ISBER Biospecimen Science Working Group Update: From *in silico* biospecimen research to external quality assessment

Fay BETSOU

BRN Symposium, Washington

24-25th February 2012

2010-2011 objectives

- Review of ISBER BPs QA QC section
- SPREC implementation in databases
- QC tool identification through data mining
- Study of robustness and reproducibility of RT RNA storage
- Study of robustness and reproducibility of viable cell shipping at frozen or RT
- Development of a Proficiency Testing program

Review of ISBER Best Practices 3rd edition

Quality Management Section

ISBER Best Practices 3rd edition
Submitted for publication

SPREC implementation

- Prostate Cancer Bioresource, Australia
- BioBIM, San Raffaele, Italy → **SPRECware tool**: SPREC coding and decoding

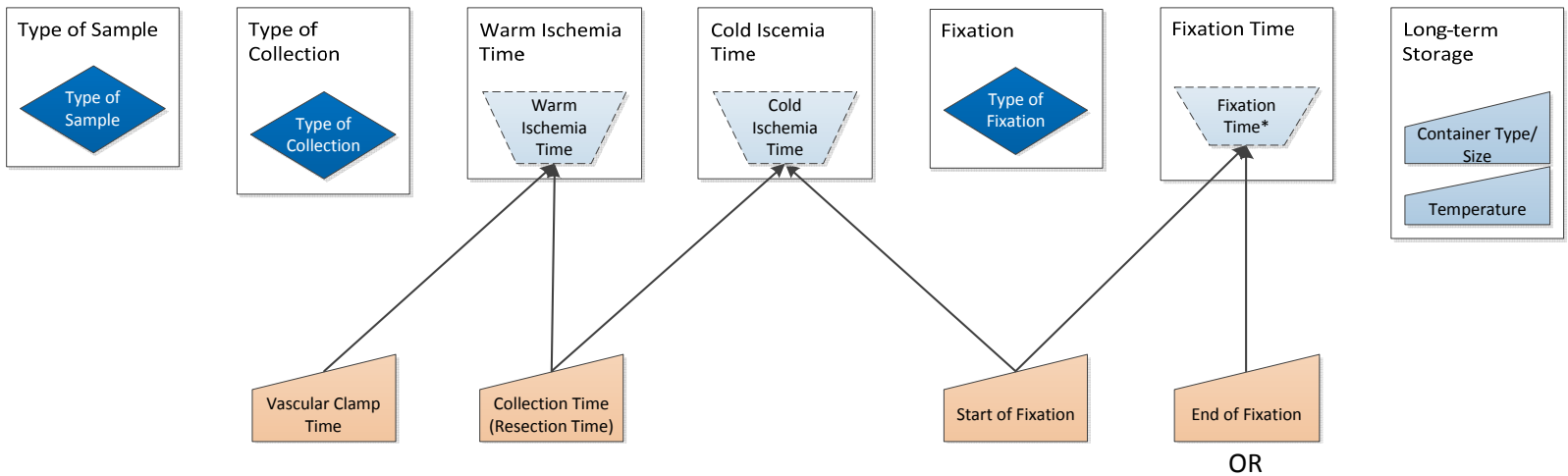
- IBBL, Luxembourg
- Seracare, USA → **SPRECalc tool**: SPREC Excel calculator
- Quintiles, USA
- Lifegene, Lifelines, TMF,...

- SPREC beyond humans... *"Standard PREanalytical Codes (SPREC): A New Paradigm for Environmental Biobanking Sectors Explored in Algal Culture Collections", Biopreservation Biobanking, 2012;4:399-410*

Fiorella GUADAGNI, Sabine LEHMANN, Erica BENSON,
Keith HARDING, Judith CLEMENTS, Kathi SHEA, Barbara GLAZER, Fay BETSOU

SPREC version 2.0

Flowchart for automatic calulation of SPREC v.2 for Solid Samples



- Standard preanalytical coding for biospecimens: Review and implementation of the *Sample PREanalytical Code, ready for submission*
  - More options
  - Implementation tools

Comparison of SPREC and BRISQ data elements

BRISQ	SPREC
Biospecimen type	Sample type
Anatomical site	-
Vital state of patients	Type of collection
Collection mechanism	Type of primary container Pre-centrifugation delay Centrifugation Second centrifugation Post-centrifugation delay Type of collection Warm ischemia time Cold ischemia time
Type of stabilization	Type of primary container Type of collection
Type of long-term preservation	Fixation / stabilization type Fixation time Long-term storage
Storage duration	-
Shipping temperature	-

Biospecimen science literature

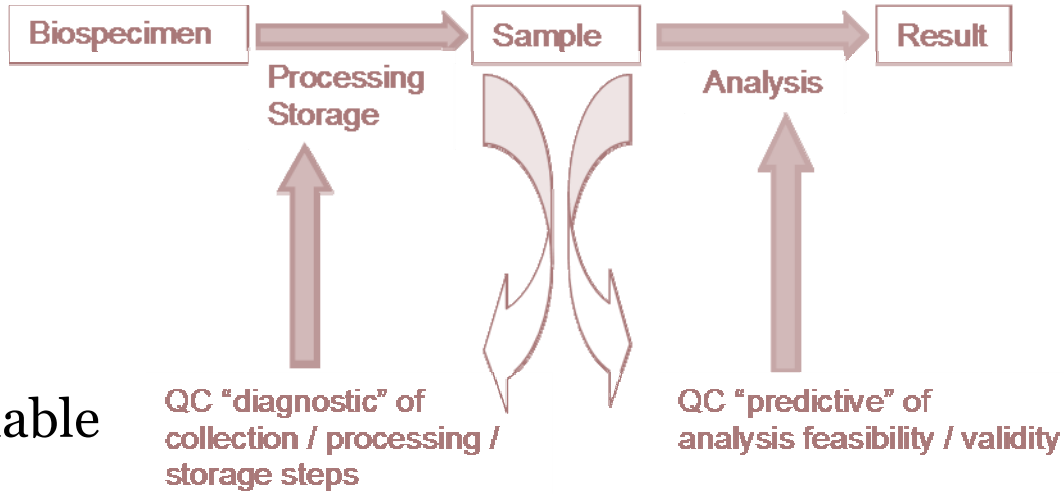
- Update of the biospecimen science literature compilation (100 new references)
  http://www.isber.org/wg/BS-WG-LitComp.html
  Rodrigo CHUAQUI, Michael BARNES, Fay BETSOU

- Long term storage and freeze-thaw stability literature information
  Elaine GUNTER

- Critical reading of ~600 publications to identify biospecimen QC tools (markers/assays) that can be used to define sample quality

Eventual implementation in PT schemes!

In silico biospecimen research



MATRIX including:

- Reference
- Type(s) of sample
- Pre-analytical variable(s)
- Range of pre-analytical variable
- Pre-analytical "threshold"
- QC tool (marker)
- QC assessment method
- Type of method (qualitative vs quantitative ; simple vs multiplex)
- Range of the QC marker
- Control samples used as baseline
- Reference (control) range

In silico biospecimen research: results

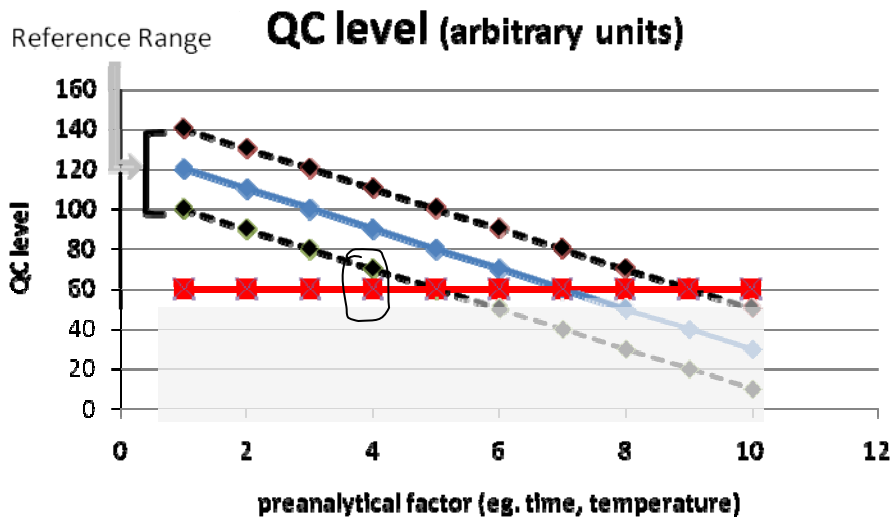
- Type of QC tool
  - Diagnostic
  - Predictive

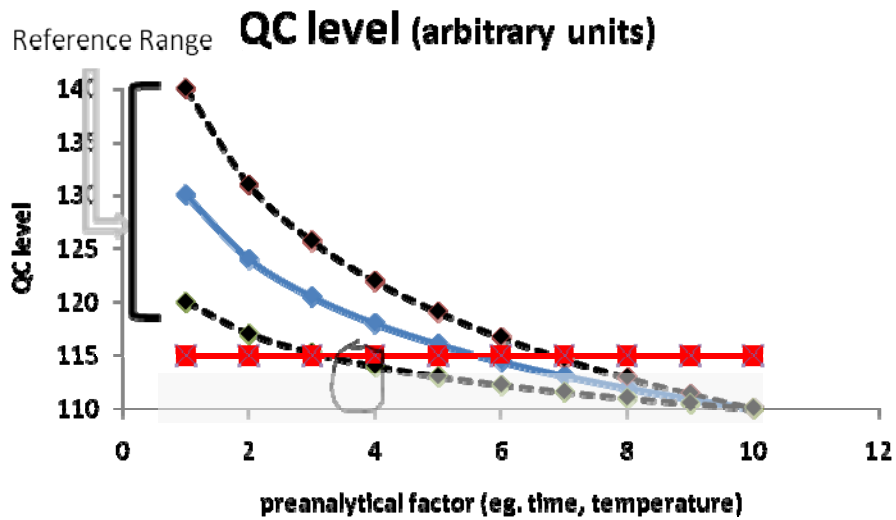
- Evidence-based

- Applicability grade
  - Immediately applicable
  - Potentially applicable
  - Not immediately applicable

- Accessibility grade
  - Readily accessible
  - Potentially accessible
  - Not immediately accessible

Models of QC tools





Examples of biospecimen molecular diagnostic tools identified

QC tool	Sample type	QC scope	Applicability grade	Accessibility grade	Future research required
1K+	Serum	Precentrifugation delay at 4°C	1	1	Plasma
2Truncated cystatin C	CSF	Storage conditions	3	3	Produce MAbs once confirmation in other sample types.
3DUSP1 expression	Prostate fresh tissue	Warm ischemia time	1	1	other tissue types
4Myosin heavy chain	Prostatic tissue	Cold ischemia	3	2	Other tissue types, ref values

1 Heins M et al. Eur J Clin Chem Clin Biochem 1995;33:231
2 Carrette O et al. Proteomics 2005;5:3060
3 Lin DW et al. J Clin Oncol 2006;24:3763
4 Jackson D et al. Proteomics 2006;6:3901

Identification of evidence-based biospecimen quality control tools, *ready for submission*

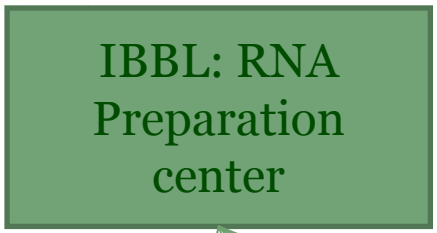
Room temperature RNA stability

Comparison of RNA stored and shipped at RT (BioMatrica, GenVault, Imagene, without stabilizer) and dry ice/-80°C

- 7 samples,
- 5 conditions,
- 5 participant labs,
- 3 testing labs

Assessment by yield, RIN,
qRTPCR (GAPDH, ACTB, IL1, ORM1, PLAUR)

Michael BARNES,
Conny MATHAY, Rodrigo CHUAQUI, Fay BETSOU,
Amy SKUBITZ, Jae-Pil JEON

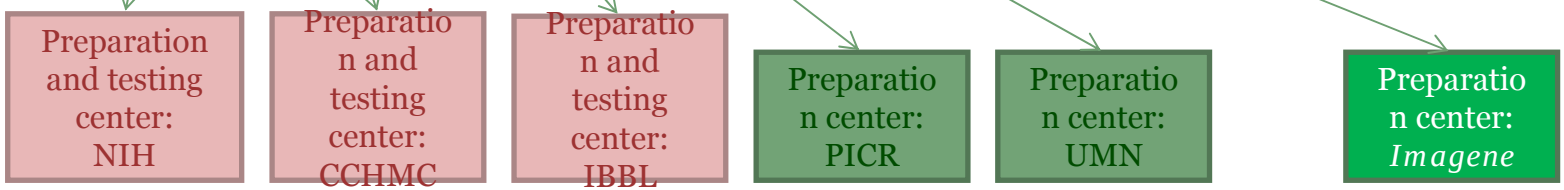


Preparation of RNA from blood:

– RNA extraction from 7 volunteer IBBL donors (QiaCube; total of 70 PaxGene blood tubes)
– Quantification and adjustment of RNA concentration: 50ng/ul
– RNA aliquoting for 5 preparation centers:
  - 3 aliquots of 65ul/donor/center→ 21 RNA samples/center
– Shipment of samples to preparation centers on dry ice

Kit preparation:

– Biomatrica RNAstable kits
– GenVault Gentegra tubes
– Tubes for dry storage without matrix
– Imagene RNAshells
– RT° shipment of kits to preparation centers



NIH: National Institutes of Health; Advanced Technology Center, Maryland
CCHMC: Cincinnati Children's Hospital, Ohio
PICR: National Biobank of Korea, Korea
UMN: University of Minnesota, Minnesota
Imagene: France

Experimental set up

GenTegra

GENVAULT

integenX

RNAstable

Biomatrica

Dry no matrix (control: RT°)

RNAshell

Imagene

Liquid no matrix (control: RT°)

Frozen (control: –80°C)

Each preparation center: 20ul liquid RNA/tube (1ug/tube)

→ 63 tubes/preparation center)

Drying (Speedvac)

2 weeks storage/ shipment at RT°

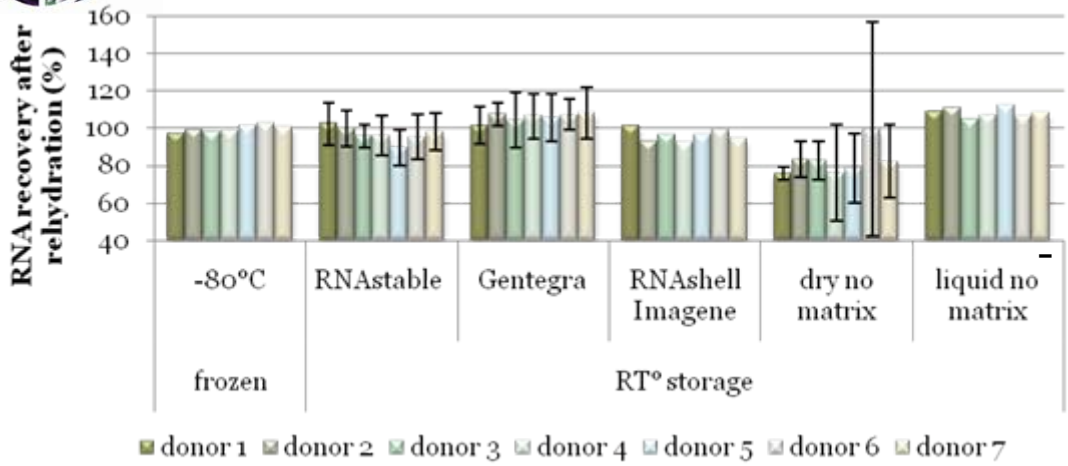
2 weeks storage/ shipment at RT°

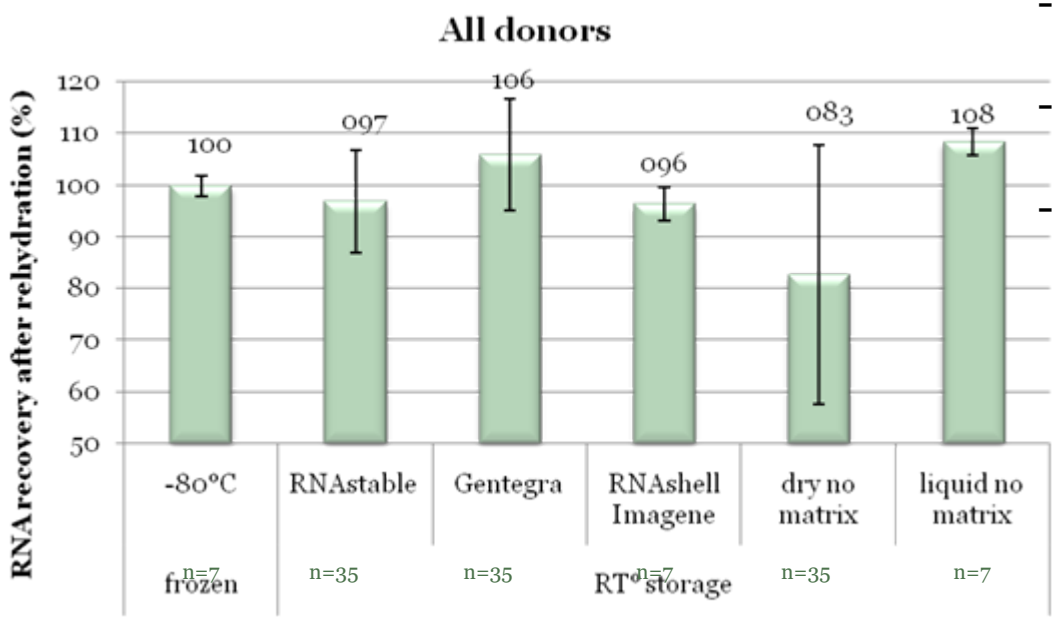
2 weeks storage/ shipment at RT°

Rehydration (testing center)

Analysis

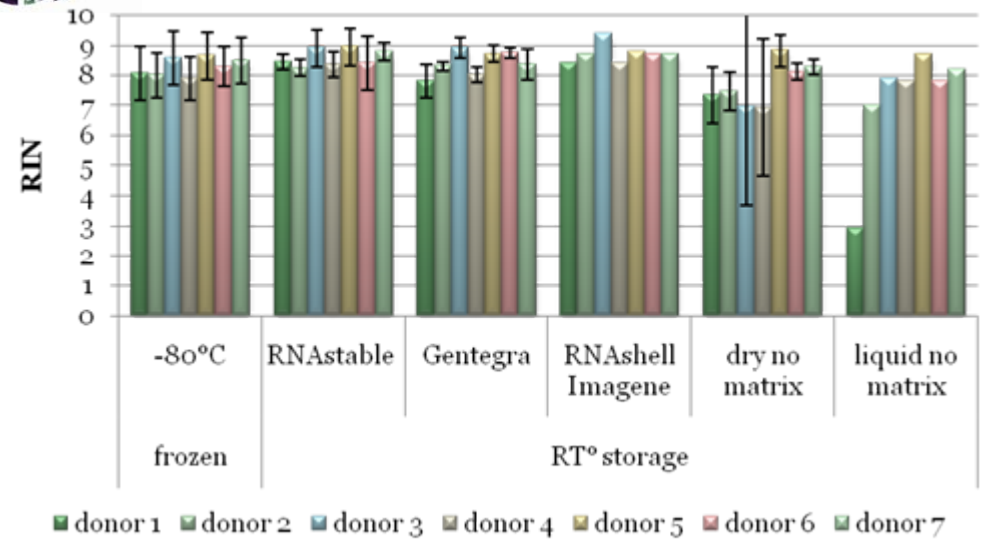
Results: RNA recovery

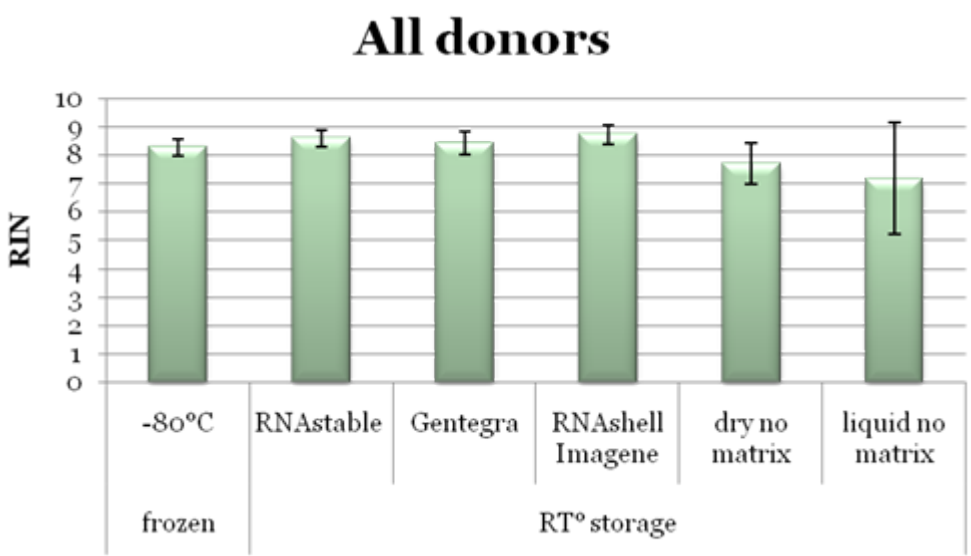




- Good RNA recovery percentages for all three RT° storage systems (spectrophotometer blanked with RNAstable + water resp. Gentegra + water)
- Almost 1µg RNA could be recovered after rehydration
- Lower RNA recovery for samples dried without matrix
- RT° storage of liquid RNA does not reduce the RNA quantity

Results: RIN





- High RIN values for all storage conditions
- RNAstable, Gentegra and RNAshell have slightly higher mean RIN values than the -80°C RNA samples.
- Slightly lower RIN values and more variability for samples dried without matrix and for liquid samples stored at RT°
- **Almost no RNA degradation when purified RNA is stored liquid for 2 weeks at RT°**

Implementation in PT schemes!

Shipment conditions and cell preservation

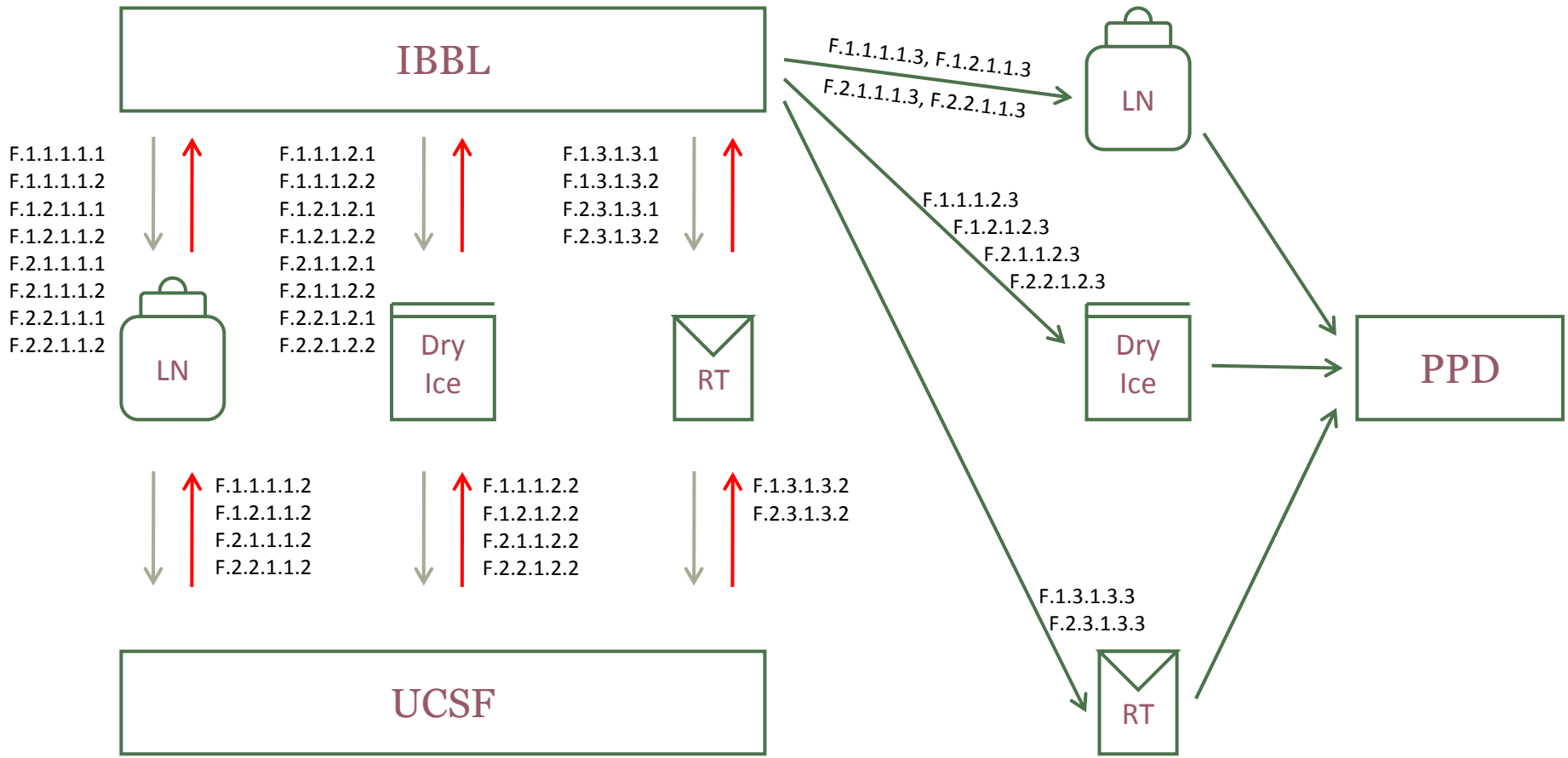
Comparison of LN, dry ice, and RT shipment
High and low viability PBMCs and Jurkat cells

- 4 samples,
- 3 media,
- 3 shipment conditions,
- 3 testing labs

Assessment by Trypan blue, CASY,
flow cytometry (Cytox, Annexin V, Hoescht), ELISPOT

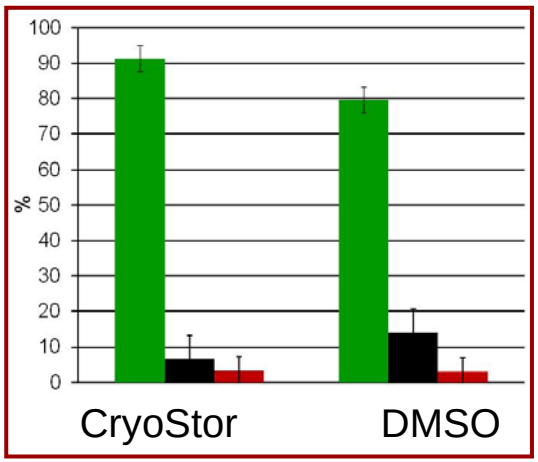
Olga KOFANOVA, Fay BETSOU,
Yvonne DE SOUZA, Kristin DAVIS, Joseph KESSLER

Shipment conditions and cell preservation

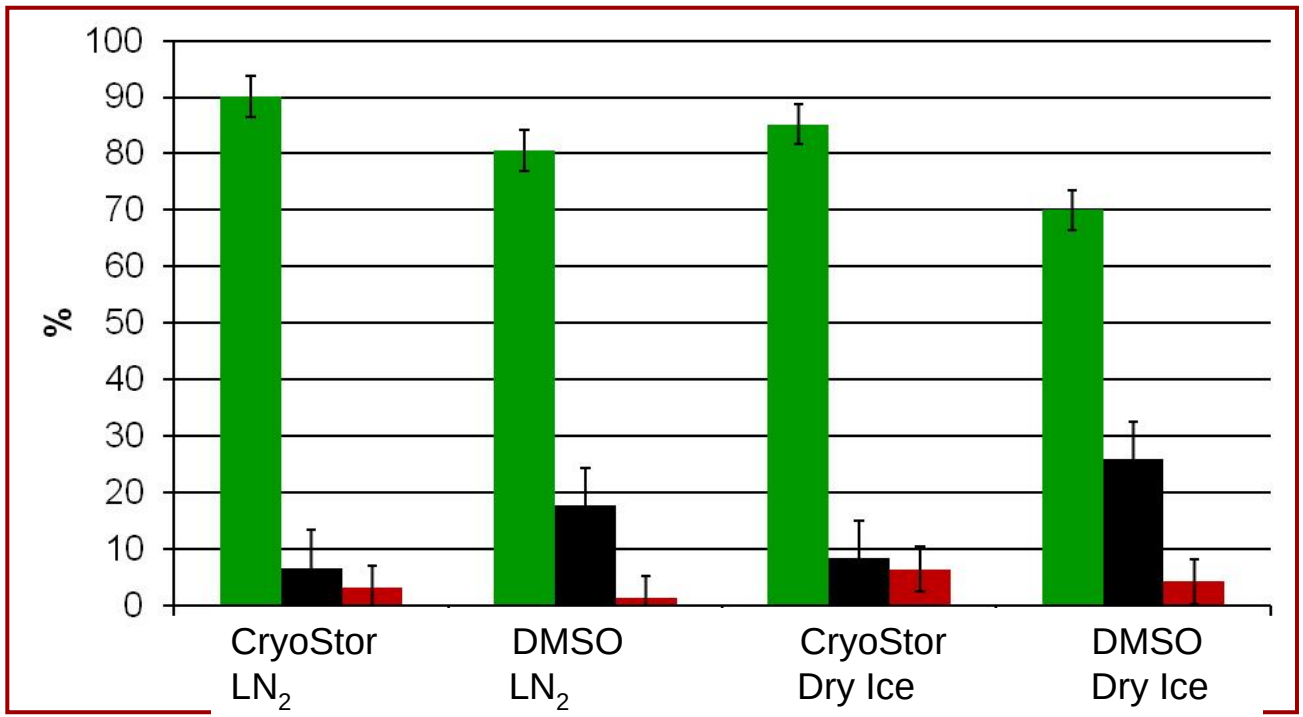


Shipment conditions and cell preservation: results

Baseline / Cells in biobank



Travelling cells



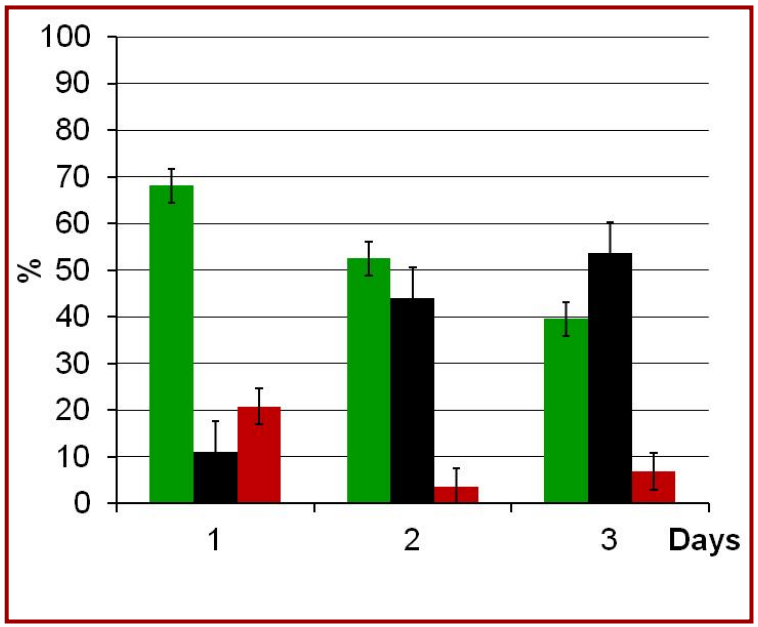
Viable cells

Dead cells

Early apoptotic cells

Shipment conditions and cell preservation: results

Room temperature transport media



Implementation in PT schemes!

Viable cells

Dead cells

Early apoptotic cells

Proficiency Testing programs

- Partnership Agreement ISBER-IBBL
- Business plan and budget
- SOPs (ISO17043:2010), User Manual, Quality Manual
- PT software implementation
- **DNA quantification and purity scheme**
- **RNA integrity scheme**

Francesca POLONI,
Garry ASHTON, Fay BETSOU, Domenico COPPOLA,
Yvonne DE SOUZA, Anne Mieke DE WIELDE, James DOUGLAS,
James ELIASON, Fiorella GUADAGNI, Elaine GUNTER, Olga KOFANOVA,
Sabine LEHMANN, Conny MATHAY, Kathi SHEA, Mark SOBEL, Gunnel TYBRING, Michele ZINC

EQA development

ISBER – IBBL partnership

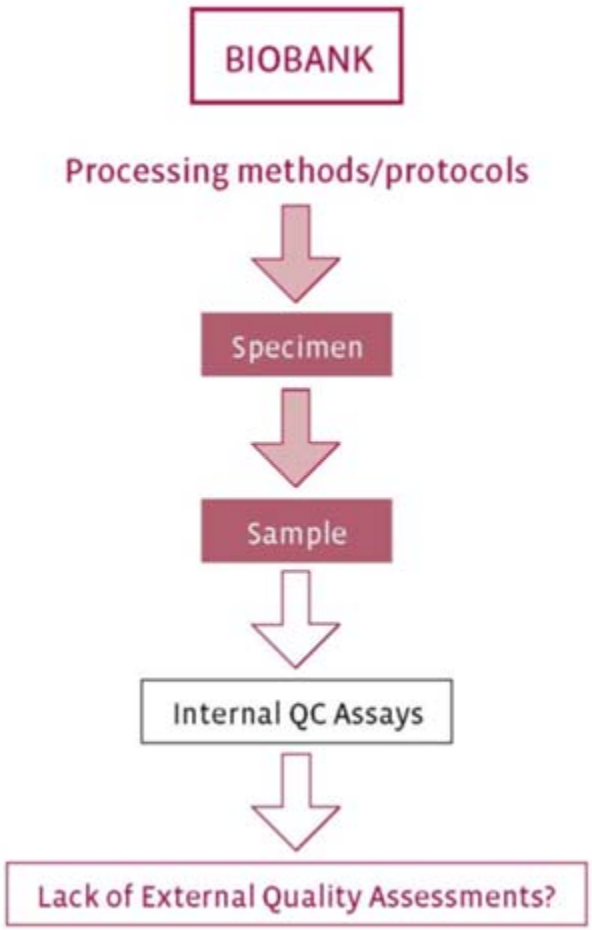
ISO/IEC 17043:2010

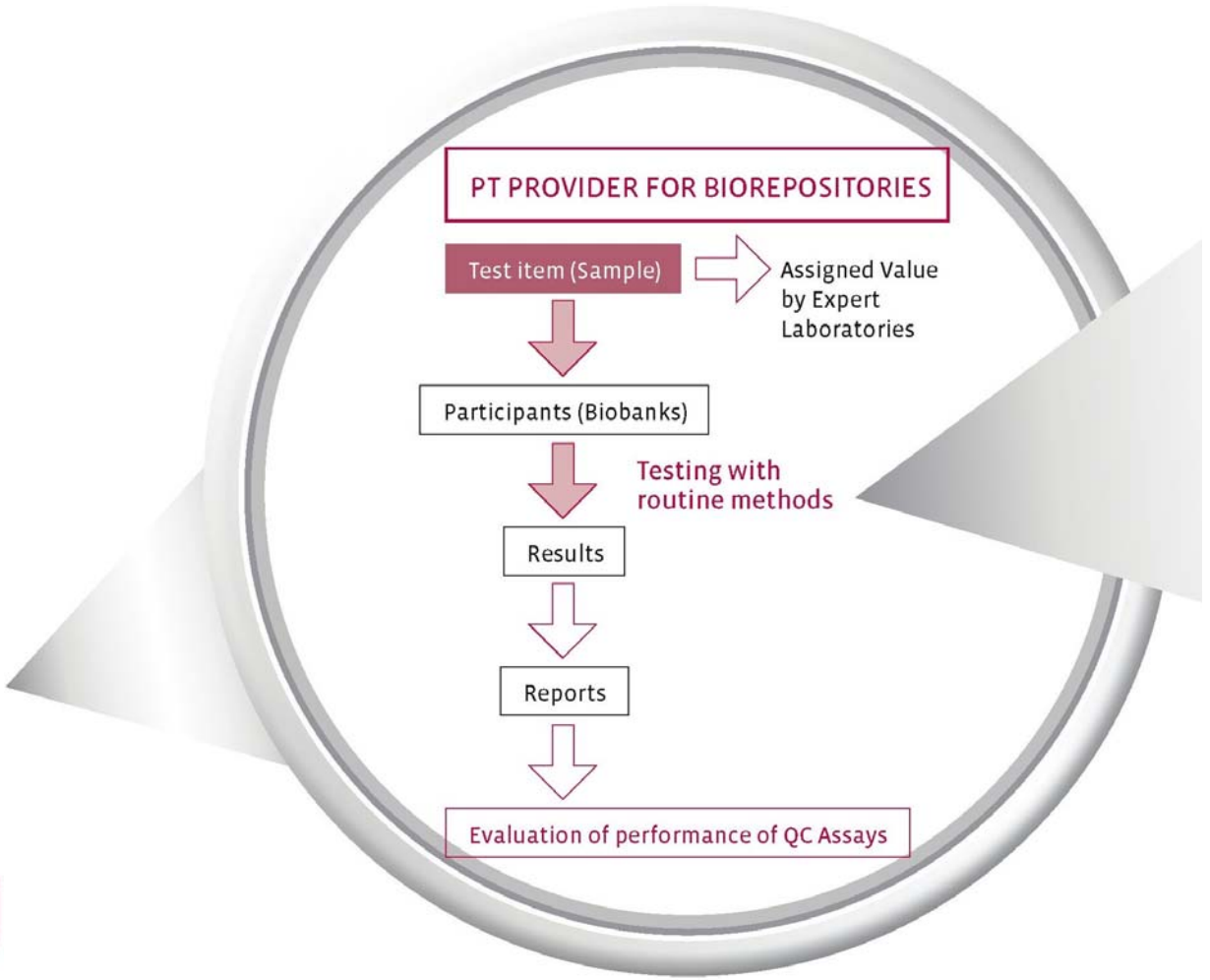
Allow biospecimen custodians to assess the accuracy of biospecimen characterization

Support biorepository accreditation initiatives

Facilitate implementation of new QC tools and evaluate their performance

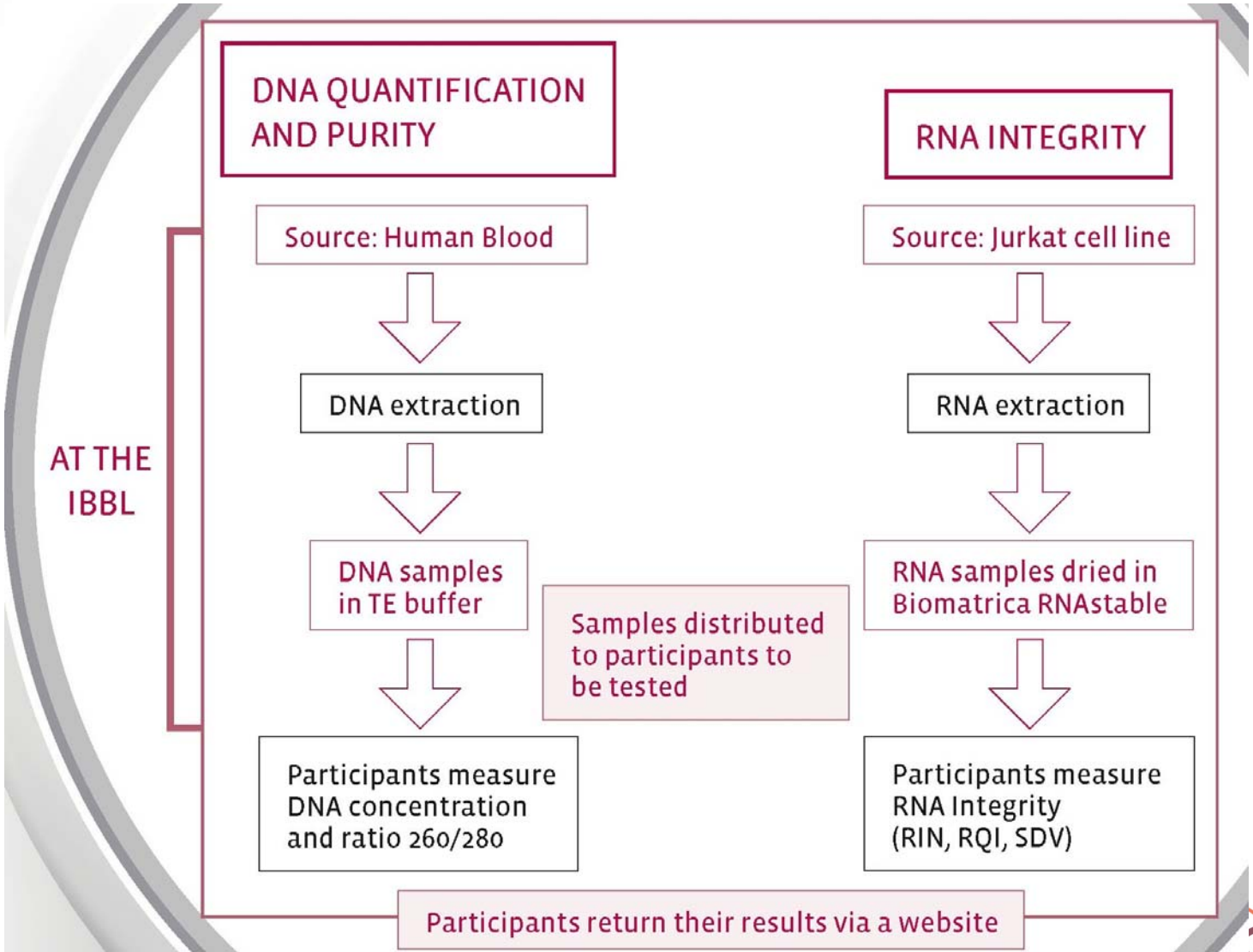
PT schemes for biorepositories





→ Highly standardized and accurately characterized biospecimens!

The first ISBER PT schemes



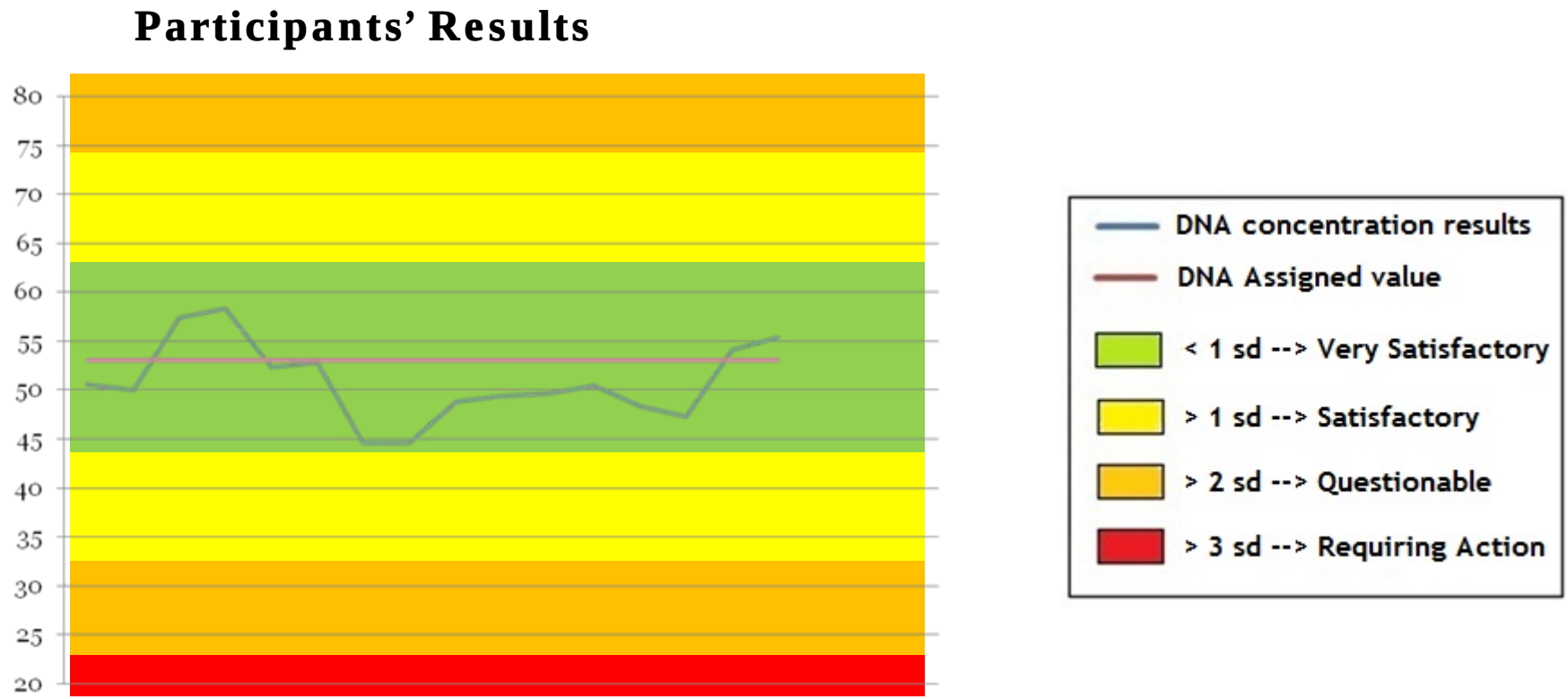
Pilot phase

- Pilot phase:
  - Run in March/April 2011
  - 3 Expert laboratories for each scheme → Assigned Value
  - 8 participants for DNA and 9 for RNA (ISBER Biospecimen Science Working Group)
- Statistical approach:
  - ISO/IEC 17043:2010: Conformity assessment – General requirements for Proficiency testing
  - IUPAC Technical Report (Pure Applied Chem 2006:78:145-196)
- PT uncertainty (i.e. standard deviation) = PT Advisory Group defined Coefficient of Variation x Assigned Value
- Z-score = (result – assigned value)/ PT uncertainty
- The scoring system is based on distance from the assigned value:

Distance from assigned value (z-score)	Consensus score	Performance
< 1 standard deviation	0	Very Satisfactory
< 2 standard deviations	1	Satisfactory
> 2 standard deviations	2	Questionable
> 3 standard deviations	3	Requiring Action

Assigned value and pilot results for the DNA concentration

Assigned value: **53.03 µg/ml**
PT uncertainty: 10.6 µg/ml

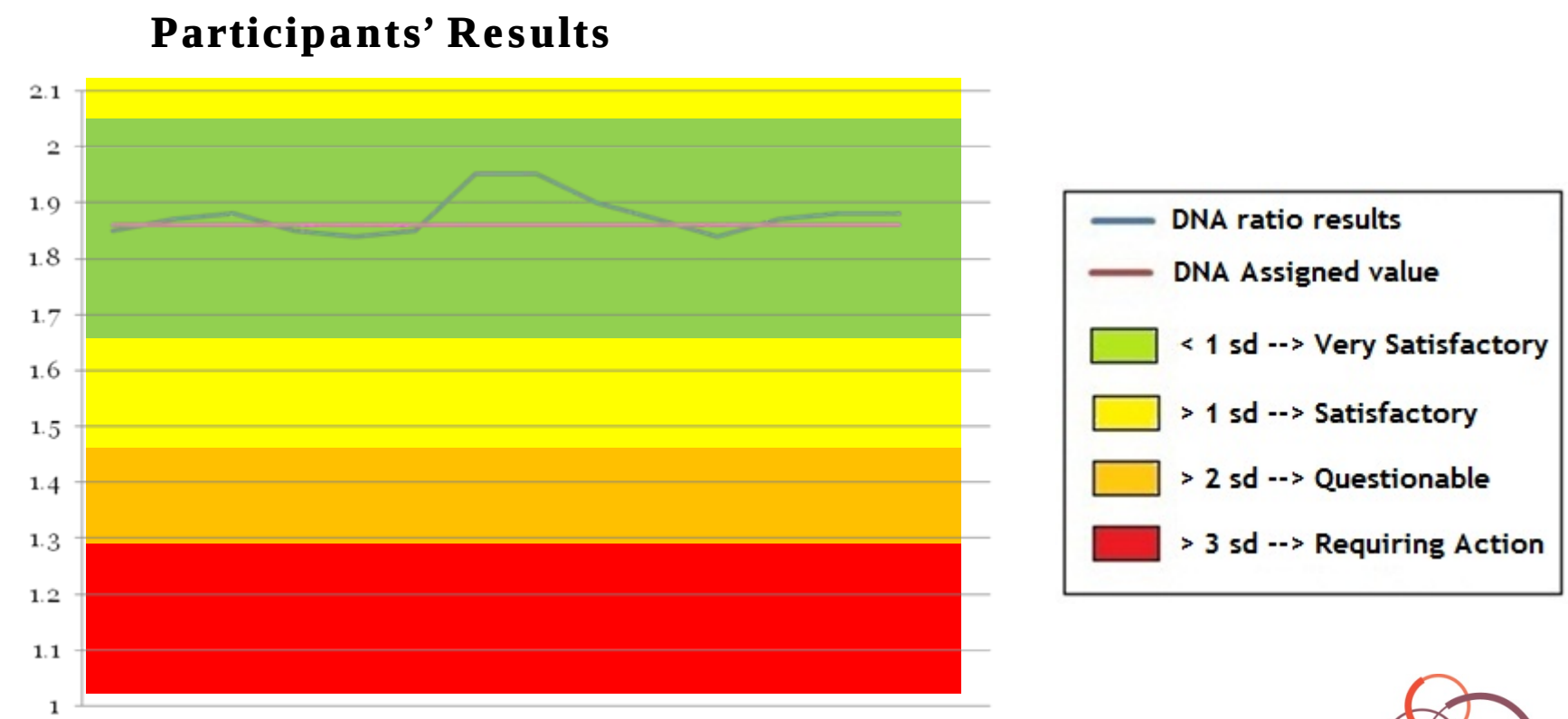


Assigned value and pilot results for the DNA ratio

Assigned value (ratio 260/280):

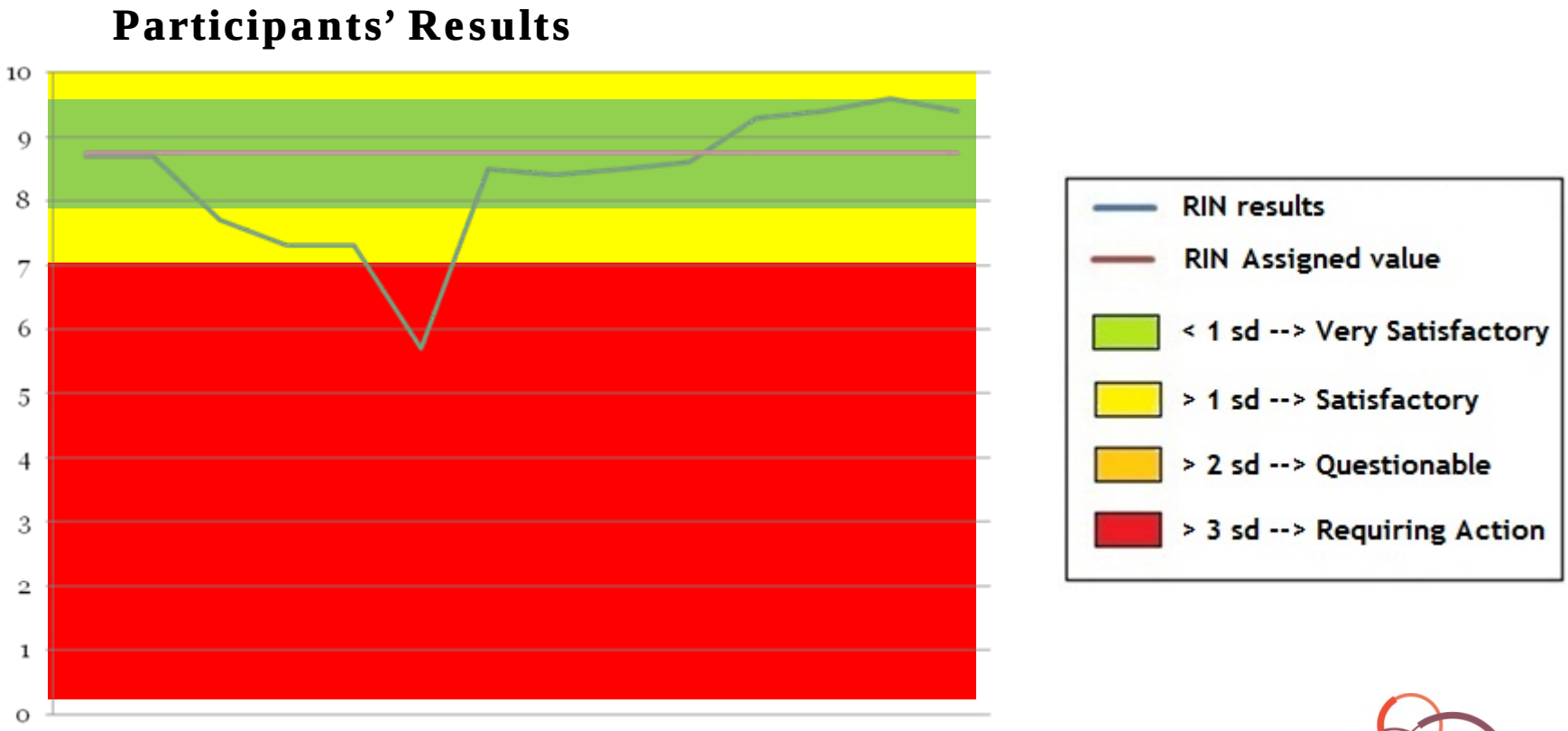
1.86

PT uncertainty: 0.19

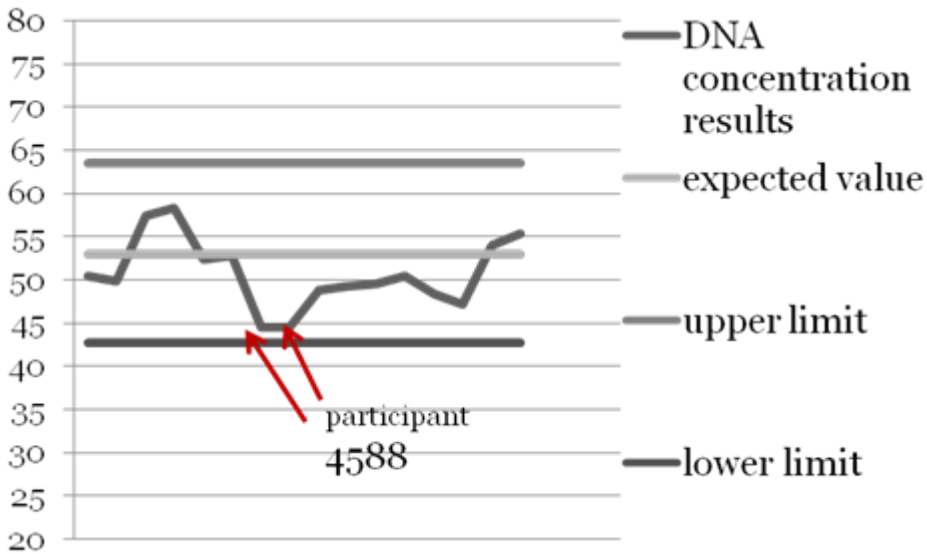


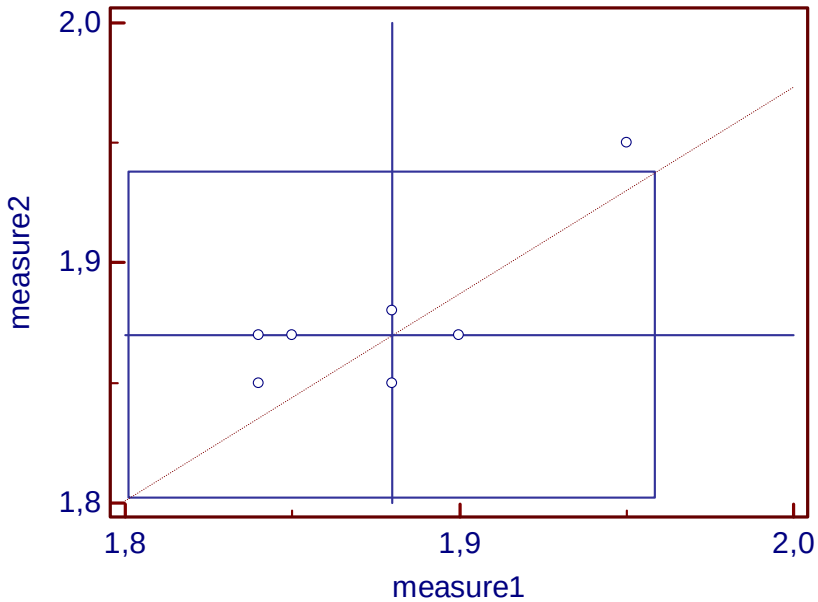
Assigned value and pilot results for the RNA integrity

Assigned value (RIN): **8.76**
PT uncertainty: 0.88

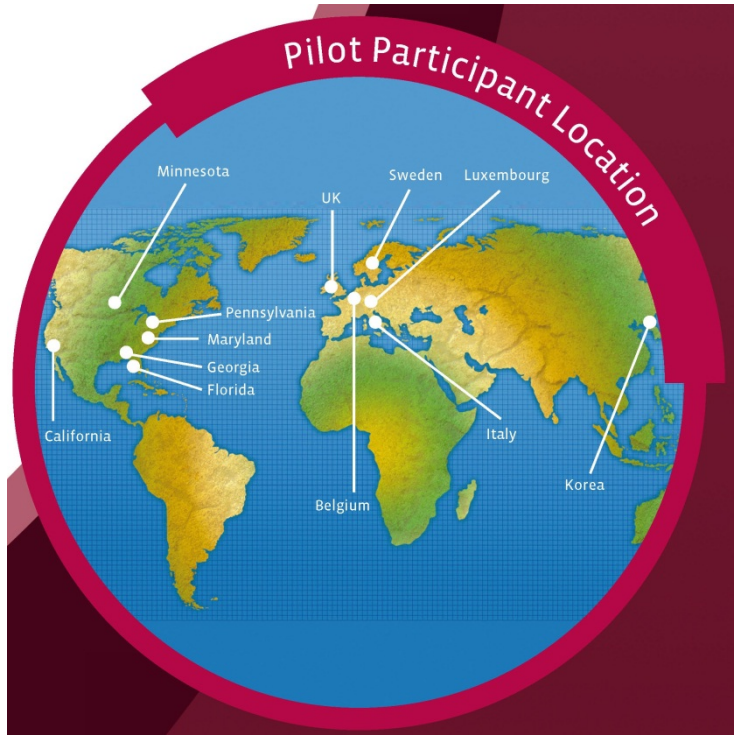


Proficiency Testing reports





PT current and future schemes



2011 PT Schemes:

DNA Quantification and Purity

RNA Integrity

40 participants

PT Schemes planned for 2012:

DNA Quantification and Purity

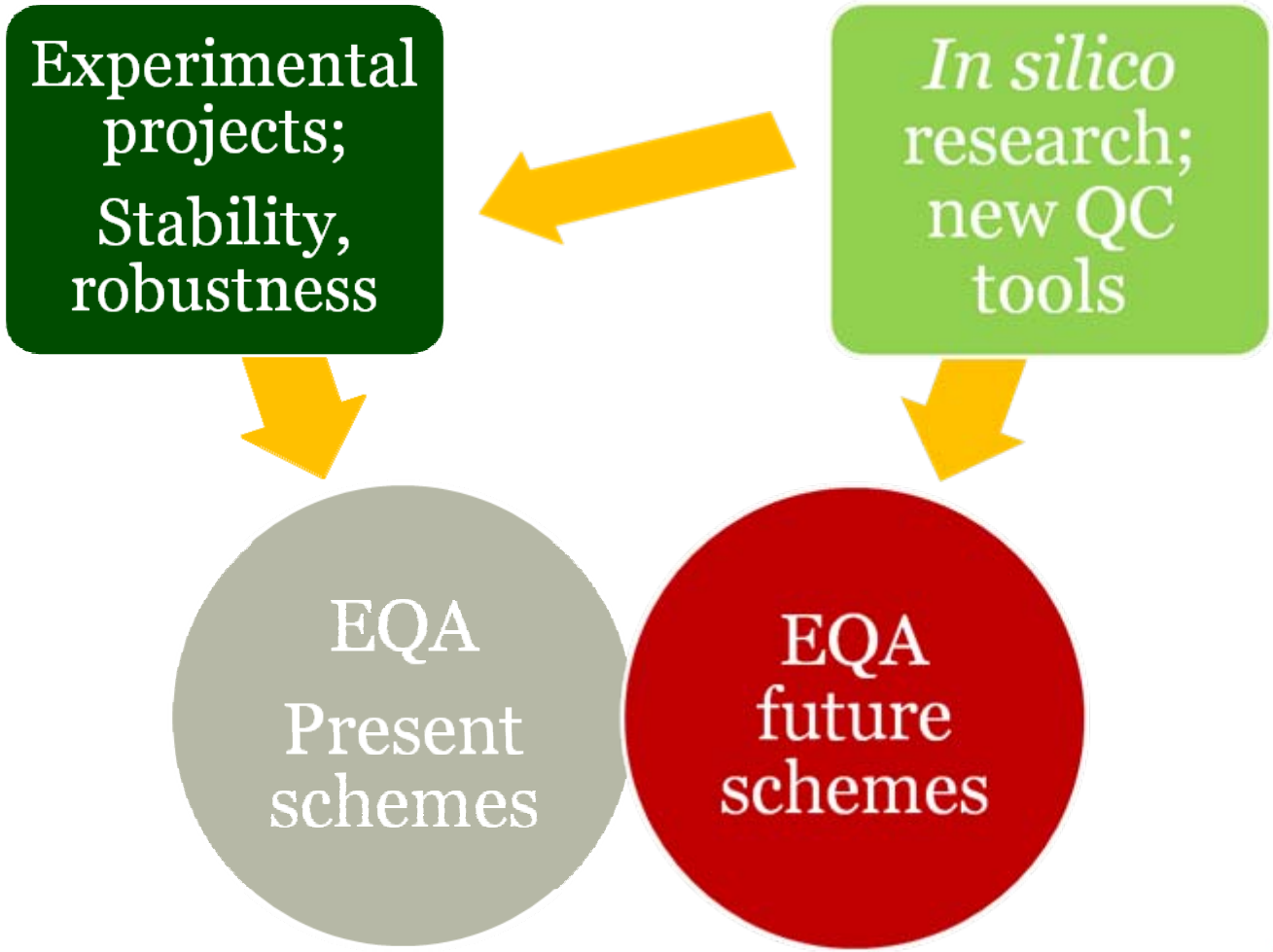
RNA Integrity

Cell Viability **new!**

Tissue Antigenicity **new!**

To know more visit www.isber.org/proficiency_testing/

Conclusions



ISBER Biospecimen Science Working Group 2011 members