

MA SC

*ICAR Sub-Committee on
Milk Analysis*



Proficiency Testing scheme interlinkage

-

International laboratory anchorage

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Outline

1- Reference in the ICAR AQA system

2- Relativeness of the reference

3- Choice for an universal reference

4- Linking PT schemes

5- Different levels of implementation

6- Application to somatic cell counting

7- Conclusion

The reference in the ICAR AQA System

👉 Objective :

- Establish the **true content** of component in DHI milk
- ICAR Guidelines :
 - a- methods **internationally standardized**
 - b- **standardized methods** tightly **fitted / anchored** to a-
- Ref methods slow => **calibration** of routine methods with reference

👉 Calibration of routine methods

- In-house calibration : individual laboratory
- Centralized calibration : interlaboratory study

👉 Secure DHI analysis thr. Quality Control :

- Internal (QC, SRMs) ; **external (CRMs, PTs)**
- **Reference** and routine methods

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What is / where is the « analytical truth » ?

« Truth » stated by reference methods but ...
is a **relative concept**



Reference methods provide results with **uncertainty !**



Precision characteristics (ISO 5725) :
repeatability, $r=2.8 sr$; reproducibility, $R=2.8 sR$,

⇒ Between lab variation $sL^2 = sR^2 - sr^2$



Deviations within limits are permitted :
> *max range between 2 laboratories $L = 2.8 sL$*
> *max lab bias from the reference $+/-2 sL$*

What is / where is the « analytical truth » ?

Component	ISO	sR	sr	sL	R	r	L	+/-2 sL
Fat g/100g	1211	0,02	0,014	0,014	0,056	0,039	0,040	0,029
Protein g/100g	8968	0,018	0,014	0,011	0,050	0,039	0,032	0,023
SCC(10^3 c/ml)	13366-2	45	20	40	126	56	113	81
SCC rel ($750 \cdot 10^3$ c/ml)	13366-2	6%	3%	5%	17%	8%	15%	10%

Ranges allowed between two laboratories chosen
at random

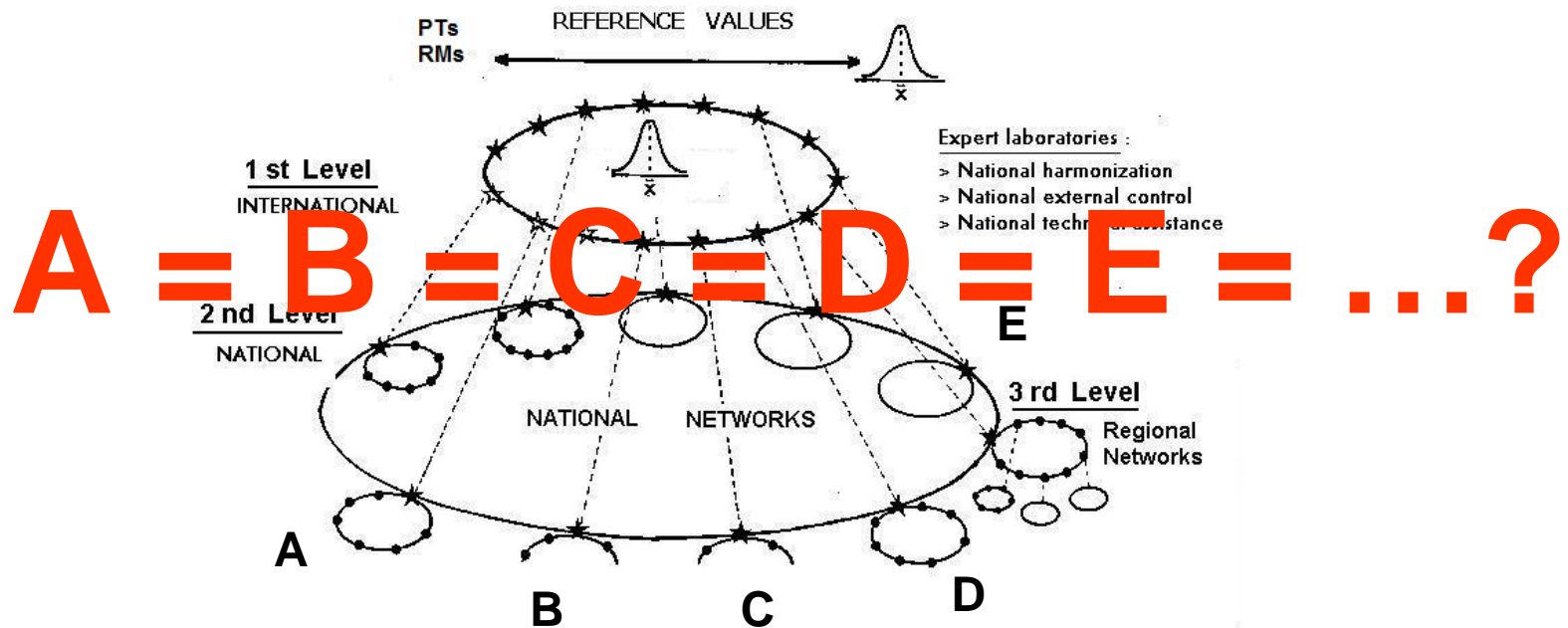
Lab bias limits allowed from the reference
for « normal laboratories »

⇒ Reduction thr. AVERAGING ⇒ laboratory network MEANS as reference

Every national network defines its own reference
None knows whether it differs from the others

... nor how far !

ICAR INTERNATIONAL REFERENCE LABORATORY NETWORK

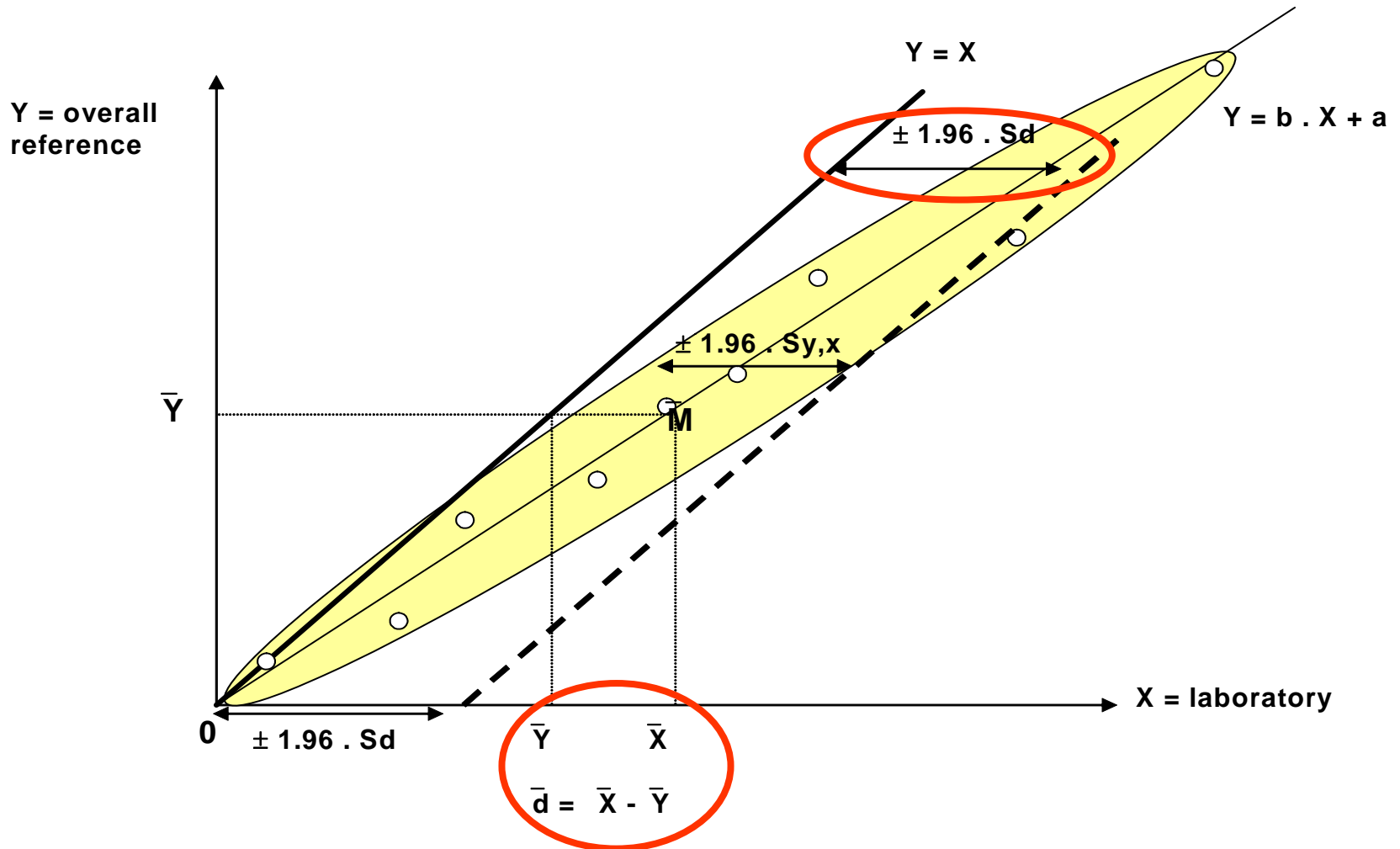


Assumption that the group gives the true reference is relative

Different conditions of interlaboratory studies (lab number, PT design and protocol, methods involved) **can influence population results** and the resulting **references.**

Recalls on calibration and PT Statistics

Statistical parameters to evaluate lab trueness performances and calibration



Trial of : 03/03/2008

Sd

12
20

225

21 laboratories
10 samples

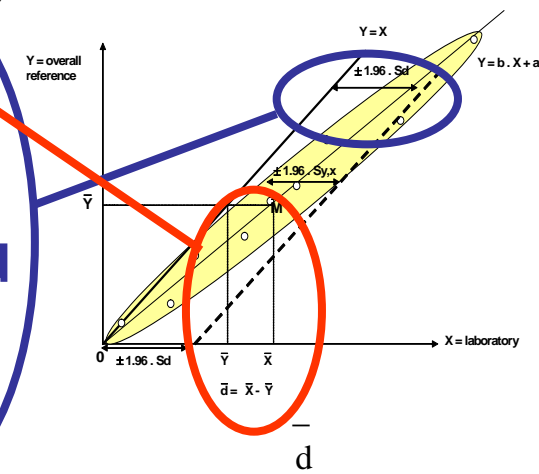
Student 5 %

Student 5 %

150

75

Statistical parameters to evaluate lab trueness performances and calibration



-150

-75

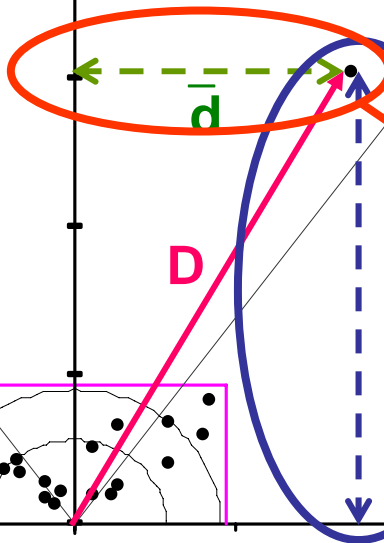
0

75

150

Target limits : $d = \pm 35 \cdot 10^3$ cells / ml of milk

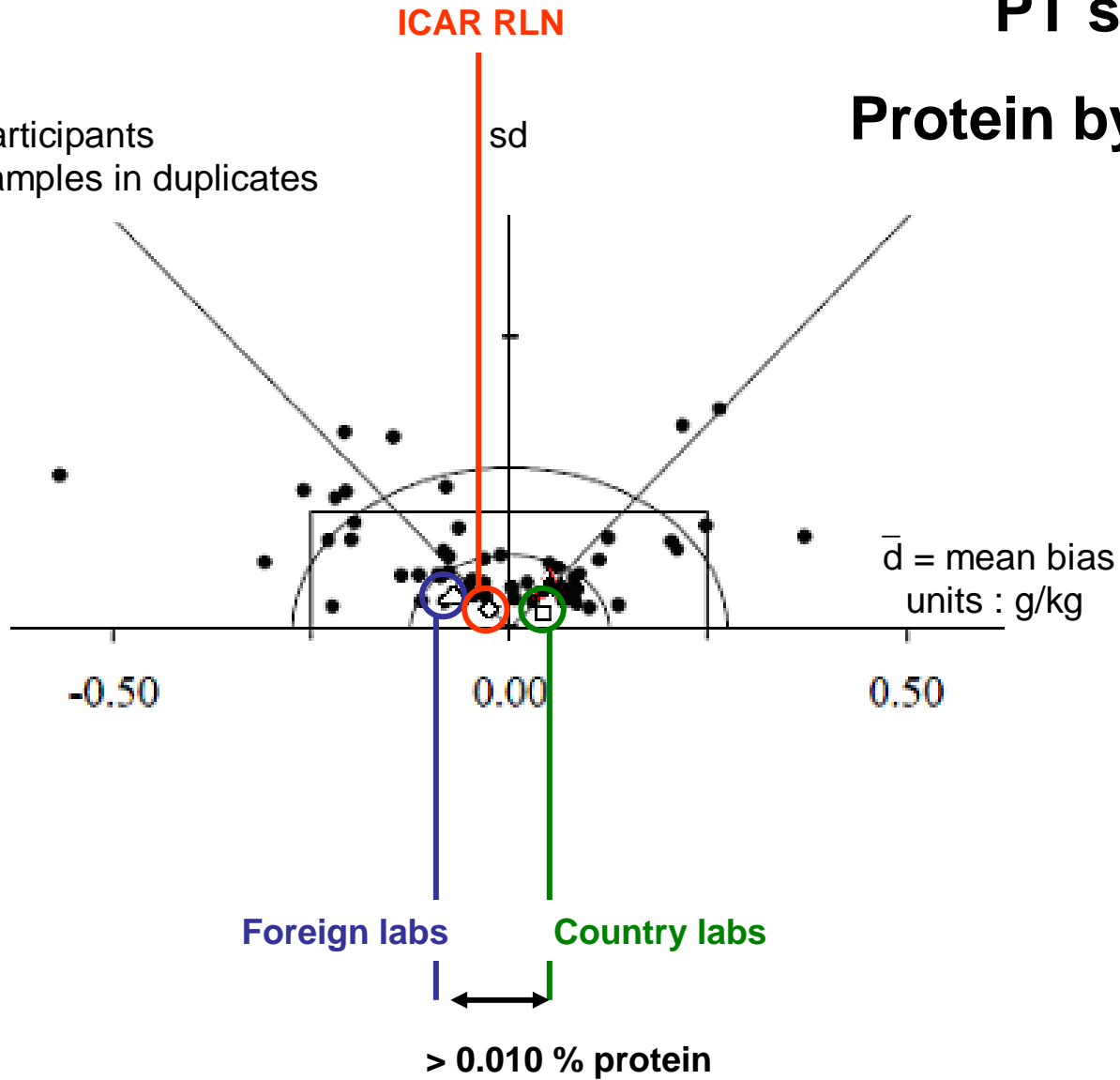
$Sd = 35 \cdot 10^3$ cells / ml of milk



PT study

Protein by Kjeldahl

66 participants
10 samples in duplicates



PT study

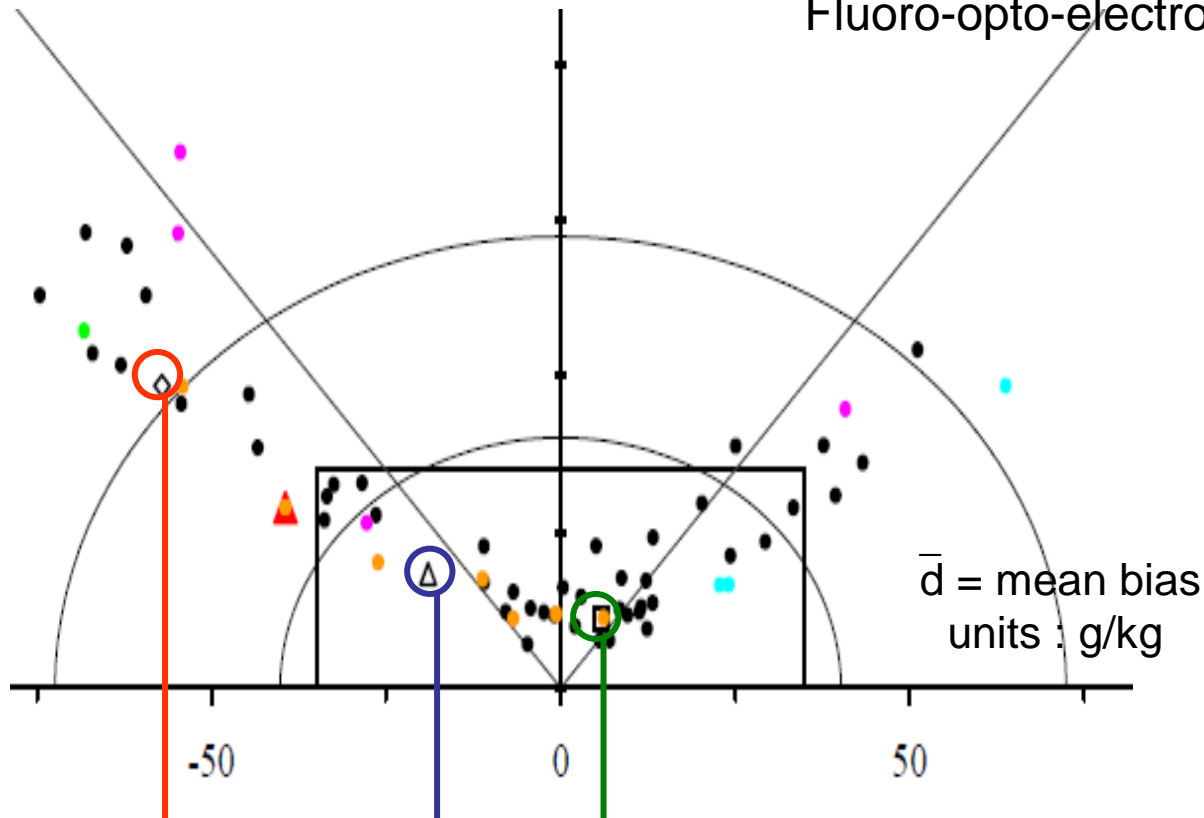
Somatic Cell Counting

Fluoro-opto-electronic methods

70 participants

10 samples in duplicates

sd



ICAR RLN

Foreign labs

Country labs

$35 \cdot 10^3$

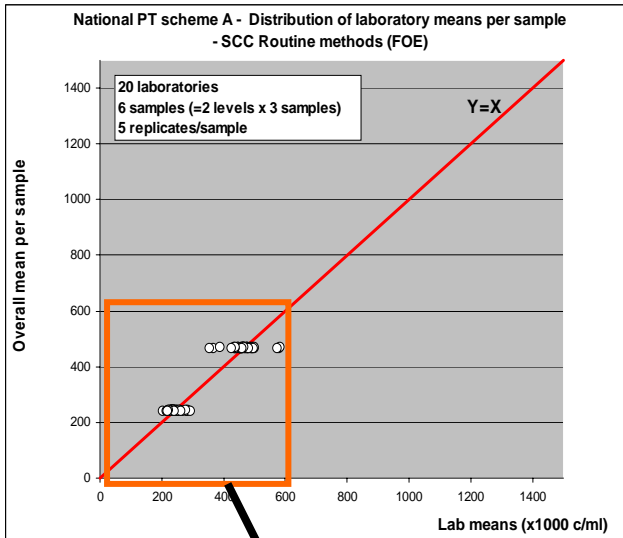
$25 \cdot 10^3$

5%

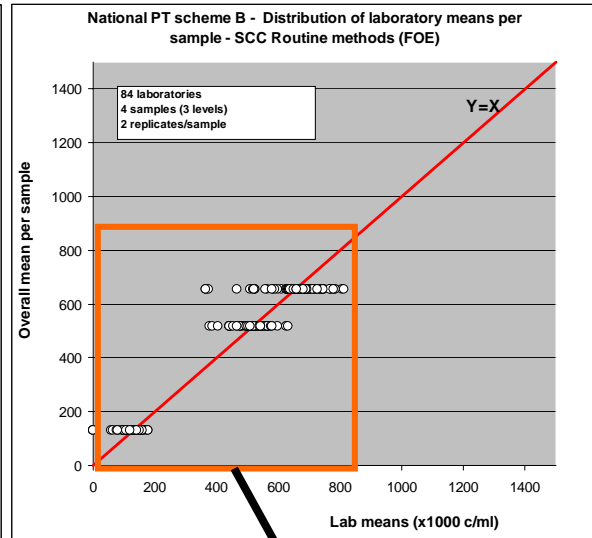
3%

Diversity in PT scheme in Europe => different references ?

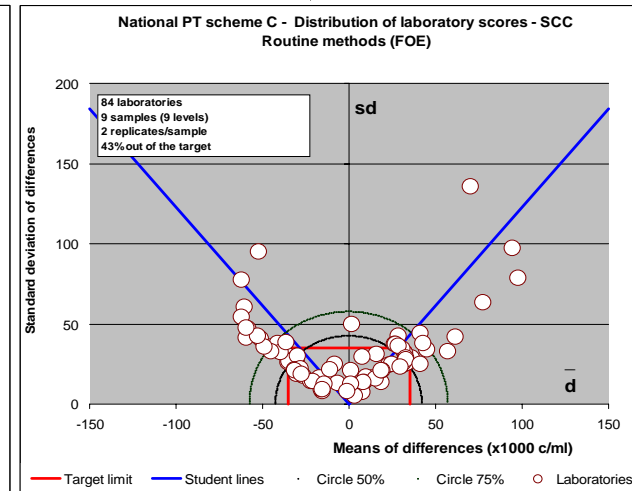
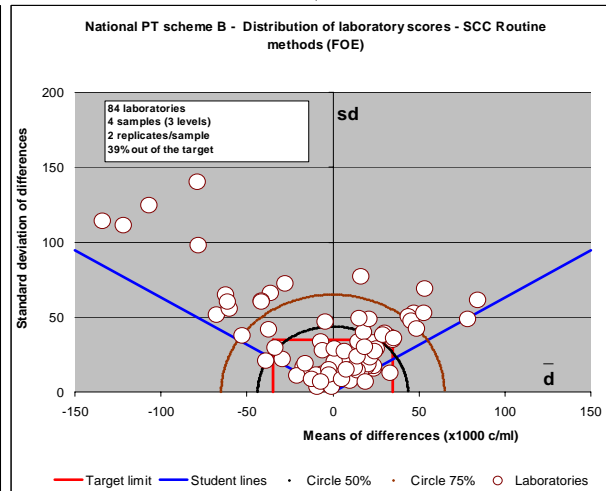
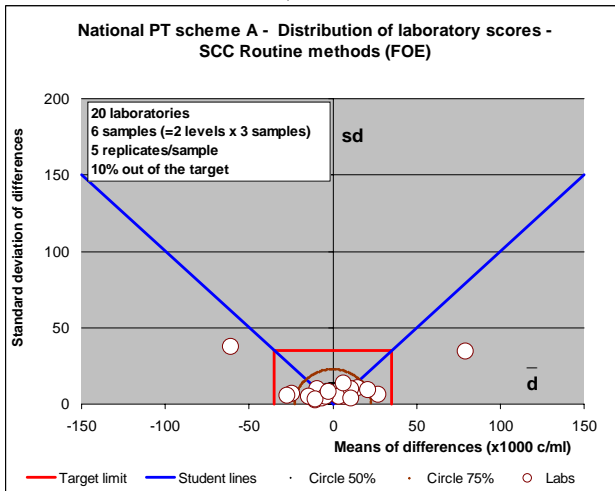
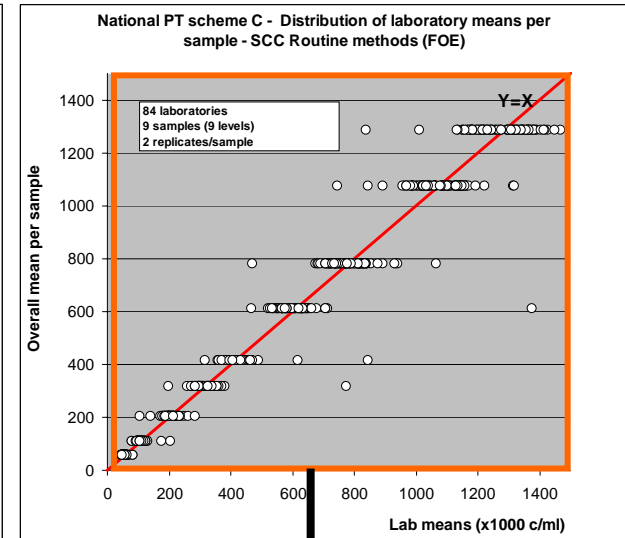
Scheme A



Scheme B



Scheme C



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Truth = agreement of all the stakeholders to reach common goals

i.e. worldwide harmonisation for ICAR



ICAR choice = International network of reference labs using reference methods

⇒ **International recognition** **representativeness (political),**

⇒ **Highest competence & training**

suitable methodology & capability (technical)

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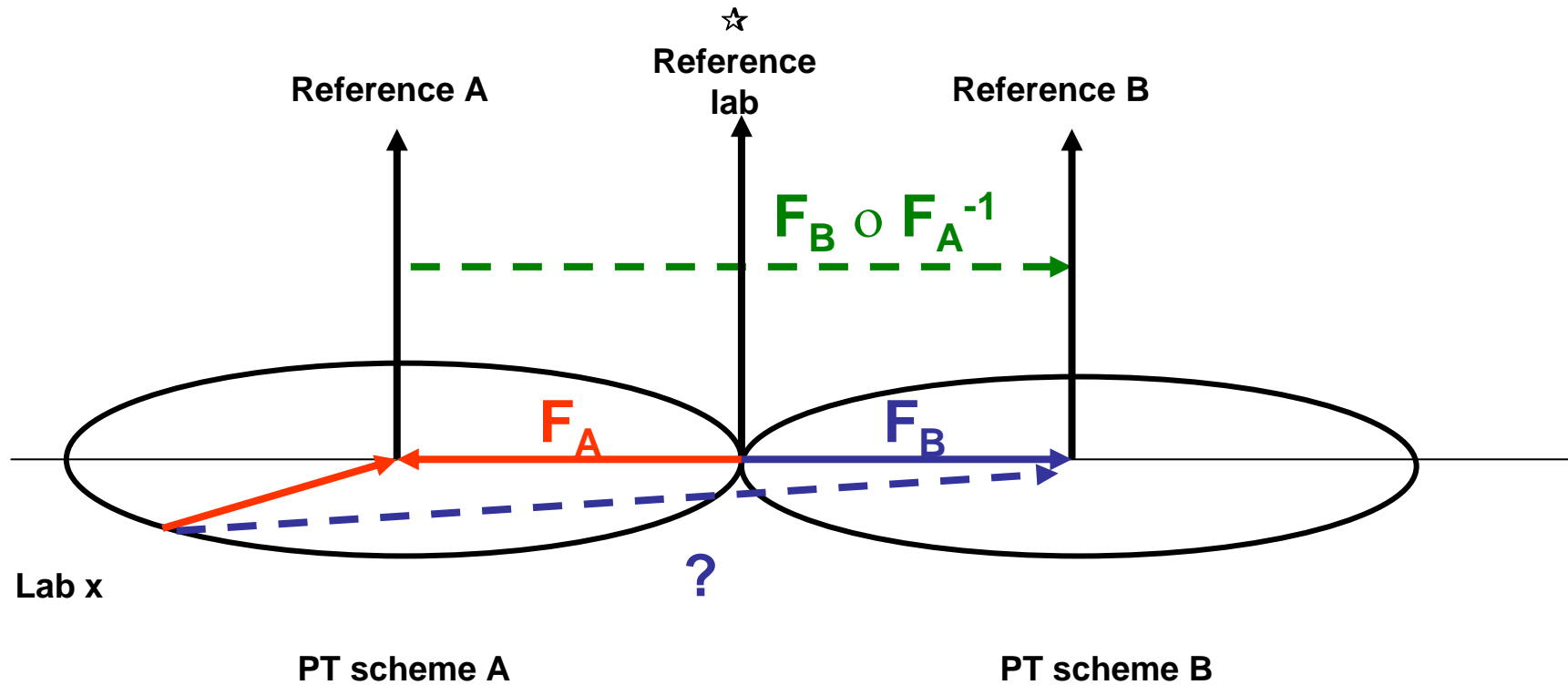
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Principle of interlinking schemes with expert laboratories

O. Leray, ICAR Session, Niagara Falls, 2008 ; IDF | ICAR – SCC RS, 2010



Existing routes

Unknown direct routes

To each route there is a specific mathematical relationship for linking

Inter-linking relation using linear equations

O. Leray, 2010 – IDF | ICAR – SCC RS project (underway)

$$F_A : y = x \cdot b_A + a_A$$

\Leftrightarrow

$$F_A^{-1} : x = y/b_A - a_A/b_A$$

$$F_B : z = x \cdot b_B + a_B$$

y = Real reference of scheme A

z = Virtual reference of scheme A
in scheme B

$$F_A^{-1} \circ F_B :$$

$$z = (y/b_A - a_A/b_A) \cdot b_B + a_B$$

$$z = y \cdot (b_B/b_A) + (a_B - a_A \cdot b_B/b_A)$$

$$\text{No level effect} \Rightarrow z = y + (a_B - a_A) = y + (\bar{d}_A - \bar{d}_B)$$

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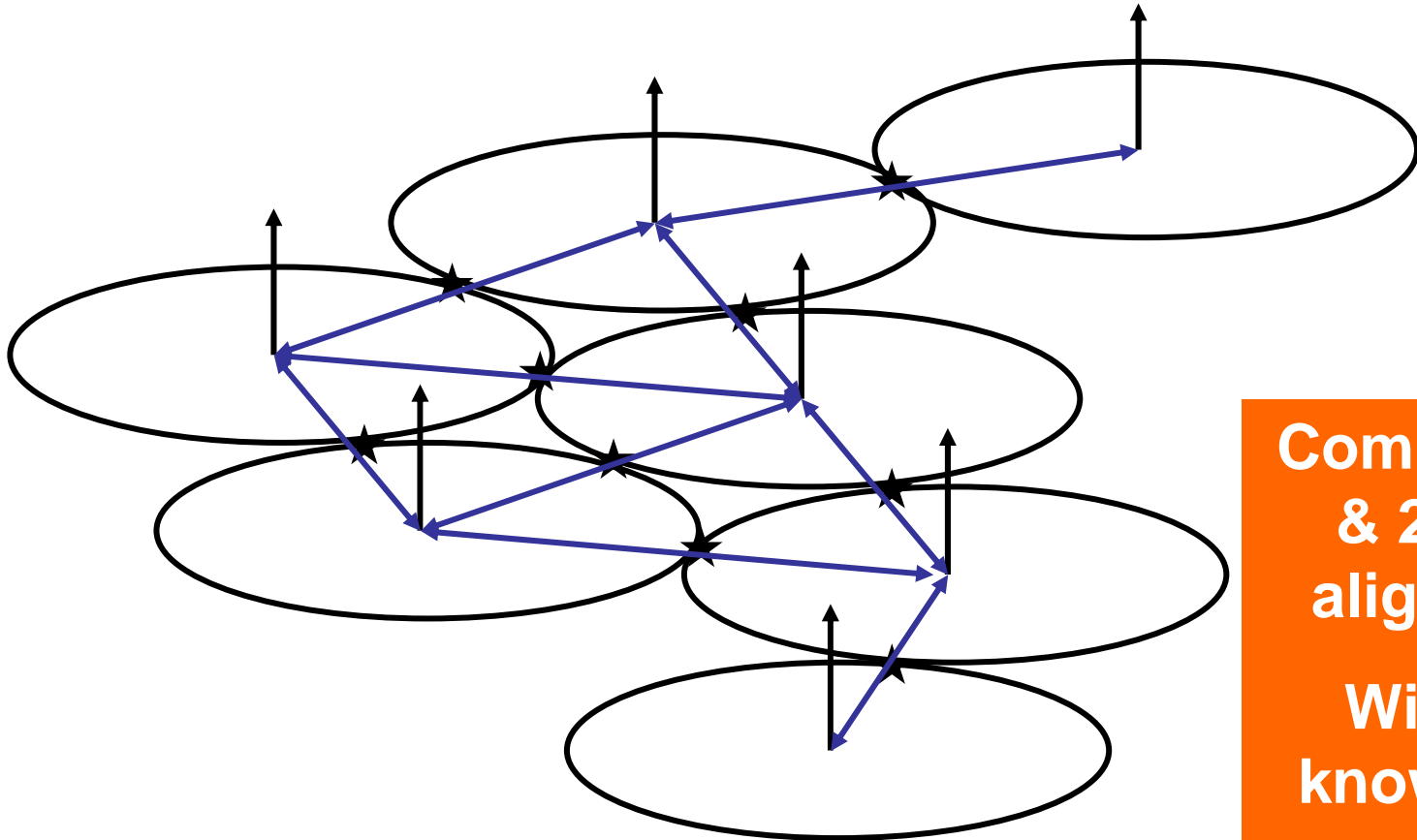
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Interlinkage of national PT schemes

\Leftrightarrow PT scheme networking

O. Leray, 2010 – IDF | ICAR – SCC RS project (underway)



↑ : Reference = Ideal consensus lab for the PT scheme

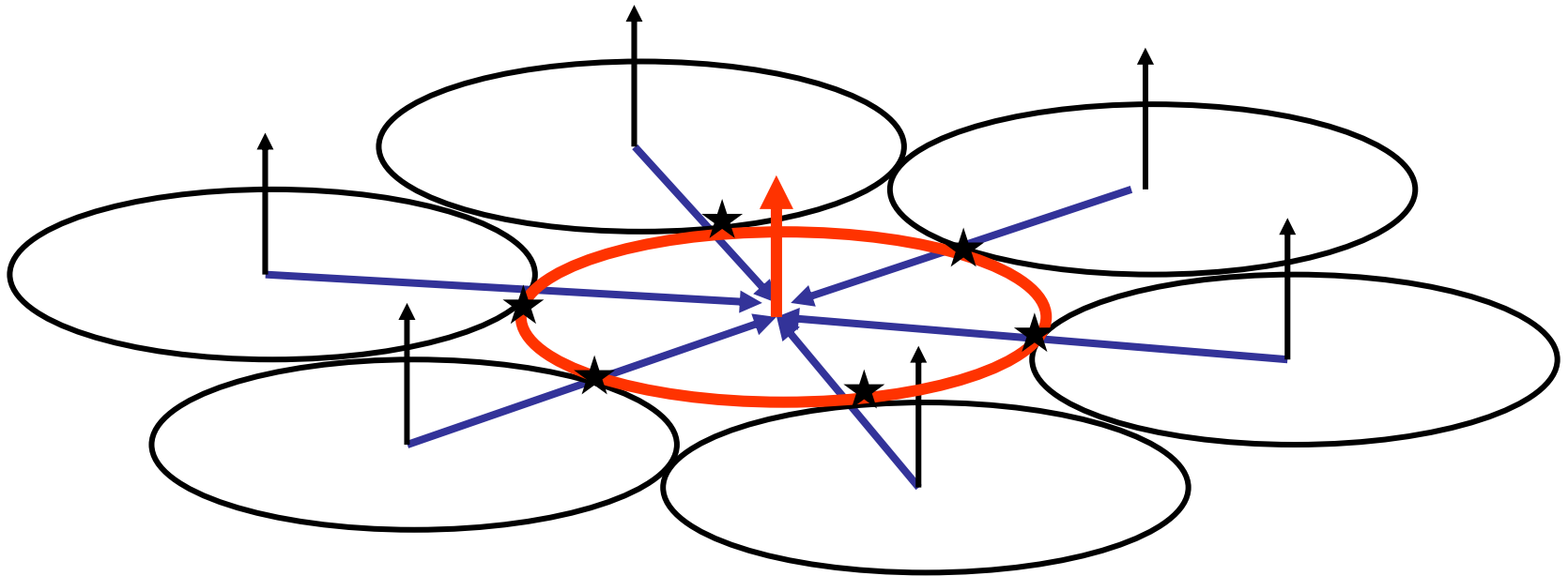
★ : Expert (reference) lab

Comparison
& 2-by-2
alignment

With no
knowledge
on where is
real « Truth »

Anchorage to an international reference scheme

O. Leray, 2010 – IDF | ICAR – SCC RS project (underway)



↑ : Reference = Ideal consensus lab for the PT scheme

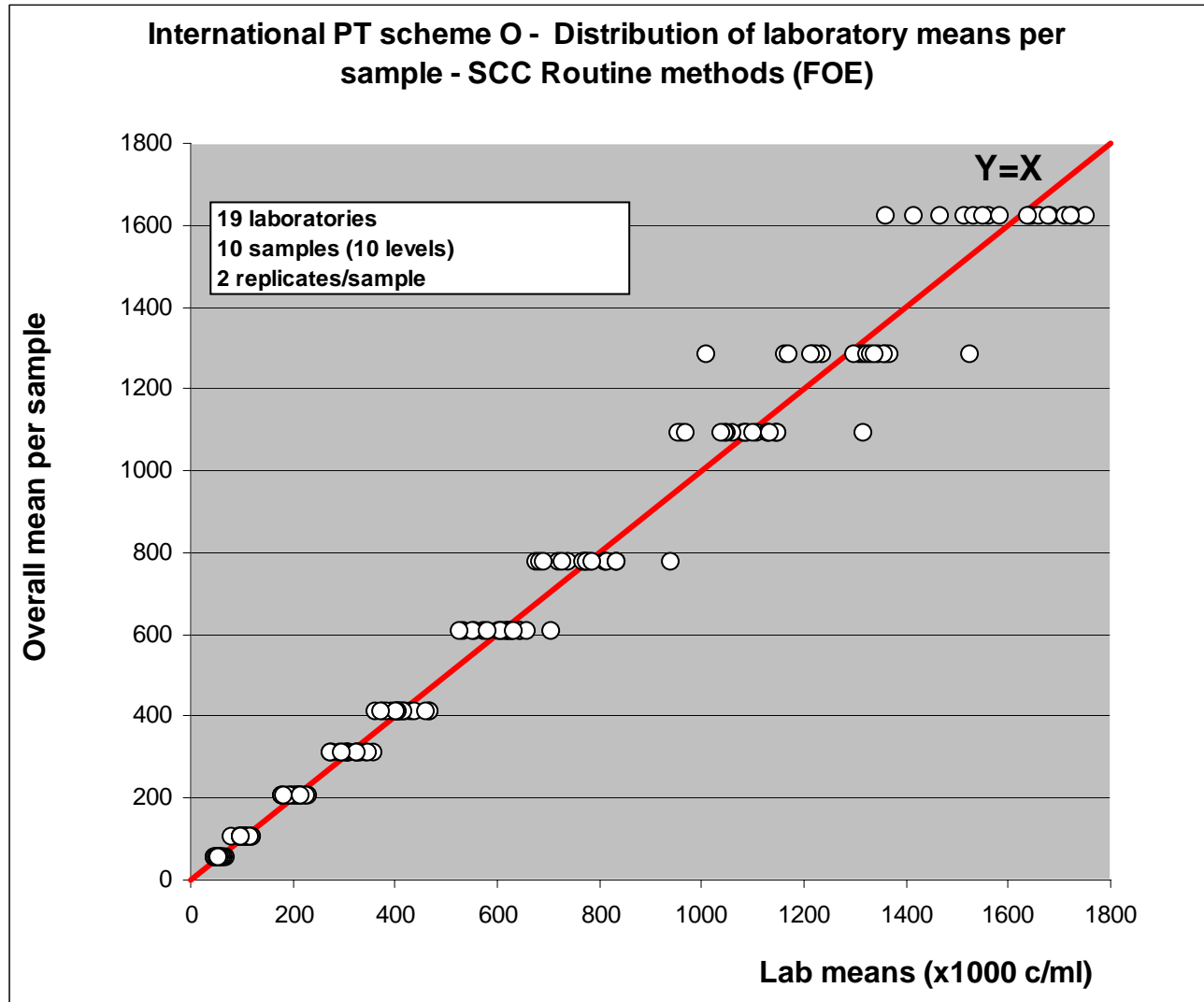
★ : Expert (reference) lab

**Alignment to a real
« Truth » stated by
consensus &
comparison**

Outline

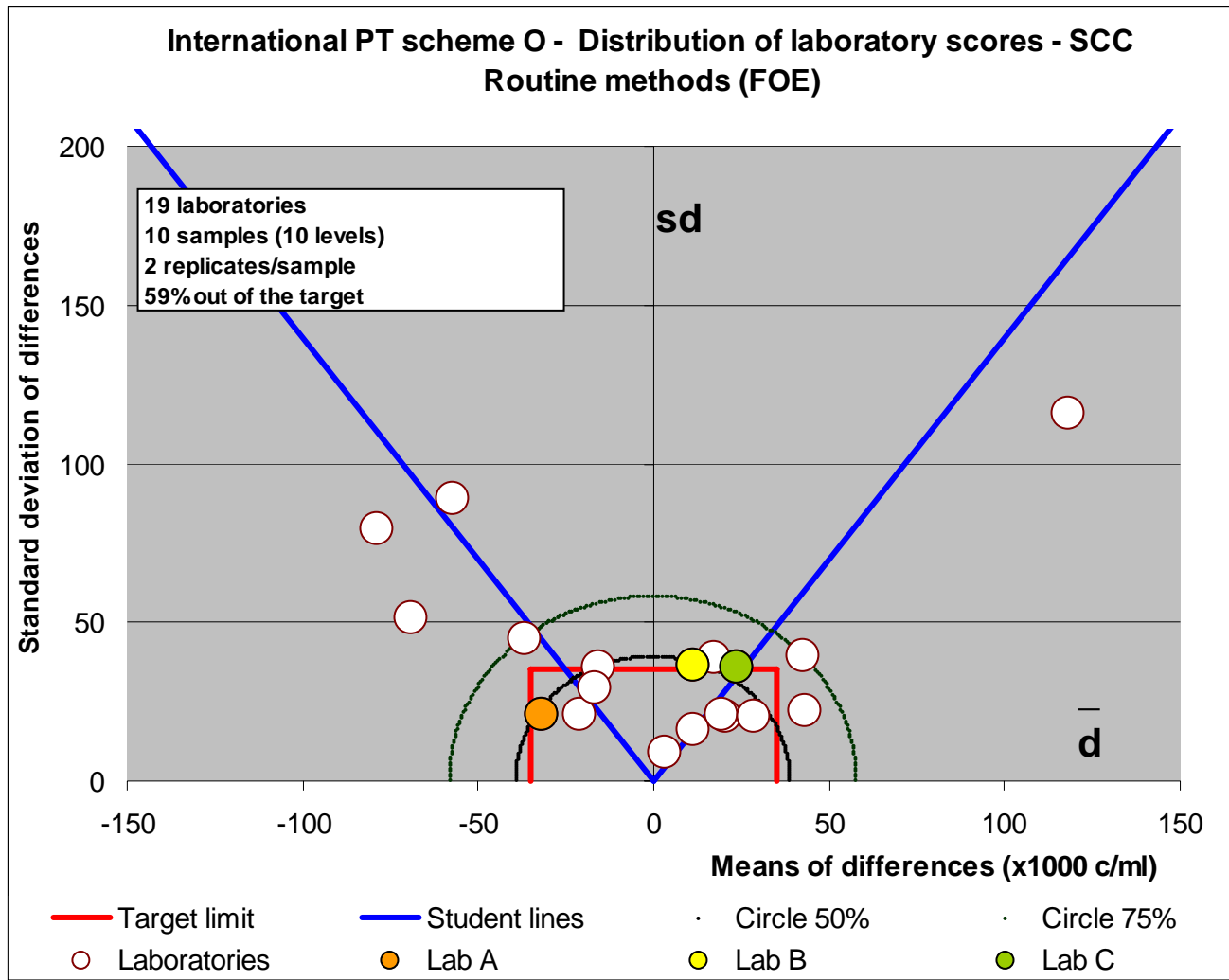
- 1- Reference in the ICAR AQA system
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SCC International PT scheme : Levels and results



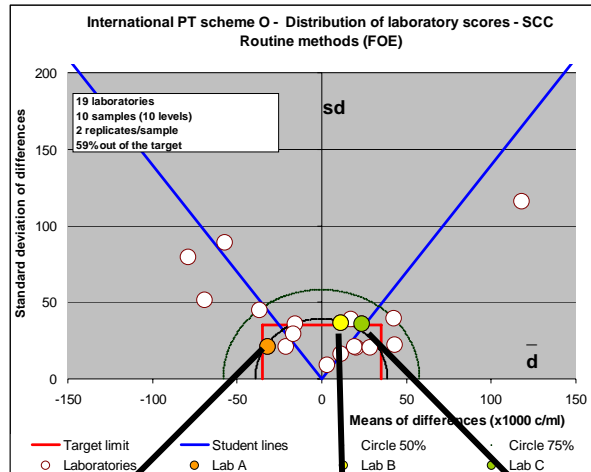
ICAR PT scheme Sept. 2009

International PT scheme : Reference Laboratory scores



International PT scheme anchorage

Scheme O

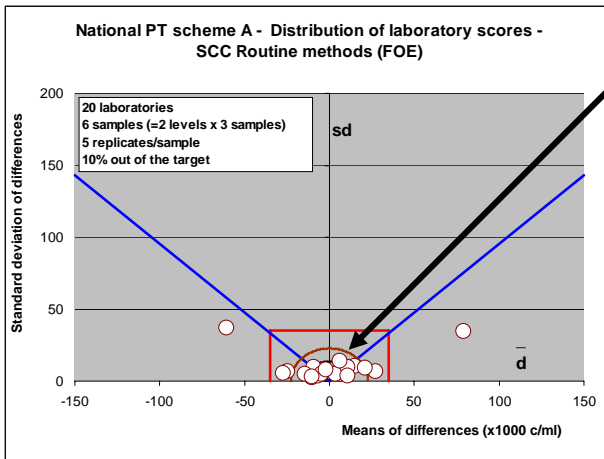


International level ⇒

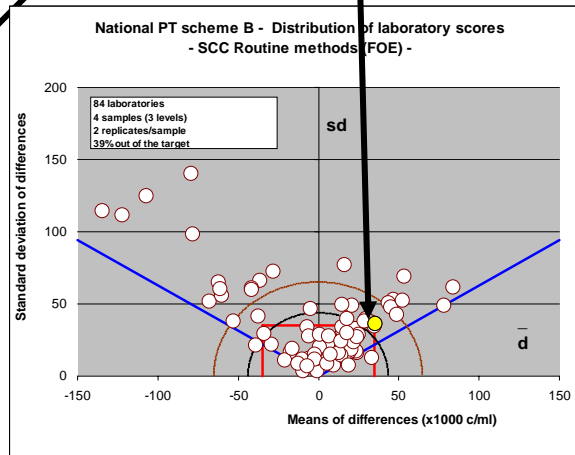
National / regional level



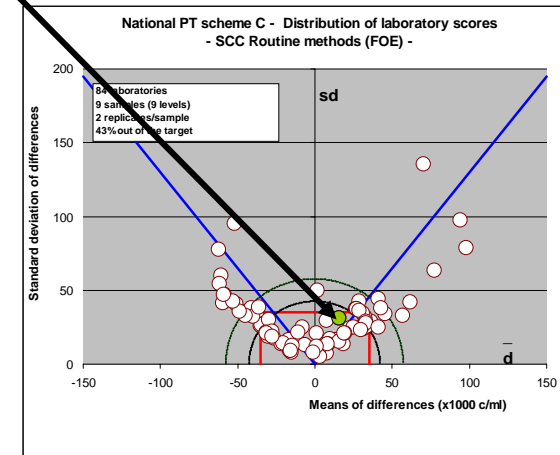
Scheme A



Scheme B

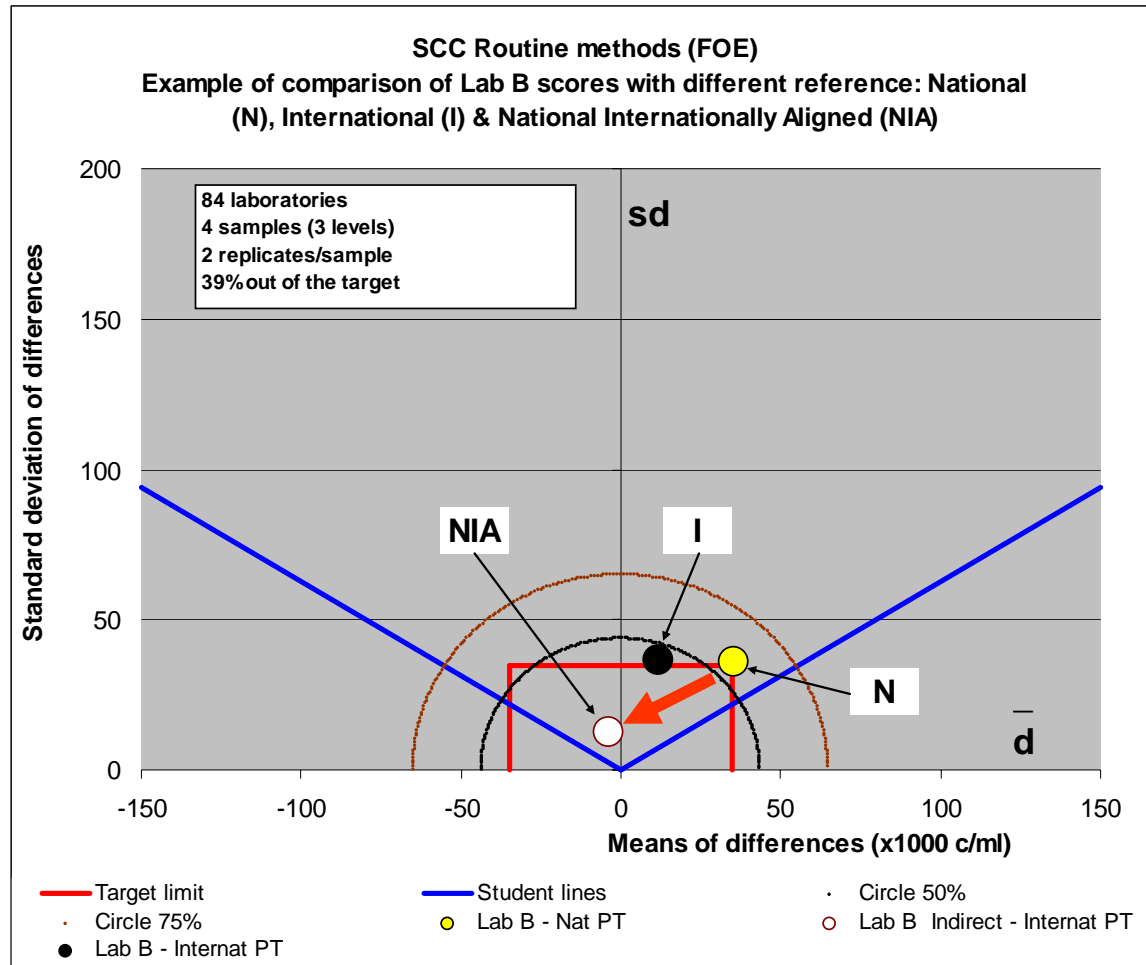


Scheme C



Alignment of reference for a national scheme B onto an international scheme :

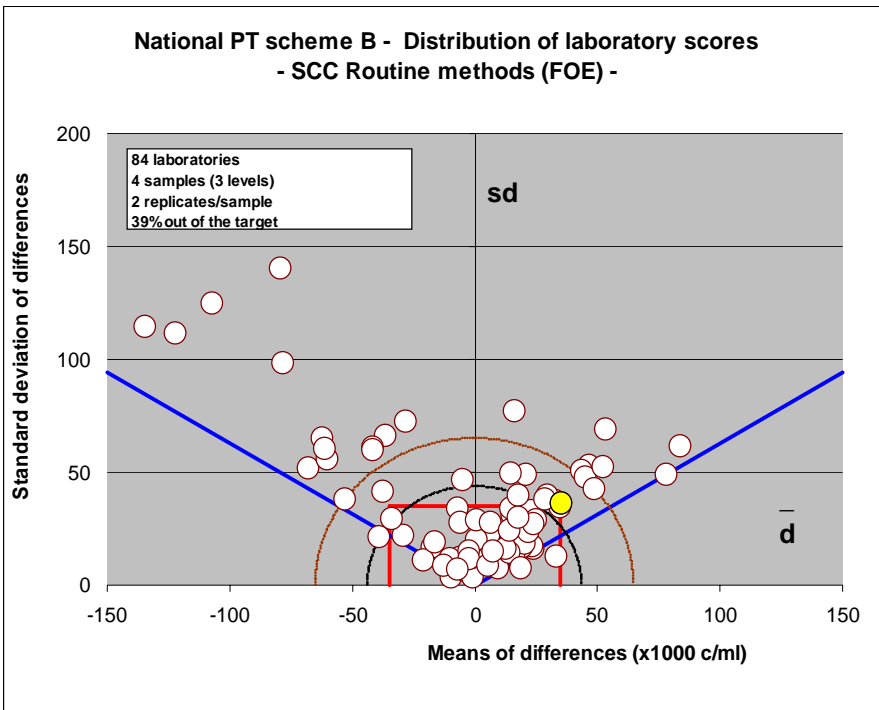
Different scores of Ref Lab B



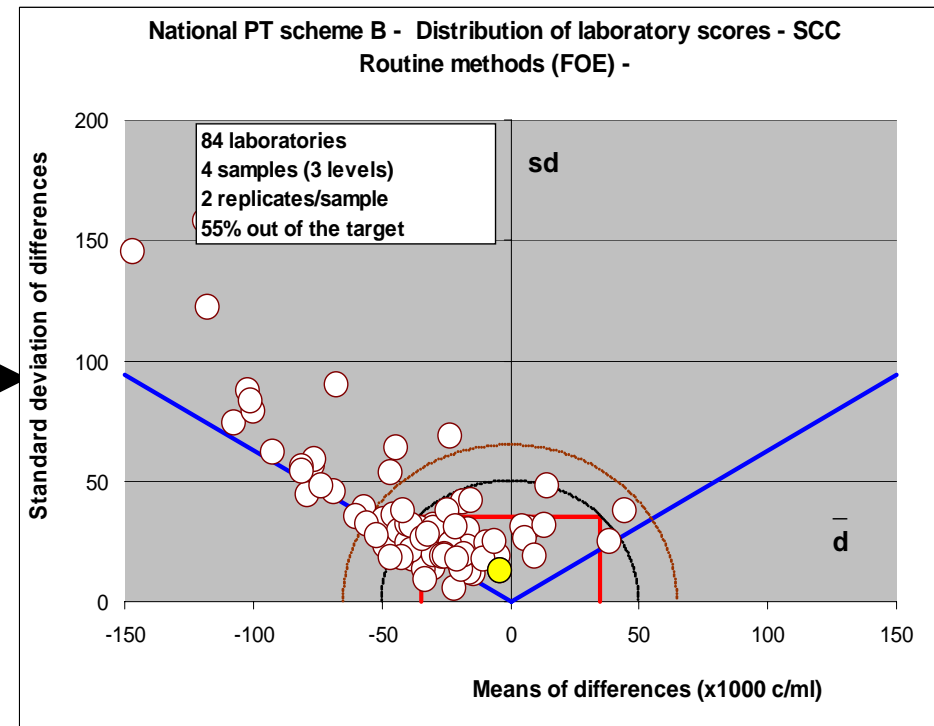
PT scheme interlinking of a national scheme B to an international scheme O

=> Evolution of labs scores of scheme B

National PT (actual)



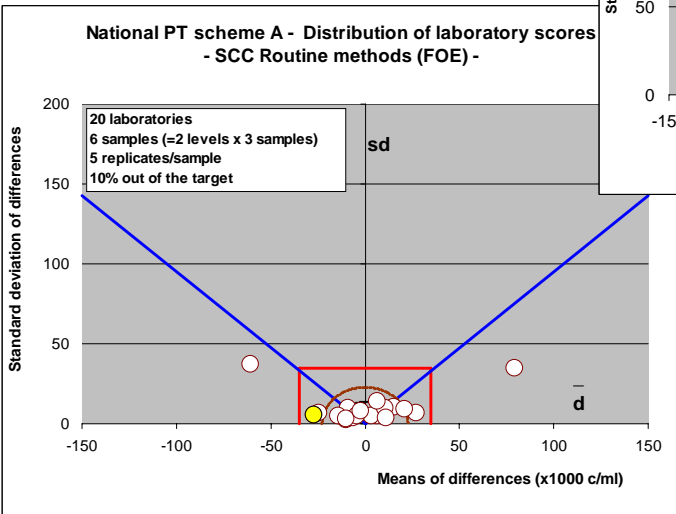
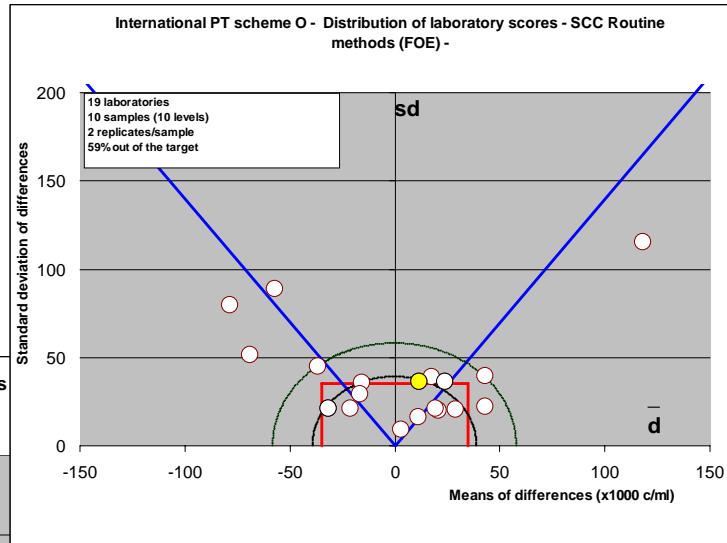
National PT Internat^ly Aligned (virtual)



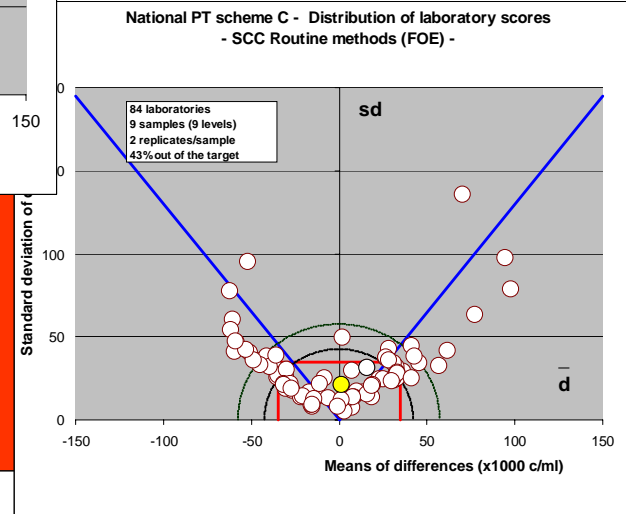
Local PT studies can show only a part of the problem if there is no external input (Reference PT or RMs) to « give the Truth »

Different locations of Lab B in 4 different schemes A, B, C & O

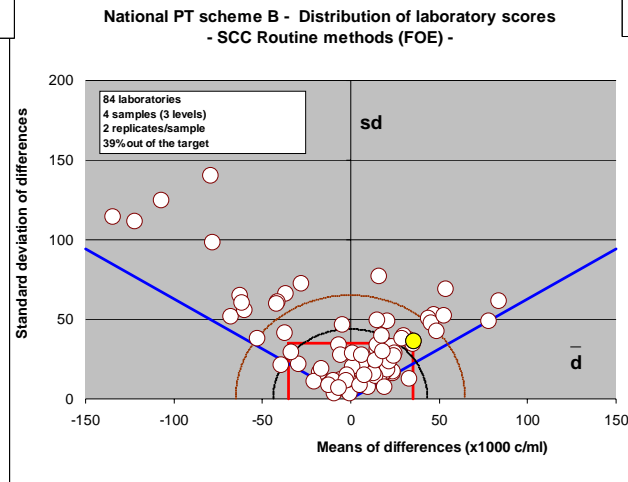
O



Ref Lab and/or references questionable ?



A

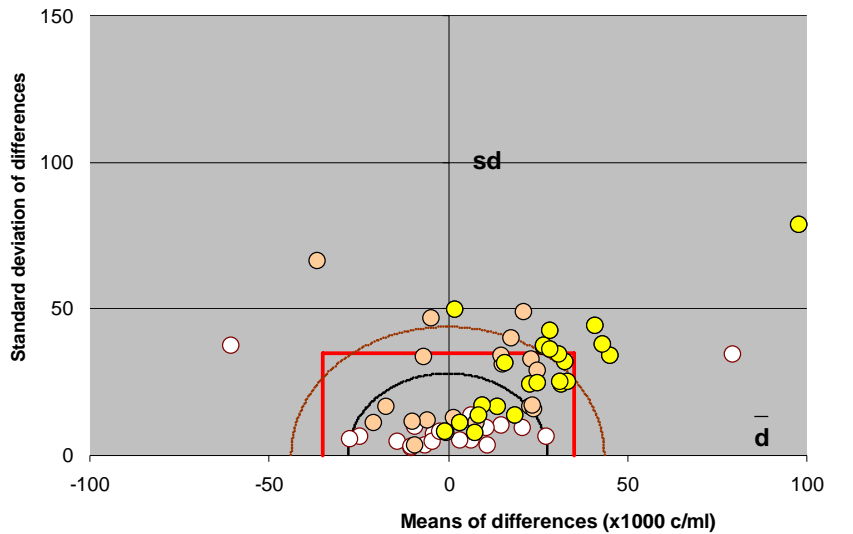


C

B

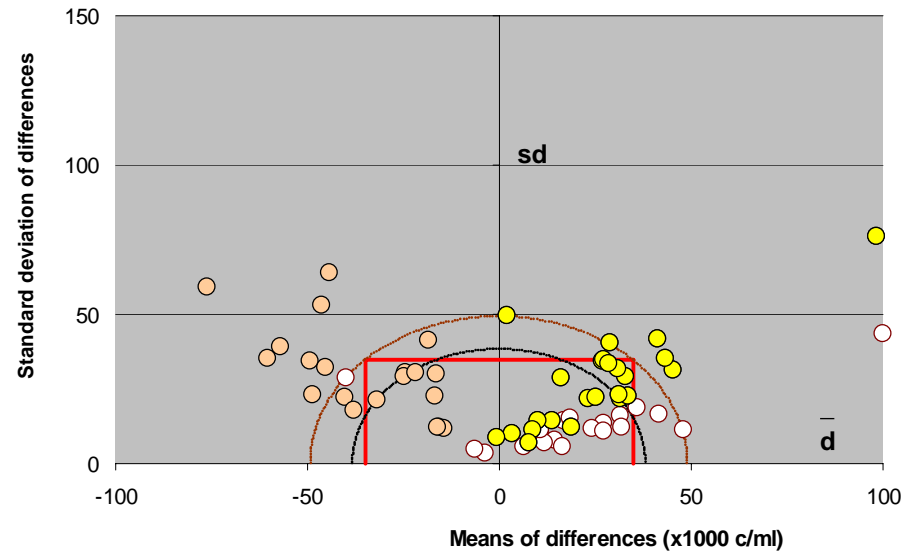
Simultaneous comparison of DHI labs of three countries (3 National PTs) in SCC

National (actual)



— Target limit · Circle 50% · Circle 75% ○ Network A ● Network B ● Network C

Internationally Aligned (virtual)



— Target limit · Circle 50% · Circle 75% ○ Network A ● Network B ● Network C

Score distributions broaden where ref lab scores differ between local and international (reference) PT

**Ref Lab and/or references
questionable ?**

RANKING LABORATORIES

National (actual)

Rank	Rank%	ID	d	sd	D
1	2%	A6	3,4	4,9	6,0
2	3%	A11	-4,5	4,9	6,6
3	5%	A7	-6,4	3,6	7,4
4	6%	B8	-0,7	7,6	7,7
5	8%	C84	-0,9	8,0	8,0
6	9%	A3	6,3	5,0	8,0
7	11%	A1	-3,9	7,1	8,1
8	13%	A19	-2,3	8,1	8,5
9	14%	B9	-9,3	3,2	9,9
10	16%	A2	-10,1	2,4	10,4
11	17%	C21	7,4	7,6	10,6
12	19%	A17	-10,2	3,1	10,6
13	20%	C22	3,2	11,1	11,5
14	22%	A14	11,0	3,4	11,5
15	23%	B21	1,6	12,6	12,7
16	25%	B2	-5,9	12,0	13,4
17	27%	A9	-9,1	9,8	13,4
18	28%	B22	7,7	11,0	13,4
19	30%	A13	10,8	9,6	14,4
20	31%	A10	-14,3	4,7	15,0
21	33%	A15	6,4	13,8	15,2
22	34%	B18	-9,9	11,7	15,3
23	36%	C14	8,6	13,8	16,2
24	38%	A4	15,0	10,2	18,1
25	39%	C13	9,7	17,0	19,6
26	41%	C2	13,6	16,5	21,4
27	42%	A16	20,8	9,2	22,8
28	44%	C15	18,6	13,6	23,0
29	45%	B13	-20,8	11,0	23,5
30	47%	B4	-17,4	16,8	24,2
31	48%	A8	-24,5	6,5	25,4
32	50%	A20	-27,2	5,7	27,8
33	52%	A5	27,3	6,4	28,0
34	53%	B6	22,8	16,6	28,2
35	55%	B19	23,8	15,9	28,6
36	56%	B24	23,6	17,2	29,2
37	58%	C8	22,9	24,2	33,3
38	59%	B7	15,1	30,8	34,3
39	61%	B17	-6,9	33,7	34,4
40	63%	C19	24,9	24,8	35,1
41	64%	C25	16,0	31,3	35,2
42	66%	B11	14,8	33,8	36,9
43	67%	B23	25,1	28,7	38,1
44	69%	C1	31,5	24,0	39,6
45	70%	C17	31,1	25,3	40,1
46	72%	B3	23,1	32,8	40,1
47	73%	C12	33,3	25,2	41,8
48	75%	B15	17,7	39,9	43,6
49	77%	C10	32,6	31,7	45,5
50	78%	C6	26,6	37,1	45,7
51	80%	C20	28,5	36,1	46,0
52	81%	C18	26,9	37,4	46,0
53	83%	C16	30,8	34,3	46,1
54	84%	B5	-4,7	46,7	46,9
55	86%	C7	1,9	49,8	49,8
56	88%	C5	28,5	42,6	51,3
57	89%	B16	20,9	48,7	53,0
58	91%	C9	45,3	34,0	56,6
59	92%	C23	43,0	38,0	57,4
60	94%	C3	41,0	44,2	60,3
61	95%	A18	-60,6	37,3	71,2
62	97%	B14	-36,6	66,3	75,7
63	98%	A12	79,3	34,6	86,5
64	100%	C11	98,0	78,5	125,5



International aligned(virtual)

Rank	Rank%	ID	d	sd	D
1	2%	A8	-3,7	3,4	5,1
2	3%	A20	-6,4	4,6	7,9
3	5%	A10	6,5	5,6	8,6
4	6%	C84	-0,7	8,7	8,7
5	8%	C22	3,4	9,9	10,4
6	9%	C21	7,6	7,2	10,5
7	11%	A9	11,7	7,0	13,6
8	13%	C14	8,8	11,5	14,5
9	14%	A2	10,7	11,0	15,3
10	16%	A17	10,6	11,3	15,5
11	17%	A7	14,4	7,8	16,3
12	19%	A11	16,3	5,8	17,3
13	20%	C13	9,9	14,6	17,6
14	22%	B23	-14,4	11,8	18,6
15	23%	C2	13,9	14,2	19,8
16	25%	B19	-15,7	12,2	19,9
17	27%	B24	-15,9	12,1	20,0
18	28%	A1	16,9	14,5	22,3
19	30%	C15	18,8	12,1	22,4
20	31%	A19	18,5	15,0	23,8
21	33%	A6	24,2	11,7	26,9
22	34%	B6	-16,7	22,7	28,2
23	36%	A15	27,2	11,1	29,4
24	38%	A3	27,1	13,3	30,2
25	39%	C8	23,1	21,7	31,7
26	41%	C25	16,2	28,8	33,0
27	42%	C19	25,1	22,1	33,5
28	44%	A14	31,9	12,0	34,1
29	45%	B3	-16,4	29,9	34,1
30	47%	A13	31,7	15,9	35,5
31	48%	B15	-21,8	30,6	37,6
32	50%	B11	-24,7	29,1	38,2
33	52%	B22	-31,8	21,3	38,3
34	53%	C1	31,7	21,5	38,3
35	55%	C17	31,3	23,1	38,9
36	56%	B7	-24,4	30,3	38,9
37	58%	A4	35,8	18,8	40,4
38	59%	C12	33,5	22,7	40,5
39	61%	B21	-37,9	17,6	41,8
40	63%	C6	26,8	34,5	43,7
41	64%	C10	32,9	29,1	43,9
42	66%	C20	28,7	33,4	44,1
43	67%	C18	27,1	35,0	44,2
44	69%	C16	31,0	31,8	44,4
45	70%	A16	41,6	16,4	44,7
46	72%	B16	-18,6	41,3	45,3
47	73%	B8	-40,2	22,3	46,0
48	75%	A18	-39,8	28,8	49,1
49	77%	A5	48,1	11,4	49,4
50	78%	C5	28,7	40,3	49,5
51	80%	C7	2,1	49,5	49,6
52	81%	B9	-48,8	23,2	54,1
53	83%	C9	45,5	31,4	55,3
54	84%	B2	-45,4	32,3	55,7
55	86%	C23	43,2	35,4	55,9
56	88%	C3	41,2	41,8	58,7
57	89%	B18	-49,4	34,2	60,1
58	91%	B4	-56,9	39,0	69,0
59	92%	B13	-60,3	35,0	69,7
60	94%	B17	-46,4	53,2	70,6
61	95%	B5	-44,2	63,9	77,7
62	97%	B14	-76,1	58,9	96,2
63	98%	A12	100,1	43,7	109,2
64	100%	C11	98,2	76,0	124,1

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Pre-requisites for international lab anchorage and intercomparison

- A international network of national expert laboratories (e.g. ICAR Reference Laboratory Network)
- Acquiring high skill and competence :
 - ⇒ High stability in performance : constant low bias & low repeatability.
 - ⇒ Tight anchorage thr. a net of RMs and PTs (QC)
- Simultaneous / close participation of expert labs in international and national PT schemes.

Anchoring labs to an international reference and comparing them on a unique scale is possible

- ↪ Existing PT schemes are appropriate tools
- ↪ PT schemes work independently with applying different protocols:
 - ⇒ To **establish new ICAR guidelines** to harmonise PT protocols thereby enable comparable and fair lab performance evaluation
- ↪ Preliminary cautions of simultaneousness, analytical stability (QC), high ref lab performance to be achieved:
 - ⇒ To **establish new ICAR guidelines** to assure trusty correspondence between PT schemes.

Thank You for your attention!