Interlaboratory variation of the INR and local system calibration

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Background

- INR is based on ISI calibration with International Standards and manual tilt tube technique.
- ISI calibration using the WHO recommended procedure is not usually possible in routine hospital laboratories.
- ISI of thromboplastin reagents differs according to the type of instrument used.

Background

- Laboratories may calibrate their local system (i.e. Instrument/reagent combination) using certified plasmas supplied by manufacturers or reference laboratories.
- Guidelines have been published on preparation, certification and use of plasmas for local system calibration.

Van den Besselaar et al. *J Thromb Haemost* 2004;2:1946

Two procedures using certified plasmas

- Modification of WHO method for ISI determination. Set of plasmas with assigned PT values using an international standard (I.S.) for thromboplastin.
- ‘Direct’ INR determination. Set of plasmas with assigned INR values. Plot of local PT versus assigned INR (log-log scales).

Different types of certified plasmas

- Plasmas derived from patients treated with vitamin K-antagonists (VKA), either single donation or pooled donations.
- Artificially depleted human plasmas, by adsorption of vitamin K-dependent factors to barium sulfate.

Different methods of preparation

- Deep-frozen plasmas. Stable for several years when stored at -70°C.
- Freeze-dried plasmas. INR may change depending on additives, method of freeze-drying, and thromboplastin/instrument combination used.

Certification

- WHO International Standards (I.S.) or European Reference thromboplastins should be used directly for value assignment.
- For use with one manufacturer's thromboplastin reagent only, certification with the calibrated reagent is acceptable ('reagent-specific' value).
- Certified plasmas should be tested for suitability with a variety of commercial thromboplastins before release for general use (validation).

Commutability

- Commutability is defined as the degree to which a material yields the same numerical relationships between results by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships for the same procedures applied to those types of material for which the procedures are intended.

Miller. *Clin Chim Acta* 2003; 327:25-37

INR interlaboratory variation: French EQA surveys using freeze-dried plasmas

Certified Plasma	Test Plasma	*N*	Usual procedure		Cert. Plasma procedure	
			Mean INR	CV (%)	Mean INR	CV (%)
VKA	VKA	758	3.41	12.3	3.38	6.2
Artific. depleted	VKA	2,542	3.38	12.4	3.39	12.1

Houbouyan & Goguel. *Am J Clin Pathol* 1997; 108:83-89

INR interlaboratory variation: North-American surveys using frozen VKA plasmas

INR test plasma	CV (%), Stated ISI	CV (%), Local ISI + MNPT	CV (%), PT/INR curve
2.15	9.0	8.5	5.8
2.44	10.4	8.2	5.0
3.58	12.5	7.6	4.0
3.73	17.0	10.4	7.8
4.80	16.0	9.0	5.0

Adcock & Johnston. *Thromb Haemost* 2002; 87:74-9

INR interlaboratory variation: Italian surveys using freeze-dried plasmas

	VKA test plasma		Artif. depl. test plasma	
	Mean INR	CV (%)	Mean INR	CV (%)
Stated ISI	4.39	11.2	4.23	10.3
Local ISI (human route)	4.09	7.0	4.02	3.9
Local ISI (rabbit route)	4.49	6.6	4.42	3.7
Local ISI (like-to-like route)	4.29	8.5	4.21	6.4

Chantarangkul et al. *Thromb Haemost* 1999; 82:1621-6

Simplified method for INR derivation: an international study using freeze-dried plasmas

Reagents	Certified INR	Before correction	Local ISI	Direct INR
Human	3.01	3.37	3.00	3.02
Bovine	2.59	2.41	2.59	2.66
Rabbit	3.81	3.38	4.05	3.91

Poller et al. *Clin Chem* 2010; 56:1608-1617

Local ISI values for one reagent (Thromborel S) generated using different commercial certified plasma sets

	Instrument: STA-R	Instrument: ACL-300	Manual
Life Therapeutics	1.19 – 1.23	1.02 – 1.20	1.23 – 1.24
Dade - Behring	1.25 – 1.30	1.04	1.08 – 1.09
Helena	1.12 – 1.15		

Favaloro et al. *Pathology* 2008; 40:277-287

Certification of plasma set for direct INR determination (Netherlands)

- One pooled normal plasma (deep-frozen).
- Five pooled VKA plasmas (deep-frozen).
- INR assignment with various commercial thromboplastin reagents by three or more laboratories.

Certification of plasma set for direct INR determination (Netherlands)

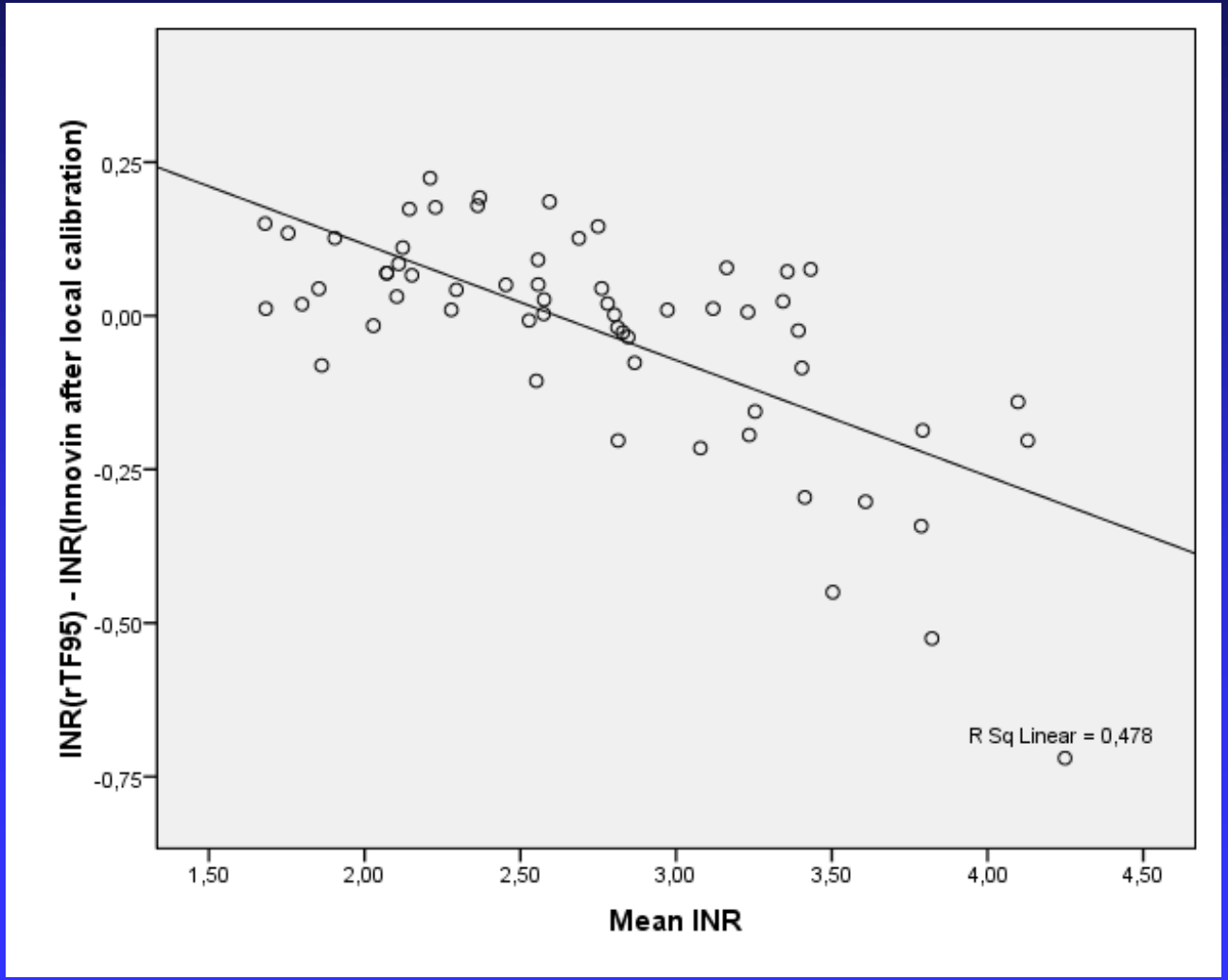
Deep-frozen plasma	INR assigned with Innovin	INR assigned with Hepato Quick	INR asigned with Recombiplastin 2G
09-0 (pooled normal)	1.05	1.02	1.01
09-1 (pooled VKA)	1.67	1.74	1.64
09-2 (pooled VKA)	2.18	2.25	2.11
09-3 (pooled VKA)	2.62	2.67	2.59
09-4 (pooled VKA)	2.97	3.00	3.03
09-5 (pooled VKA)	3.47	3.43	3.40

Validation of certified plasmas

- Each set of certified plasmas must be validated before release.
- Validation should be performed with 10 or more fresh plasmas from patients on long-term VKA treatment.
- Calculate relative INR difference for fresh plasmas: $\Delta = 2 \times (INR_R - INR_C)/(INR_R + INR_C)$
- Mean relative difference Δ should 0.1 or less.

Van den Besselaar et al. *J Thromb Haemost* 2004; 2:1946

Validation of 6 certified deep-frozen plasmas with reagent-specific INR values for Innovin



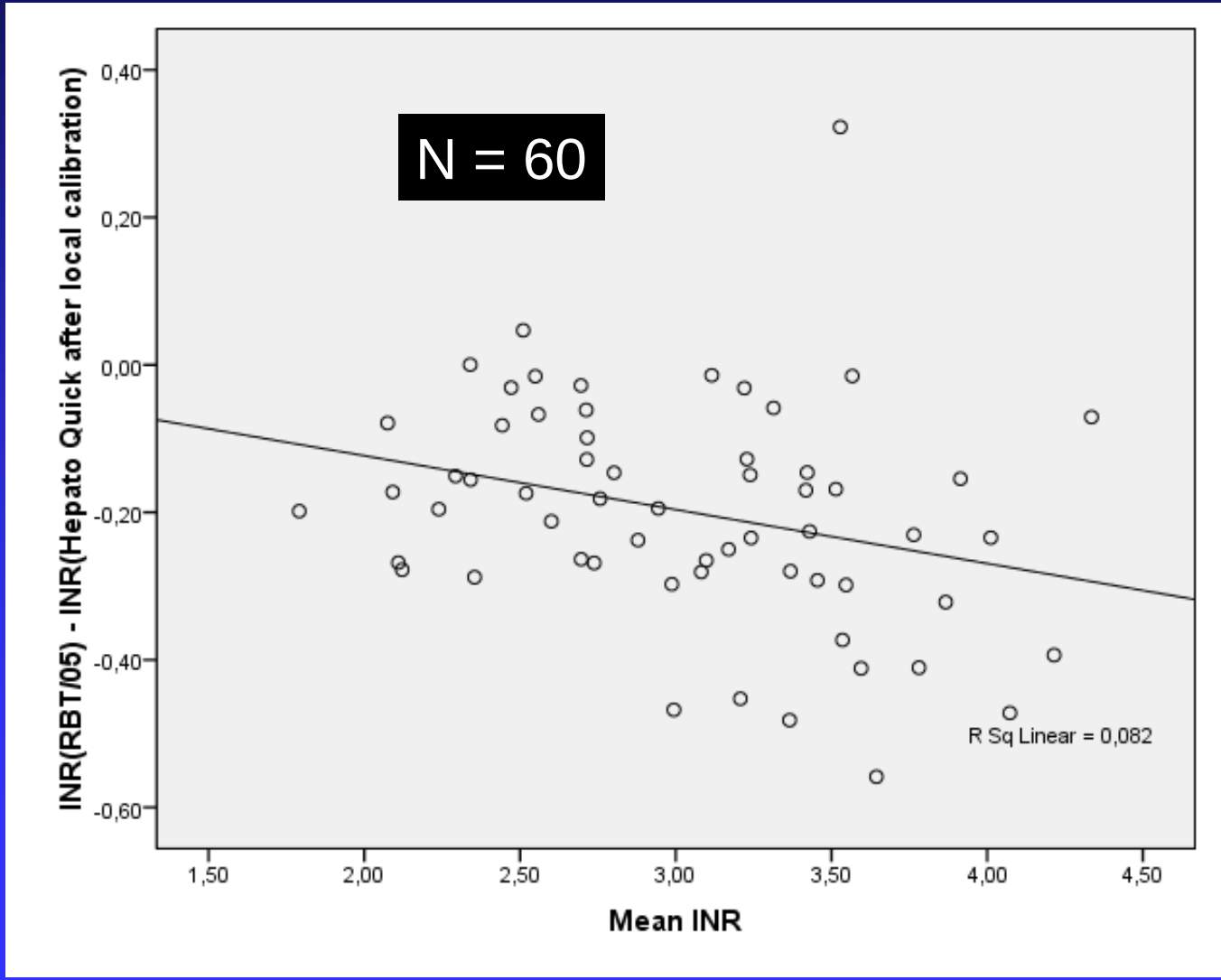
Validation of certified plasmas with reagent-specific INR values for Innovin

Mean INR of 60 fresh plasmas with I.S.: 2.73

Mean INR with Innovin after local calibration: 2.76

Relative INR deviation = (2.73 – 2.76)/2.75 = - 0.009

Validation of 6 certified deep-frozen plasmas with reagent-specific INR values for Hepato Quick



Validation of certified plasmas with reagent-specific INR values for Hepato Quick

Mean INR of 60 fresh plasmas with I.S.: 2.94

Mean INR with Hepato Quick after local calibration: 3.14

Relative INR deviation = (2.94 – 3.14)/3.04 = - 0.07

Conclusions

- Local system calibration may reduce inter-laboratory variation of INR.
- Magnitude of reduction depends on type of calibration plasma and test plasma.
- Many certified plasma sets have not been validated.
- Freeze-dried, artificially depleted plasmas are not commutable.

Conclusions

- Indiscriminate use of certified plasmas may lead to wrong results.
- The use of plasmas with ‘reagent-specific’ values may avoid the problems of non-commutability and should be explored further.