

SPIDIA-RNA: First 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* 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

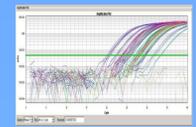
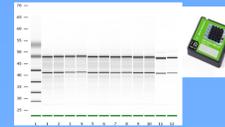
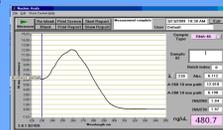
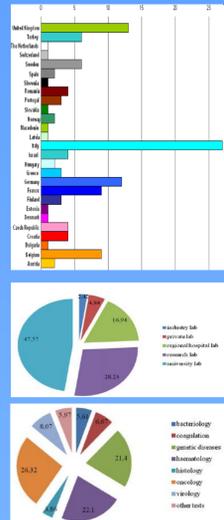
102 laboratories were recruited from the EQA by the European federation of Laboratory Medicine (EFLM) support.

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 IL1 β , IL8, c-Fos and GAPDH gene expression)
- **qPCR interferences** (by Kineret® software – analysis of qPCR kinetics)



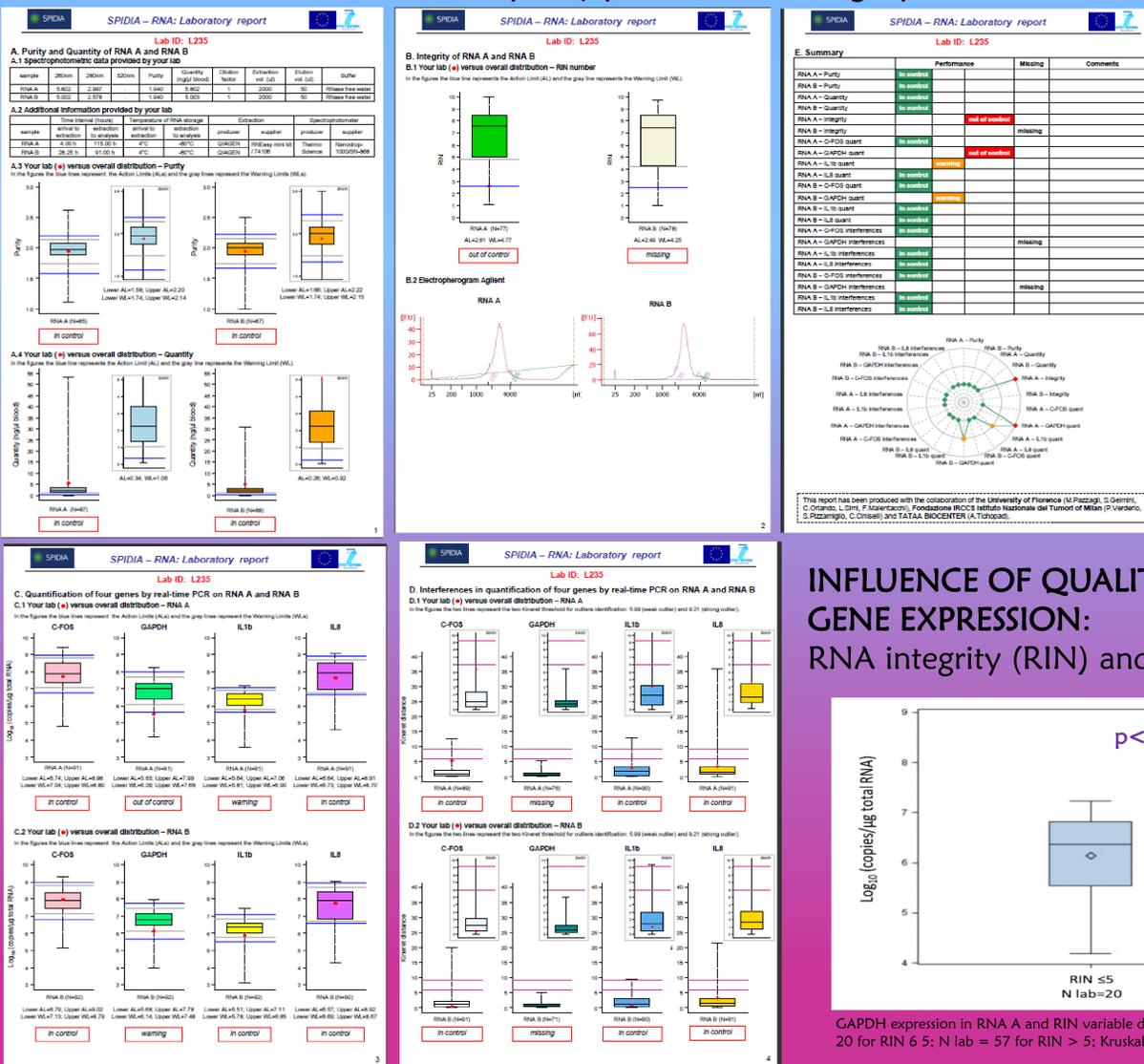
..and developed a REPORT for the participants containing the performance and the comparison of each RNA quality parameter among the other laboratories (consensus mean).

RESULTS

Questionnaire results

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

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



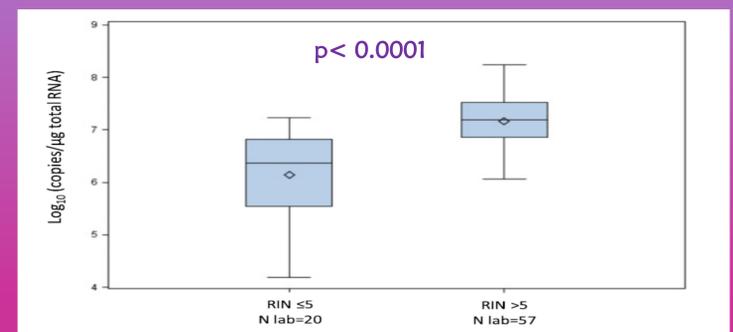
Overall performances

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 ≤ 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 dramatic 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 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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