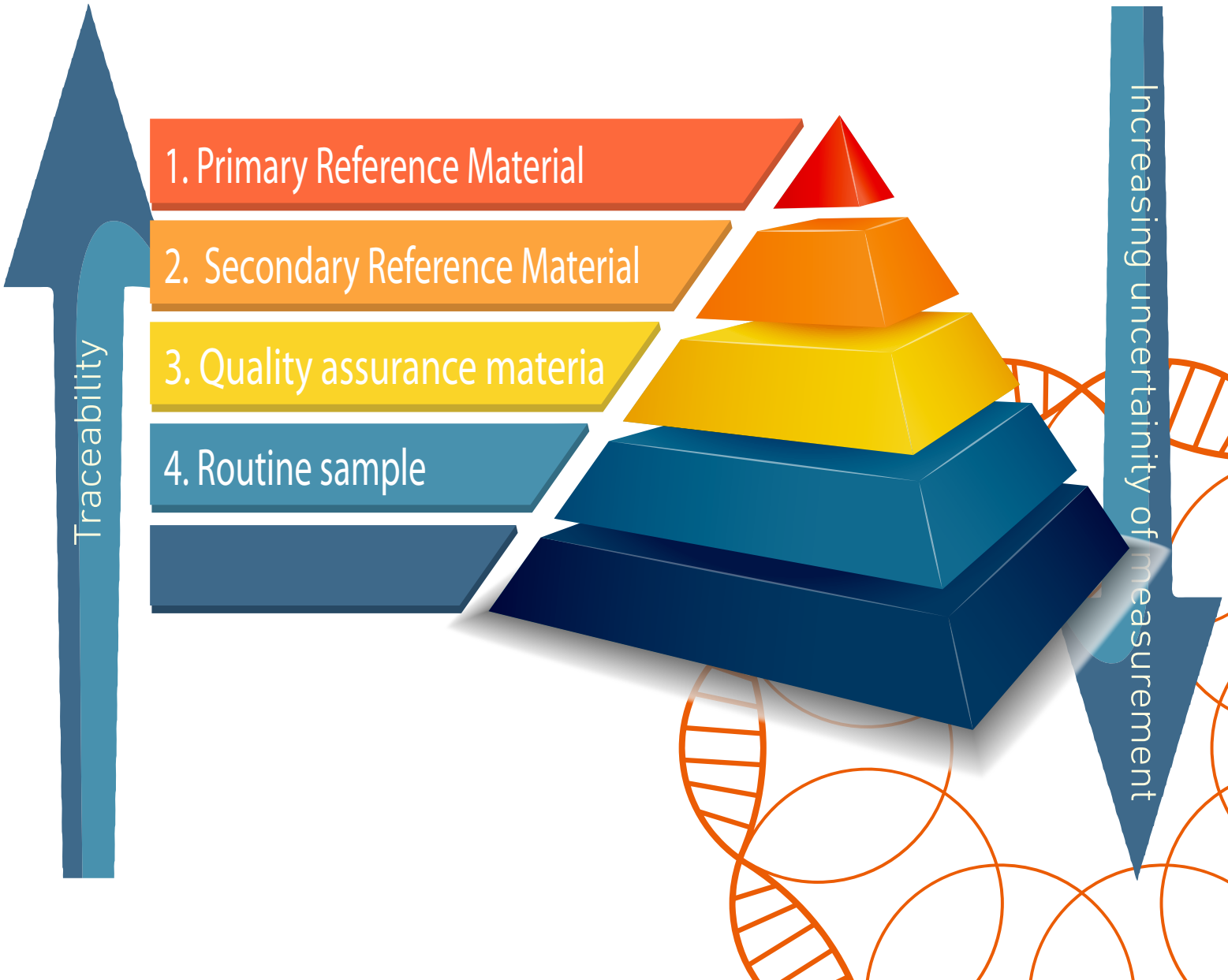
THE GLOBAL STANDARD
FOR LIVESTOCK DATA

Network. Guidelines. Certification.

ICAR Proficiency Test (PT) SCC

Traceability with SCC primary reference material



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September 2020

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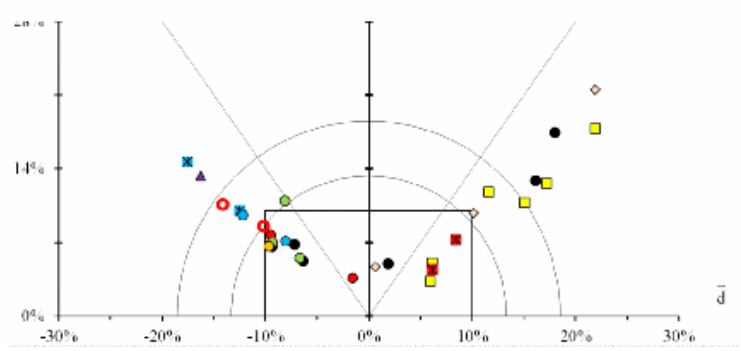
1. Introduction

Dear Participant,

Since 2009 ICAR, with International Dairy Federation (IDF), joined the project 'Reference System for Somatic Cell counting' to build up a better equivalence for the parameter somatic cell in milk. With the ICAR PT we received over the year a clear picture that shows a large distribution around the ICAR PT assign value. The bias distribution around ICAR PT assign value (AV), is quite constant over the years showing a maximum bias of plus and minus 20 % for the mean range tested.

Figure 1 Example of an ICAR PT bias distribution. Different colors represent different secondary reference materials used



The main reason of this static situation is due to the fact the laboratories always used the same secondary reference materials (SRM) and that these materials were not traceable to each other or with a primary reference material.

Thanks to the fruitful collaboration between IDF, ICAR and EU JRC (EU Join Research Center), in February 2020 the EU JRC launched a new certified reference material (CRM ERM®-BD001) for somatic cell counting in milk. This primary reference material will serve in the future to anchor the SRM !

During the ICAR PT September 2020, ICAR organized tests to compare the ICAR PT Assign Value (AV) with the CRM. ICAR asked the collaboration of an expert and qualified laboratory, Milchprüfring Bayern (DE) to set two master instruments with a calibration adjustment linked with the CRM.

Two fluoro optoelectronic instruments have been dedicated to this task and the calibration was adjusted with 5 CRM samples.

The ICAR PT samples have been analysed with these two instruments and the traceable assign values (AVT) with CRM have been calculated following the approach reported in the publication (Kuselman et al, 2002).

2. CRM preparation

CRM ERM®-BD001a (low concentration level) and CRM ERM®-BD001b (high concentration level) have been diluted following the instruction indicated on the ERM certificate and schematized in table 1

Table. 1 CRM - 5 samples dilution Certified values and expanded uncertainty

Dilution CRM ERM®-BD001a ml	15	11,25	7,5	3,75	
Dilution CRM ERM®-BD001b ml		3,75	7,5	11,25	15
	CRM ERM®-BD001a				CRM ERM®-BD001b
CRM ERM®-BD001a %	100	75	50	25	0
CRM ERM®-BD001b %	0	25	50	75	100
	CRM1	CRM2	CRM3	CRM4	**CRM5**
CRM scc*1000/ml	**62**	338	614	890	**1166**
UCRM scc*1000/ml	**6**	21	40	59	**79**

Each sample have been analyzed by two fluoro opto electronic instrument in 15 replicates each.
The instruments repeatability, linearity and calibration have been evaluated according ISO 13366-2.

2.1 Verification master instruments repeatability

The two instruments have been checked for repeatability. The results were below the limits reported in the ISO 13366-2 and its interpolation for the concentration tested. Instrument 1 repeatability resulted slightly higher than the limit of ISO 13366-2 sr_{CRM1}=8% for the low sample with a concentration of 62*1000 cells/ml

Table. 2 Instrument repeatability

		CRM1	CRM2	CRM3	CRM4	CRM5
CRM	value	62	338	614	890	1166
Instr. 1	mean	67	336	626	905	1177
Instr. 1	sr	4,74	12,84	11,15	16,33	17,95
Instr. 1	sr %	8	4	2	2	2
Instr. 2	mean	62	340	627	918	1184
Instr. 2	sr	1,92	5,54	8,90	12,57	14,91
Instr. 2	sr %	3	2	1	1	1
ISO 13366-2	sr%	6	5	3	3	3

2.2 Verification master instrument linearity

The instrument linearity was checked according ISO 13366-2. The ratio between the range tested and the residual range was below 2% for both instruments
De/DC%Instr.1=1,04 and De/DC%Instr.2=1,41

Table. 3 Instrument linearity

Linearity Instrument 1	CRM1	CRM2	CRM3	CRM4	CRM5	Limit
Mean residual	2,47	-7,09	3,49	4,41	-3,28	
SD	4,74	12,84	11,15	16,33	17,95	
Minimum	57	312	608	883	1143	
Maximum	73	356	643	938	1202	
D = Max-Min	16	44	35	55	59	
N	15	15	15	15	15	
Mean Max - Mean Min	1110	De/DC (%)	1,04		positive	2%
Mean residual Max - Mean residual Min	11,49					

Linearity Instrument 2	CRM1	CRM2	CRM3	CRM4	CRM5	Limit
	CRM1	CRM2	CRM3	CRM4	CRM5	
Mean residual	0,35	-4,06	0,67	9,46	-6,41	
SD	1,92	5,54	8,90	12,57	14,91	
Minimum	60	329	612	895	1166	
Maximum	66	347	641	941	1205	2%
D = Max-Min	6	18	29	46	39	
N	15	15	15	15	15	
Mean Max - Mean Min	1122	De/DC (%)	1,41		positive	
Mean residual Max - Mean residual Min	15,87					

2.3 Verification master instrument calibration

The mean results obtained on the CRM samples have been compared with the certified values. An ordinal least-squares method regression was applied considering a constant distribution of residuals over the calibration range. Because the characterization process included 14 results from ISO 133661 and 14 results from ISO 13366-2 the error in the calculated reference is negligible. The reference values were plotted on x-axis and instrumental results on y-axis.

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Table. 4 Instrument calibration verification

	Instr. 1	CRM	Bias
CRM1	67	62	-5
CRM2	336	338	2
CRM3	626	614	-12
CRM4	905	890	-15
CRM5	1177	1166	-11

slope	1,0105	1,6849	intercept
se(slope)	0,00657	4,781104	se(intercept)
r2	0,99987	5,7354	Standard Error
F	23644	3	df(residual)
SS	777778	98,6858	ss(residual)

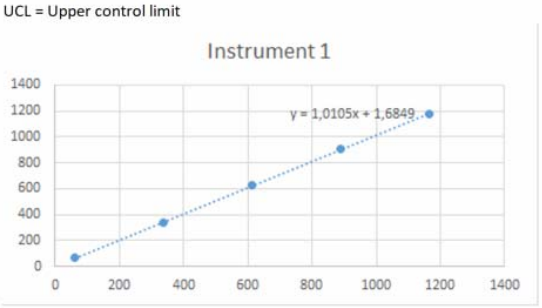
t(n-1,0.975)	2,77645	
LCL	0,99	-11,59
UCL	1,03	14,96

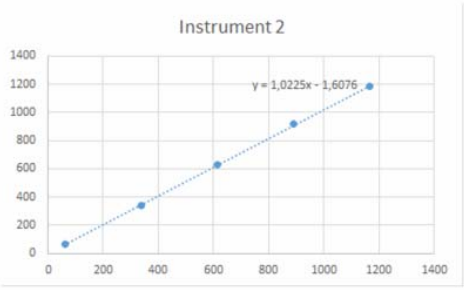
	Instr. 2	CRM	Bias
CRM1	62	62	0
CRM2	340	338	-2
CRM3	627	614	-13
CRM4	918	890	-28
CRM5	1184	1166	-18

slope	1,0225	-1,6076	intercept
se(slope)	0,008039	5,848548	se(intercept)
r2	0,999815	7,01595	Standard Error
F	16179,37	3	df(residual)
SS	796406	147,6707	ss(residual)

t(n-1,0.975	2,77645	
LCL	1,00	-17,85
UCL	1,04	14,63

LCL = Low control limit
UCL = Upper control limit



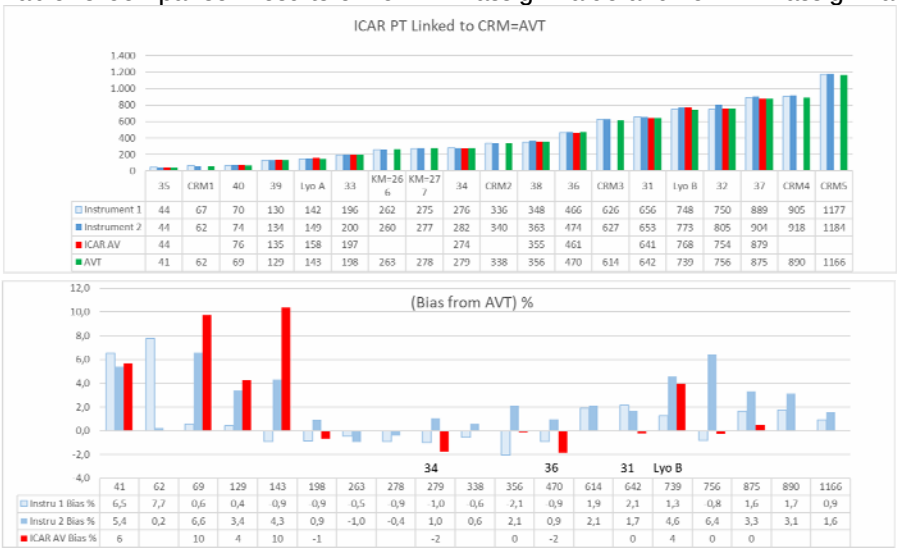


For both instruments the slope and intercepts were not statistically different from zero and one respectively.
This calibration setting was adapted and the instruments classified as master instruments to characterize the ICAR PT samples.

3. Calculation of ICAR PT Assign Value Traceable to the CRM (AVT)

The ICAR PT samples were analysed in 15 replicates on each of the two instruments immediately after the EU CRM and the calibration verification. Applying a comparative approach for secondary reference material characterization as reported by Kuselman et al, 2002 a traceable assign value (AVT) was calculated for each ICAR PT sample.
Complete details on this approach will be provided in a dedicated guideline that IDF and ICAR are going to publish.
In table 5 the mean results obtained with Instrument 1, Instrument 2, the ICAR PT assign value September 2020 and assign value traceable with CRM (AVT) are reported. The samples are ordered by concentration.

Table. 5 Comparison results of ICAR PT assign value and ICAR PT assign value traceable with CRM



	35	CRM1	40	39	Lyo A	33	KM=266	KM=277	34	CRM2	38	36	CRM3	31	Lyo B	32	37	CRM4	CRM5
Instrument 1	44	67	70	130	142	196	262	275	276	336	348	466	626	656	748	750	889	905	1177
Instrument 2	44	62	74	134	149	200	260	277	282	340	363	474	627	653	773	805	904	918	1184
ICAR AV	44		76	135	158	197			274		355	461		641	768	754	879		
AVT	41	62	69	129	143	198	263	278	279	338	356	470	614	642	739	756	875	890	1166

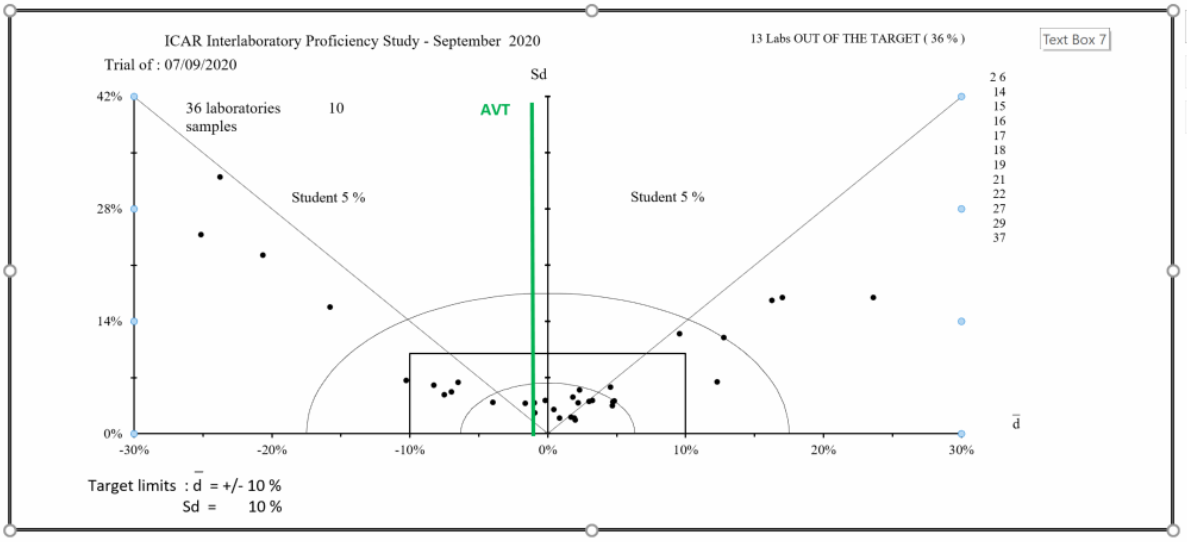
	41	62	69	129	143	198	263	278	279	338	356	470	614	642	739	756	875	890	1166
Instru 1 Bias %	6,5	7,7	0,6	0,4	0,9	0,9	0,5	0,9	1,0	0,6	2,1	0,9	1,9	2,1	1,3	0,8	1,6	1,7	0,9
Instru 2 Bias %	5,4	0,2	6,6	3,4	4,3	0,9	-1,0	-0,4	1,0	0,6	2,1	0,9	2,1	1,7	4,6	6,4	3,3	3,1	1,6
ICAR AV Bias %	6		10	4	10	-1			-2		0	-2		0	4	0	0		

Samples, CRM1,KM=267,KM=277,CRM2,CRM3,CRM4 and CRM5 were reported because used only by the laboratory as quality assurance tools.

4. Conclusions

The comparison between the ICAR PT assign value (AV) and the ICAR PT assign value traceable to the CRM (AVT) shows that the mean difference between AV and AVT is 2,4%.
In figure 1 the green line represents the AVT (assign value calculated on the traceable results)

Figure. 1 ICAR PT September 2020 laboratories performance



The expectation is that, gradually, the secondary reference material producers will anchor their characterization process with the CRM following the traceability chain. Finally, the users should adjust the instruments calibration accordingly. The standard deviation distribution should decrease over the time.

5. Bibliography

ISO 13366-2|IDF 148-1:2006 Milk - Enumeration of somatic cells - Part 2: Guidance on the operation of fluoro-optoelectronic counters.

Kuselman, I., Weisman, A. & Wegscheider, W. 2002. Traceable property values of in-house reference materials. Accred Qual Assur 7 p122-124.

EU Joint Research Centre. 2020. Certification report : ERM®-BD001. https://crm.jrc.ec.europa.eu/p/ERM-BD001