3rd Annual Biospecimen Research Network (BRN) Symposium:
Advancing Cancer Research Through Biospecimen Science
March 24-25, 2010, Bethesda, Maryland, USA

Standard Operating Pre-Analytical Procedures for Protein Profiling and DNA Studies: the Experience of the Interinstitutional Multidisciplinary BioBank (BioBIM)

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Istituto di Ricovero e Cura a Carattere Scientifico

BioBIM - Interinstitutional Multidisciplinary BioBank

The BioBIM is an organized collection of biological samples obtained from individuals attending several Italian Institutions. The BioBIM operates under the auspices of the IRCCS San Raffaele, Rome, Italy, a private Scientific Institution certified by the Italian Minister of Health.

The BioBIM is ISO certified.

AIMS

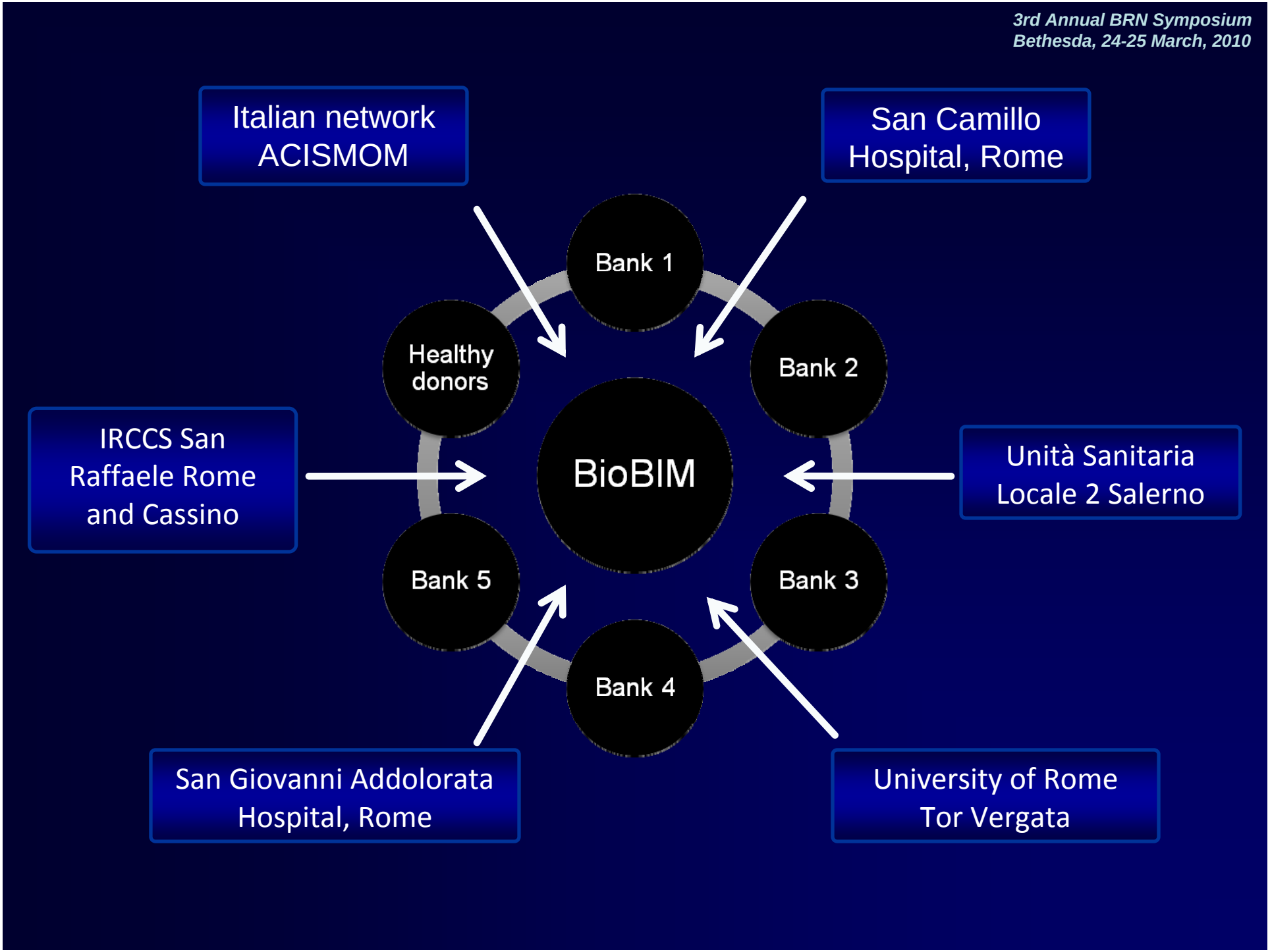
The Inter-Institutional Multidisciplinary BioBank will operate with the aims of:

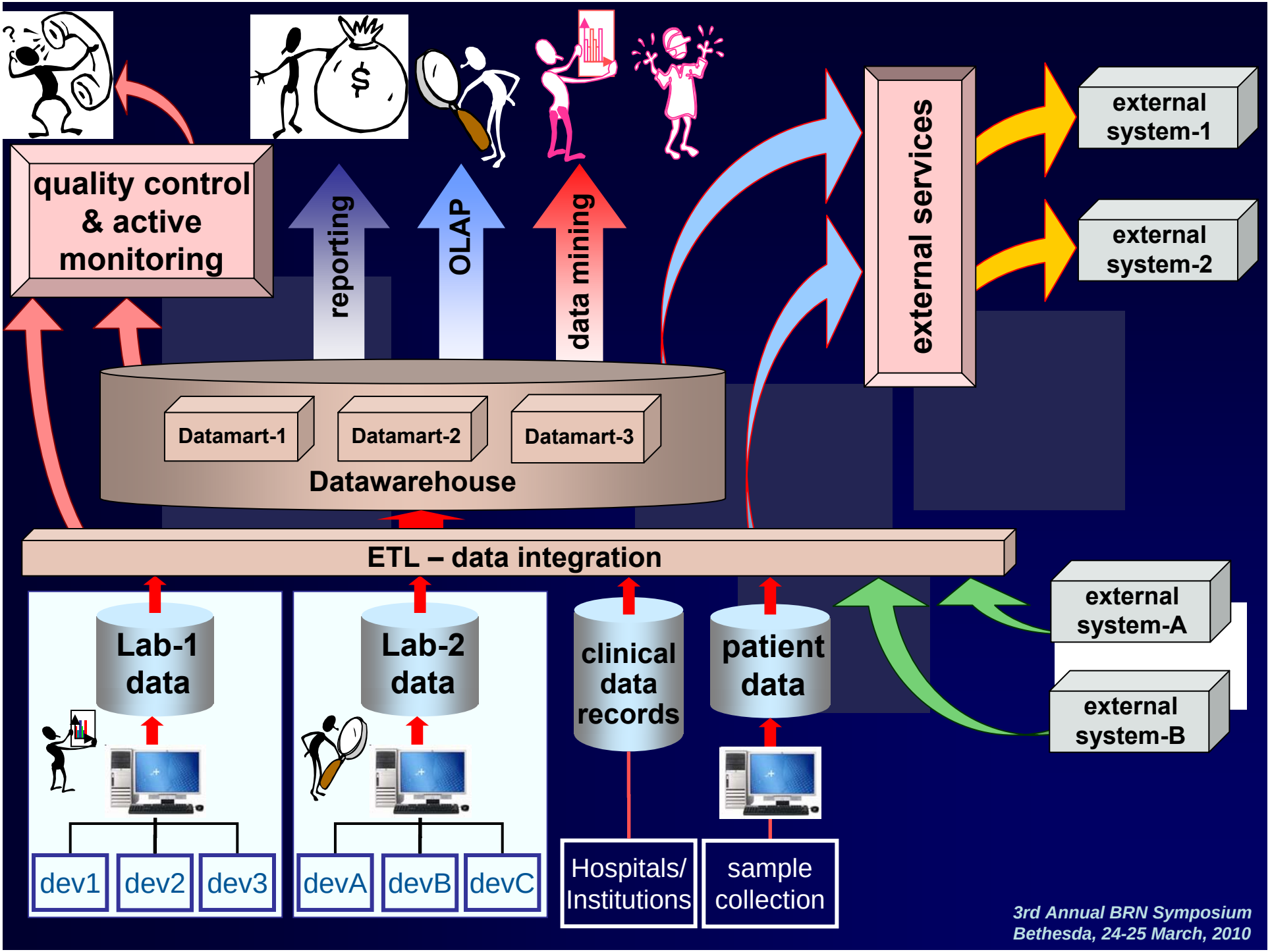
- collecting and analyzing high-quality biological specimens from all participating Units, in an efficient, organized and accessible manner using leading-edge technologies.
- connecting professionals with each other across traditional hospital or laboratory departments to provide a multidisciplinary research structure properly embedded into European ethical and legal frameworks.
- facilitating translational research enabling us to apply biomarker discovery to clinical outcome studies and novel targets for therapy.

Five different disease-based biorepositories have been structured, devoted to:

1. ***Cancer diseases***
2. ***Cardiovascular and respiratory diseases***
3. ***Neurodegenerative diseases***
4. ***Developmental disabilities***
5. ***Rare diseases***

Healthy volunteers are also recruited


Italian network ACISMOM
San Camillo Hospital, Rome
Bank 1
Healthy donors
Bank 2
IRCCS San Raffaele Rome and Cassino
BioBIM
Unità Sanitaria Locale 2 Salerno
Bank 5
Bank 3
Bank 4
San Giovanni Addolorata Hospital, Rome
University of Rome Tor Vergata


quality control & active monitoring
reporting
OLAP
data mining
external services
external system-1
external system-2
Datamart-1
Datamart-2
Datamart-3
Datawarehouse
ETL – data integration
Lab-1 data
Lab-2 data
clinical data records
patient data
external system-A
external system-B
dev1
dev2
dev3
devA
devB
devC
Hospitals/ Institutions
sample collection
3rd Annual BRN Symposium
Bethesda, 24-25 March, 2010

Clinical Center Network

Check-in Biological Samples

Automation
Pre-Analytical Phase

Automation
Research activities

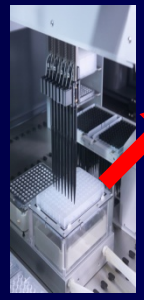
BioBIM

TLA Diagnostic area

Genomics

Proteomics

Metabolomics



BIOMARKERS DISCOVERY

3rd Annual BRN Symposium
Bethesda, 24-25 March, 2010

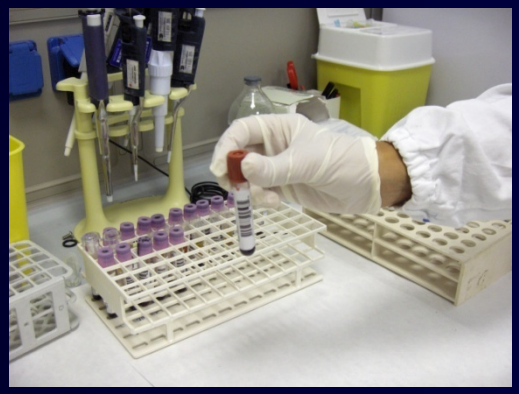
Key point - Pre-analytical phases: Sample Processing and Storage Procedures

Possible approach: Total Lab Automation

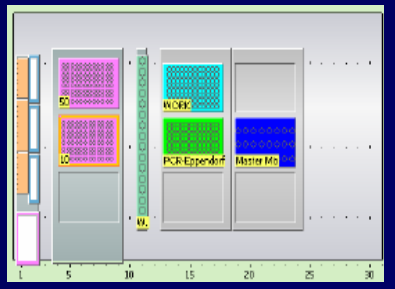
All blood samples are initially identified by the Tecan FE500 robot that sorts samples for diagnostic routine and samples dedicated to the BioBIM

Total Lab Automation

Sample distribution



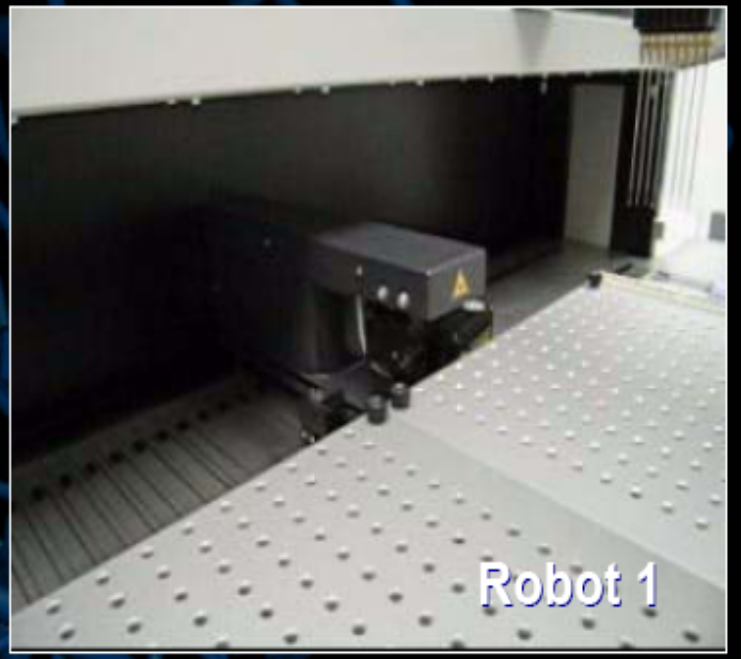
Robotic Station TECAN FE500



Robotic Station Freedom Evo 150

Total Lab Automation

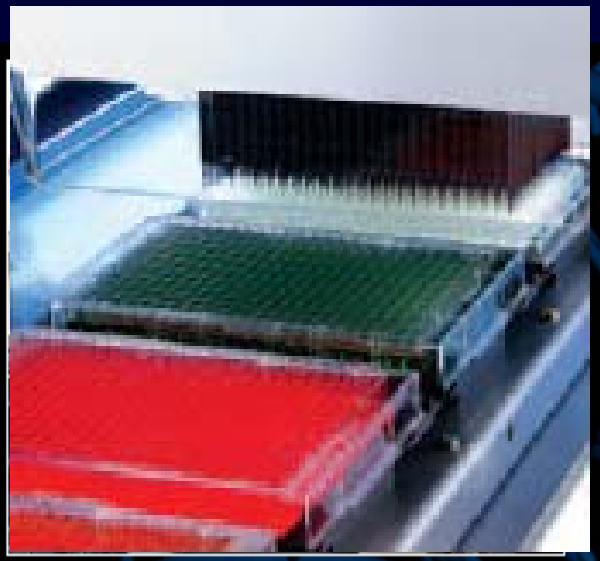
Sample identification and recording

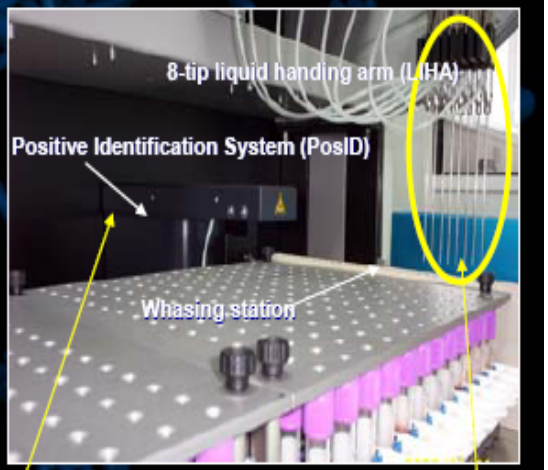


Automatic barcode reading and recording of samples received in an internal database.

Total Lab Automation

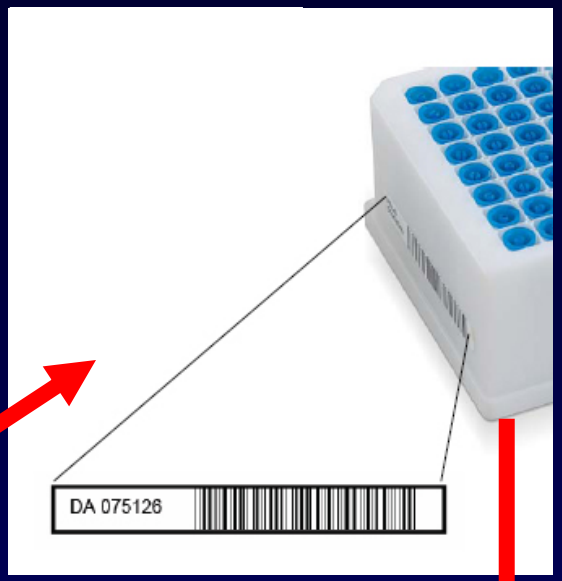
Blood storage for biological bank



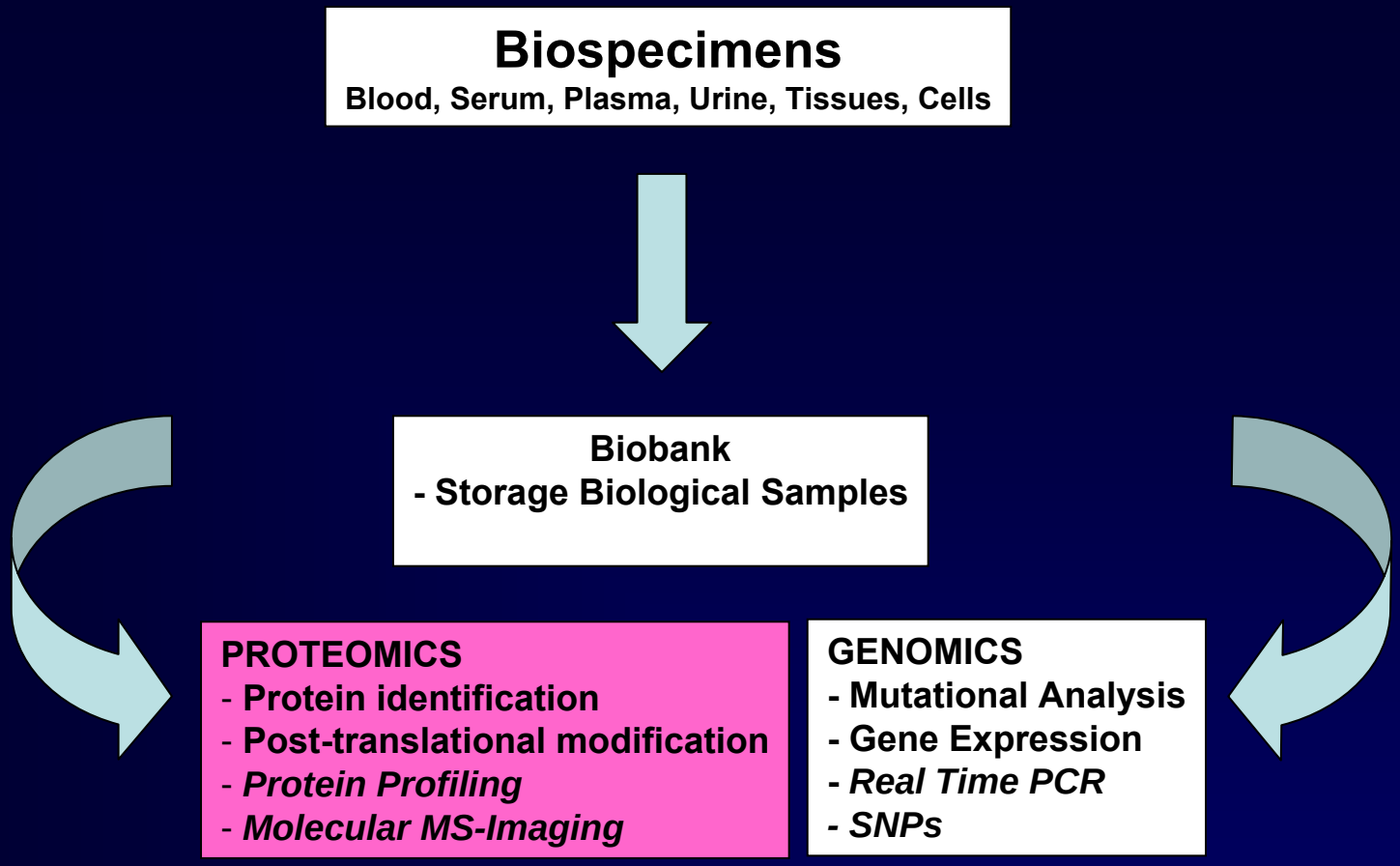


Needles cross the tube

Barcode Reader



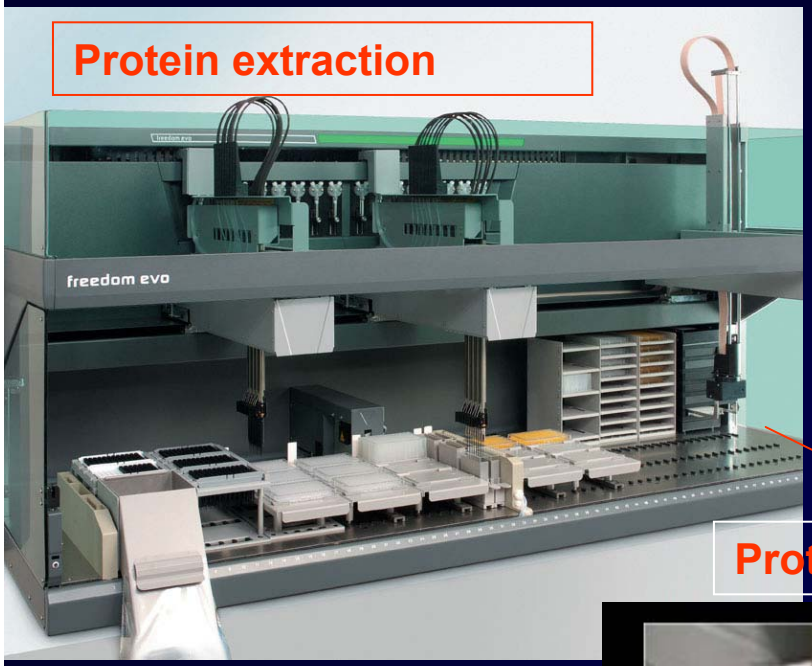
Robotic Station Tecan Freedom Evo 100 and 150



BIOMARKERS DISCOVERY

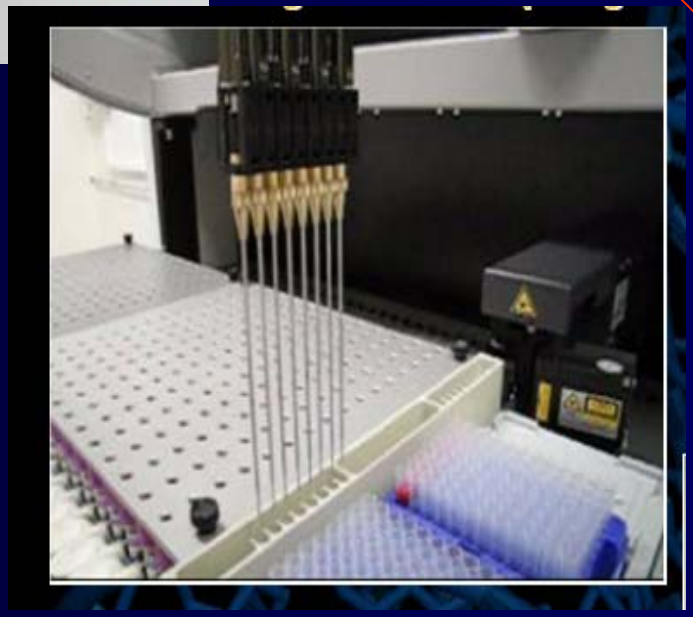
Laboratory Robotization for proteomics highthroughput analysis

Protein extraction, purification and MS analysis



Protein caracterization

Protein purification



Protein profiling

Standard Operating Pre-Analytical Procedures for Protein Profiling

Methodological Approach

- Comparison between manual *vs* robotized analytical processing for protein profiling (Low Molecular Weight proteins)

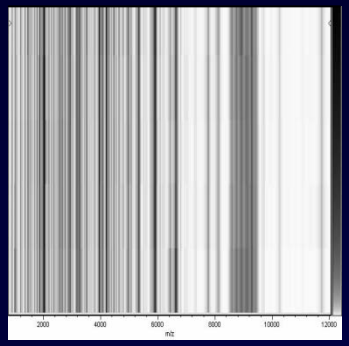
Pre-analytical conditions

- Addition of protease inhibitors
- Time before processing
- Storage temperature

Automated Procedure
(peaks 68, average CV 14.1 %)

Manual Procedure
(peaks 57, average CV 18.2 %)

A



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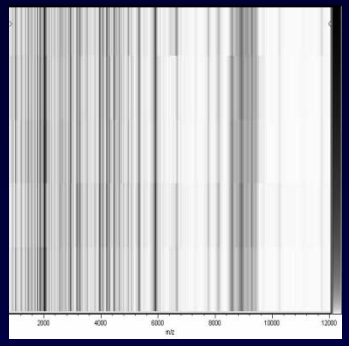
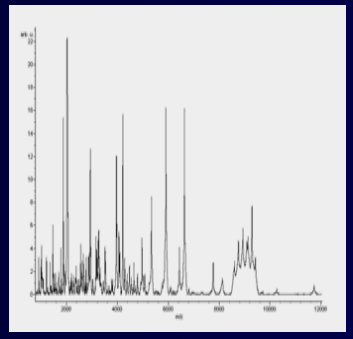


C



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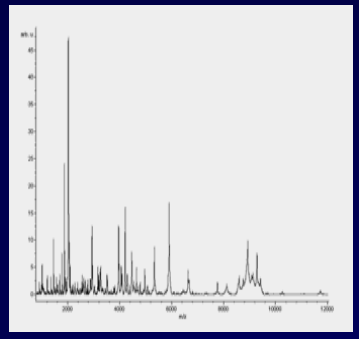

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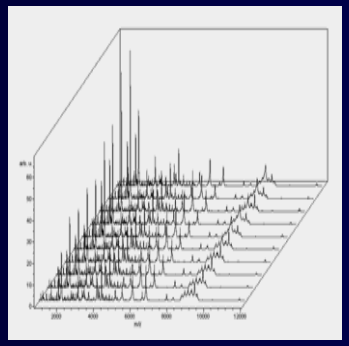
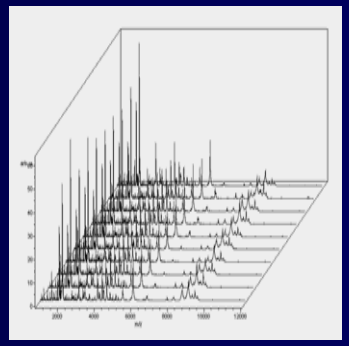
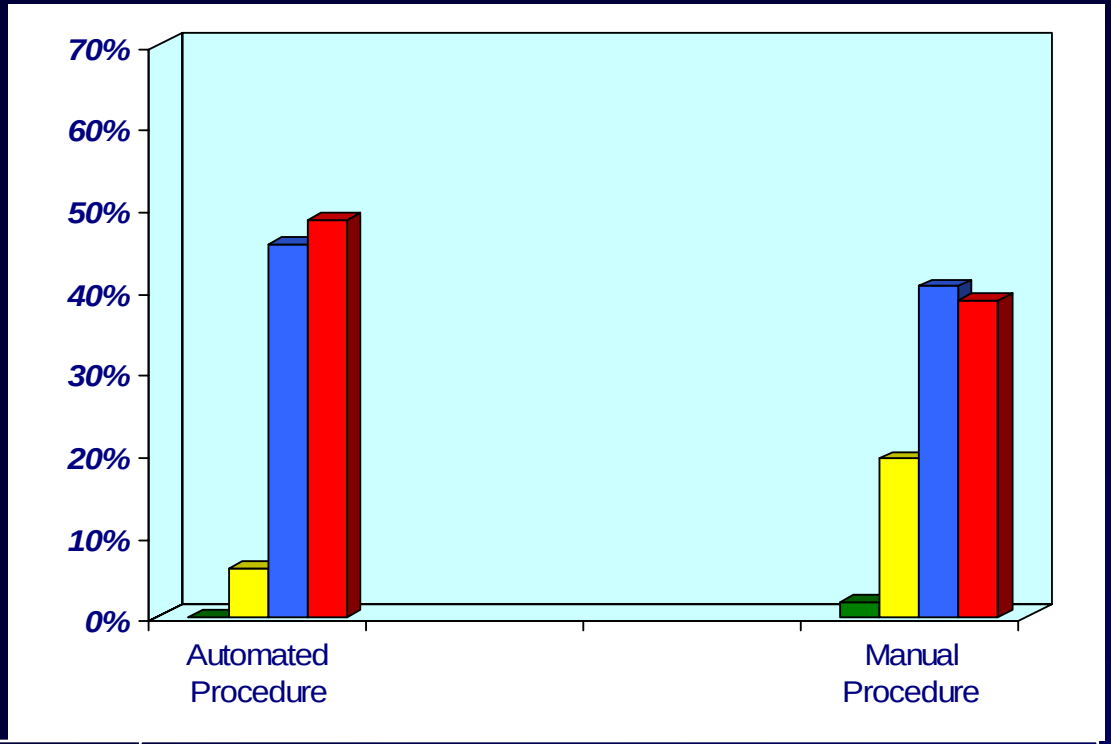


F





CV> 50%	0%	1.75%
CV 30-50%	5.90%	19.30%
CV 10-30%	45.60%	40.35%
CV< 10%	48.50%	38.60%

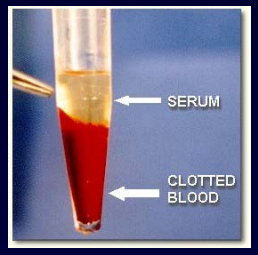
Blood samples from six consenting healthy donors

24 to 42 years, 3 males and 3 females

Eight Vacutainer tubes with clot activator from each subject.

Four blood samples were treated with protease inhibitor-EDTA free

1 our of clotting time

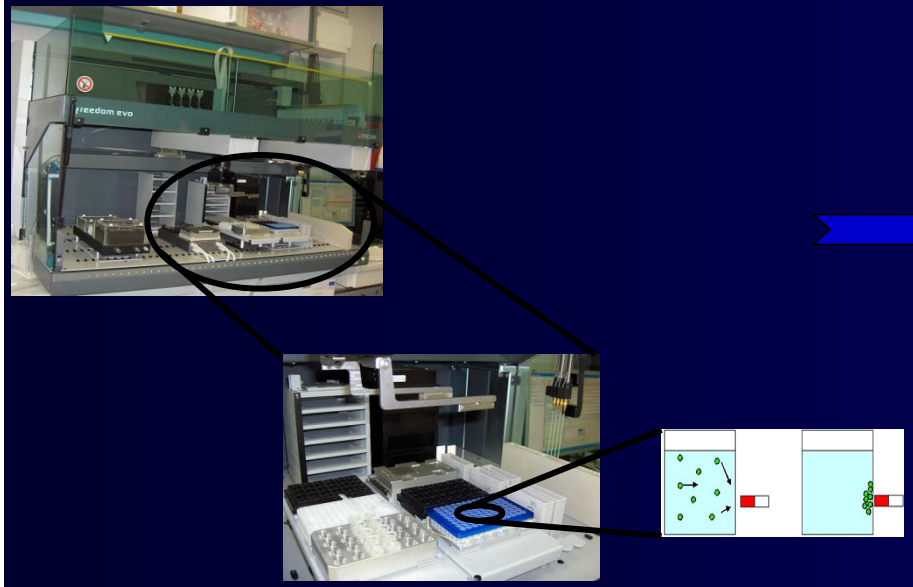


Protease inhibitor were added in some serum samples

HIL test of serum samples on an Architect c8000 system (Abbott, Irving TX USA)

The tests confirmed the absence of haemolysis in serum samples

Automated serum Low Molecular Weight protein/peptides (LMW) purification

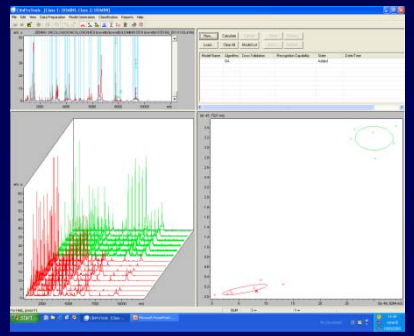


LMW serum proteome purification by C18 magnetic beads

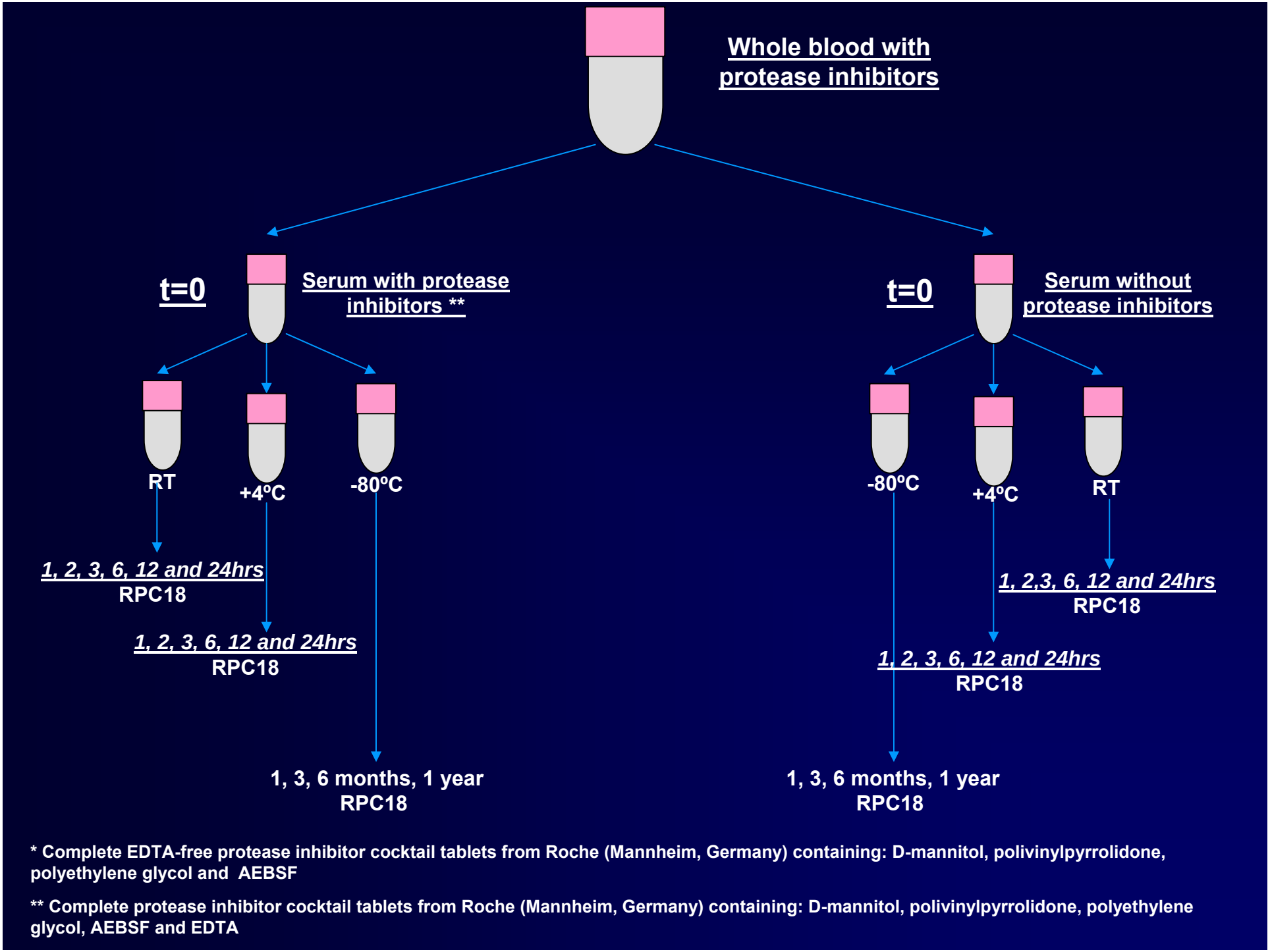
MALDI/MS analisys of LMW

ClinProt system

ClinProTools software analysis

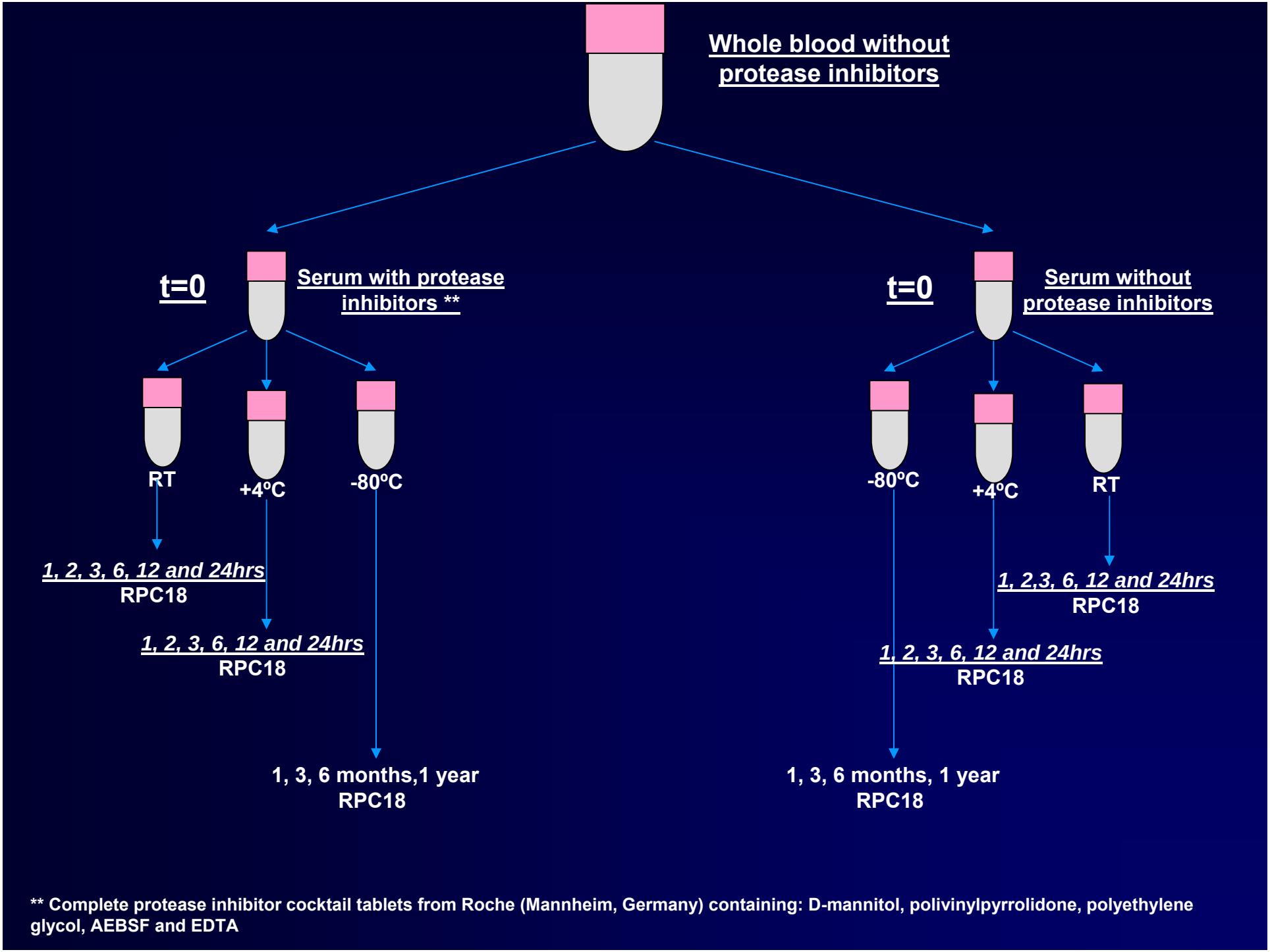


The average peak intensity, SD and CV (%) for each corresponding peak was calculated.



* Complete EDTA-free protease inhibitor cocktail tablets from Roche (Mannheim, Germany) containing: D-mannitol, polivinylpyrrolidone, polyethylene glycol and AEBSF

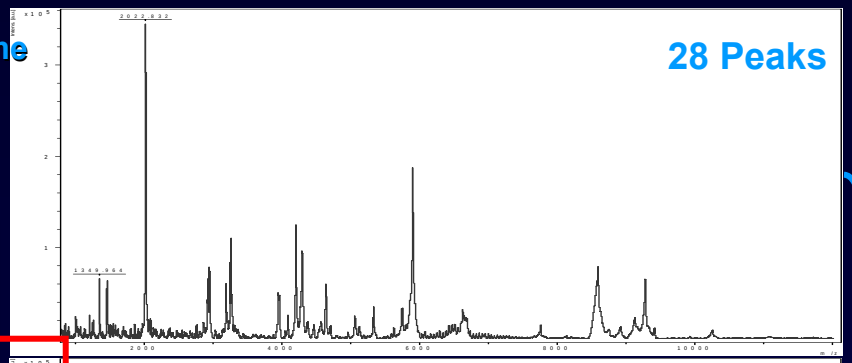
** Complete protease inhibitor cocktail tablets from Roche (Mannheim, Germany) containing: D-mannitol, polivinylpyrrolidone, polyethylene glycol, AEBSF and EDTA



** Complete protease inhibitor cocktail tablets from Roche (Mannheim, Germany) containing: D-mannitol, polivinylpyrrolidone, polyethylene glycol, AEBSF and EDTA

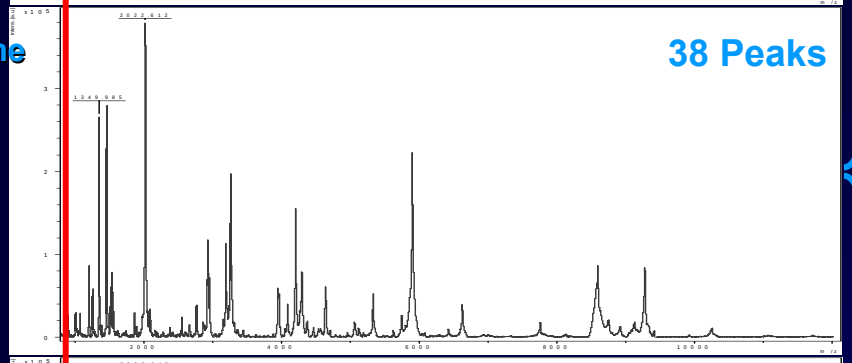
Clotting times

Clotting Time
30 min.



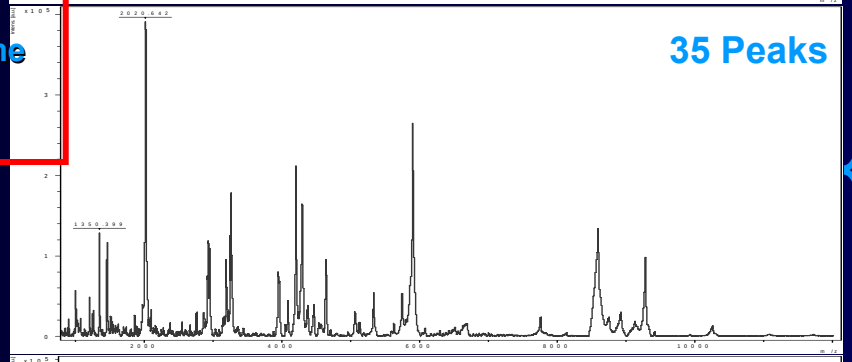
28 Peaks

Clotting Time
60 min.



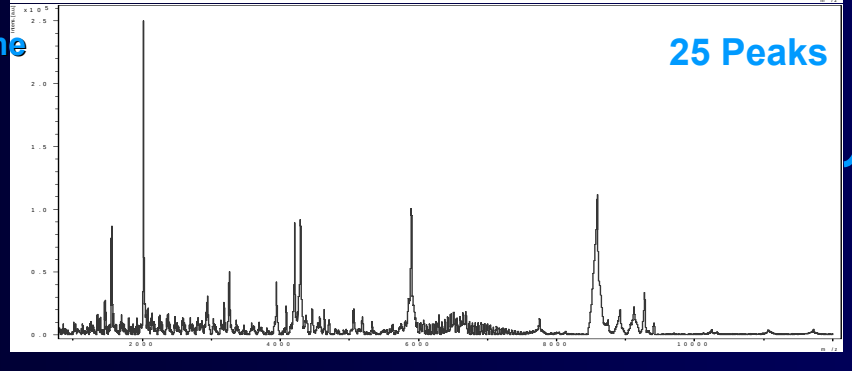
38 Peaks

Clotting Time
120 min.



35 Peaks

Clotting Time
240 min.



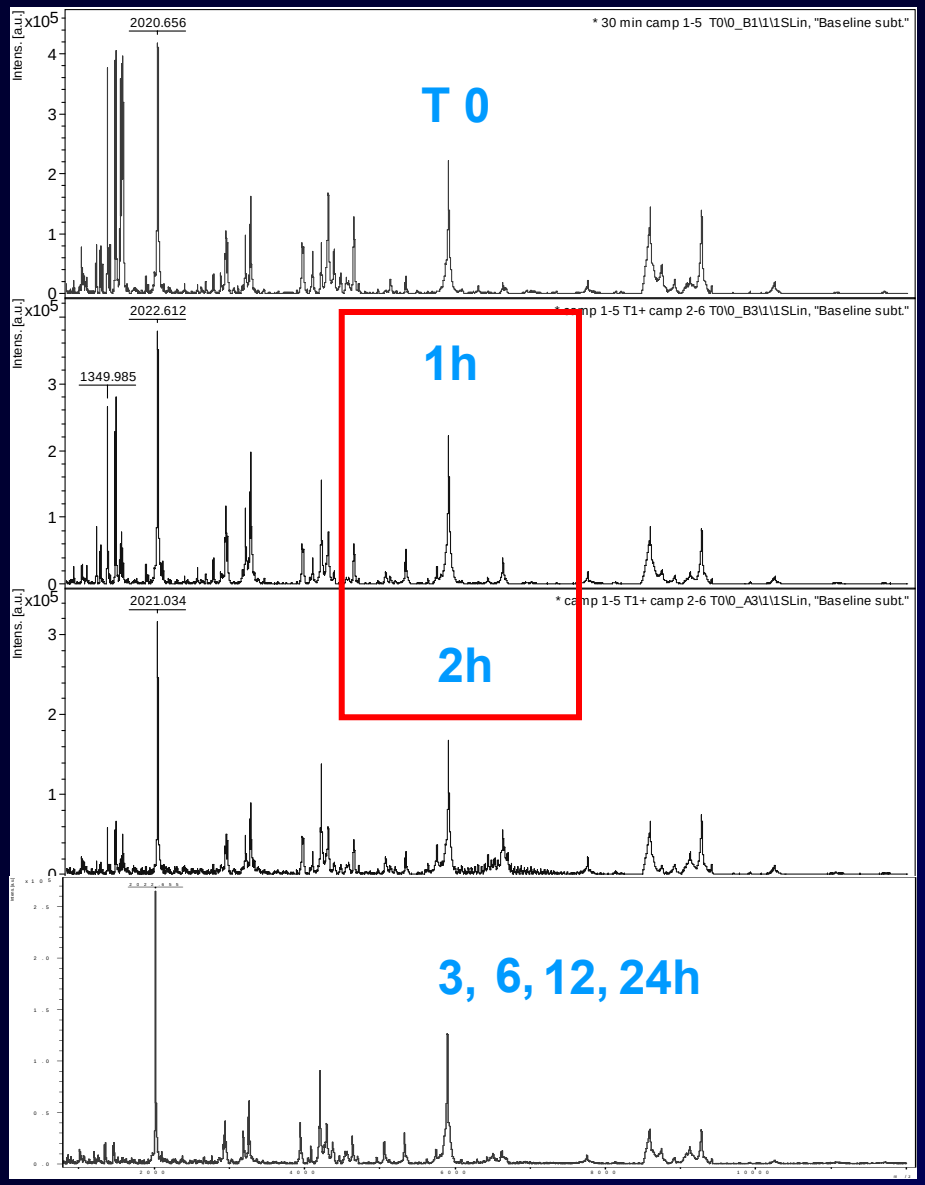
25 Peaks

Ave. CV
32.81%

Ave. CV
18.35%

Ave. CV
28.38%

LMW protein profiles from serum (w/o PI addition on whole blood) stored at +4 C up to 24 hrs



Ave. CV < 16%

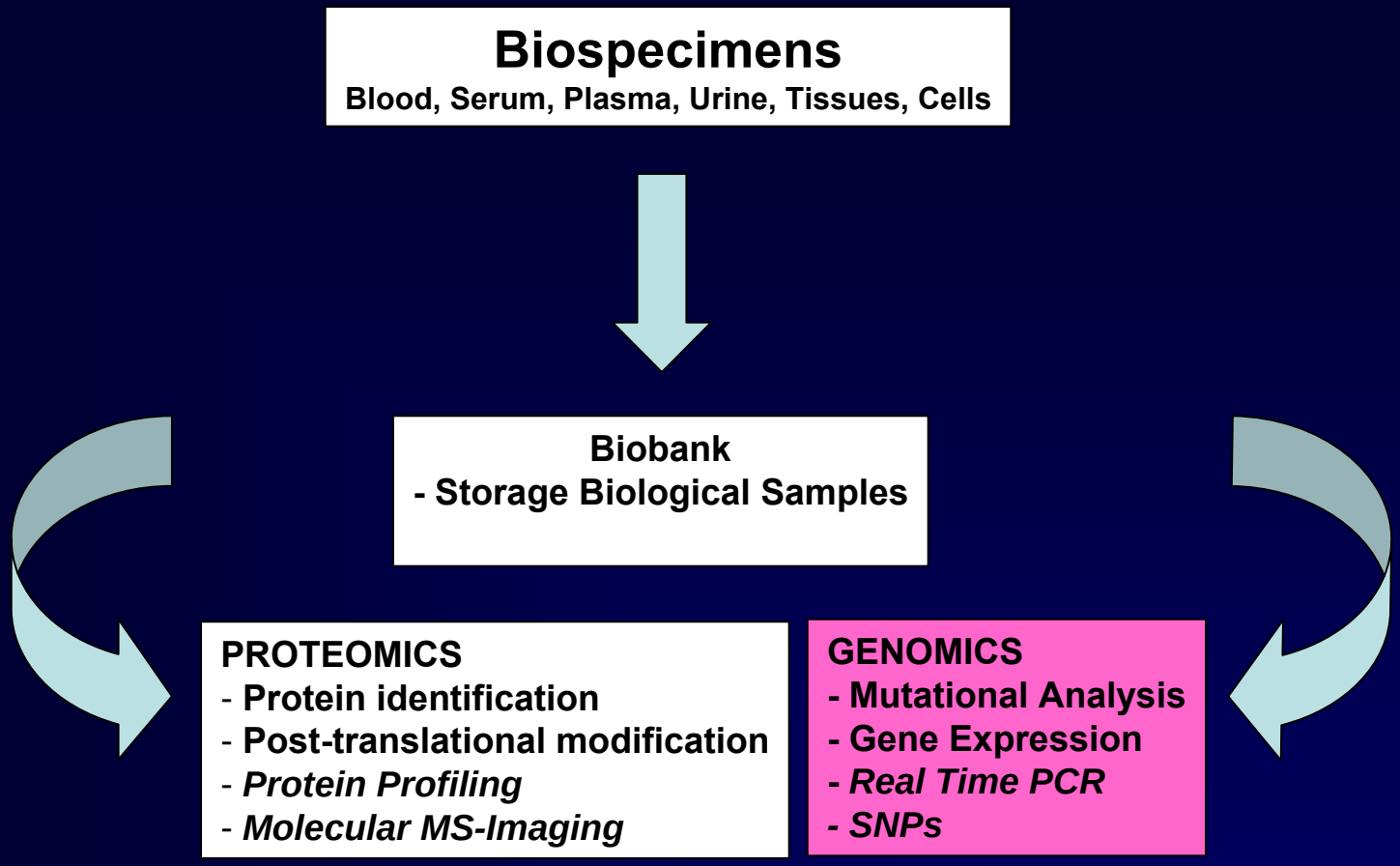
Ave. CV < 26%

3h
Ave. CV 30-36%

6h
Ave. CV 25-28%

12h
Ave. CV 25-27%

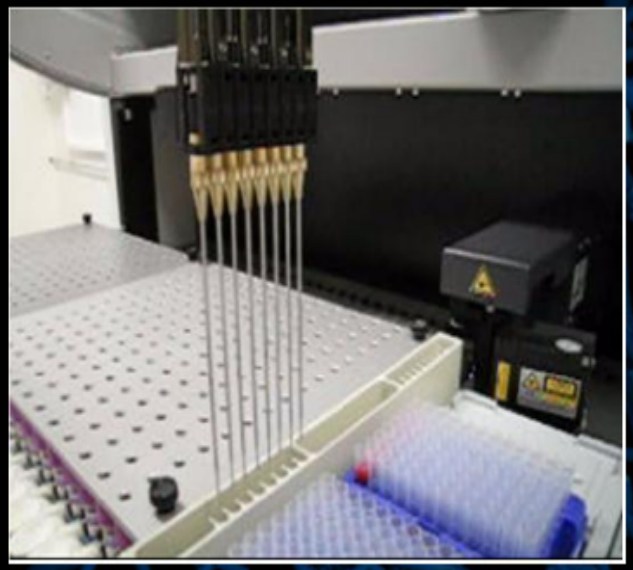
24h
Ave. CV 29-33%



BIOMARKERS DISCOVERY

Laboratory Automation for genomic high-throughput analysis

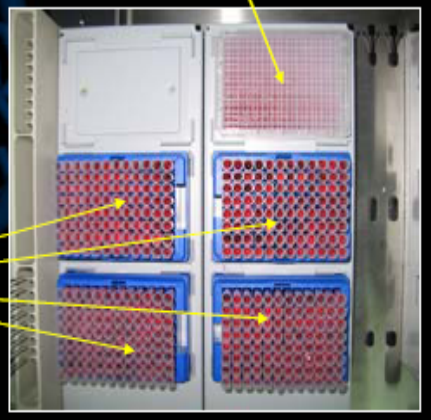
DNA extraction, purification and sequence analysis



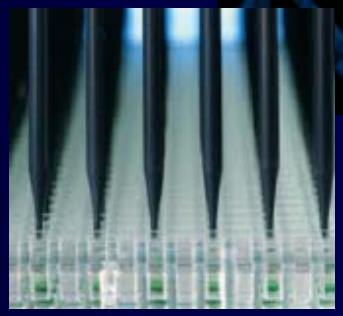
1 Template of samples

+

384 plate for DNA extraction (20µL / well)



Blood bank:
96 wells plate
(950 µL / sample)



Standard Operating Pre-Analytical Procedures for DNA Analysis

Methodological Approach

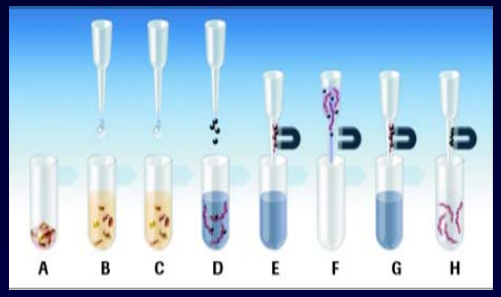
- Comparison between manual *vs* robotized DNA extraction

Pre-analytical conditions

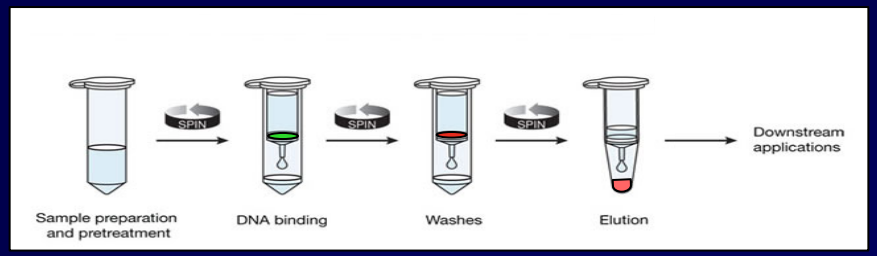
- Different anticoagulants
- Time before processing
- Storage temperature

Different DNA extraction methods

Automated - DNA Isolation Kit



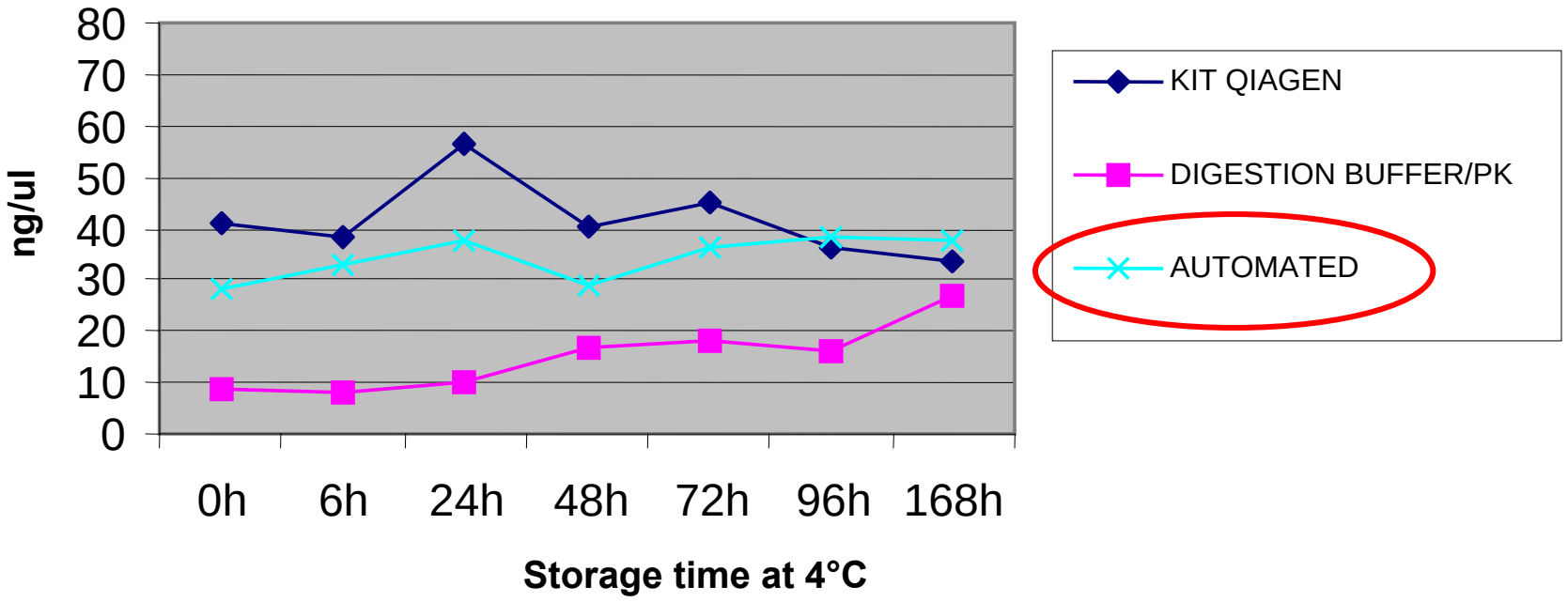
Manual QIAmp DNA Blood Mini Kit



In house Digestion Buffer / Proteinase K kit

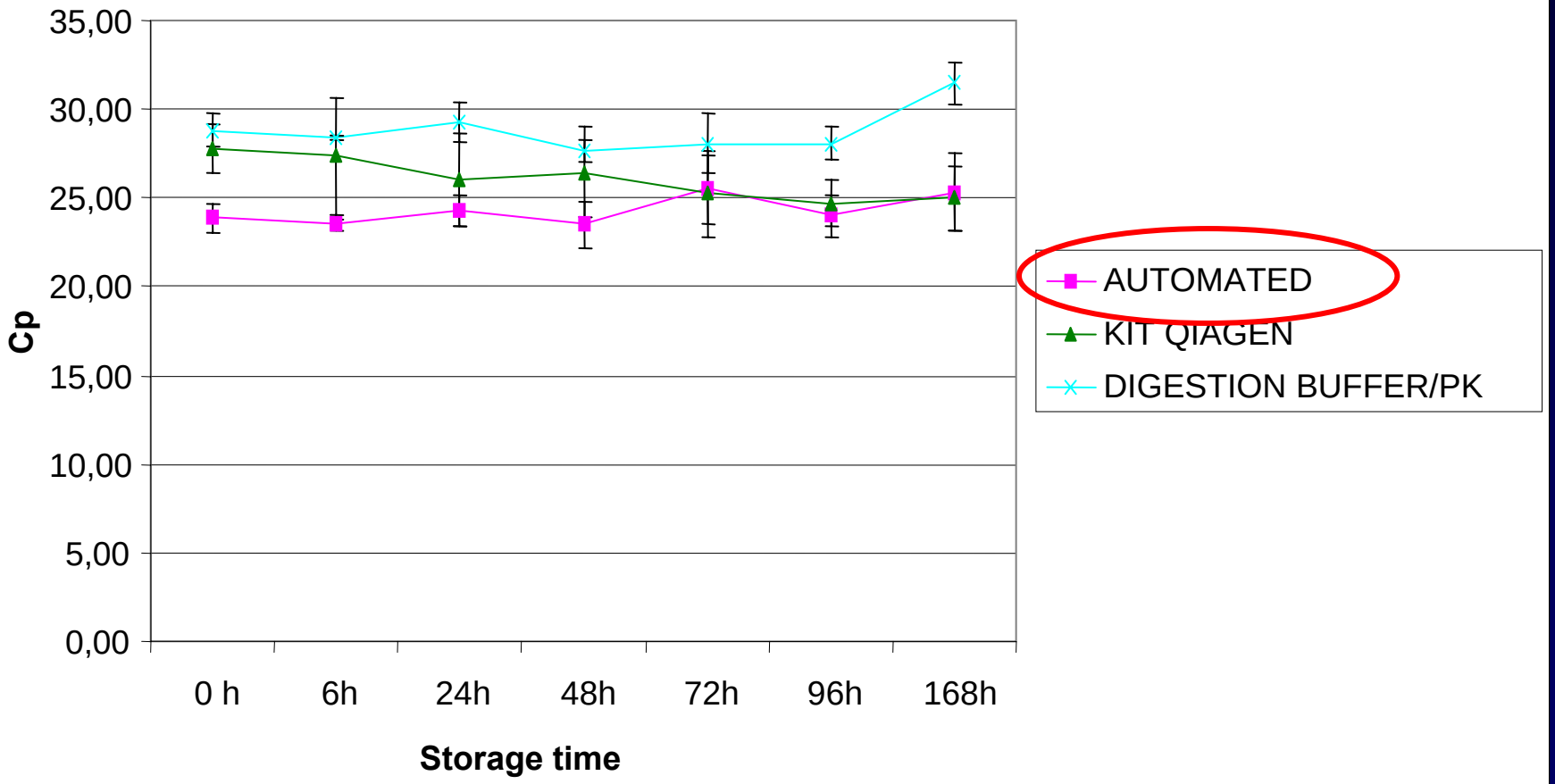
DNA recovery ng/µl

(Nanodrop spectrophotometric quantification 260 nm)



Evaluation of DNA quality/integrity

(Real –Time PCR, ex9a APC gene, 186bp)



Any temperature

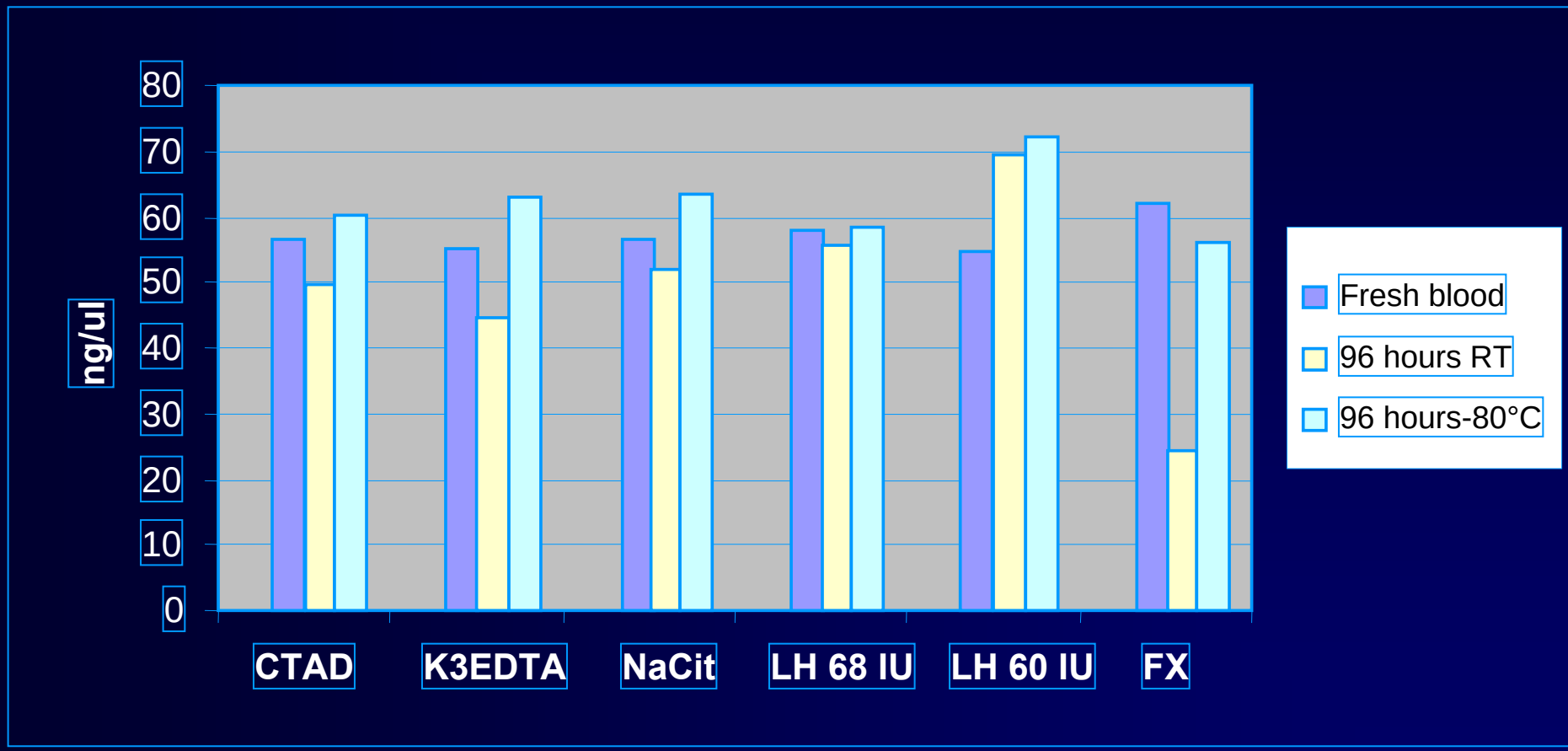
Anticoagulants

BD Vacutainer Venous Blood Collection Tubes

Anticoagulant	Closure color	Volume
CTAD (Buffered sodium citrate theorphylline adenosine dipyridamole)	**Light blue**	4,5 ml
K3EDTA (K3 Ethylenediaminetetraacetic Acid)	**Lavender**	3 ml
NaCit (Sodium Citrate, Silicone coated)	**Light blue**	4,5 ml
LH 68 IU (Lithium Heparin 68 IU)	**Green**	4 ml
LH 60 IU (Lithium Heparin 60 IU)	**Grey**	3 ml
FX (Sodium Fluoride Oxalate)	**Light grey**	2 ml

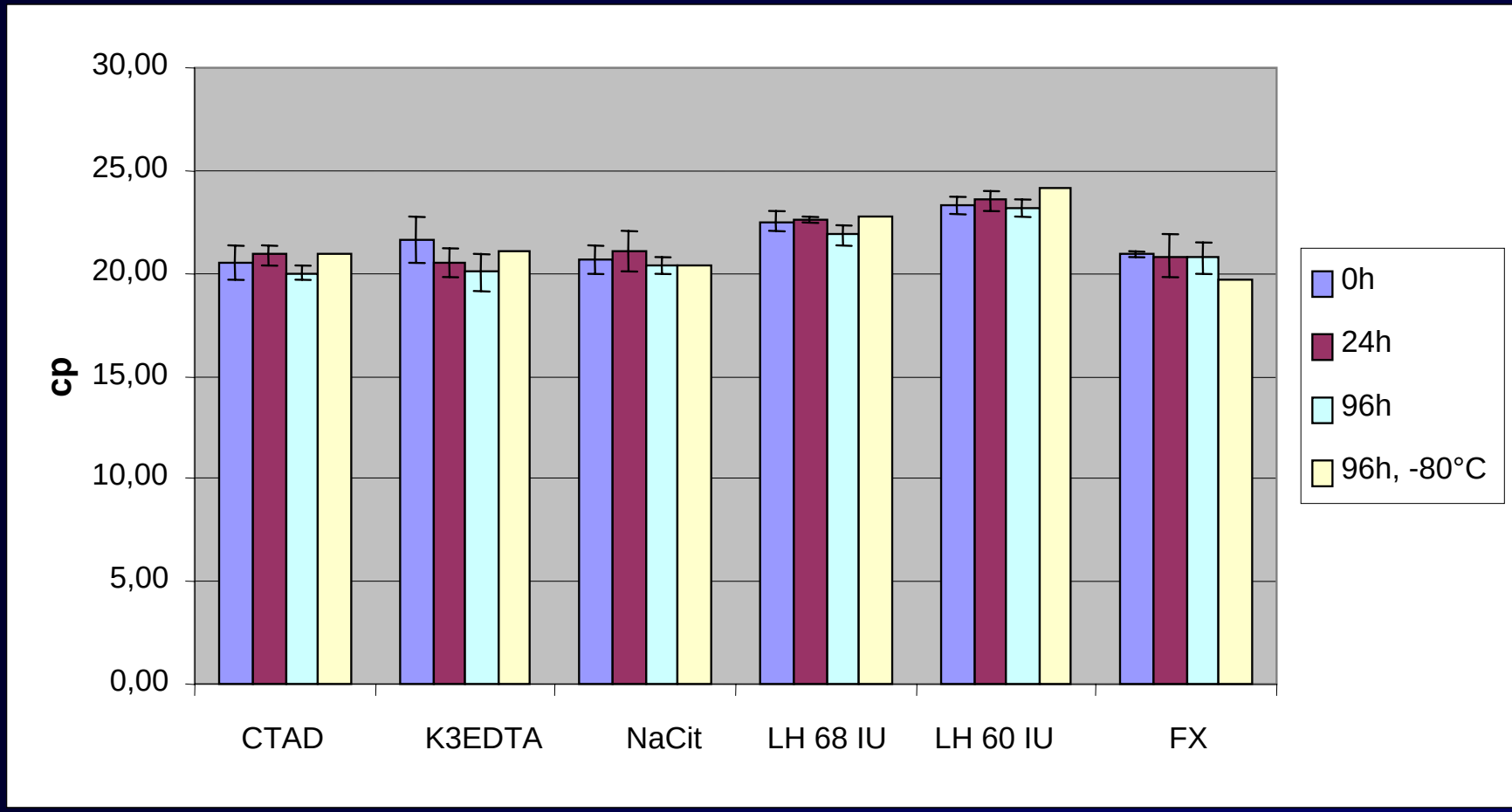
DNA recovery ng/µl

(Automated extraction - Nanodrop spectrophotometry 260 nm)



Quantitative Real –Time PCR

(ex9a APC gene, 186bp)



These data were obtained also in all the other storage conditions

Conclusions

Automation of the pre-analytical phases of proteomics as well as genomic studies might be one approach to improve standardization and homogeneity of analytical results

LMW protein profiling

1. **Automation allowed an increased LMW peak recovery of 16.2%, as well as a better reproducibility of results compared to manual procedure;**
2. **Clotting times up to 2 hours and serum storage (with or w/o PI addition) up to 2 hrs at +4 °C did not significantly modified the LMW protein profiling results;**
3. **Storage at −80 °C up to 1-year, without PI addition resulted in not significant changes in serum LMW profiling.**

Conclusions (2)

DNA analyses

1. **Automation of DNA extraction allowed the best recovery and reproducibility of the results compared to manual procedures;**

2. **All six anticoagulant evaluated, with the exception of LH show similar results.**

3. **RT up to 168 hrs can be considered a good storage condition for samples to be used for DNA studies.**

In conclusion, preanalytical phases are crucial and require specific focus and traceability. However, we should not underestimate other parameters like, cellular population and the values of analytes that can be tested only on fresh samples. All these information should represent the package data linked to a sample stored in a biorepository.

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World Biobanking Summit
23-25 September, Edinburgh, Scotland