately after arrival (24h after blood collection); RNA D had to be extracted 24h after RNA C from Tube D (48h after blood collection) storing Tube D at Room temperature (RT) or +4°C. The extracted RNAs have been sent back to SPIDIA-facility in dry ice.

RNA QUALITY PARAMETERS analysis performed at SPIDIA facility:
- Yield & Purity (by Nanodrop spectrophotometer)
- Integrity (RIN score, by Agilent Bioanalyzer 2100)
- qPCR Interferences evaluation (by Kineret® software)
- mRNA Stability (by absolute qPCR analysis of IL1β, IL8, c-FOS and GAPDH)

EXPRESSION OF TNFRS and FOSB BIOMARKERS (by relative qPCR)
These 2 biomarkers (identified by SPIDIA W.P. 1.3) are indicators of ex vivo changing in stored K2EDTA blood samples.

CONCLUSIONS
- Participants were proficient in the pre-analytical aspects of specimen handling for RNA analysis. The distribution of the overall proficiency ratings was similar within the 2 donors with almost 40% of laboratories receiving "in control" assessments for all the considered quality parameters.
- Analysis of the gene expression demonstrated that pre-analytical factors significantly affected the quantity of some gene transcripts (data not shown) and of the 2 biomarkers (FOSB and TNFRS) relative to TO depending on blood collection tube (PAX Gene tubes or EDTA tubes) and on time & temperature blood storage.
- Our results demonstrated that the use of PAX Gene tubes allows reliable gene expression analysis within 48 h from blood collection.
- The results of the two SPIDIA RNA EQAs studies have been proposed for use in the development of a Technical Specification by the European Committee for Standardisation (CEN).