SPIDIA-RNA: Second External Quality Assessment for the pre-analytical phase of blood samples used for RNA based analyses

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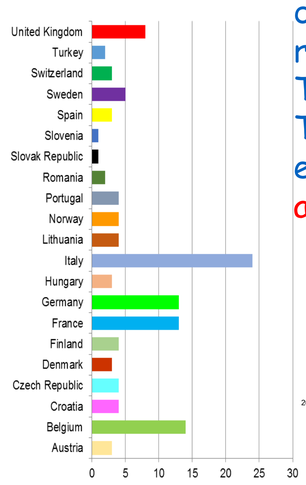
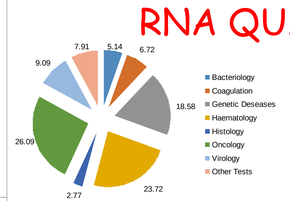
The diagnostic use of *in vitro* molecular assays can be limited by the lack of guidelines for collection, handling, stabilisation and storage of biosamples. One of the goal of the EC funded project SPIDIA (Standardisation and improvement of pre-analytical procedures for *in vitro* diagnostics, www.spidia.eu) 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.

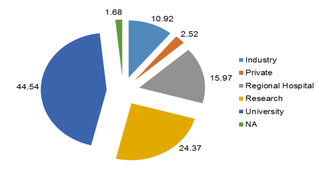
119 laboratories were recruited from 21 European countries by the announcement published on European federation of Laboratory Medicine (EFLM) website. At deadline, 109 laboratories (92 %) returned extracted RNA to the SPIDIA facility (Clinical Biochemistry Laboratory, University of Florence).



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 T0 control and proficiency specimens. The participating laboratories were therefore randomized into two groups, each group receiving proficiency specimens associated with one donor

They received 2 blood samples (Tube C & Tube D) with or without stabiliser (PAXgene Blood RNA tube® or K_2EDTA, 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.

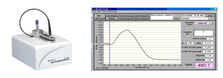




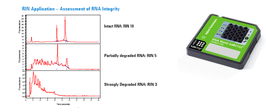
RNA QUALITY PARAMETERS analysis performed at SPIDIA facility:

- Yield & Purity (by Nanodropspectrophotometer)
- 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)

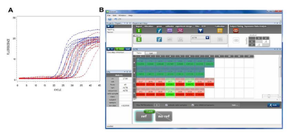
Yeld & Purity



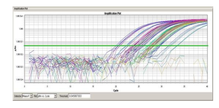
RNA Integrity: RIN



qPCR interferences: Kineret analysis



Gene expression analysis



EXPRESSION of TNFRS and FOSB BIOMARKERS (by relative qPCR)

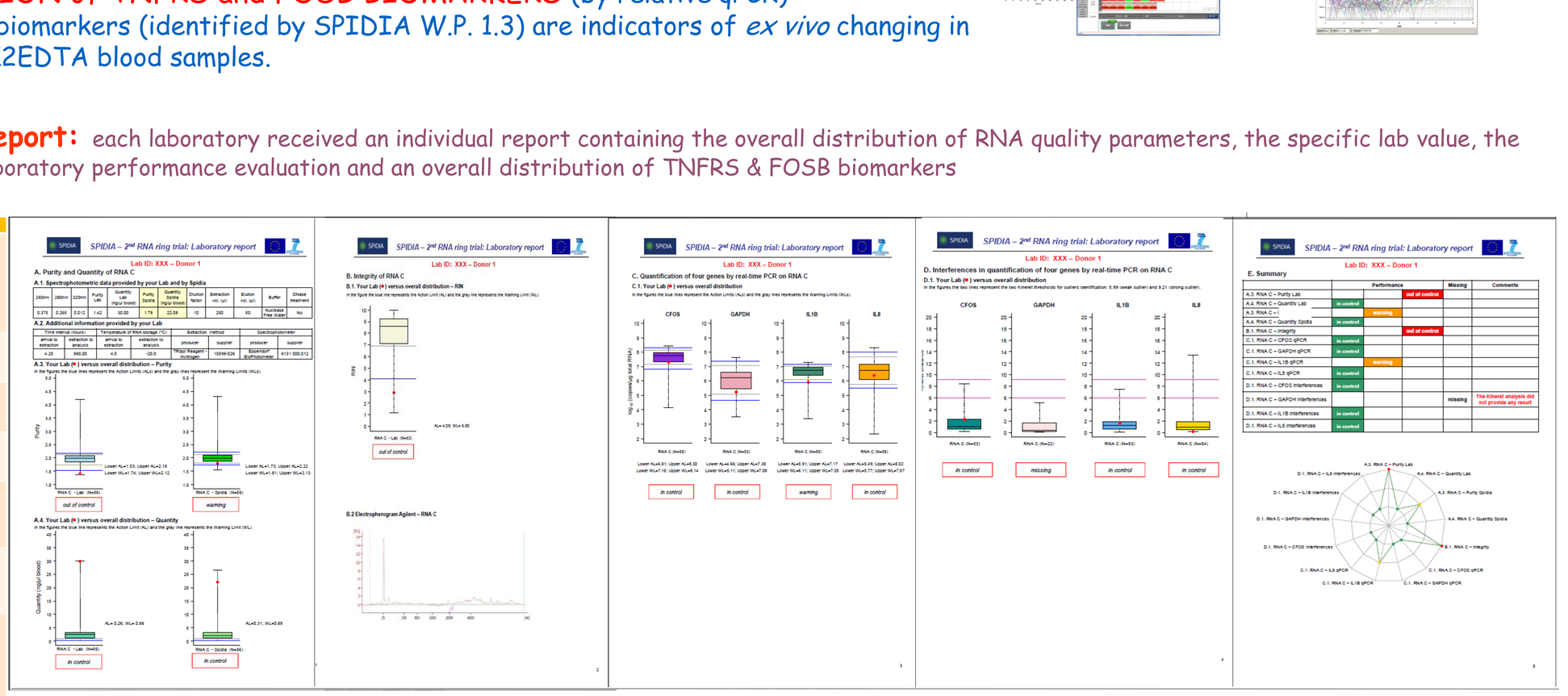
These 2 biomarkers (identified by SPIDIA W.P. 1.3) are indicators of *ex vivo* changing in stored K2EDTA blood samples.

Questionnaire

Report:

each laboratory received an individual report containing the overall distribution of RNA quality parameters, the specific lab value, the laboratory performance evaluation and an overall distribution of TNFRS & FOSB biomarkers

Questions		N	%
1 - In which tube do you usually perform blood collection?	K_2EDTA Tube	61	66
	NaCitrate Tube	1	1
	PAXgene blood RNA tube	19	21
	Tempus Tube	1	1
	Li-Heparine Tube	-	-
	Other	10	11
2 - Do you collect the blood on your own or do you receive the blood collected from elsewhere?	Collect	7	7
	Receive	53	58
	Collect and receive	32	35
3 - How many milliliters of blood do you collect?	1 mL ≤ mL ≤ 5 mL	21	54
	5 mL ≤ mL ≤ 10 mL	14	36
	>10 mL	4	10
4- How many milliliters of blood do you receive?	1 mL ≤ mL ≤ 5 mL	42	49
	5 mL ≤ mL ≤ 10 mL	32	38
	>10 ml	11	13
5 - How long is the usual time interval between the blood collection/receipt and the RNA extraction?	≤ 12 h	39	42
	12 h < h ≤ 24 h	29	32
	24 h < h ≤ 480 h	15	16
	>480 h	9	10
6- Only for those that receive blood from elsewhere: at what temperature is the collected blood usually delivered to you?	-80°C	3	4
	-20°C	4	5
	4°C	25	29
	RT	53	62
7 - At what temperature you store the collected blood before RNA extraction?	-80°C	10	11
	-20°C	12	13
	4°C	55	60
	RT	14	15
	missing	1	1
8 - What is the procedure for RNA extraction?			
a.Do you use a kit?	Yes	79	86
	No	13	14
If yes, what is the kit?	Magnetic Bead	5	6
	PAXgene	19	25
	Precipitation	5	6
	Silica	41	52
	Missing	9	11
a.The procedure is:	Automatic	22	24
	Manual	69	75
	Missing	1	1
9 - How many milliliters of blood do you use for RNA extraction?	≤ 5 mL	66	72
	>5 mL	22	24
	missing	4	4
11 - Do you evaluate the yield and purity of extracted RNA?	Yes	80	87
	No	12	13
If the answer is "Yes": what is the method?	Total RNA integrity	1	1
	RiboGreen	1	1
	Spectrophotometer	74	92
	Total RNA integrity+ Spectr	2	3
	Other	2	3
12 - How long is the usual time interval between the RNA extraction and concentration evaluation?	≤ 6 h	79	86
	6 h < h ≤ 24 h	5	6
	>24h	4	4
	missing	4	4
13 - What kind of analysis do you usually perform on your extracted RNA?	Microarray evaluation	1	1
	RIN evaluation	3	4
	RT-PCR	9	11
	RT-qPCR	64	74
	RIN + RT-qPCR	2	2
	RIN + RT-PCR/qPCR	2	2
	RT-qPCR +microarray	1	1
	RT-qPCR/PCR+microarray	1	1
	RT-PCR/qPCR	3	4
14- How long is the time interval between the RNA extraction and analysis of RNA?	≤ 6 h	21	23
	6 h < h ≤ 24 h	34	37
	24 h < h ≤ 120 h	22	24
	120 h < h ≤ 240 h	3	3
	>240 h	11	12
	missing	1	1
15- At what temperature do you usually store the extracted RNA?	-80°C	74	80
	-20°C	18	20
16- For how long do you usually store RNA?	No storage	2	2
	Days	4	5
	Months	12	13
	Years	74	80
17- Is your laboratory accredited to perform molecular diagnostic tests?	Yes	49	53
	No	42	46
	missing	1	1
If yes, which kind of accreditation do you have?	Academic	5	10
	ISO 15189	22	45
	Institutional	7	14
	Regional	3	6
	Other	12	25



Overall performances

	DONOR 1 (n=56)	DONOR 2 (n=53)
1: all in control or warning performance	44.6 %	41.5 %
2: only one out of control and/or missing performance	28.6 %	30.2 %
3: labs with two or more "out of control" and/or two or more "missing" performance	26.8 %	28.3 %

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.

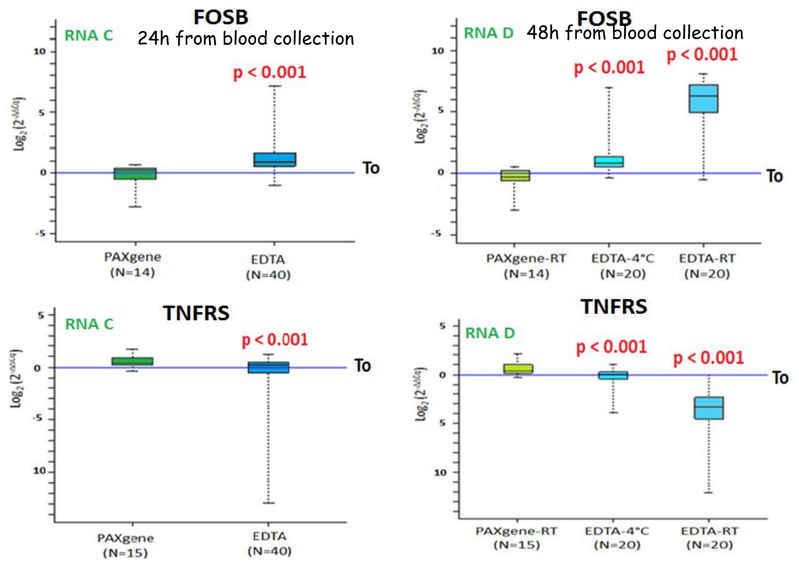
- Analysis of the gene expression demonstrated that pre-analytical factors significantly affected the quantity of some gene transcripts (data not shown) and of the 2 biomarkers (FOSB and TNFRS) relative to T0 depending on blood collection tube (PAX Gene tubes or EDTA tubes) and on time & temperature 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).

Influence of storage condition & blood collection tube on gene expression

Relative quantification of the up- (FOSB) and down-(TNFRS) regulated biomarkers, selected to monitor ex vivo gene expression changes.



The horizontal blue line is plotted in corrispondence of log2 (RQ)=0

1) SPIDIA-RNA: first external quality assessment for the pre-analytical phase of blood samples used for RNA based analyses. *Pazzagli M, Malentacchi F, Simi L, Orlando C, Wyrich R, Günther K, Hartmann CC, Verderio P, Pizzamiglio S, Ciniselli CM, Tichopad A, Kubista M, Gelmini S. Methods. 2013 Jan;59(1):20-31.*