Assignment of Values to Reference Standards in Haematology

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Reference Standards and Materials

Available:

- **Coagulation factors and inhibitors - ISTH**
- **Blood group serology - ISBT/ICSH**
- **Platelet and red cell antibodies –ISBT/ICSH/ISTH**

Traceability:

- **Complex biologicals mostly measured in arbitrary units eg IU - where appropriate, some are assigned with SI units, some are qualitative and do not have any values assigned**
- **Primary standards establish by WHO**
- **Secondary standards directly traceable to WHO primary standards**

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World Health Organization, Technical Report Series, No. 800, 1990

Annex 4

GUIDELINES FOR THE PREPARATION, CHARACTERIZATION AND ESTABLISHMENT OF INTERNATIONAL AND OTHER STANDARDS AND REFERENCE REAGENTS FOR BIOLOGICAL SUBSTANCES

(Revised 1989)

1989 Guidelines

- Assessment of need and procurement of materials
- Distribution into final containers
- Processing of filled ampoules
- International collaborative studies
- Detailed information to be provided to WHO
- Establishment of an international biological standard or reference reagent

Quality of Final Product I

- With a few exceptions, international standards usually in heat-sealed glass ampoules
  - No exchange of gases and moisture
  - Greater stability over time
- Precision of fill - < 0.25%; 0.05 –0.07% achieved
- Majority are freeze-dried, filled with inert gas before sealing
- Secondary desiccation (for some materials)
- Residual moisture: <1%; <0.05% with secondary desiccation
- Residual oxygen content: <45 μmol/L

Quality of Final Product II

- No declared shelf life
- Stability monitoring :
  - accelerated degradation study at elevated temperatures
    - ampoules stored at +4, +20,+ 37, +45 and +56°C
    - At various time-points, activities compared with -150°C ampoules
    - Fit in Arrhenius equation for prediction of loss of activity
  - real time degradation monitoring
    - Ampoules at storage temperature (-20°C) against ampoules kept at ultra-low temperatures (-150°C)

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Accelerated Degradation Studies on 4th IS FVIII/vWF Plasma – ampoules stored at +4, +20,+ 37, +45 and +56°C compared with -150 °C ampoules

Study	Time points years	Labs	Method	Predicted loss at -20°C (%/year)
Single time-point, multi-lab	0.63	a	1-stage	0.001
		b	"	0.263
		c	Chromogenic	0.021
		d	"	0.022
Multi-time point, single lab	0.34, 0.55, 1.06, 2.30, 3.76, 4.66	c	Chromogenic	0.007

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Real Time Studies on the 4th IS FVIII/vWF Plasma

Ampoule/Assay	Potency of -20°C ampoules as % -150°C ampoules	
	3.75 yrs	4.66 yrs
1	102	102
2	96	101
3	101	96
4	103	97
Mean (%CV)	100 (3.1)	99 (3.0)

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Actual “Shelf-Life”

FVIII Concentrate	5 years
FVIII/VWF Plasma	5 years
Protein C Plasma	18 years
Unfractionated Heparin	15 years
Antithrombin Concentrate	7 years

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Quality of Final Product III

- No declared uncertainty of measurement

But

- Collaborative study reports do contain information on 95% confidence limits based on potency estimates from:
  - individual laboratory
  - all laboratories
- Potency estimates and confidence limits can be calculated for different method types, but this is not always helpful for the intended use of WHO biological international standard

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New Revision of WHO Guidelines – In Progress

General consideration

- **Traceability Path**
  - **Relationship of the UNIT of proposed standard to the previous units of the same material**
  - **Evaluation of the extent of the continuity of the IU**
- **Uncertainty of Measurement**
  - **CV of the fill**
  - **Evaluation of the requirements of uncertainty in the context of the traceability path**

Specific for IVD reference materials

- **ISO 17511 principles**
- **Commutability**

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Coagulation Factors & Inhibitors

- **Definition of unit**
- **Continuity of unit**
- **Like vs like**
- **Multi-methods**

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Units of Coagulation Factors and Inhibitors

1 International Unit =

Amount or activity in 1mL of

“average fresh normal plasma”

As defined by pools of fresh plasma collected from normal donors in labs participating in international collaborative study, according to a defined protocol (normally > 200 donors overall)

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Coagulation Factors & Inhibitors

- Definition of unit
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Continuity of Unit

- Long-term use of same standard
  - Dependent on batch size and demand
  - Assuming no significant degradation
- Replacement by similar material
  - Calibrate against preceding standard
  - Same lab, same methods?
- Cross check against “normal plasma” as well as previous standards
  - For plasma standards
  - Assuming unchanging values in normal population

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Coagulation Factors & Inhibitors

- Definition of unit
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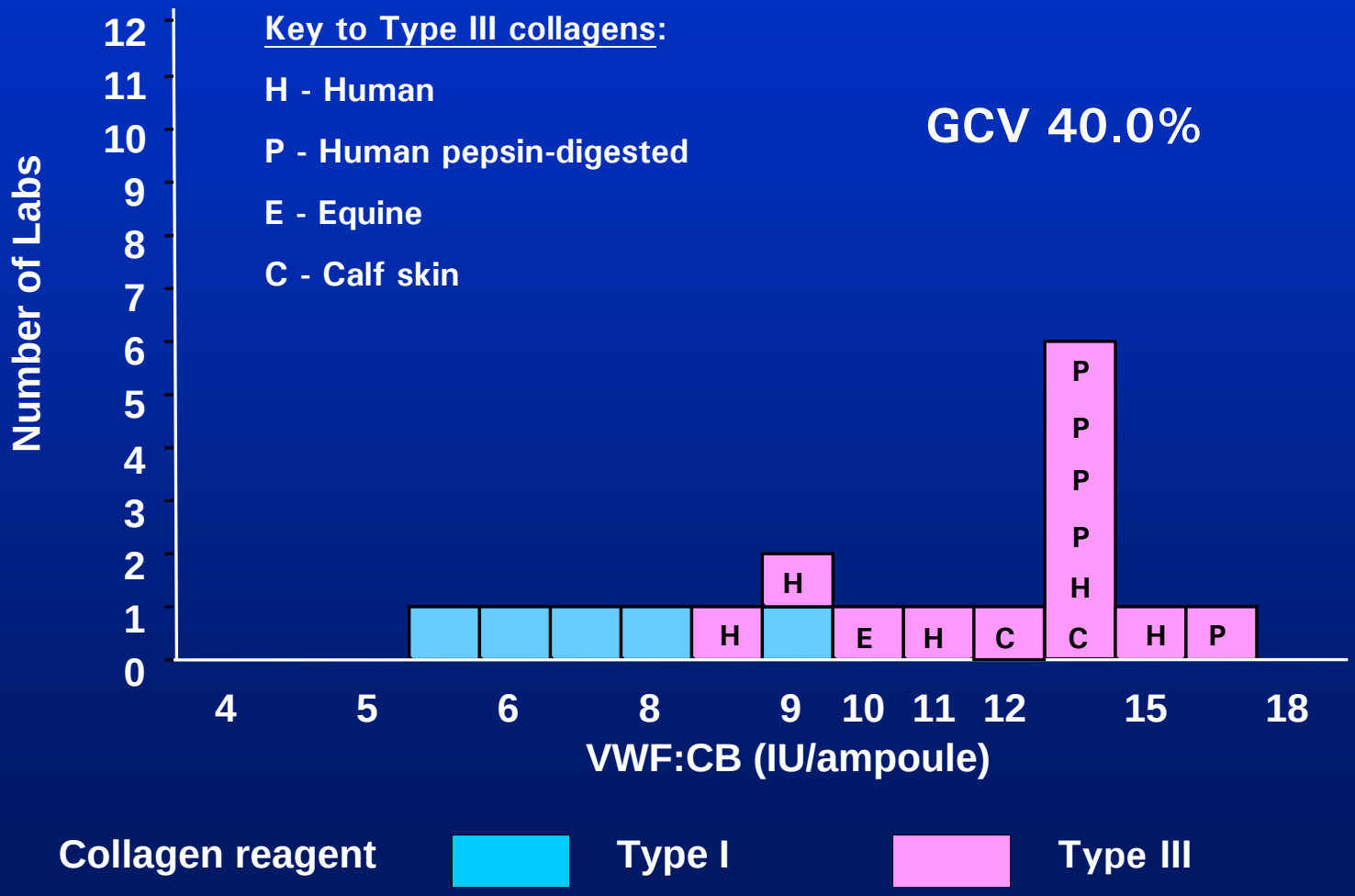
Importance of “Like vs Like”

- To minimise “matrix” effects
- Concentrate standards for assay of concentrate products
- Plasma standards for assay of patients’ plasma
- Concentrate vs plasma:
  - large variability between labs
  - differences between methods

VWF: Collagen binding

Estimates for concentrate C relative to the 4th IS Plasma



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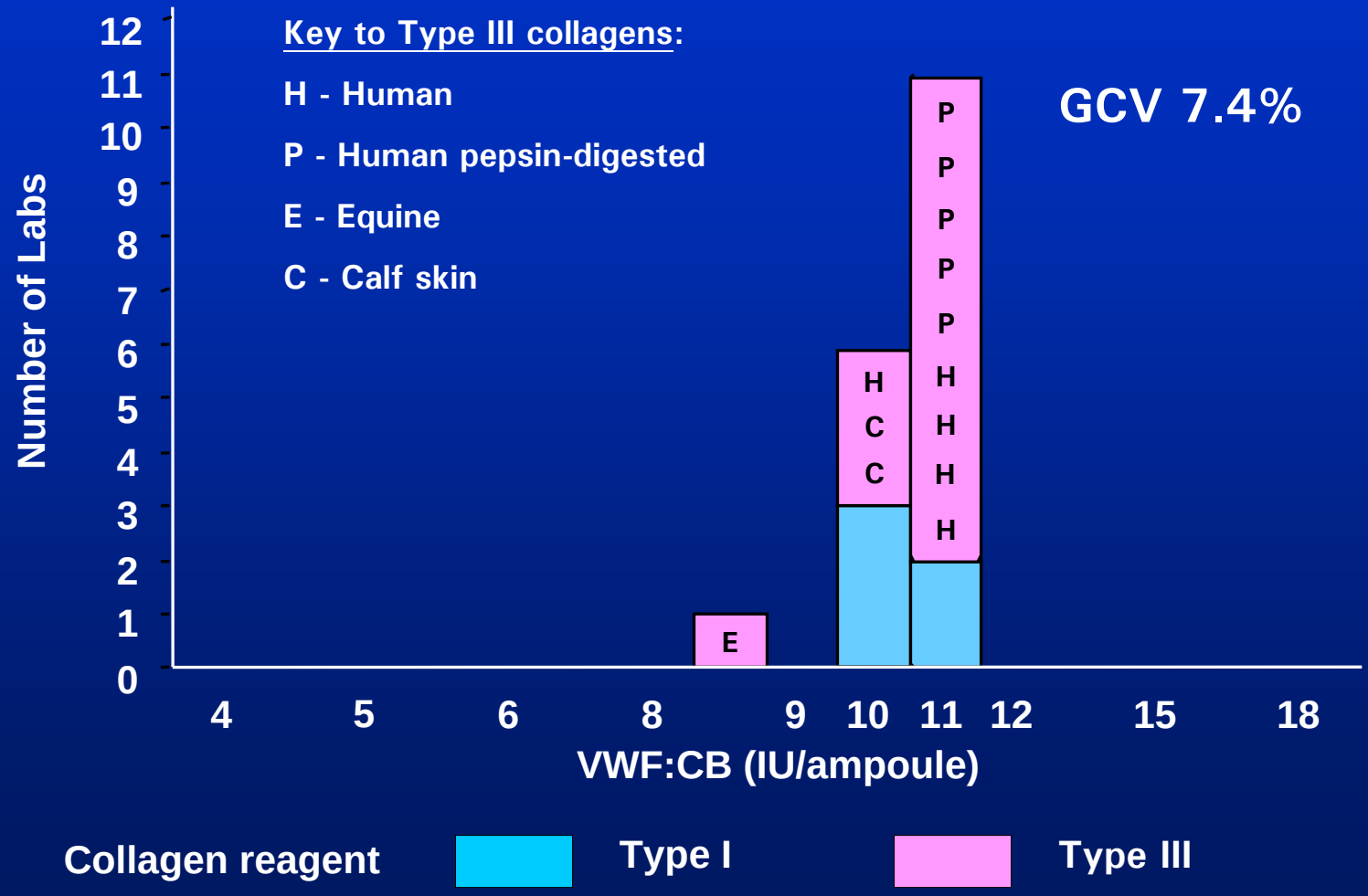
VWF: Collagen binding

Estimates for concentrate C relative to 1st IS VWF Concentrate



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Coagulation Factors & Inhibitors

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“All Methods” Approach

- WHO Standards should be suitable for use with all current methods
- “Reference methods” not easily definable for coagulation factors/inhibitors
- Some prescription of methodology in collaborative studies etc:
  - Pre-dilution of concentrates with specific buffers or deficient plasma

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Exceptions to Combined Potency with All Methods

- Antigen and biological activity – always separate

- Different biological activities if related to differences in mechanism of action, eg:
  - ristocetin co-factor and collagen binding activities of vWF
  - anti-Xa and anti-IIa activities of LMW heparin

Assigned Potencies (IU/Ampoule) of 5th IS FVIII/vWF Plasma

FVIII:C	0.68
FVIII:Ag	0.94
VWF:RCo	0.78
VWF:CB	0.94
VWF:Ag	0.91

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Route for Establishment of WHO Biological International Standards and Reference Preparations

- Collaborative study: multi-centre; multi-methods
- Participants comments and approvals on analysis of results and recommendations
- For some materials, review and approval by appropriate expert/professional organisations eg International Society for Thrombosis and Haemostasis (ISTH)
- Review and approval by the Expert Committee on Biological Standardisation (ECBS)

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Role of ISTH in Establishment of WHO Standards in Haemostasis & Thrombosis

- ISTH Scientific & Standardisation Committee (SSC)
- SSC Annual Meetings – 19 Subcommittees
- SSC involvement at all stages of standards establishment
  - proposals, methodology, evaluation of results

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Role of ISTH in Establishment of WHO Standards in Haemostasis & Thrombosis

Formal process

- Report to Subcommittees
- Approval (with comments) by Subcommittee Chair, co-chairs and members (up to 30 members)
- Comments and approval by attendees of the Subcommittee meeting (up to 200 people)
- Discussion and presentation of comments at SSC business meeting – Official ISTH approval
- Approval send by ISTH to WHO/ECBS
- ECBS reviews study design, analysis of data and recommendation of the proposed assigned values

☺ Approval by ECBS

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