Standardization Activities of the Paul-Ehrlich-Institut

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National and International Integration

Federal Ministry of Health and Social Security

PEI

Paul-Ehrlich-Institut

Federal Agency for Sera and Vaccines

BfArM

Federal Institute for Medicines and Medical Devices

BzgA

Federal Centre for Health Education

DIMDI

German Institute for Medical Documentation and Information

RKI

Robert Koch Institute

and:

BVA (Federal Social Insurance Authority)

BSG (Federal Social Court)

EMEA:
European Medicines Evaluation Agency

CHMP:

Committee for Medicinal Products for Human Use

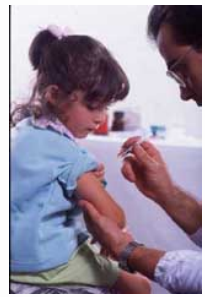
CVMP:

Committee for Medicinal Products for Veterinary Use

Tasks of the Paul-Ehrlich-Institut

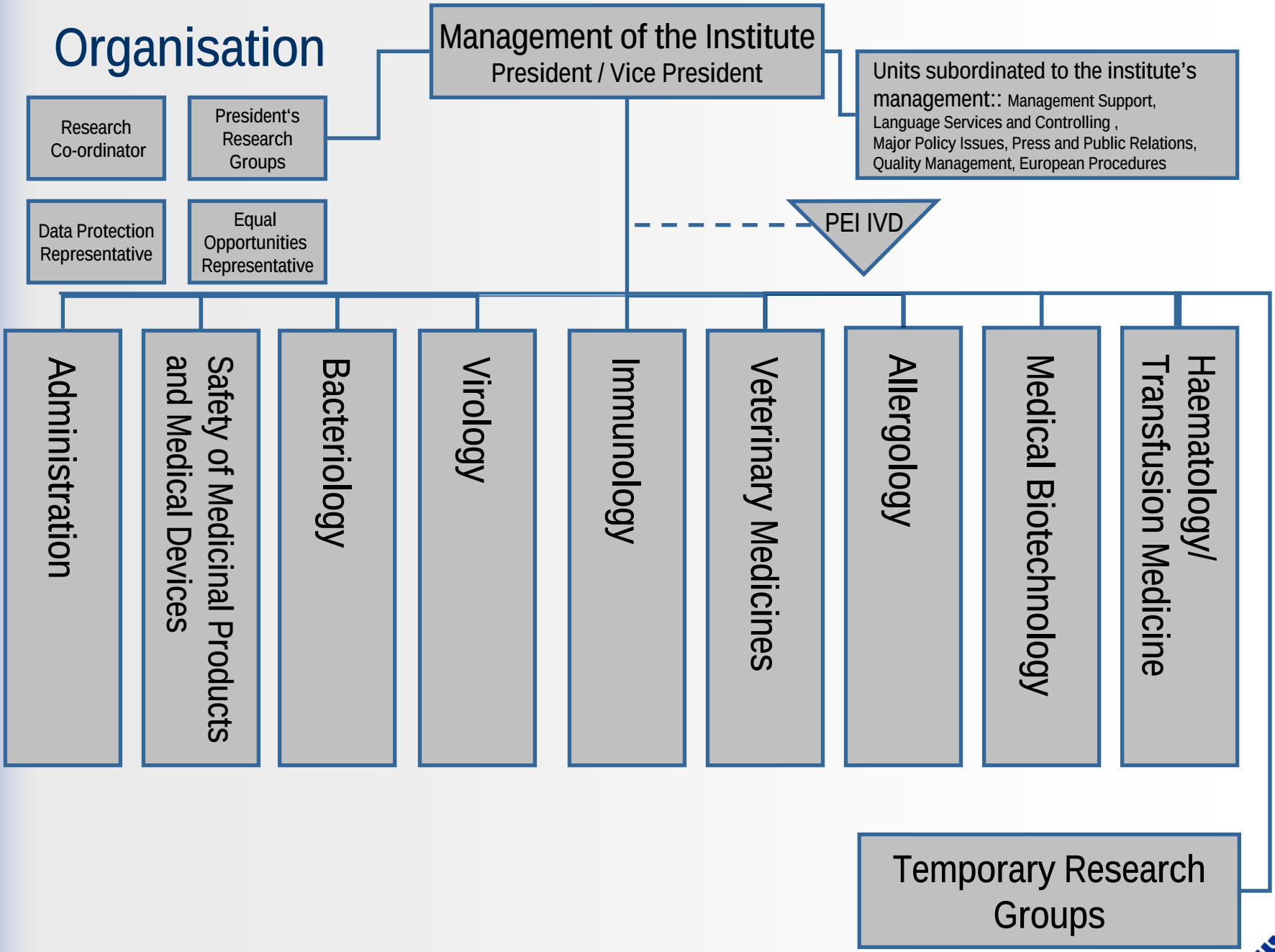
- Granting of the marketing authorisation
  (medicinal products for human use)
  - Vaccines and sera
  - Blood and blood products
  - Allergens (test and therapy allergens)
  - Gene transfer medicinal products
  - Somatic and xenogeneic cell therapy products

- Batch release
  - Vaccines and sera
  - Allergens
  - Blood products
  - Plasma pools



- Post marketing surveillance

Organisation



Paul-Ehrlich-Institut

- The PEI employs approx. 630 staff members
- Out of these, approx. 200 work as scientists
- The PEI processes more than 200 marketing authorisations and 7,000 batch tests/year.

History of the Paul-Ehrlich-Institut

1972: The PEI becomes a senior federal authority

1994: Responsibility for blood and blood products

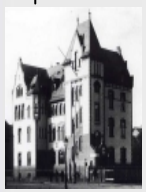
2000: IVD test laboratory (CE)

2004: GCP Directive

1896: Institute for Sera Examination & Sera Research

1906: Georg-Speyer-House

1900



1899: Royal Institute of Experimental Therapy

1947: Paul-Ehrlich-Institut

1993: PEI in Langen

2000

today

Tasks of the Paul-Ehrlich-Institut on the IVD field

National Drug Law 

1972 - 2000

- Approval, batch testing and post marketing surveillance of in vitro diagnostic devices (high and medium risk infectious markers)

IVD Directive (IVDD) 

Since 2000

- IVD test lab for Annex 2 List A, (B) markers
  - Evaluation studies
  - Verification of manufactured products

Standardization Tasks of the Paul-Ehrlich-Institut

Law on the establishment of a Federal Agency for Sera and Vaccines (07/11/1972)

Article 1

- ……
- Development of standard preparations for biologicals
- ……

1972 – 2005 Development of numerous PEI standards

- Immunoglobulins, vaccines
- Allergens
- IVDs
  - Serological assays: HBsAg, anti-HBc, anti-HCV, anti-HIV-1/2,...
  - NAT assays

Nucleic Acid Amplification Techniques (NAT)

Need for NAT standardization

- Virus transmissions by blood and blood products
- Sensitive NAT methods
- Manufacturer-defined unitages (copies, genome equivalents, ...)
- Regulations (HCV NAT) defining minimal sensitivity

- Common Technical Specifications (CTS) of IVDD refer to International Standards

Standardization Activities of the PEI

NAT standardization

- WHO International Standards (SoGAT)
- Biological Reference Preparations (EDQM)
- PEI Reference Preparations

Nucleic Acid Amplification Techniques (NAT)

NAT standardization: Biological standards

- WHO International Standards (SoGAT)
  - HCV RNA, HIV-1 RNA, HBV DNA
  - B19V DNA, HAV RNA
- Biological Reference Preparations (EDQM)
  - PEI as Project Leader for establishment of BRPs for HCV RNA and B19V DNA
- PEI Reference Preparations
  - HCV RNA, HIV-1 RNA, HBV DNA

Nucleic Acid Amplification Techniques (NAT)

NAT standardization

- WHO International Standards (SoGAT)
  - IU/ml
  - For calibration of secondary standards
  - For calibration of assays (CTS)
  - Validation studies
- Biological Reference Preparations (EDQM)
  - Calibrated against WHO IS (IU/ml)
  - NAT testing performed as OMCL (plasma pools)
- PEI Reference Preparations
  - Calibrated against WHO IS (IU/ml)
  - Validation of blood screening and diagnostic assays
  - Run controls for blood screening in-house assays
  - Verification of manufactured products (PEI-IVD)

Nucleic Acid Amplification Techniques (NAT)

NAT standards: Desired Features

- Suitable for all NAT methods: PCR, TMA, NASBA, branched DNA, ...
- Suitable for standardization of extraction, amplification and detection
- Cover measuring range of quantitative NATs
- Reflect target material (as far as possible)
- One common unitage
- Feasibility of replacement

Nucleic Acid Amplification Techniques (NAT)

Establishment of an European Pharmacopoe Biological Reference Preparation for HCV RNA

Background

- CPMP requirement for plasma pool HCV NAT
- OMCLs confirm manufacturer‘s testing (batch release)
- Need for a common reference material

Biological Reference Preparation HCV RNA (PhEurBRP)

WHO IS (96/790)	BRP Candidate Material
HCV RNA pos plasma	
HCV genotype I	
anti-HCV pos	anti-HCV neg
diluted in neg plasma pool	
100,000 IU/ml	4,000 – 10,000 geq/ml
freeze dried (0.5 ml)	

Biological Reference Preparation HCV RNA (PhEurBRP)

Collaborative calibration study

26 Participants (Europe, USA)

OMCLs (9)

Plasma derivatives manufacturers (11)

Diagnostic labs (6)

Biological Reference Preparation HCV RNA (PhEurBRP)

Collaborative calibration study

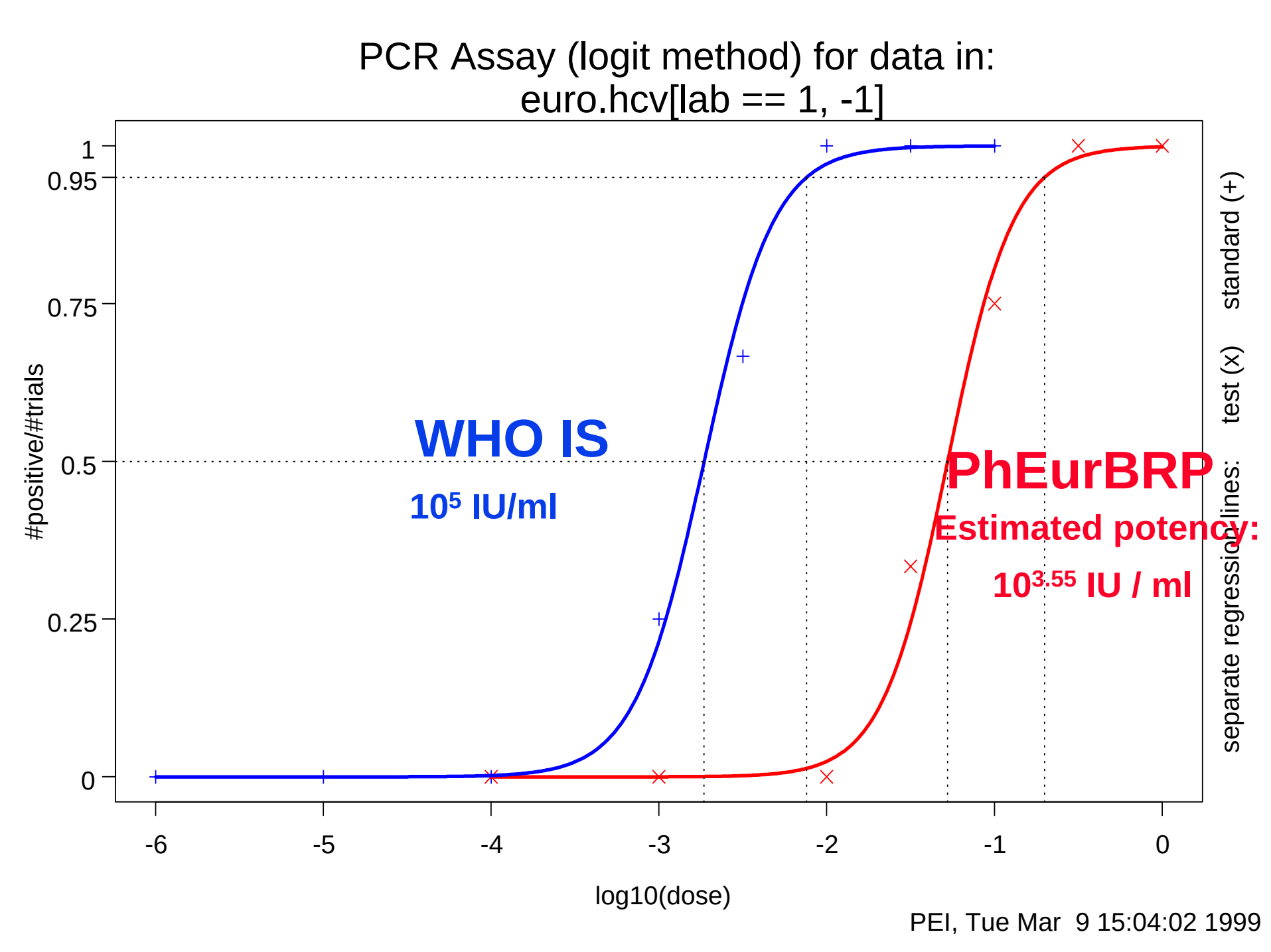
* Calibration against WHO standard
* Assignation of unitage (IU/ml)
* Suitability for OMCLs‘ plasma pool testing

Biological Reference Preparation HCV RNA (PhEurBRP)

Collaborative calibration study

Protocol

* 4 vials of candidate material and WHO IS
* Routine NAT
* Dilution series (0.5 $\log_{10}$) around end point
* Statistical evaluation, potency calculation
* Independent from methodical sensitivities


PCR Assay (logit method) for data in:
euro.hcv[lab == 1, -1]
#positive/#trials
1
0.95
0.75
0.5
0.25
0
-6
-5
-4
-3
-2
-1
0
log10(dose)
separate regression lines: test (x) standard (+)
WHO IS
10^5 IU/ml
PhEurBRP
Estimated potency:
10^3.55 IU / ml
PEI, Tue Mar 9 15:04:02 1999

Biological Reference Preparation HCV RNA (PhEurBRP)

Collaborative calibration study

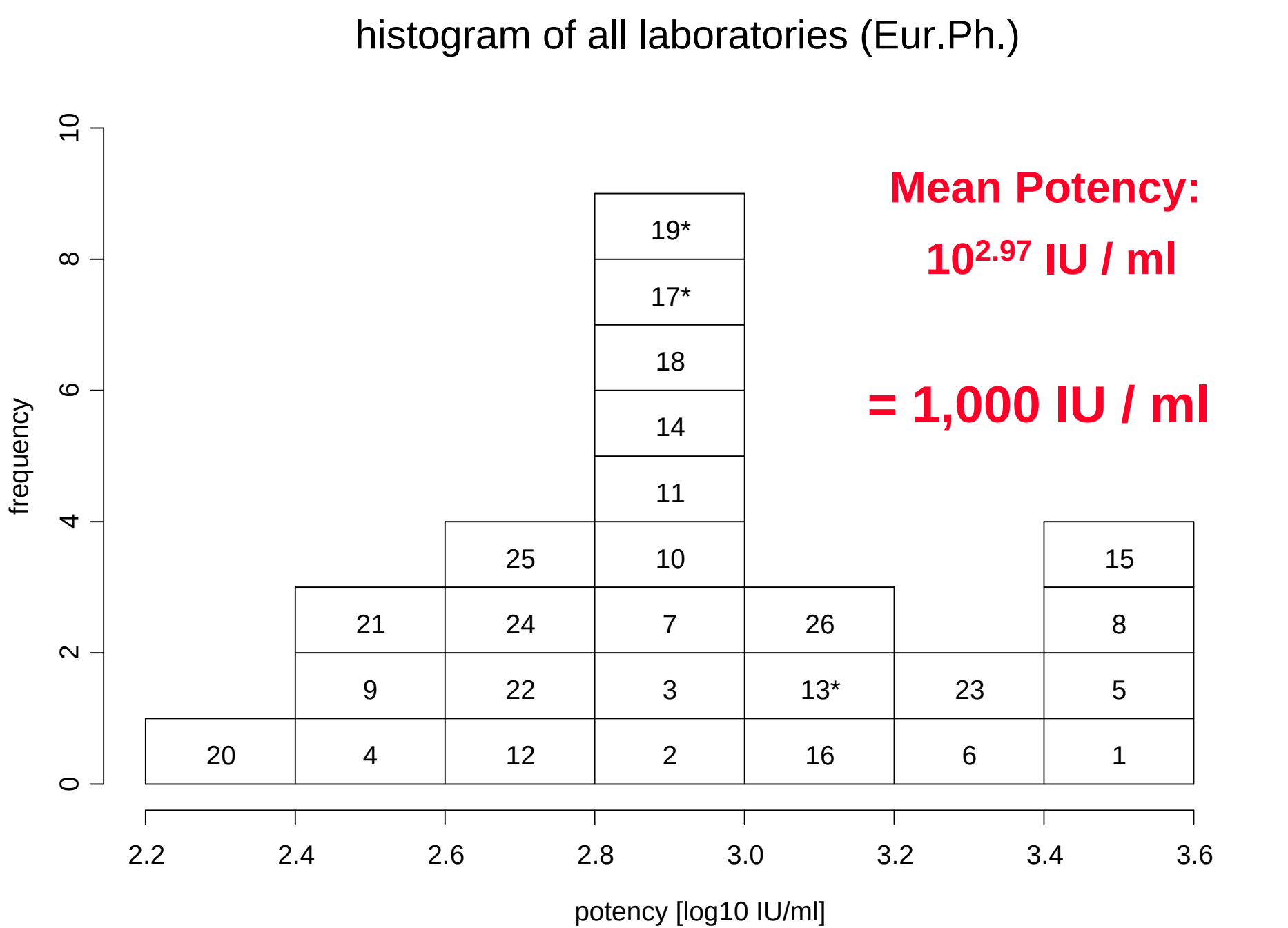
Results

* 26 „valid“ data sets

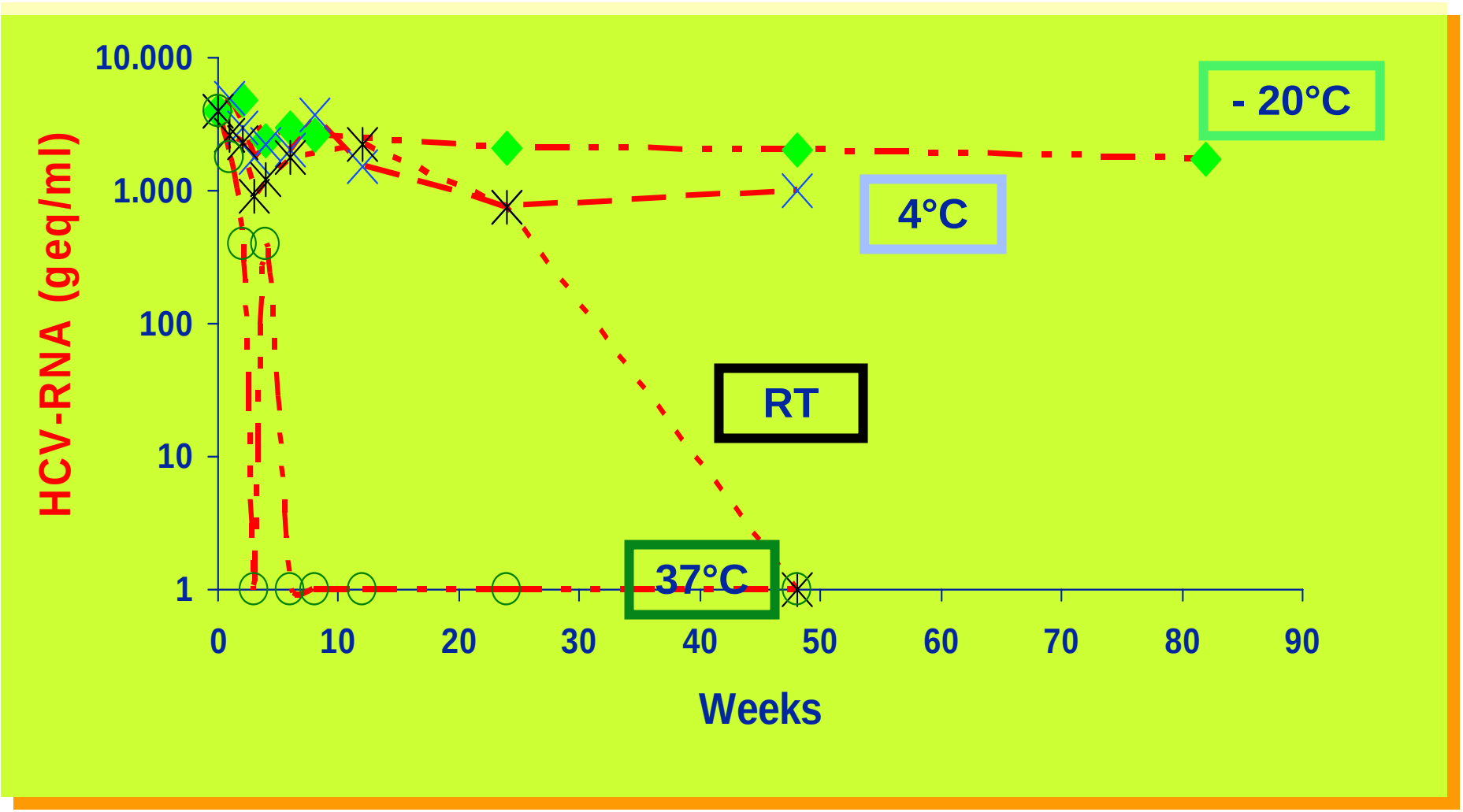
* 23 parallel curves, 3 non-parallel curves

* Mean estimated potency $10^{2.97}$ IU / ml for PhEurBRP

  (independent from inclusion of non-parallel curves)


histogram of all laboratories (Eur.Ph.)
Mean Potency:
10^2.97 IU / ml
= 1,000 IU / ml
frequency
0
2
4
6
8
10
19*
17*
18
14
11
25
10
15
21
24
7
26
8
9
22
3
13*
23
5
20
4
12
2
16
6
1
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
potency [log10 IU/ml]

Ph. Eur. BRP HCV RNA
Stability at Different Storage Temperatures



Standardization Activities of the PEI

06/06/2005

PEI becomes WHO Collaborating Centre for Quality Assurance of Blood Products and in vitro Diagnostic Devices

10/27/2005

WHO Expert Committee on Biological Standardization (ECBS)

Approval of PEI Work Plan on e.g.

- HBV DNA and HBsAg genotype (A-H) panel
- Anti-HCV mono-specific antibodies panel
- Anti-HBc IS
- Network training on IVD regulatory issues

Biological and Synthetic NAT Standards

NAT International (Biological) Standards

- Inherent variability (biologicals)
- Unknown extent of variability
- No traceability to SI units
- Arbitrary units
- Replacement standards difficult to calibrate

NAT Methods

- Different principles
- High variation, fast improvements
- No „reference method“

Biological and Synthetic NAT Standards

Reference System

..... consists of

Reference Material

Reference Method

Reference Laboratory

..... provides

Traceability, e.g. to SI units

Quantification of Uncertainty

Biological and Synthetic NAT Standards

Reference System for next generation NAT Standards

..... could consist of

* Reference Material

  Well-characterized synthetic Nucleic Acid:

  Plasmids, in-vitro transcripts

  Which sequences? Which geno/subtypes?

* Reference Method

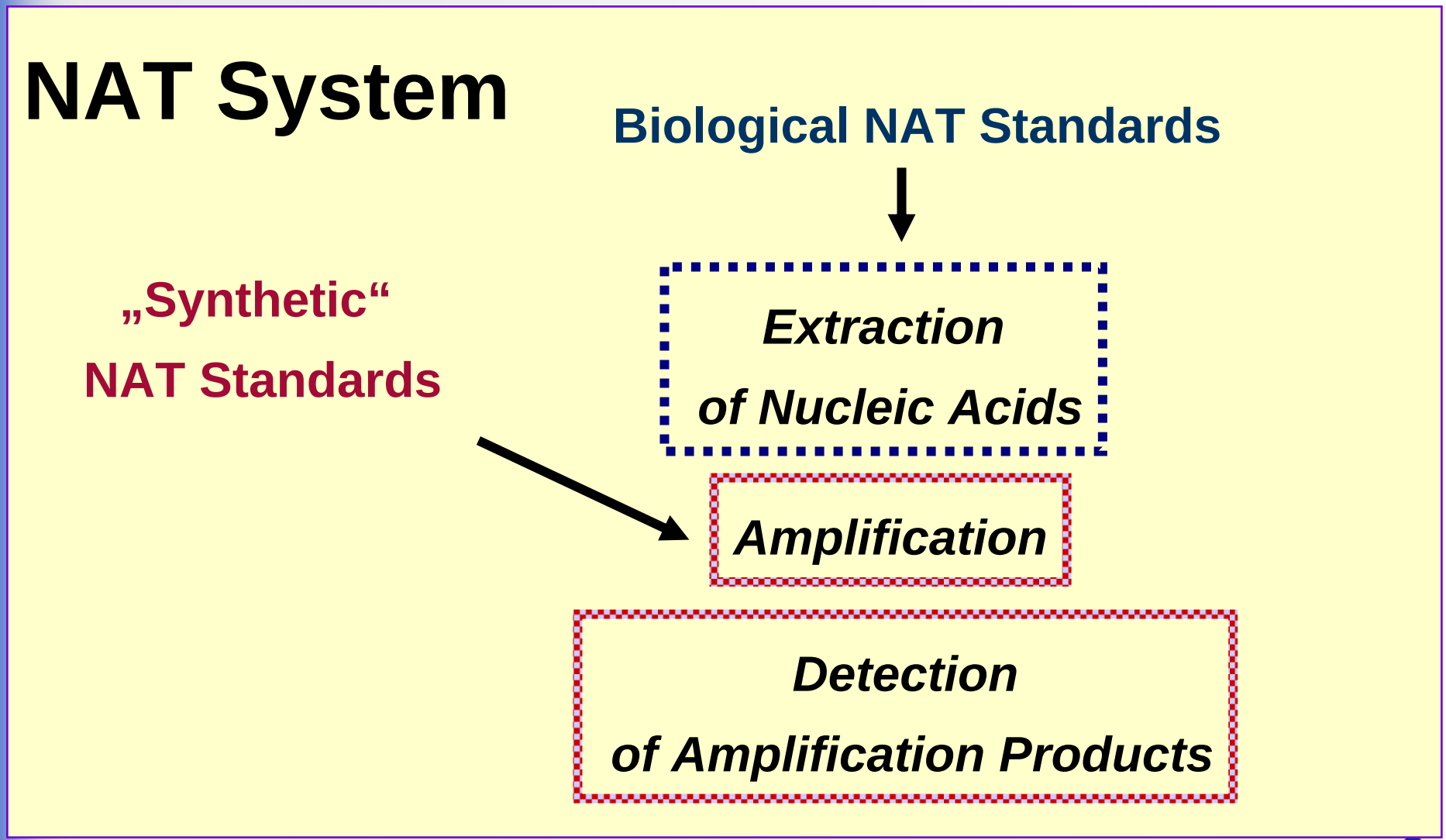
  NAT-independent quantitation method (SI units)

  e.g. phosphate quantitation, OD, dephenylamine measurement

* Reference Laboratory

  Qualification?

Biological and Synthetic NAT Standards



NAT Standardization

European Study Group on Viral Hepatitis (EUROHEP)

Standardization of laboratory methods
(Report from 1994)

- Quality control of HBV detection methods
- Quantitation of HBV DNA in two Eurohep reference plasma preparations
  - Proof of the NAT method (using rHBV DNA)

NAT Standardization: Synthetic Material

rHBV DNA (Chrion): Certificate of analysis

- HBV genomic fragment of subtype adw2 (3200 bp cloned)
- purification check (High Performance Capillary Electrophoresis and agarose gel)
- Quantitation of the purified fragment (optical density at 260 nm, phosphate analysis, and diphenylamine measurement)
- Quantitation in µg/mL

NAT Standardization: Synthetic Material

Titration of recombinant HBV DNA using nested PCR (limiting dilution)

Dilution	HBV DNA (10 µl)	HBV geq (10 µl)	PCR Positive
10^{-7}	100 ag	29	6/6
10^{-8}	10 ag	2.9	6/9
10^{-9}	1 ag	0.29	1/7

rHBV DNA (Chiron Corp., 2 µg HBV DNA/ml) = **5.80 x 10^{11} HBV geq/ml**

PPU_{50} (maximum likelihood): $10^{8,22\pm0,34}$ /10µl = **2.32 x 10^{11} HBV geq/ml**

Synthetic NAT Standards

- Standardization of amplification/detection procedures
- Geno/subtype-specific
- Would fulfill criteria of JCTLM (traceability to SI units)
- On-going studies (IVD manufacturer)
- Open question: Standardization of the whole NAT procedures?
  - Including sample preparation = viral nucleic acid extraction
  - Synthetic standards don not represent the complete virus

Thank you for your attention!

Biological Reference Preparation HCV-RNA (PhEurBRP)

	10^{-1}	$10^{-1.5}$	10^{-2}	$10^{-2.5}$	10^{-3}	$10^{-3.5}$
WHO IS	+ + + +	+ + + +	+ + + +	+ + + +	+ + - +	+ - - -
PhEur	+ + + +	+ + + +	- + - +	- - + -	- - - -	- - - -