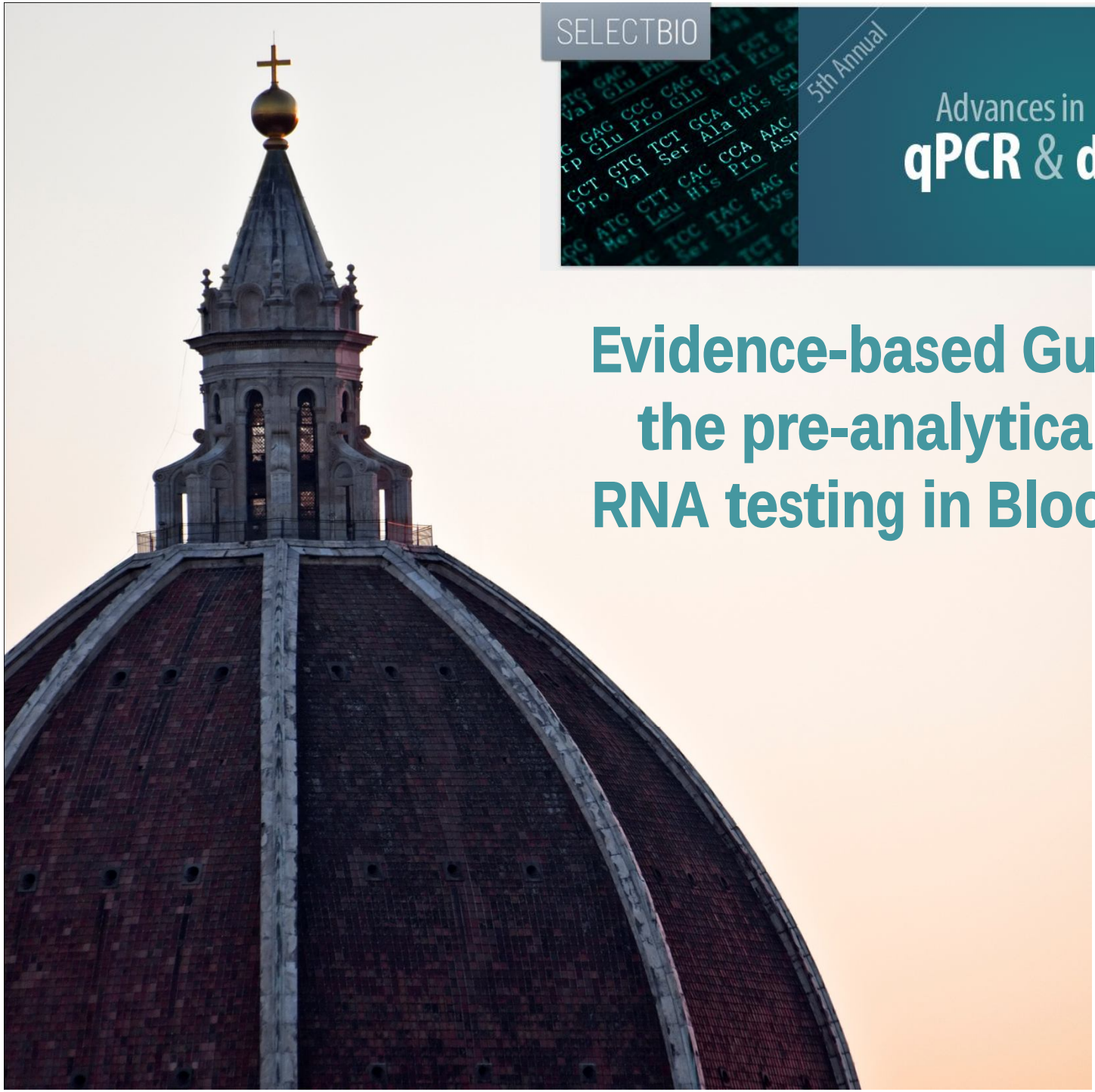


SELECTBIO

5th Annual

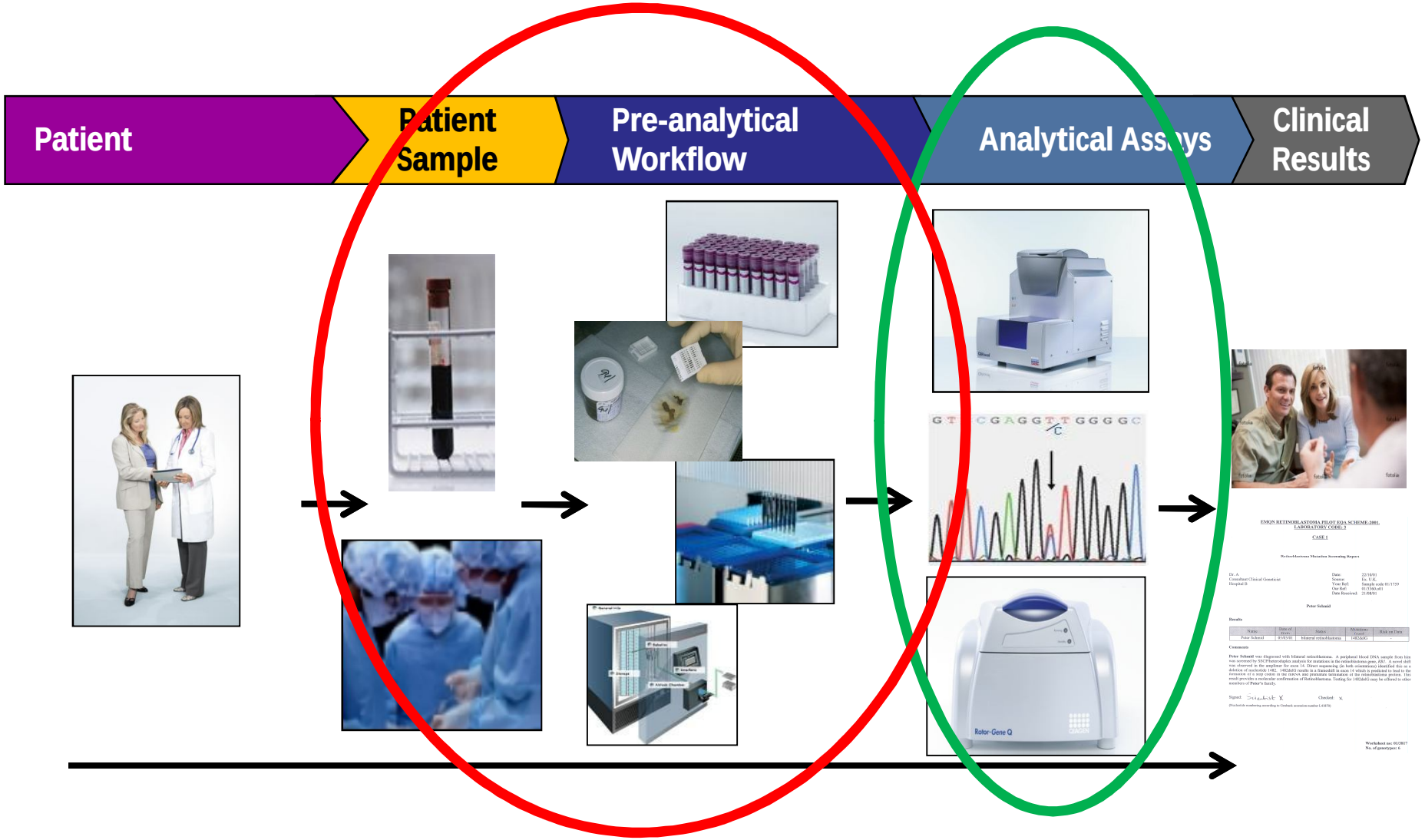
Advances in
qPCR & dPCR

ELA
European Lab Automation
BARCELONA
SPAIN
14 - 15 MAY
2014

Evidence-based Guidelines for the pre-analytical phase of RNA testing in Blood Samples

Francesca Malentacchi
University of Florence

Laboratory Workflow



Pre-analytical phase of blood sample

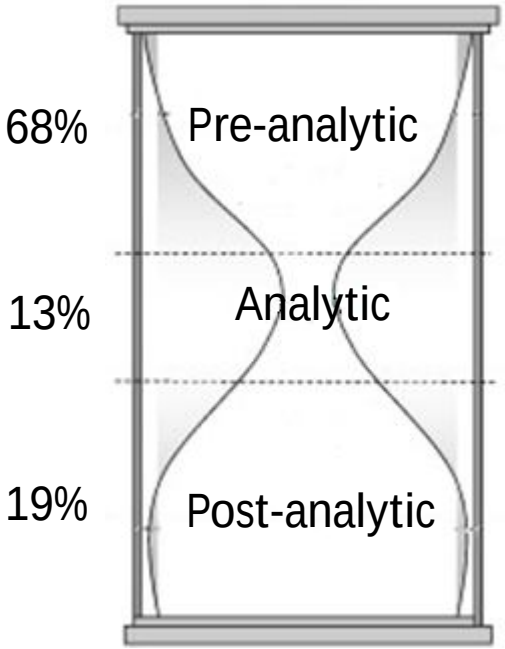
Analytical - Phase

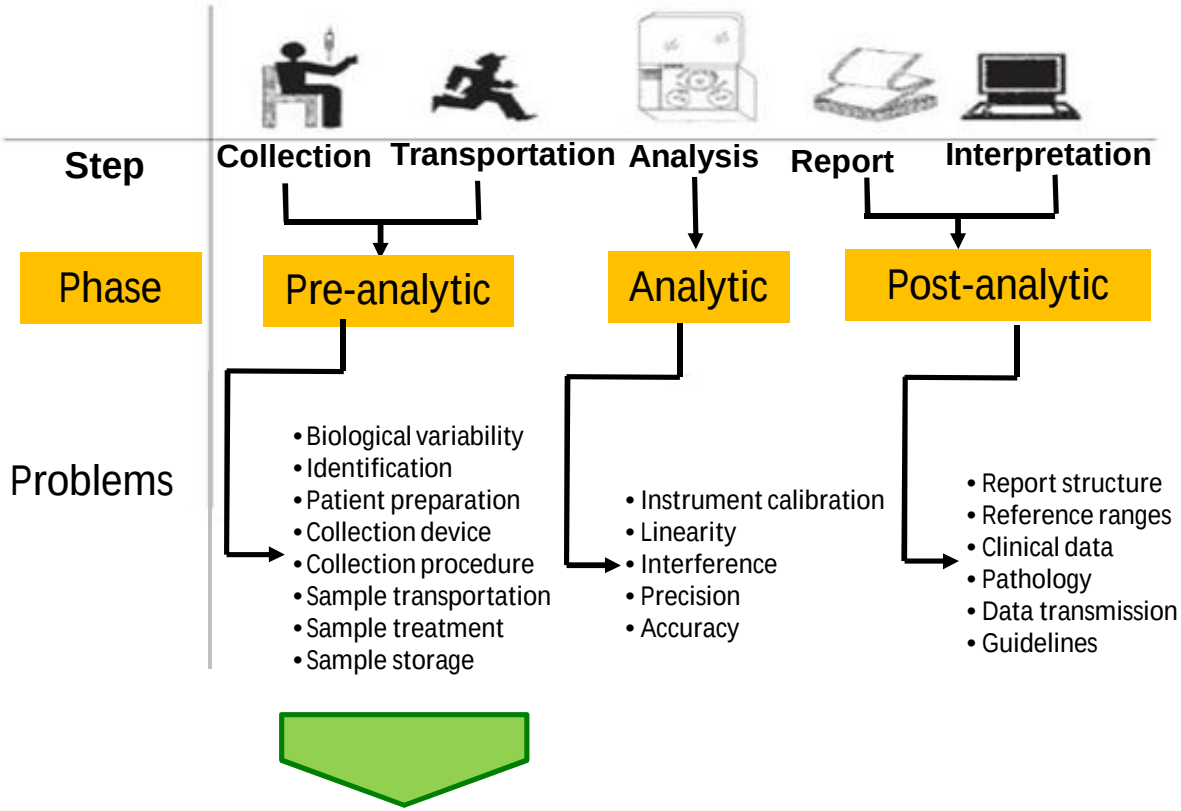
Pre-analytical Phase

no widespread knowledge on the role of this phase on RNA analysis

Influence in the analytical results

Laboratory workflow





OUTSIDE Lab

INSIDE Lab

- **BLOOD COLLECTION (collection device, identification)**
- **BLOOD STORAGE (time & temperature, treatment)**
- **BLOOD SHIPPING (transportation)**
- **RNA EXTRACTION PROCEDURE**

Lippi G. *et al.*. Preanalytical quality improvement: from dream to reality. Clin Chem Lab Med. 2011; 49:1113-26;
Plebani M. Exploring the iceberg of errors in laboratory medicine. Clin. Chem. Acta. 2009; 404: 16-23;
Lippi G et al. la variabilità preanalitica. RIMeL/IJLaM 2006; 2:24-31

Role of pre-analytical phase

...a Pan-European question...

SPIDIA

(Standardisation and improvement of Pre-analytical procedures for *In vitro* DIAgnostics)

SPIDIA is a four-year large-scale integrating project that responds to the FP7-HEALTH-2007-B call for proposals in the following topic: **HEALTH-2007-1.2-5** – Standardisation and improvement of pre-analytical procedures for *in vitro* diagnostics. The proposed research and standardisation activities cover all steps from **creation of evidence-based guidelines (through pan-European quality assurance schemes, EQAs)** to **creation of tools for the pre-analytical phase** to testing and optimisation of these tools through the **development of novel assays and biomarkers**. All the activities focus on the **validation of the translational research providing tools for the pre-analytical phase of *in vitro* diagnostics.**

Role of pre-analytical phase

...a Pan-European question...

SPIDIA

(Standardisation and improvement of Pre-analytical procedures for *In vitro* DIAgnostics)

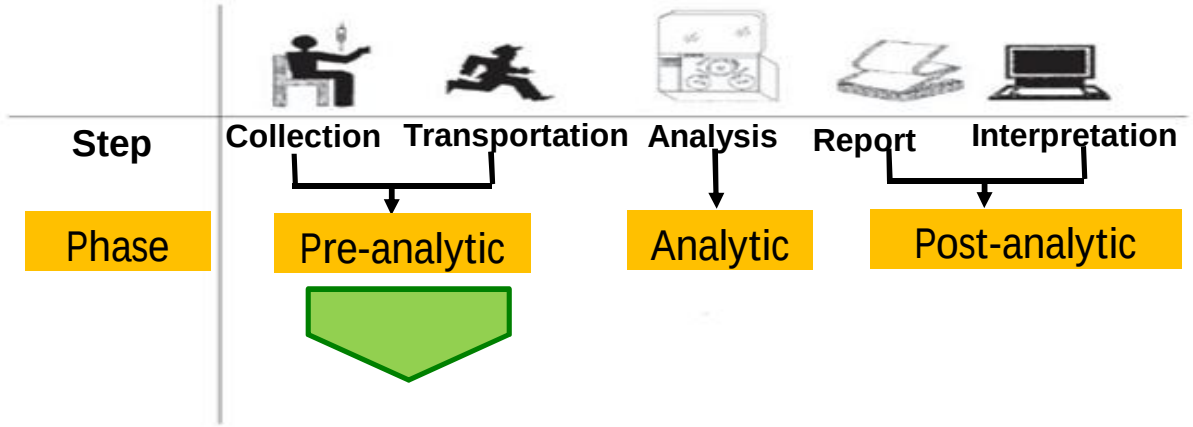
External Quality Assessment (EQA)

..for the evaluation of pre-analytical phase in blood sample:

- RNA
- Circulating cell free DNA (ccfDNA)
- Genomic DNA (gDNA)

SPIDIA-RNA
External Quality Assessments

SPIDIA-RNA EQAs: Purposes



SPIDIA-RNA EQAs

- BLOOD COLLECTION (collection device, identification)
- BLOOD STORAGE (time & temperature, treatment)
- BLOOD SHIPPING (transportation)
- RNA EXTRACTION PROCEDURE

BLOOD COLLECTION TUBE
BLOOD STORAGE (time & temperature) between collection and RNA extraction
RNA EXTRACTION

SPIDIA-RNA EQAs: Model

1. Active involvement of high number of laboratories performing molecular methods from different European countries

with the support of the ***European Federation of Clinical Chemistry Laboratory Medicine***; www.efcclm.eu

2. Collection of information about areas of competence, facilities, expertise, accreditation of participating laboratories

about 50% were accredited laboratories for molecular diagnostics, within them about 25% were certified ISO15189

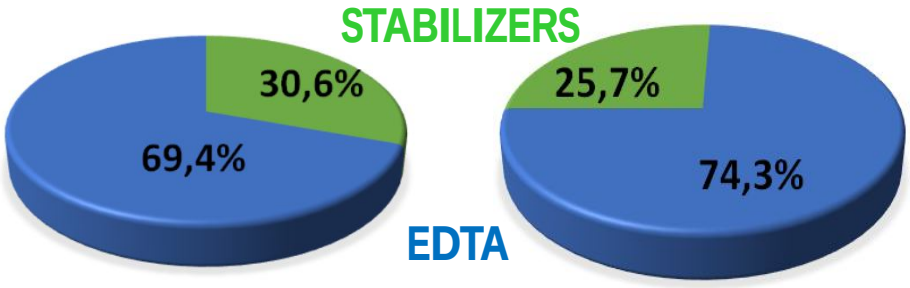
3. Programs: implementation of two External Quality Assessment (EQAs) focused on the evaluation of the pre-analytical phase of blood samples used for RNA based analyses

SPIDIA-RNA EQAs: Recruitment

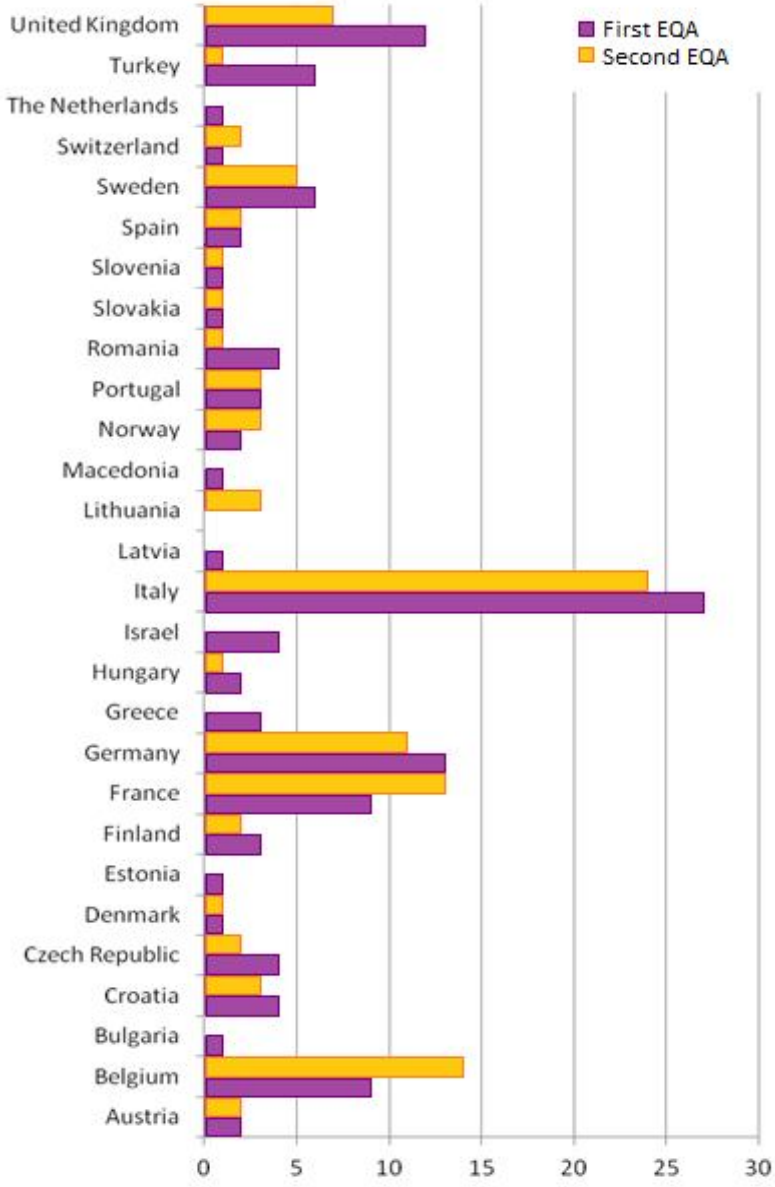
2 runs: First on May 2010
Second on May 2012

1st RNA EQA	
sent	back
102	93
91.2%	

2nd RNA EQA	
sent	back
119	109
91.5%	



Blood sample collection tube requested by the Participants



SPIDIA-RNA: Sample Challenge

1. only ***real*** blood samples were used to monitor the performance of the pre-analytical phase

2. appropriate precautions (time intervals, temperature, etc) were adopted for the collection and shipment

(due to the well known instability of some transcripts)

3. time-course experiments were implemented at SPIDIA facilities in order to **compare** the quality parameters of the participants (i.e. time zero (t0) of blood collection)

SPIDIA-RNA: EQA scheme

SPIDIA facilities

- Collection and shipping of ***real*** blood samples
- Shipping of the same blood samples to all participants, following selection of blood collection tube performed by laboratories

PARTICIPANT LABORATORIES

WHAT THEY HAVE DONE:

- They extracted RNA from blood samples
- They measured the concentration of extracted RNA
- They performed the RNA shipping to SPIDIA facility
- They filled the questionnaire
- They filled a «result form» (with details on storage conditions of the challenge blood samples plus details on their own reagents/procedures for RNA extraction)

WHAT THEY HAVE RECEIVED FROM SPIDIA:

- A detailed report of their performance
- Certificate of participation

SPIDIA

Standardization and Improvement of Generic Pre-analytical Tools and Procedures for In-Vitro Diagnostic

SEVENTH FRAMEWORK PROGRAMME

UNIVERSITÀ DEGLI STUDI FIRENZE

DIPARTIMENTO DI SCIENZE BIOMEDICHE SPERIMENTALI E CLINICHE

Certificate of Participation

This is to confirm that the
Laboratory **name laboratory**
Directed by **head of department**
Responsible Investigator: **responsible investigator**
city, **country**
has participated in the

SPIDIA-RNA Program 2nd RING TRIAL

Dr. Uwe Oelmueller
Coordinator of the SPIDIA Project

Prof. Mario Pazzagli
Leader of WP 1.2
Evidence-based Quality Guidelines for the pre-analytical phase of Blood Samples

Florence, 30th September 2012

SPIDIA-RNA EQAs scheme

SPIDIA facilities

- Send to all the participants two ***real*** blood samples:
  - PAXgene blood RNA tube™
  - K_2EDTA
- Blood was collected from several donors by pre-filled K_2EDTA bags under controlled temperature
- Blood was aliquoted immediately in:
  - empty tubes (K_2EDTA)
  - PAXgene blood RNA tube™
- Blood was shipped at controlled temperature (2-8°C) using dedicated shipping boxes.

Participant laboratories

- RNA had to be extracted:
  sample 1: immediately (24 h after blood collection)
  sample 2: 24h after sample 1 (48h after blood collection)
- RNA had to be sent back to SPIDIA facilities in dry-ice

Critical points of the SPIDIA-RNA EQAs model

- First approach to evaluate the performance of the pre-analytical phase by a specifically designed EQA
- Pan-European panorama (due to the high number of participating laboratories) about reagents and facilities used for the pre-analytical phase

– THE RESULTS CAN BE AFFECTED:
  - by post-analytical errors (mistakes performed by the participants filling the “result form”)
  - by the heterogeneity of the reagents used by the participants
  - by the technical skills of the personnel involved in the study

SPIDIA check to overcome the “laboratory” post-analytical error

Samples	A. Purity and Quantity of RNA A and RNA B	checking
RNA A RNA B		– Calculation of Purity and Quality values by using the raw data reported by each Lab – Check of the reported extraction and elution volumes according to the used extraction procedure

sample	260nm	280nm	320nm	Purity	Quantity (ng/µl blood)	Dilution factor	Extraction vol. (ul)	Elution vol. (ul)	Buffer
RNA A	0.051	0.025	0.001	2.083	0.600	1	5000	30	-
RNA B	0.114	0.055	0.000	2.073	1.368	1	5000	30	-

– 13 Labs reported discordant results with respect to the recalculated ones for Purity and/or Concentration in at least one sample (possible errors in reporting absorbance values and/or in dilution factors)

–25 Labs reported an extraction volume different from that suggested by the standard protocol of the kit

– 6 Labs reported both discrepancies

Calculation was performed as:
- Purity =A260/A280
- Quantity =(A260*40*dilution factor*elution volume)/extraction volume
For the lab that provided also the absorbance A320 we also computed:
- Purity =(A260-A320)/(A280-A320)
- Quantity =[(A260-A320)*40*dilution factor*elution volume]/extraction volume

SPIDIA check to overcome the “laboratory” post-analytical error

Samples	A. Purity and Quantity of RNA A and RNA B	checking
RNA A RNA B		– Calculation of Purity and … values by using the … reported by each … the reported … traction and elution volumes according to the used extraction procedure

sample	260nm	280nm	320nm	Purity	Quantity (ng/µl blood)	Dilution factor	Extraction vol. (ul)	Elution vol. (ul)	Buffer
RNA A	0.051	0.025	0.001	2.083	0.600	1	5000	30	
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Relevant role of Post-analytical phase

– 13 Labs reported dis… …ed ones for Purity and/or Concentration in at least one sample (possibl… …or in dilution factors)

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SPIDIA-RNA
External Quality Assessments: laboratory performance

Samples evaluation at SPIDIA facilities

1. RNA quality parameters

a. BY KNOWN QUALITY PARAMETERS

- **Quantity and Purity**
- **RNA integrity**

b. BY ADDITIONAL QUALITY PARAMETERS

- **Presence of qPCR interferences**
- **Evaluation of expression of selected «variable» genes** (the "unstable" ones were developed by SPIDIA WP1.3) (only in the 2nd EQA)
- **Presence of DNA contamination in RNA sample** (for investigational purpose)

2. Analytical test

c. BY SPECIFIC qPCR TESTINGS TO MIMIC THE ANAYTICAL PHASE

- **Evaluation of expression of selected genes**

Comparison to time zero (To) value in order to identify critical steps in the pre-analytical phase that can significantly affect the results

RNA QUALITY PARAMETERS

Known parameters

1. QUANTITY and PURITY by UV spectrophotometric measurements

2. Total RNA integrity by RIN (Bionalyzer 2100, Agilent Technologies)

Additional parameters

3. qPCR inteferences by Kineret software

4. DNA contamination by qPCR (RNase P – intron - single copy gene)

5. mRNA stability – expression profile of selected genes by RT-qPCR

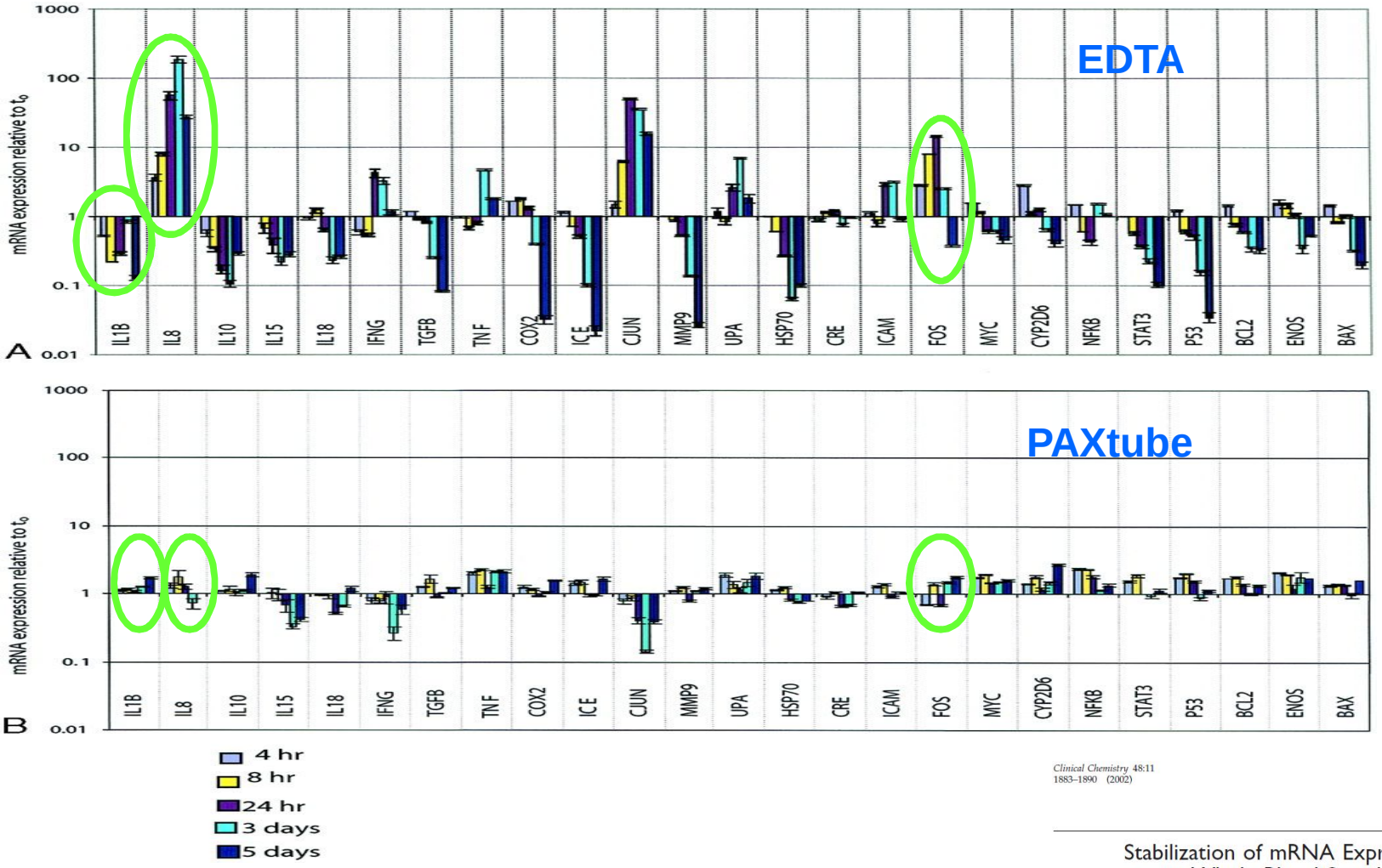
   - housekeeping: PPIB, GUSB (for relative quantification)
   - «unstable genes»: FOSB, TNFRS (by relative quantification)

Specific qPCR testings (to mimic the performance in RT-qPCR analytical assays)

6. mRNA expression profile of selected genes by RT-qPCR: **GAPDH, IL1β, IL8, C-FOS*** (by absolute quantification)

* (Rainer L. et al. Clin. Chem. 2001)

Specific qPCR testings: IL1β, IL8, C-FOS



Clinical Chemistry 48:11
1883–1890 (2002)

Molecular Diagnostics and Genetics

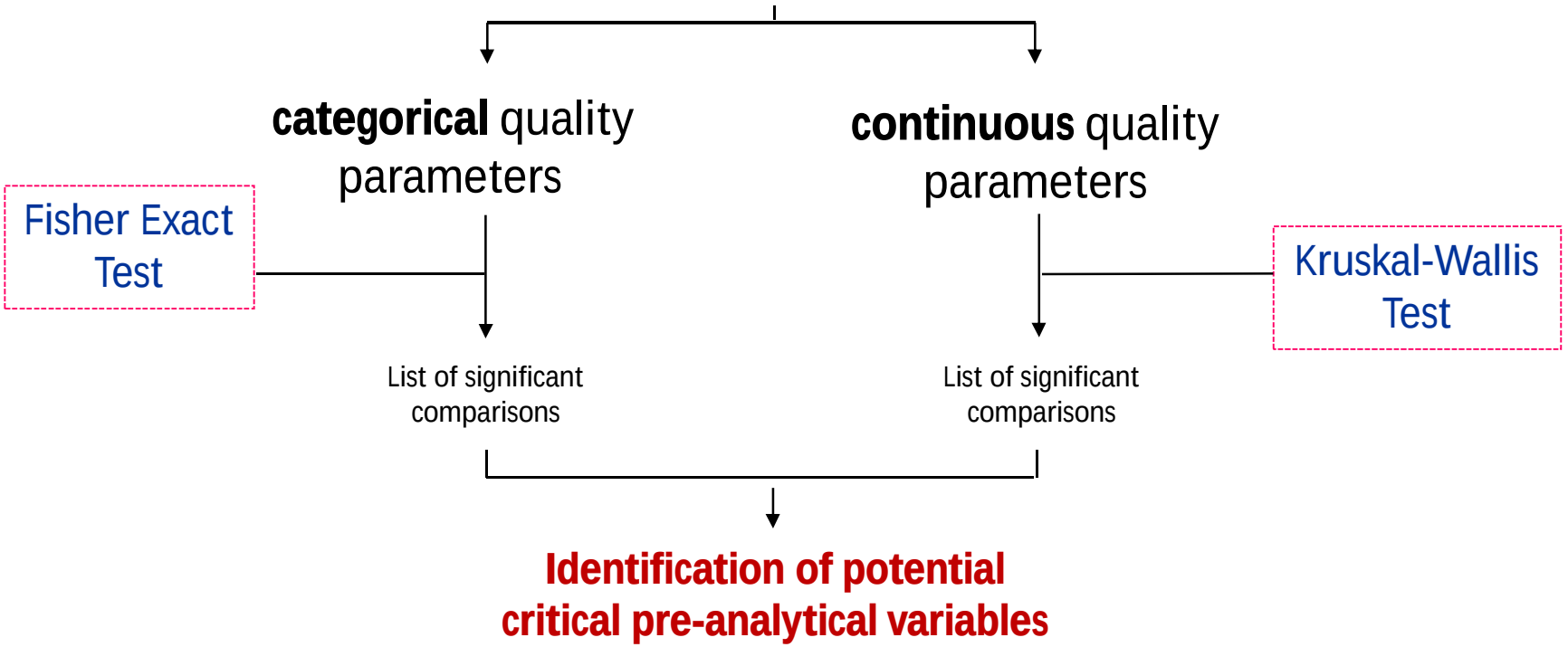
Stabilization of mRNA Expression in Whole Blood Samples

Lynne Rainen,[1*] Uwe Oelmueller,[2] Stewart Jurgensen,[3] Ralf Wyrich,[2] Cynthia Ballas,[1] Jim Schram,[3] Chris Herdman,[3] Danute Bankaitis-Davis,[4] Nancy Nicholls,[4] David Trollinger,[4] and Victor Tryon[4]

Samples evaluation at SPIDIA facilities statistical approach

IDENTIFICATION OF CRITICAL PRE-ANALYTICAL VARIABLES

Quality parameters vs pre-analytical factors



Agresti A. Categorical Data Analysis. 1990;
Hollander M, Wolfe DA. Nonparametric Statistical Methods, 1999

Samples evaluation at SPIDIA facilities
statistical approach

EVALUATION OF LABORATORY PERFORMANCE

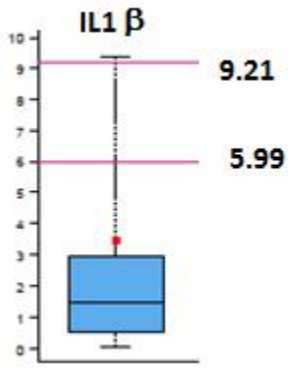
Laboratory specific report

Quality parameters

PCR kinetics interferences

↓

Kineret software



Quantity, purity, RIN and qPCR data

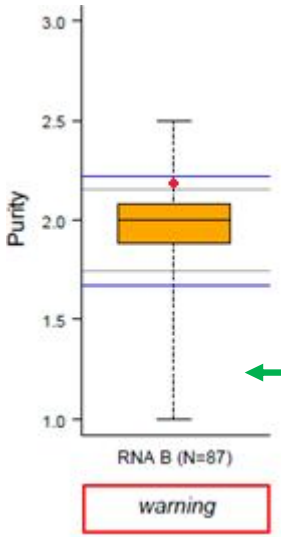
↓

1. outlier detection (M-statistic)
2. identification of specific bootstrap centiles from outlier-free distribution

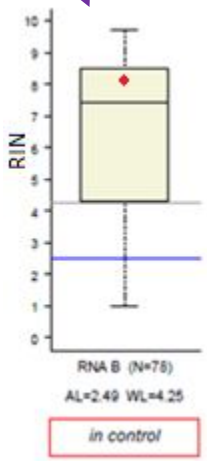
↓

bootstrap centile :

- 2.5th and 97.5th → two sided Action Limit (ALs)
- 10th and 90th → two sided Warning Limit (WLs)
- 5th and 20th → one sided AL and WL



Lower AL=1.66; Upper AL=2.22
Lower WL=1.74; Upper WL=2.15



strong outlier

weak outlier

in control

evaluation of laboratory performance

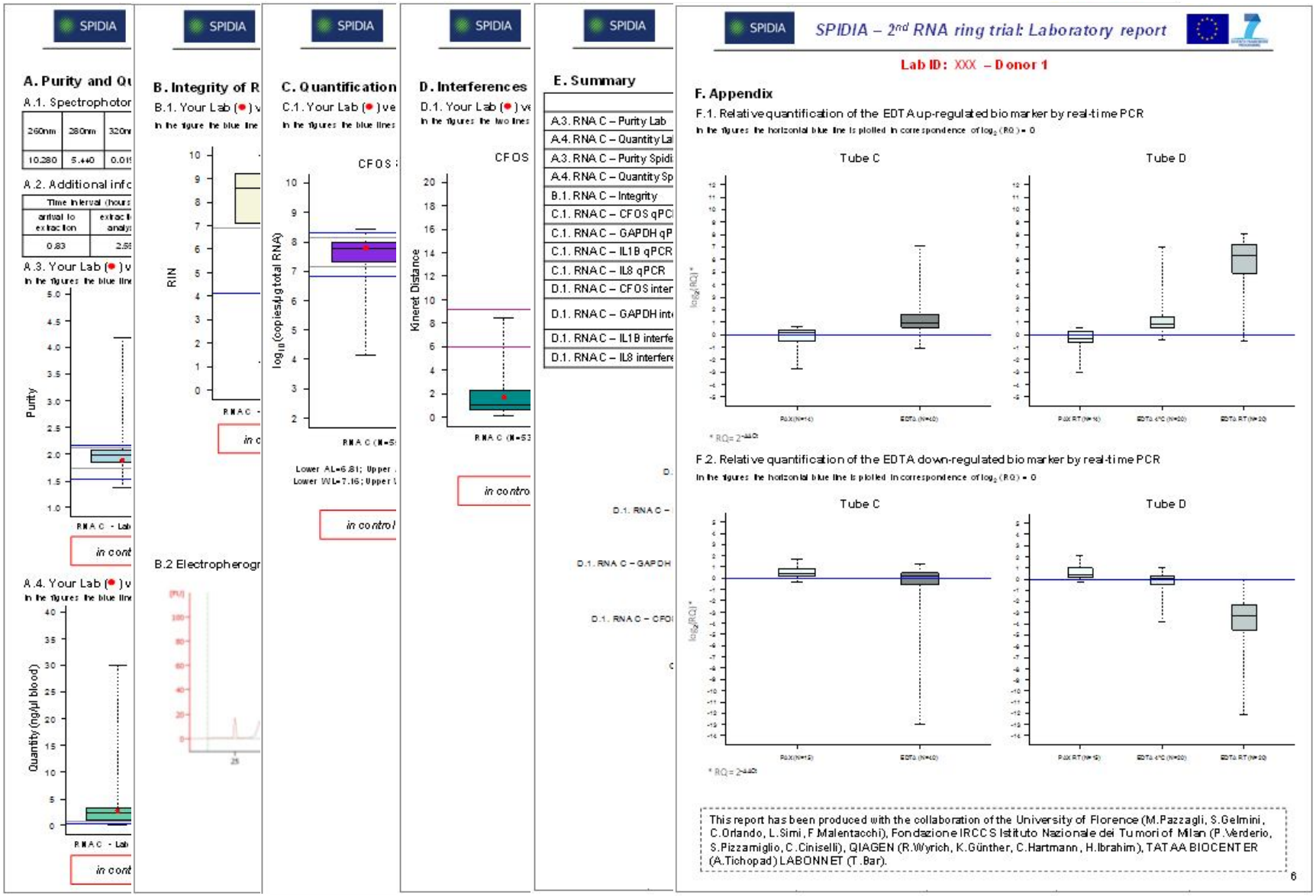
out of control

warning

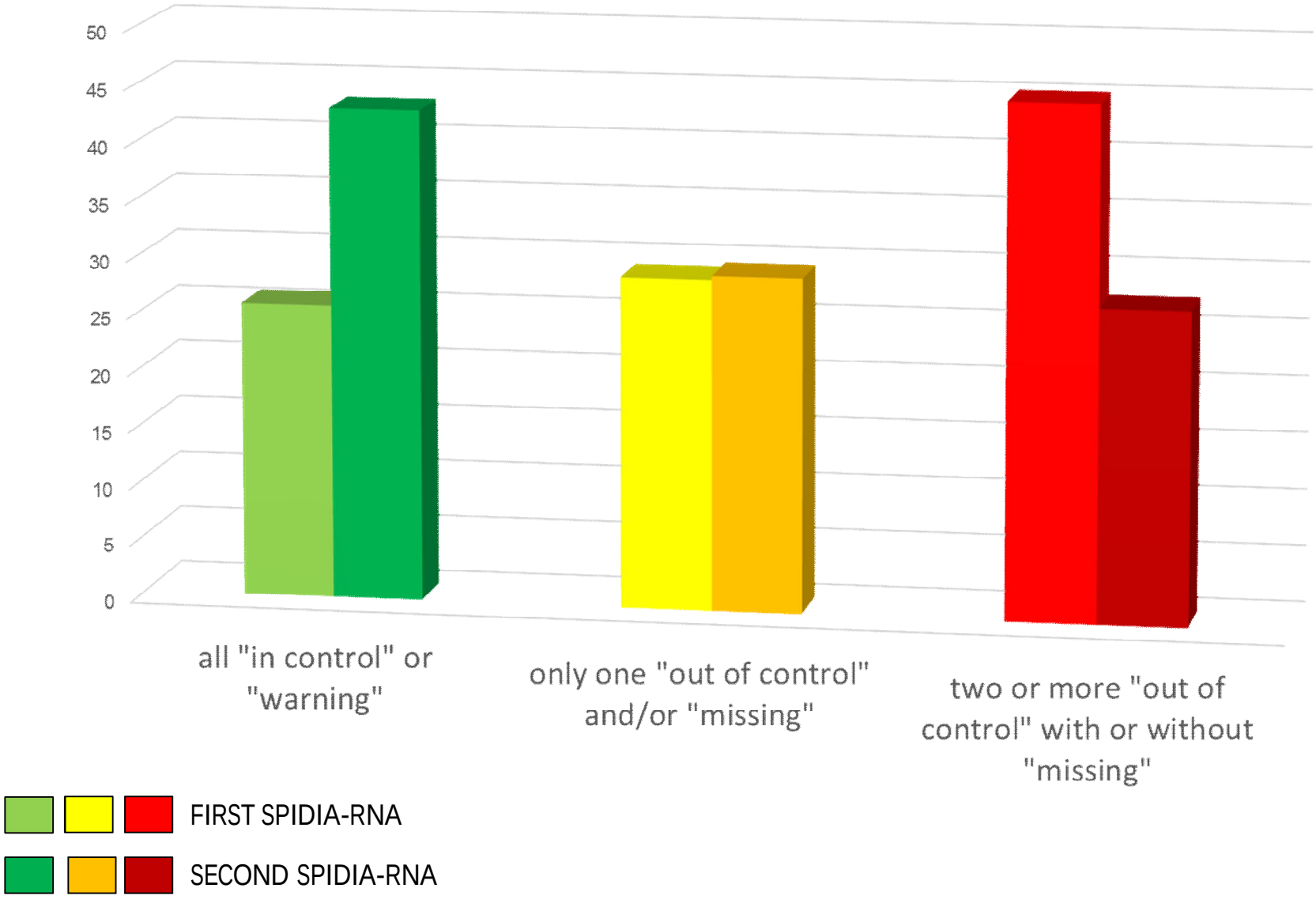
in control

Pazzagli M et al. Methods 2013; 59:20-31;
Malentacchi F et al. Clin Chim Acta. 2013;424:274-86;
Tichopad A et al. Methods. 2010;50:308-12

Samples evaluation at SPIDIA facilities laboratory specific report

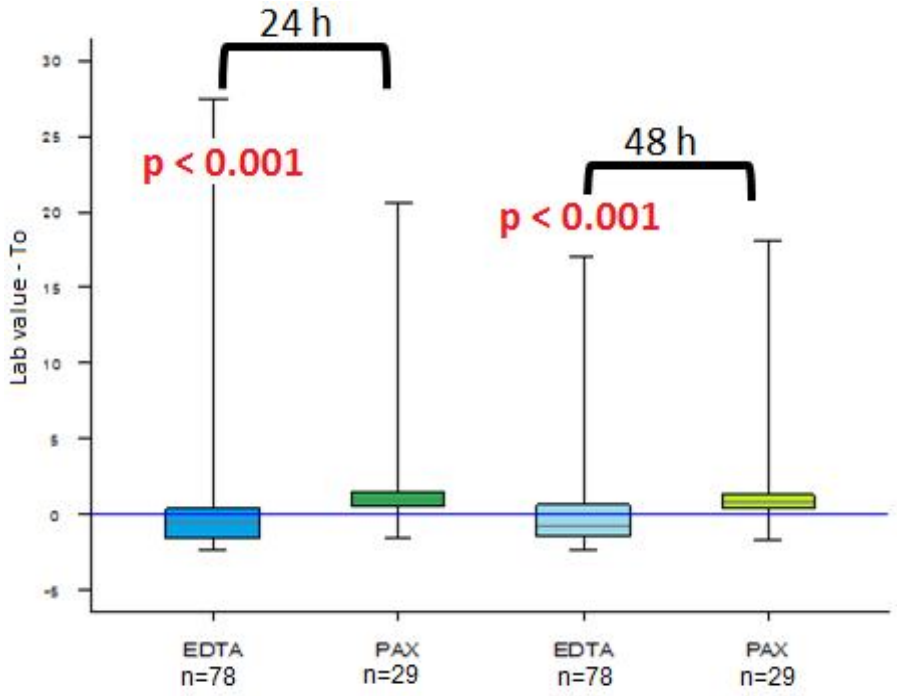


Samples evaluation at SPIDIA facilities overall performance



SPIDIA-RNA
External Quality Assessments: pre-analytical variables and RNA quality parameters

RNA quality parameters
mRNA quantity (known parameters)



BLOOD COLLECTION TUBE AND BLOOD STORAGE CONDITION

Stabilizer versus unstabilizer

Time storage: 24h versus 48h

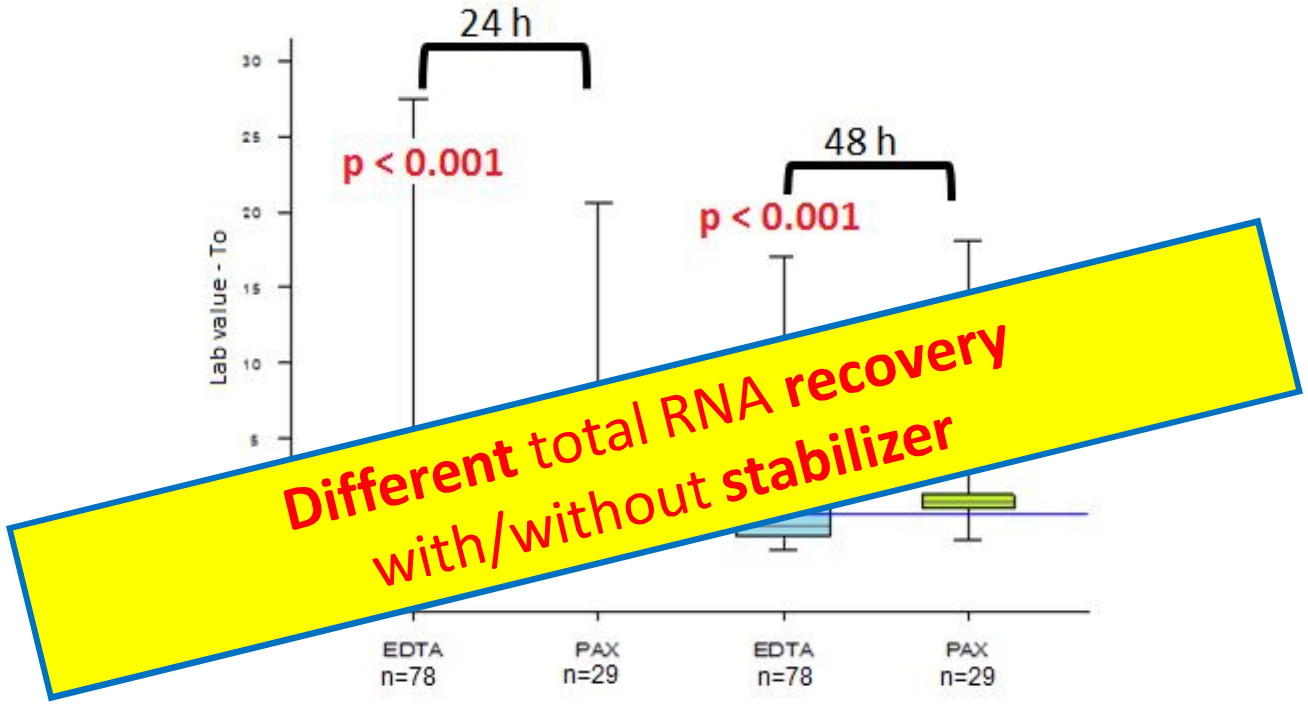
Different recovery depending on presence of stabilizer

Samples extracted within 24h or at 48h after blood collection

To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit

Lab value, To = (ng/µl blood) by Kruskal-Wallis Test, normalised to T0

RNA quality parameters
mRNA quantity (known parameters)



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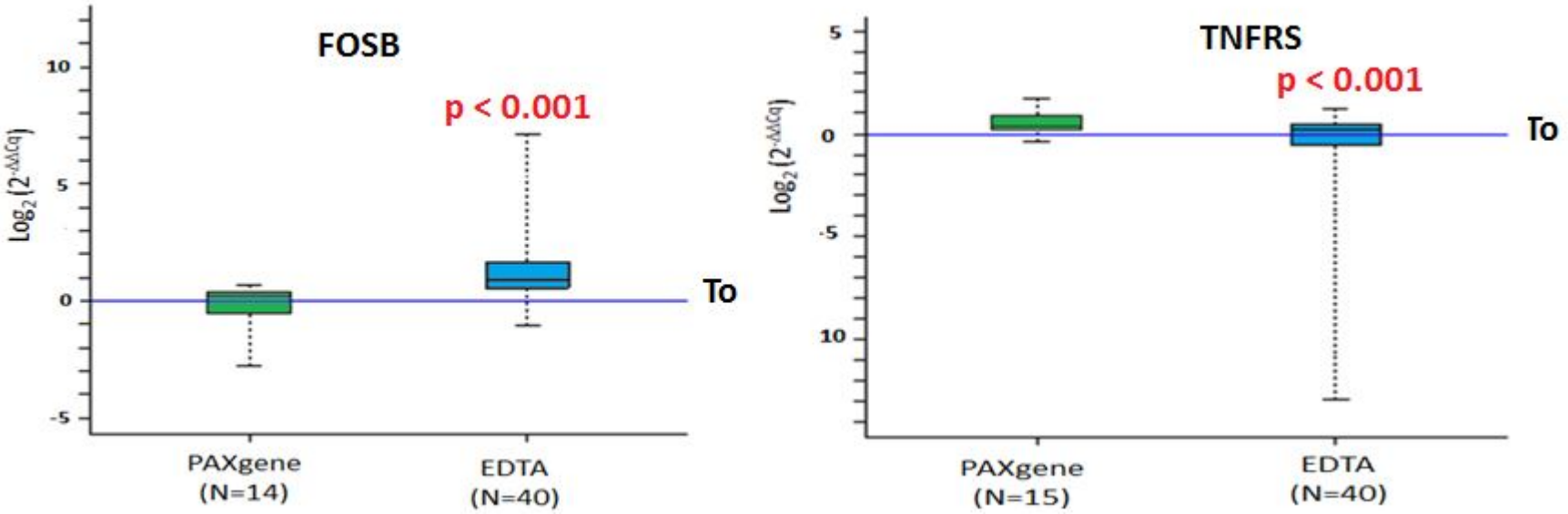
Lab value, To = (ng/µl blood) by Kruskal-Wallis Test, normalised to T0

RNA quality parameters

mRNA stability by selected «unstable genes» (additional parameters)



BLOOD COLLECTION TUBE

Stabilizer versus unstabilizer

Changes in gene expression in EDTA tube (compare to To) ⇒
Influence of the stabilizer

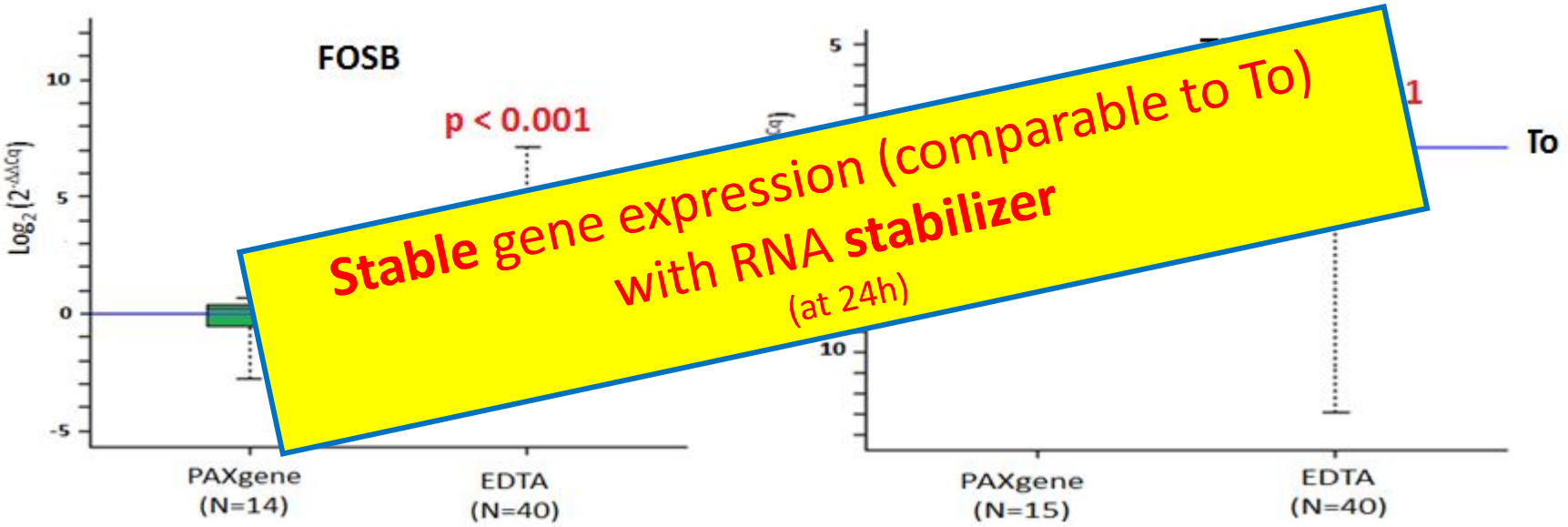
Samples extracted up to 24h after blood collection

To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit

$\Delta\Delta Cq = \Delta Cq_{\text{sample 24h}} - \Delta Cq_{To}$; $\Delta Cq = Cq_{\text{target gene}} - Cq_{\text{(gemetric mean housekeepings)}}$ by Kruskal-Wallis Test, normalised to T0

RNA quality parameters

mRNA stability by selected «unstable genes» (additional parameters)



BLOOD COLLECTION TUBE

Stabilizer versus unstabilizer

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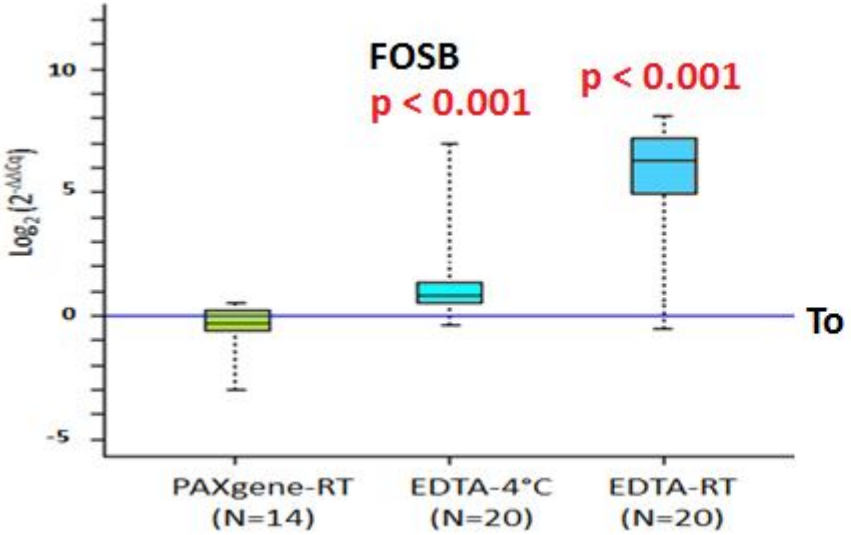
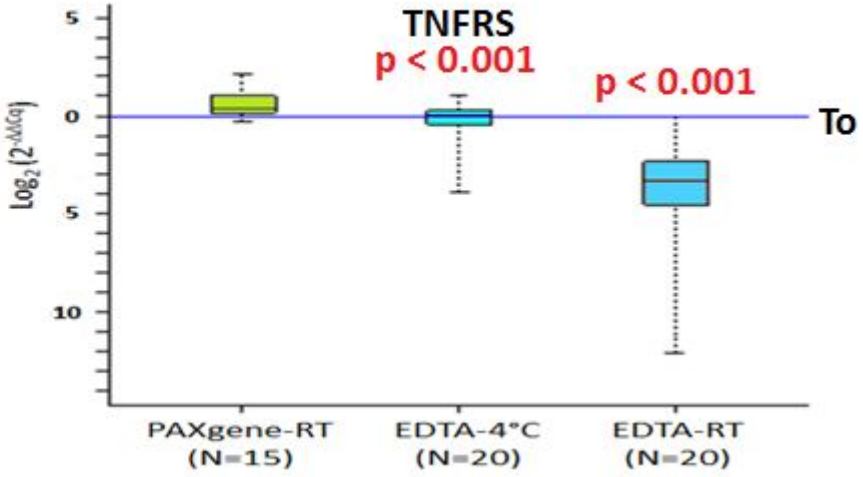




BLOOD COLLECTION TUBE AND BLOOD STORAGE CONDITION

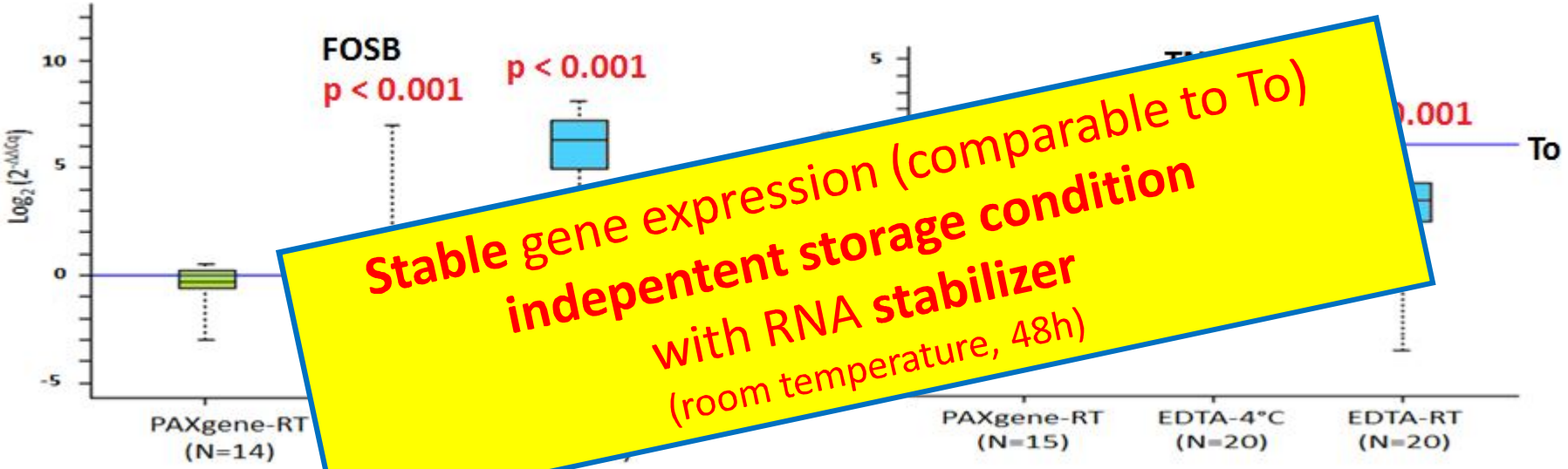
Stabilizer at Room Temperature versus unstabilizer at 4°C
Stabilizer at Room Temperature versus unstabilizer at Room Temperature
Unstabilizer at Room Temperature versus unstabilizer at 4°C

Changes in gene expression in EDTA tube indipendently of temperature storage (compare to To) ⇒ Influence of the stabilizer

Samples extracted at 48h after blood collection
To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit
$\Delta\Delta Cq = \Delta Cq_{\text{sample 24h}} - \Delta Cq_{To}$; $\Delta Cq = Cq_{\text{target gene}} - Cq_{\text{(gemetric mean housekeepings)}}$ by Kruskal-Wallis Test, normalised to T0

RNA quality parameters

mRNA stability by selected «unstable genes» (additional parameters)



Stable gene expression (comparable to To)
indepentent storage condition
with RNA **stabilizer**
(room temperature, 48h)

BLOOD COLLECTION TUBE AND BLOOD STORAGE CONDITION

Stabilizer at Room Temperature versus unstabilizer at 4°C
Stabilizer at Room Temperature versus unstabilizer at Room Temperature
Unstabilizer at Room Temperature versus unstabilizer at 4°C

Changes in gene expression in EDTA tube indipendently of temperature storage (compare to To) ⇒ Influence of the stabilizer

Samples extracted at 48h after blood collection
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Other additional parameters

DNA contamination on RNA samples

"genomic DNA (gDNA) contamination is an inherent problem during RNA purification that can lead to non-specific amplification and aberrant results in reverse transcription quantitative PCR (RT-qPCR)…Since gDNA contamination levels are frequently not uniform between samples …mainly affected if the qPCR assays can not be design spanning exons."

(Correction of RT-qPCR data for genomic DNA-derived signals with ValidPrime, Laurel at al. NAR 2012)

Samples evaluation at SPIDIA facilities

DNase treatment in RNA samples extracted from participants

...from the «result form»

DNase treatment		
	Overall evaluation	
	N. lab	Percentage
Yes	30*	27.8%
No	78	72.2%
Missing	1	
Total	109	100%

DNAse treatment performed by all the PAXgene RNA extraction kit

* Including 24 using PAXgene RNA extraction kit

Samples evaluation at SPIDIA facilities

DNase treatment in RNA samples extracted from participants

...from the «result form»

DNase treatment		
		Percentage
Y	30*	**27.8%**
N	78	**72.2%**
Missing	1	
Total	109	**100%**

Few laboratories perform the DNase treatment

DNAse treatment performed by all the PAXgene RNA extraction kit

* Including 24 using PAXgene RNA extraction kit

Samples evaluation at SPIDIA facilities

DNA contamination in RNA samples extracted from participants

DNA contamination in RNA samples*		
	N. sample	Percentage
no DNA contamination	54	**24.8%**
Low (<10 copies/ng RNA)	16	**7.3%**
Medium (10<copies/ng RNA <1000)	124	**56.9%**
High (>1000 copies/ng RNA)	24	**11.0%**
total	218	**100%**

...addictional quality parameters by qPCR (RNase P – intron - single copy gene)

* All the RNA samples were analysed (RNA C and RNA D = 218 samples)

Samples evaluation at SPIDIA facilities

DNA contamination in RNA samples extracted from participants

DNA contamination in RNA samples*		
	N. sample	Percentage
no DNA contamination	54	**24.8%**
Low (<10 copies/ng RNA)	16	
Medium (10<co...	124	**56.9%**
High (>1000 copies/ng RNA)	24	**11.0%**
total	218	**100%**

Presence of DNA in RNA samples

...addictional quality parameters by qPCR (RNase P – intron - single copy gene)

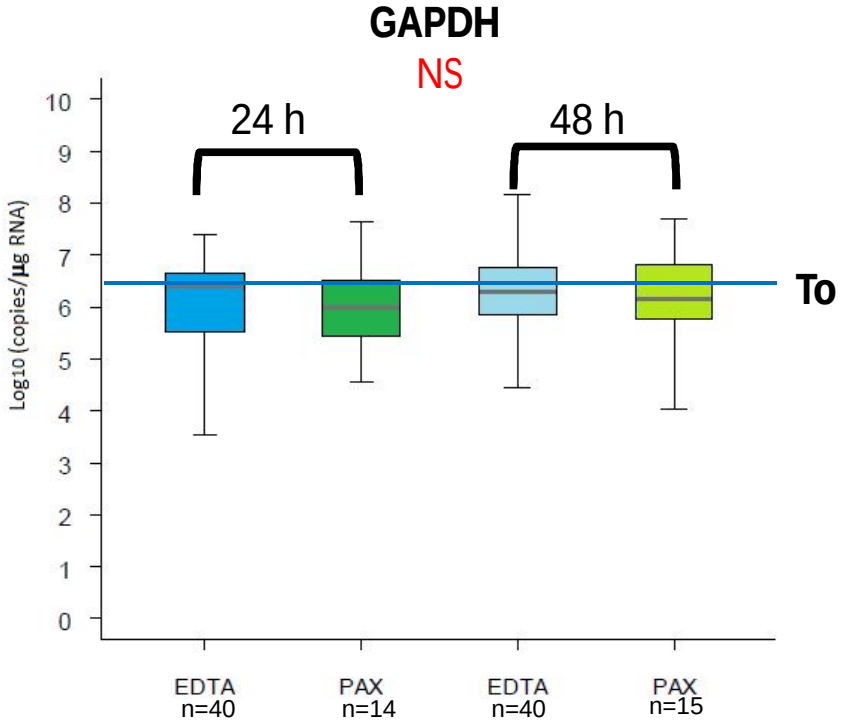
* All the RNA samples were analysed (RNA C and RNA D = 218 samples)

SPIDIA-RNA
External Quality Assessments: simulation of analytical testing
Do the pre-analytical variables influence a RT-qPCR based assay?

Expression of GAPDH and IL8 by RT-qPCR

Advanced in qPCR & dPCR, Barcelona, 14 May 2014

RNA quality parameters
Blood collection tubes and storage condition



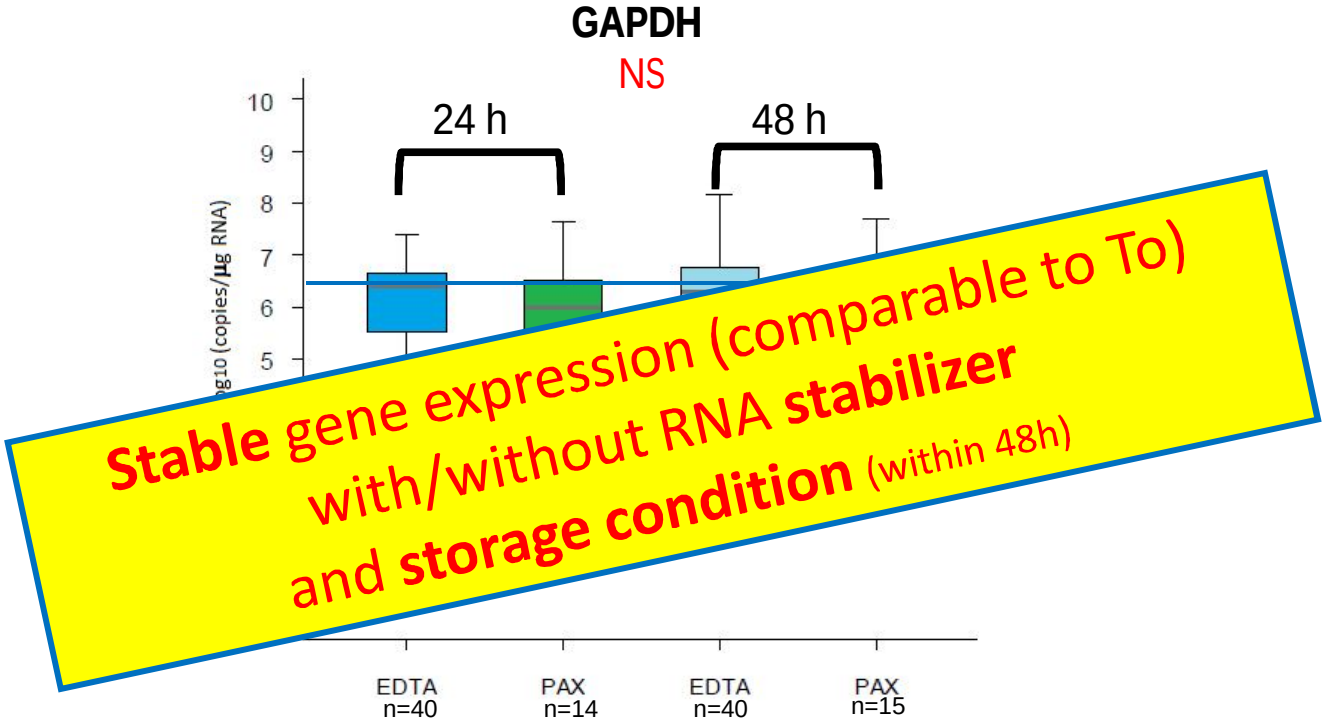
All the samples had gene expression close to To

No significant changes in the results of RT-qPCR-based analytical test

Samples extracted within 24h or at 48h after blood collection
To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit

by Kruskal-Wallis Test, normalised to T0

RNA quality parameters
Blood collection tubes and storage condition



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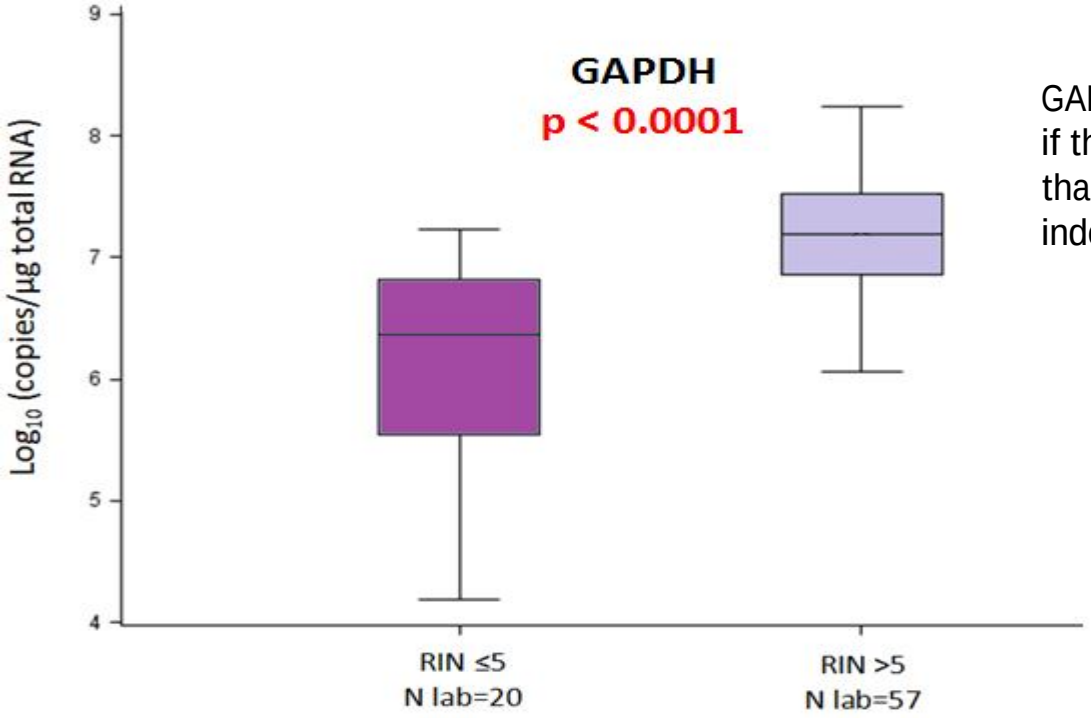
RNA quality parameters
RNA integrity



GAPDH levels are 10 fold higher if the RNA is "intact" (RIN > 5) than is fragmented (RIN <5) independently of time storage

Different gene expression depenfing on the integrity of RNA

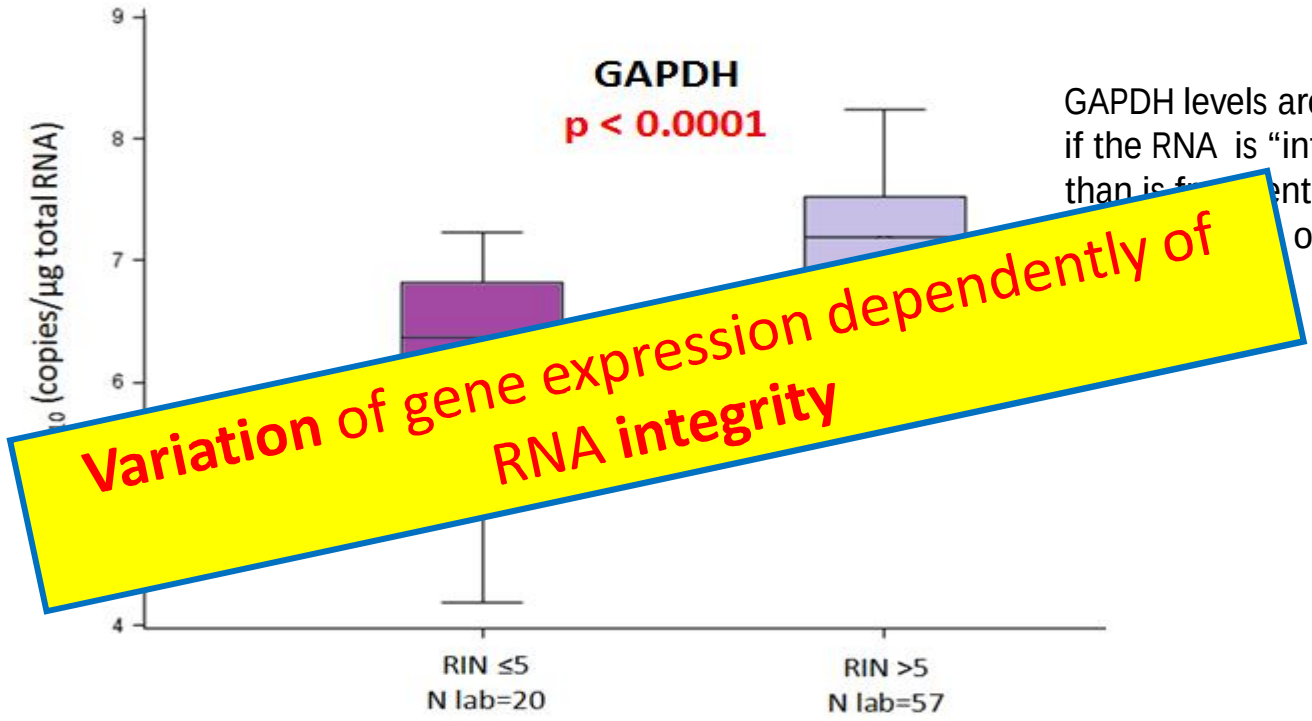
The integrity of RNA (by RIN, cutoff =5) can influence by the results of RT-qPCR-based analytical test

RIN cutoff = 5*

*S. Fleige,et al. Biotechnol. Let 28 (2006) 1601-13

by Kruskal-Wallis Test

RNA quality parameters
RNA integrity



GAPDH levels are 10 fold higher if the RNA is "intact" (RIN > 5) than is f…ented (RIN <5) … of time storage

Variation of gene expression dependently of RNA **integrity**

Different gene expression depenfing on the integrity of RNA

The integrity of RNA (by RIN, cutoff =5) can influence by the results of RT-qPCR-based analytical test

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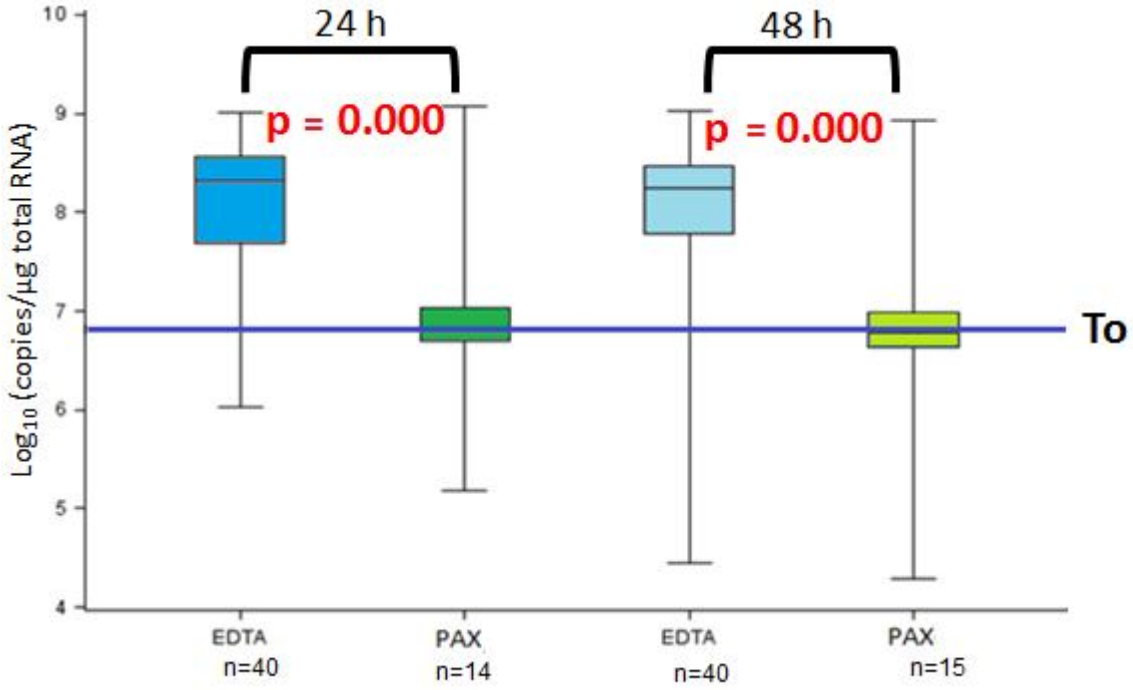
Influence on RT-qPCR based assay
Blood collection tubes and storage condition

IL 8



IL8 levels are 10 fold higher than those measured at To when the blood sample is collected without stabilizer and stored at 24 h/48 h

The presence of stabilizer in the blood collection tube maintains the gene expression close to To

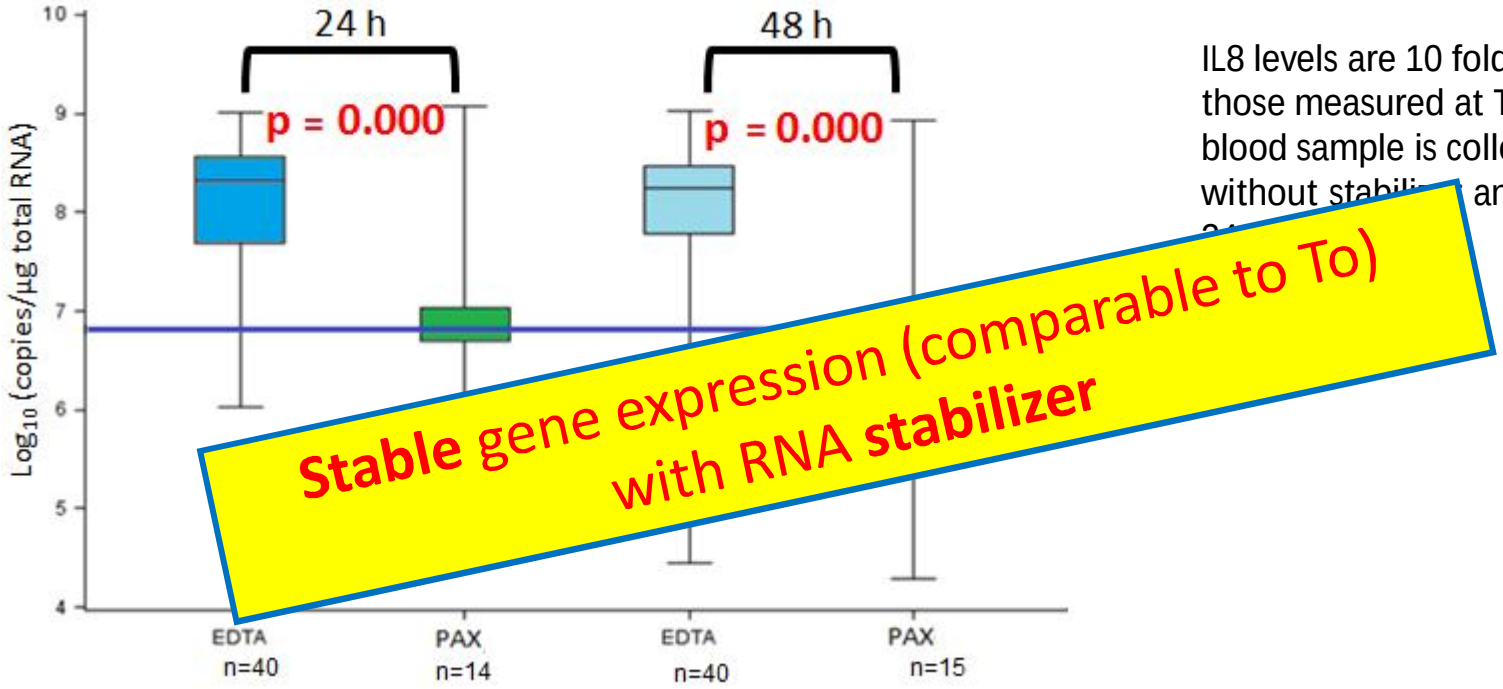
The absence of stabilizer in the blood collection tube and its time storage condition can influence the results of RT-qPCR-based analytical test

Samples extracted within 24h or at 48h after blood collection
To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit

by Kruskal-Wallis Test

Influence on RT-qPCR based assay
Blood collection tubes and storage condition

IL 8



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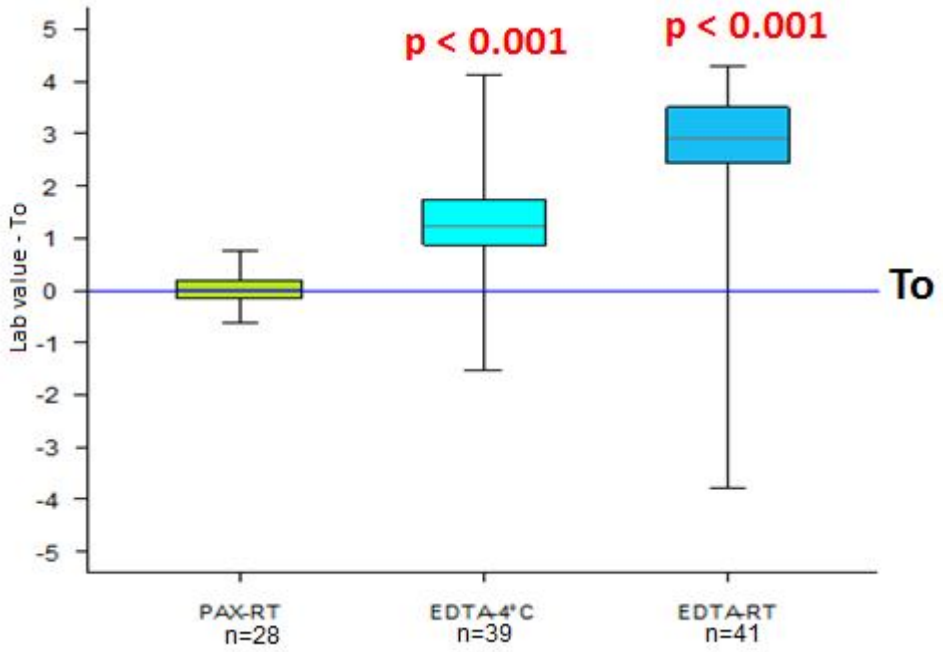
Influence on RT-qPCR based assay
Blood collection tubes and storage condition
IL 8



IL8 levels are 10-1000 fold higher than those measured at T0 when the blood sample is collected without stabilizer and stored at +4°C/RT for 48 h

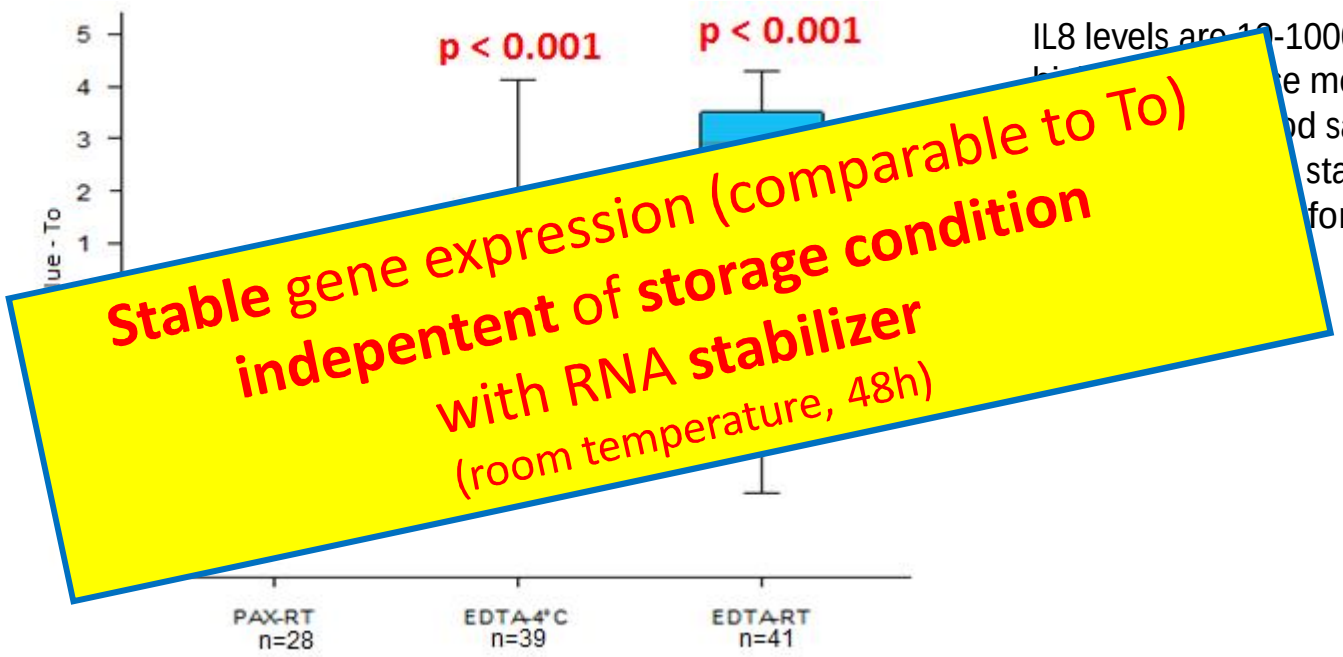
The presence of stabilizer in the blood collection tube maintains the gene expression close to To

The absence of stabilizer in the blood collection tube and its temperature storage condition can influence the results of RT-qPCR-based analytical test

Samples extracted within 24h or at 48h after blood collection
To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit
Lab value, To = $\log_{10}$(copies/µg RNA)

by Kruskal-Wallis Test, normalised to T0

Influence on RT-qPCR based assay
Blood collection tubes and storage condition
IL 8



IL8 levels are 10-1000 fold
e measured at
od sample is
stabilizer and
for 48 h

Stable gene expression (comparable to To)
indepentent of **storage condition**
with RNA **stabilizer**
(room temperature, 48h)

The presence of stabilizer in the blood collection tube maintains the gene expression close to To

The absence of stabilizer in the blood collection tube and its temperature storage condition can influence the results of RT-qPCR-based analytical test

Samples extracted within 24h or at 48h after blood collection
To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit
Lab value, To = $\log_{10}$(copies/µg RNA)

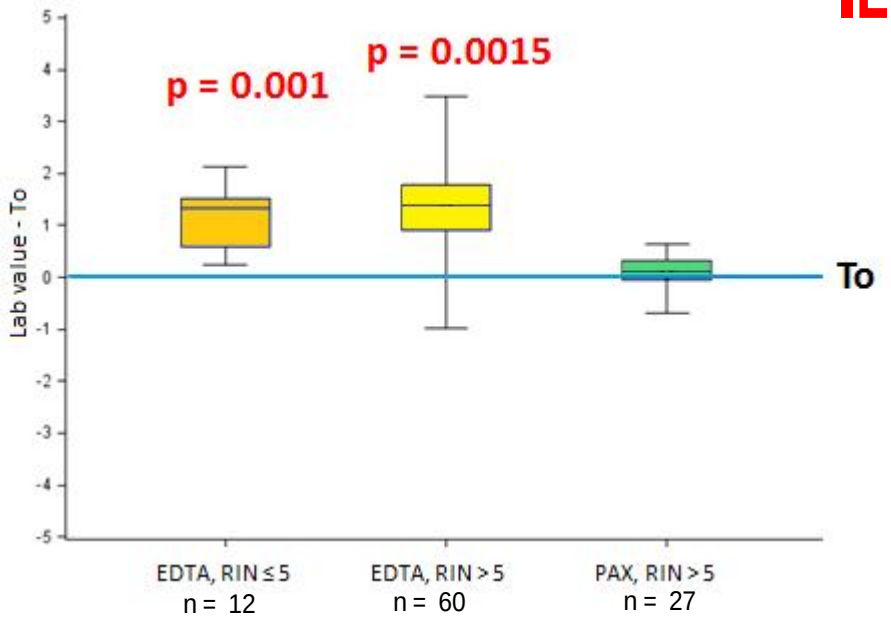
by Kruskal-Wallis Test, normalised to T0

Influence on RT-qPCR based assay

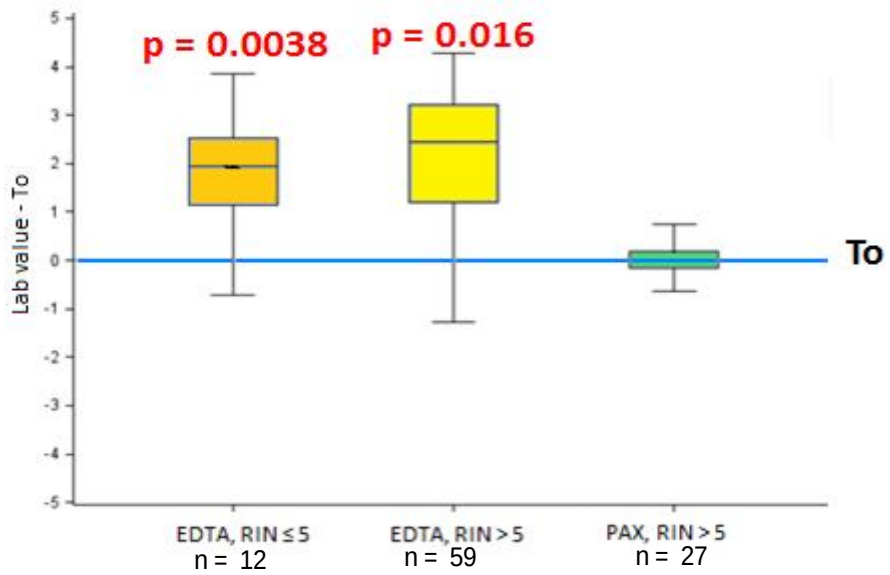
RIN

IL 8

Sample extracted at 24h



Sample extracted at 48h



IL8 RNA levels measured in EDTA blood collection tubes are 10-100 fold higher than those measured at T0 independently by the RIN value

Different values depending on the RIN value

The presence of stabilizer in the blood collection tube maintains the RNA integrity (more than 5)

The absence of stabilizer in the blood collection tube and its temperature storage condition can influence the results of RT-qPCR-based analytical test

To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit

Lab value, To = $\log_{10}$(copies/µg RNA)

by Kruskal-Wallis Test, normalised to T0

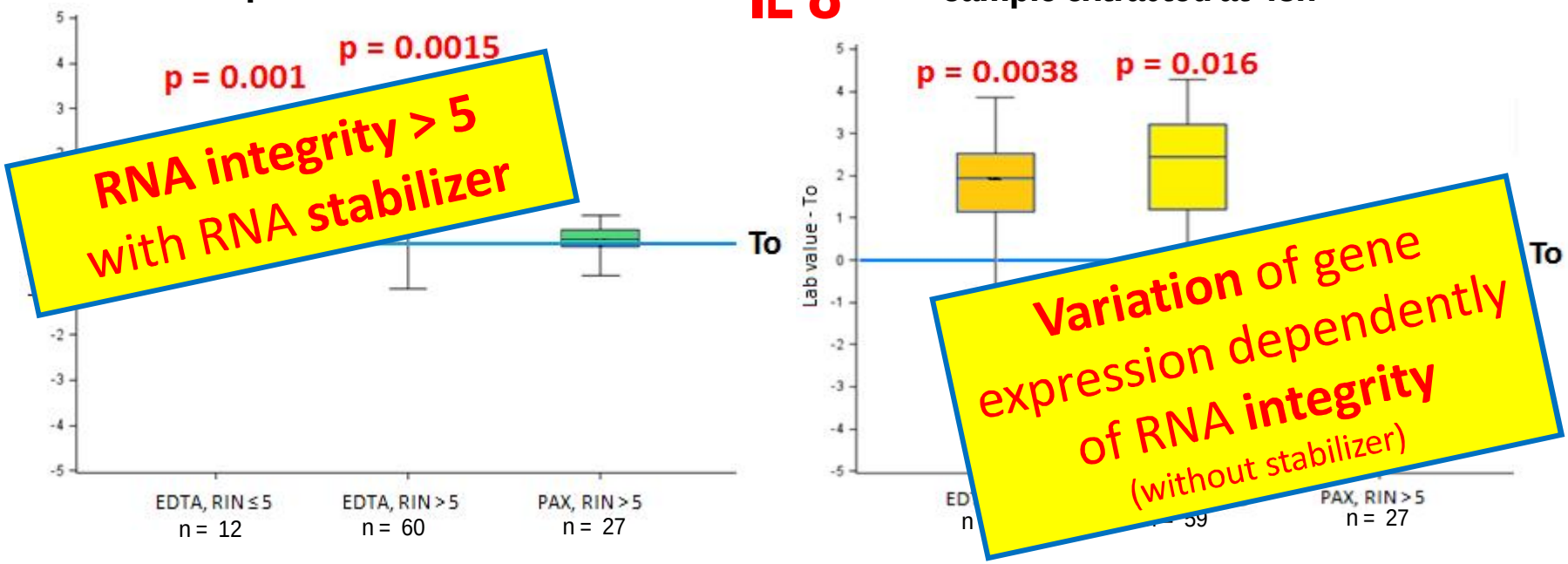
Influence on RT-qPCR based assay

RIN

IL 8

Sample extracted at 24h

Sample extracted at 48h



RNA integrity > 5 with RNA stabilizer

Variation of gene expression dependently of RNA **integrity** (without stabilizer)

IL8 RNA levels measured in EDTA blood collection tubes are 10-100 fold higher than those measured at T0 independently by the RIN value

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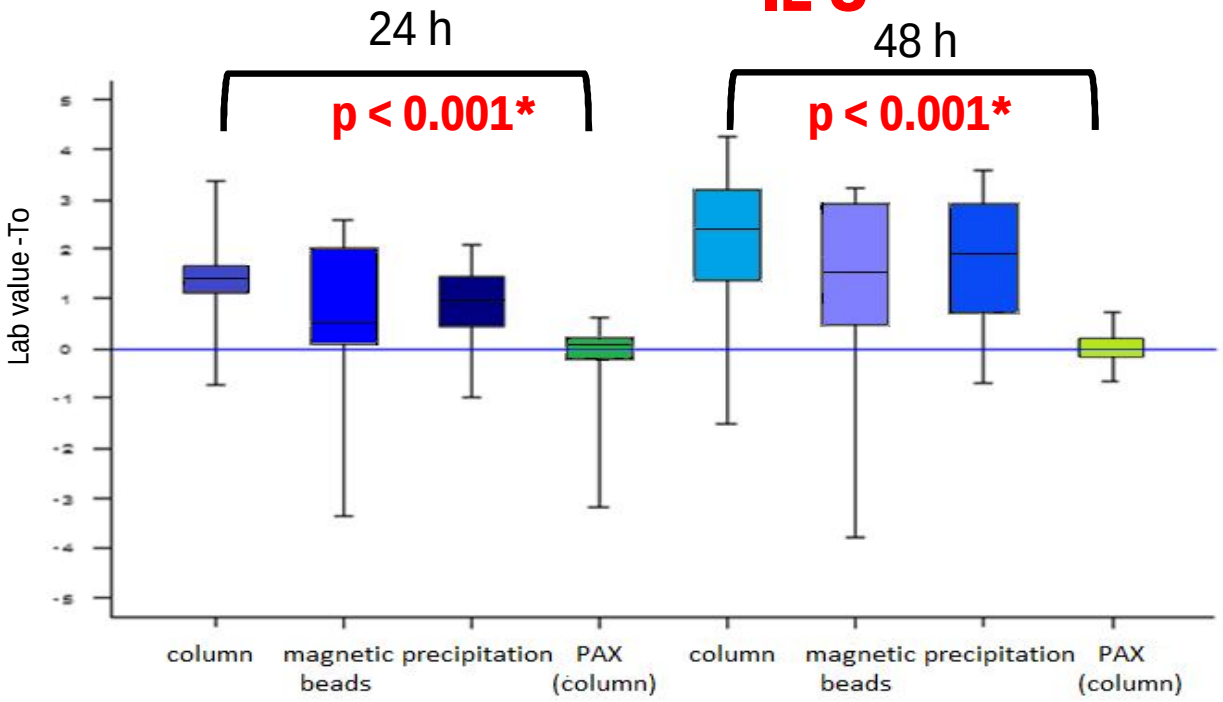
by Kruskal-Wallis Test, normalised to T0

Influence on RT-qPCR based assay

Extraction methods

IL 8



IL8 RNA levels are mainly influence by presence of RNA stabilizer rather than extraction methods

* Referred to the comparison between PAX and each of the other thre methods (from EDTA)

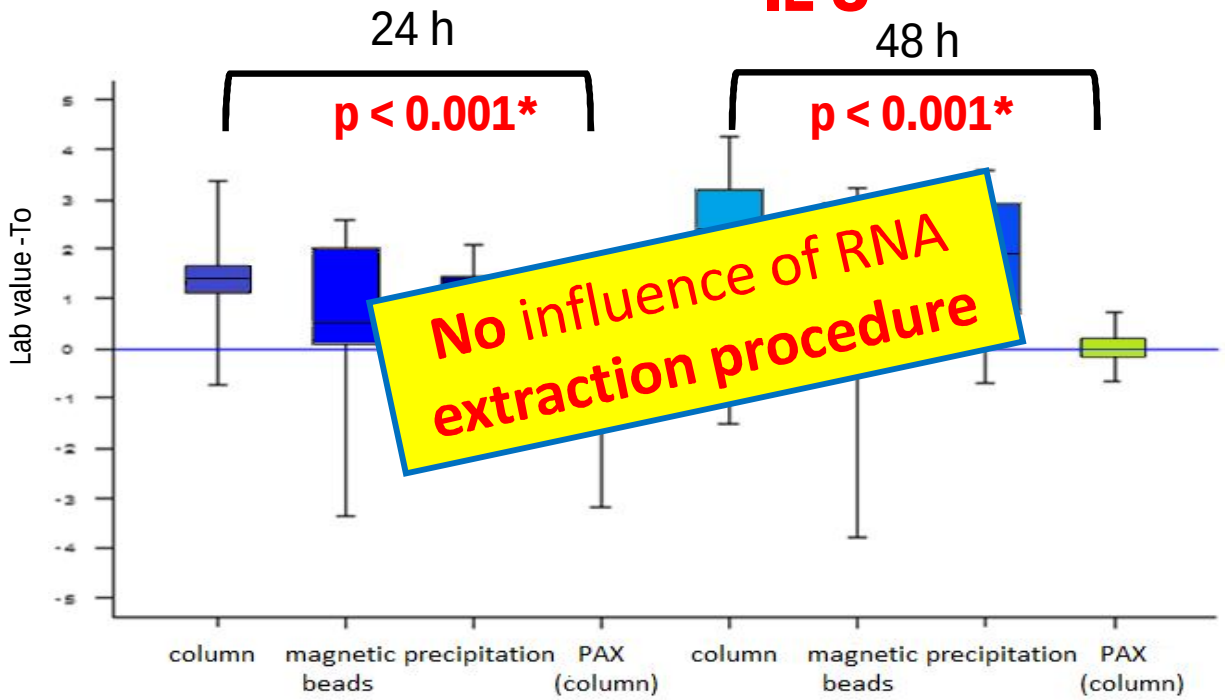
The presence of stabilizer in the blood collection tube maintains the mRNA expression close to To, within the RNA extracted from blood collected without stabilizer no significant differences were observed

The extraction method itself doesn't influence the results of RT-qPCR-based analytical test

To (Time zero) blood collected in PAXgene blood RNA tube and immediately extracted by PAXgene blood RNA kit
Lab value, To = $\log_{10}$(copies/µg RNA)

Influence on RT-qPCR based assay
Extraction methods

IL 8



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SPIDIA-RNA EQAs: tools for evidence-based guidelines

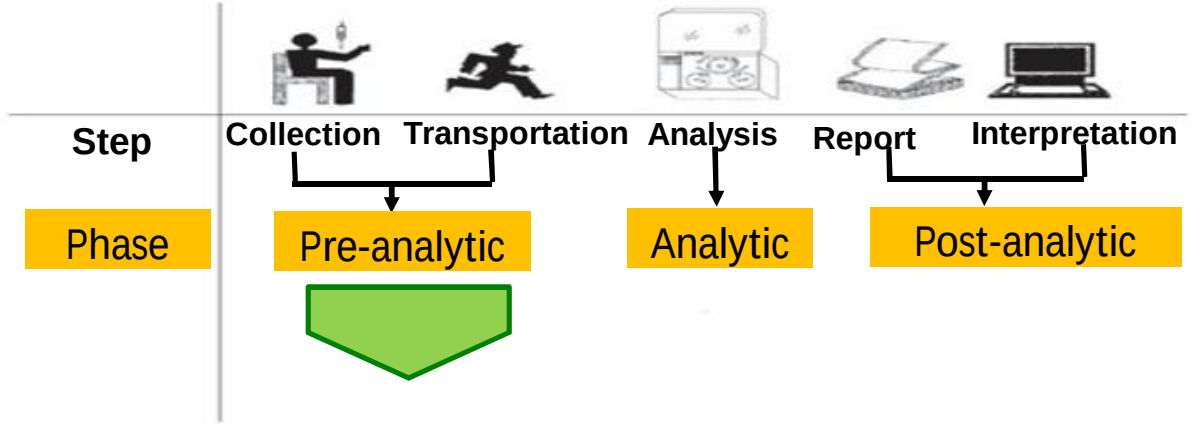
STATEMENT:

- Gene expression in BLOOD SAMPLES may be affected by several factors that can induce or repress gene expression or lead to degradation of RNA if it is not handled properly

EQAs evidence-based results:

- Most of the laboratories do not take care of the use and importance of DNase treatment
- The presence/absence of a RNA stabilizer in the blood collection tube influences the evaluation of mRNA profile
- The presence/absence of a RNA stabilizer in the blood collection tube influences the integrity of RNA
- The used of blood collection tube containing stabilizer allows to preserve gene expression and RNA integrity, maintaining the gene expression and the RNA integrity close to the profile of the patient (at the moment of blood collection)
- Participate to External Quality Assessment Programmes

SPIDIA-RNA EQAs: Purposes



SPIDIA-RNA EQAs

- BLOOD COLLECTION (collection device, identification)
- BLOOD STORAGE (time & temperature, treatment)
- BLOOD SHIPPING (transportation)
- RNA EXTRACTION

BLOOD COLLECTION TUBE
BLOOD STORAGE (time & temperature) between collection and RNA extraction
RNA EXTRACTION

SPIDIA-RNA EQAs: Purposes

BLOOD COLLECTION TUBE
BLOOD STORAGE (time & temperature) between collec ion
RNA EXTRACTION

Take home message

BLOOD COLLECTION TUBE

The use of blood collection tube containing a RNA STABILIZER is RECOMMENDED

BLOOD STORAGE - TIME

1) Blood collection tube with a RNA stabilizer: MANIFACTURER'S INSTRUCTIONS
2) Blood collection tube without a RNA stabilizer: IMMEDIATELY*

BLOOD STORAGE - TEMPERATURE

1) Blood collection tube with a RNA stabilizer: MANIFACTURER'S INSTRUCTIONS (room temperature)
2) Blood collection tube without a RNA stabilizer: IMMEDIATELY*

RNA EXTRACTION

Treatment with DNase

SPIDIA-RNA EQAs: Purposes

BLOOD COLLECTION TUBE
BLOOD STORAGE (time & temperature) between collectio[illegible]on
RNA EXTRACTION

Take home message

BLOOD STORAGE - TIME

1) Blood collection tube with a RNA stabilizer: MANIFACTURER'S INTRU[illegible]ION
2) Blood collection tube without a RNA stabilizer: IMMEDIATELY*

BLOOD STORAGE - TEMPERATURE

1) Blood collection tube with a RNA stabilizer: MANIFACTURER'S INTRUCTION (room temperature)
2) Blood collection tube without a RNA stabilizer: IMMEDIATELY*

IMMEDIATELY* It is necessary to perform VALIDATION STUDIES to monitor and verify the RNA QUALITY of the TARGET GENE(s) depending on "TIME & TEMPERATURE" STORAGE CONDITION

SPIDIA results have been provided to CEN*/TC 140 WG3 "*In vitro Diagnostic Medical Devices*" as an input and a potential basis for a technical work on European Standards.

A Technical Specification (TS) Document:

Molecular in-vitro diagnostic examinations — Specifications for pre-examination processes for blood — Cellular RNA

is under development and it should be released within the end of the 2014

(* CEN = European Committee for Standardization)

Acknowledgements

SPIDIA WP 1.2 partners:

- **QIAGEN**
- **UNIFI**
- **IRCCS Milano**
- **TATAA biocenter**
- **Labonnet**

SPIDIA-RNA participants (202 laboratories)

SPIDIA WP 1.3

SPIDIA team

UNIFI team

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thanks