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

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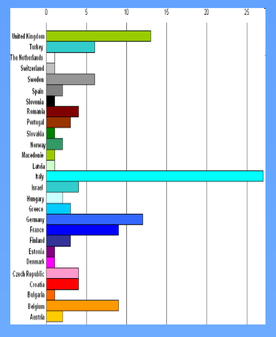
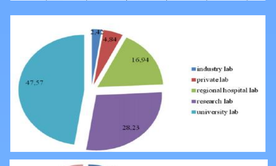
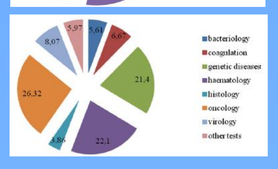
The diagnostic use of *in vitro* molecular assays can be limited by the lack of guidelines for collection, handling, stabilisation and storage of patient specimens. One of the goal of the EC funded project SPIDIA (Standardisation and improvement of pre-analytical procedures for *in vitro* diagnostics, www.spidia.eu) was the implementation of a pan-European External Quality Assessment (EQA).

METHODS

102 laboratories were recruited fro the EQA by the European federation of Laboratory Medicine (EFLM) support.

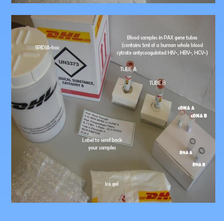






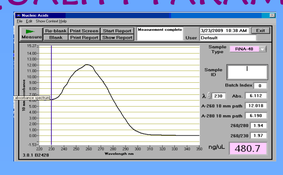
SPIDIA-UNIFI collected the blood from 7 donors, pooled and sent the same samples to all participants.

Participants received 2 blood samples with or without stabiliser (PAXgene Blood RNA tube® or K2EDTA, they chose the tubes during the enrolment) and performed RNA extraction following their own procedure, a questionnaire and result form to collect the data. The RNAs were sent back to SPIDIA-UNIFI in dry ice.

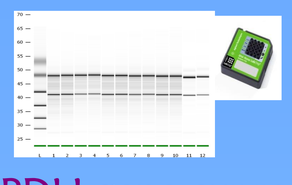


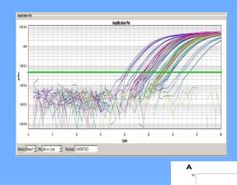
SPIDIA-UNIFI performed the "RNA QUALITY PARAMETERS" analysis as follow:

- **Yield & purity**
  (by spectrophotometer- Nanodrop)

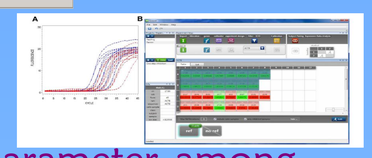


- **Integrity**
  (by Agilent Bioanalyzer 2100, RIN RNA Integrity Number)





- **mRNA stability**
  (absolute quantification by qPCR of IL1β, IL8, c-Fos and GAPDH gene expression)
- **qPCR interferences**
  (by Kineret® software – analysis of qPCR kinetics)



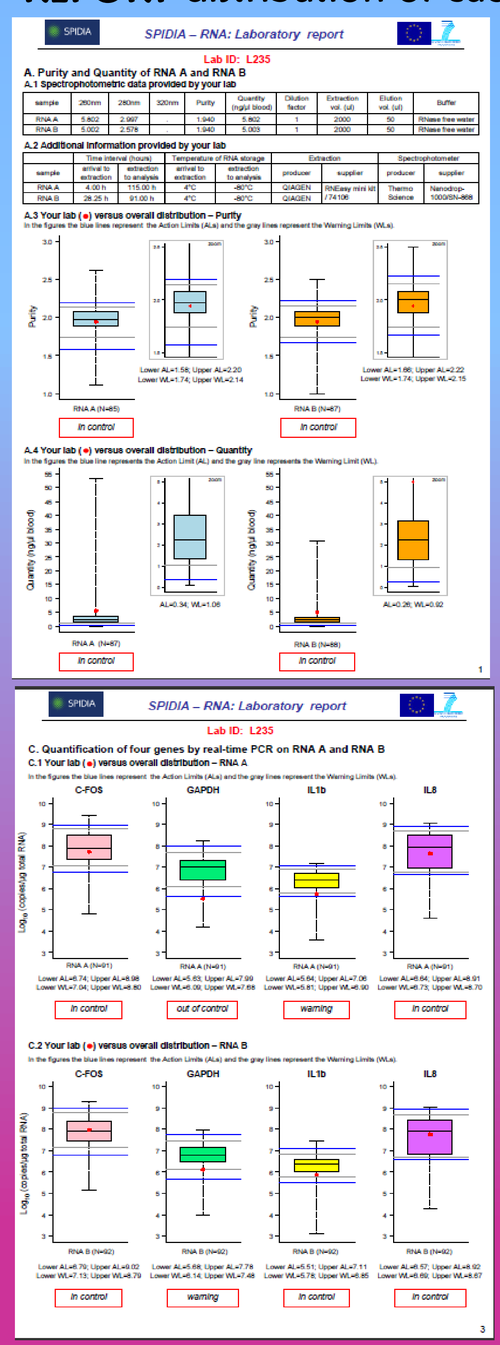
..and developed a REPORT for the participants containing the performance and the comparison of each RNA quality parameter among the other laboratories (consensus mean).

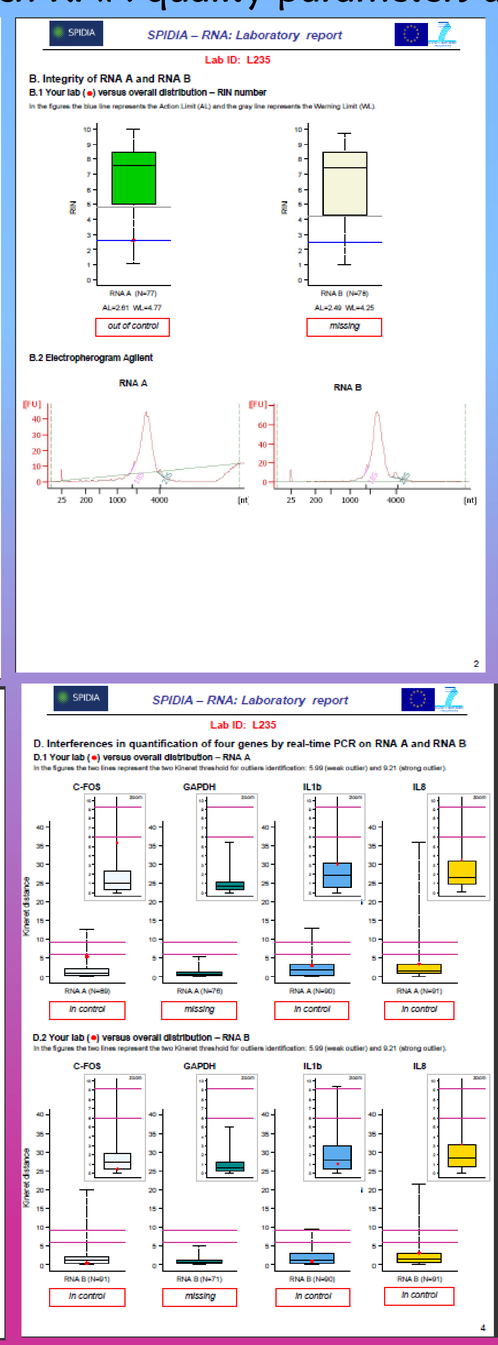
RESULTS

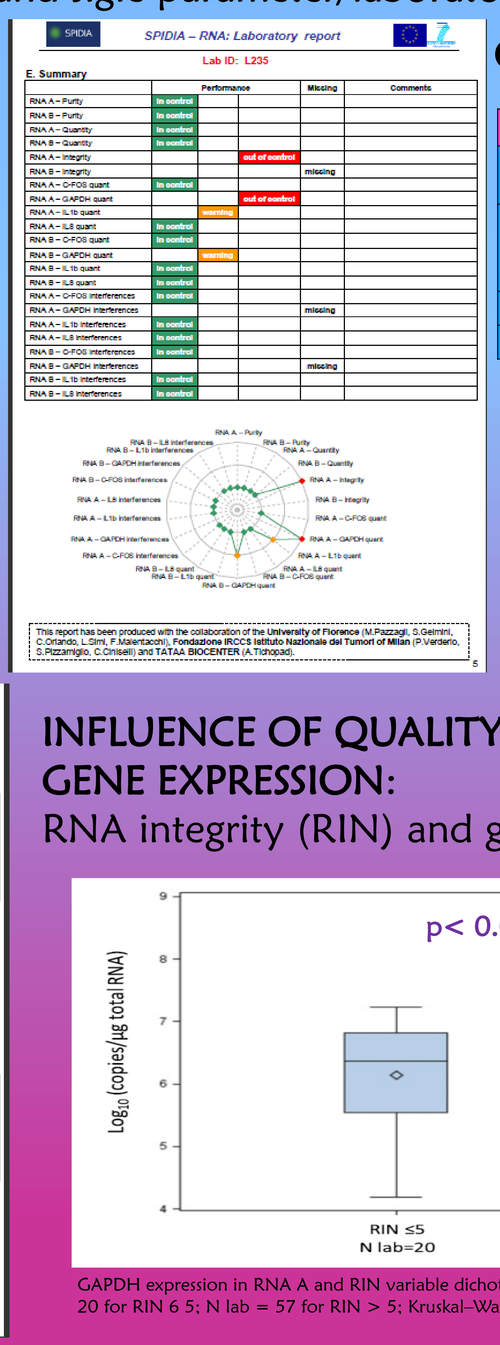
Questionnaire results

Questions		% of labs
1 - In which tube do you usually perform blood collection?	K_2EDTA	66%
	NaCitrate	2%
	LiEparine	1%
	PAXgene blood RNA tube	23%
	other	8%
2 -How many milliliters of blood do you collect?	1ml<x<2.5ml	7%
	2.5ml≤x<5ml	41%
	5ml≤x≤10ml	43%
	>10ml	9%
3 - How long is the time interval between the blood collection and the RNA extraction?	≤12h	54%
	12<x≤24h	33%
	>24h	13%
4- At what temperature is stored the collected blood?	-80°C	10%
	-20°C	12%
	4°C	53%
	Room temperature	25%
5 - What is the procedure for RNA extraction?		
Do you use a kit?	Yes	84%
	No	16%
The method to isolated RNA is based on…	Silica membrane	89%
	Magnetic beads	5%
	precipitation	6%
6- How many microliters do you use to resuspend/elute the extracted RNA from blood?	≤ 50µl	66%
	50µl<x≤ 100µl	28%
	>100 µl	6%
7 - Do you evaluate the concentration of extracted RNA?	Yes	88%
	No	22%
What is the method?		
	Spectrophotometer	90%
	picoGreen	2%
	RIN	8%
8 - How long is the time interval between the RNA extraction and concentration evaluation?	≤6h	85%
	6h<x≤24h	13%
	>24h	2%
9 - What kind of analysis do you usually perform on your extracted RNA? (multiple answers)	rt-PCR	5%
	rt-qPCR	40%
	microarray	1%
	rt-PCR+rt-qPCR	40%
	RIN+rt-PCR	7%
	RIN+rt-PCR+rt-qPCR	3%
	RIN+microarray	1%
	RIN+rt-qPCR+microarray	2%
	Rt-PCR+qrt-PCR+microarray	1%
10 - How long is the time interval between the RNA extraction and the analysis of RNA?	≤6h	23%
	h6<x≤24h	44%
	24h<x≤5days	23%
	>5days	10%
11 - At what temperature do you usually store the extracted RNA?	-80°C	84%
	-20°C	16%
	4°C	-
	Room temperature	-
12 - For how long time do you usually store RNA?	No storage	5%
	Days	8%
	Months	85%
	years	2%

REPORT distribution of each RNA quality parameters and sigle parameter/laboratory performance







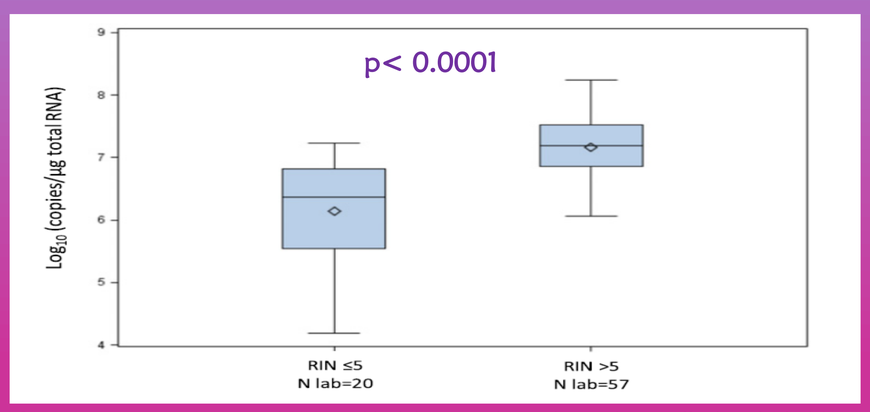
Overall performances

Categories	N	%
all in control or warning performance	24	25.81
only one out of control and/or missing performance	27	29.03
other	42	45.16
Total	93	100

Categories:
all in control or warning performance: labs with all performances "in control" or "warning";
only one out of control and/or missing performance: labs with only one "out of control" or labs with only one "missing" or labs with one "out of control" and one "missing" performance;
other: labs with two or more "out of control" and/or two or more "missing" performance. Also, labs with two or more "missing" and one "out of control" and viceversa.

INFLUENCE OF QUALITY PARAMETER ON GENE EXPRESSION:

RNA integrity (RIN) and gene expression (GAPDH)



GAPDH expression in RNA A and RIN variable dichotomized according to the cut-off value of 5 (N lab = 20 for RIN 6 5; N lab = 57 for RIN > 5; Kruskal–Wallis test, p < 0.0001).

The median value of RIN and PURITY are closed to expected high quality RNA. No drammatic gene expression changing within 72h of blood storage. Few qPCR interferences (none for GAPDH). RIN value (cut off=5) influences specific gene expression.

CONCLUSION

The results of this EQA will be used to to enhance a second Pan-European EQA.

The results of both EQAs will be the basis for the implementation of evidence-based guidelines for blood sample managing to obtain good quality RNA sample

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