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 goals 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 for 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**

- In which tube do you usually elute the extracted RNA?
  - Yes
  - No
- Is the method to isolated RNA in dry ice?
  - Yes
  - No
- Do you choose an RNA extraction method based on the RNA purity or other factors?
  - RNA purity
  - Other factors
- Which method do you usually use for RNA quantification? 
  - Nanodrop
  - Other
- How many milliliters of blood do you usually collect blood?
  - <20 µl
  - 20-200 µl
  - >200 µl
- How many hours do you usually collect blood? 
  - <24h
  - 24-96h
  - >96h
- How many milliliters of EDTA do you usually use for RNA extraction?
  - <20 µl
  - 20-200 µl
  - >200 µl
- How many hours do you usually perform RNA extraction after blood collection?
  - <24h
  - 24-96h
  - >96h
- How many hours do you usually perform the RNA extraction procedure?
  - <24h
  - 24-96h
  - >96h
- How many hours do you usually store RNA?
  - <24h
  - 24-96h
  - >96h

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

**Overall performances**

<table>
<thead>
<tr>
<th>Categories</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>All in control or warning performance</td>
<td>24</td>
<td>25.81</td>
</tr>
<tr>
<td>Only one out of control or missing performance</td>
<td>27</td>
<td>29.85</td>
</tr>
<tr>
<td>Other</td>
<td>42</td>
<td>46.16</td>
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<tr>
<td>Total</td>
<td>93</td>
<td>100</td>
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**INFLUENCE OF QUALITY PARAMETER ON GENE EXPRESSION:**

RIN integrity (RIN) and gene expression (GAPDH)

\[ p < 0.0001 \]

GAPDH expression vs. RIN (R and B) with biological variation from the cutoff value of 5. 50 lab ≥ 70 for RNA ≥ 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 quality RNA sample.