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Taiwan Join the ICAR Proficiency Test of Cow Raw Milk

台灣參加國際畜政聯盟牛乳乳質分析熟練測試

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What is Proficiency Testing?

The ISO definition of laboratory proficiency testing is:

"Determination of laboratory testing performance by means of inter laboratory comparisons"

PT is a test of accuracy by comparing the laboratory results with the 'true' value.

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Taiwan Join the ICAR Proficiency Test of Cow Raw Milk

- Laboratory performance proficiency testing of analytical method performance has become an integral part of third-party performance certification programs for laboratories around the world.
- Proficiency testing results indicate if the analytical performance of a laboratory on a particular method and analyte are within some normative performance criteria and provides a comparison to the performance of other laboratories.
- The Proficiency Tests that are organised twice a year by ICAR and participated for the milk analysis laboratories in global Laboratories Reference Network (LRN) since 2016.

Proficiency Tests for the milk analysis laboratories

The following information are available in ICAR Proficiency Test:

- Information about the ICAR Proficiency Test
  - Results of the Proficiency Test organised in September 2018
  - Results of the Proficiency Test organised in March 2018
  - Procedures and information to join the ICAR Proficiency Test and call for 2018
  - Results of the Proficiency Test organised in September 2017
  - Results of the Proficiency Test organised in March 2017
  - Procedures and information to join the ICAR Proficiency Test and call for 2017
  - Results of the Proficiency Test organised in December 2016: new parameters (PAG, BHB and DNA microorganisms)
  - Results of the Proficiency Test organised in March 2016
  - Information about the Proficiency Test run in September 2016
  - Results of the Proficiency Test organised in September 2016

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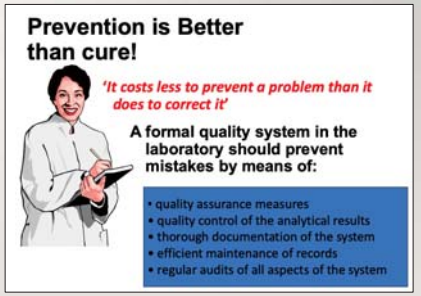
Proficiency Testing and ISO 17025

- The philosophy behind the quality assurance section of ISO/IEC 17025 is to firstly ensure that a single analyst within a laboratory is able to consistently reproduce the same result on the same sample.
- Secondly, the result produced by this analyst should reflect the result that would have come from any other analyst in the laboratory.
- Thirdly, any results from the laboratory as a whole should reflect the results that are agreed upon by many other laboratories.



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Why PT Participate?

- Requirements of certification and accreditation body.
- Proof to management of competence.
- Proof to higher authorities and clients of competence.
- Opportunity for increasing understanding of quality issues in a test.
- Opportunity for comparison of methodologies with other labs.
- Increase confidence of laboratory.

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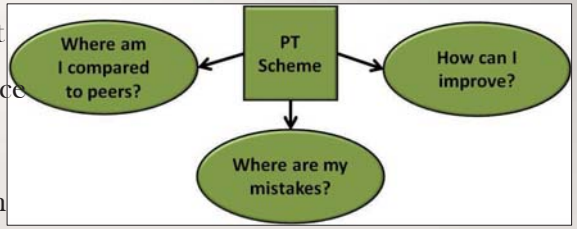
Benefits of PT Participation

- Monitor & improve measurement
- Demonstrate analytical competence
- Develop and train analysts
- Method and instrument validation



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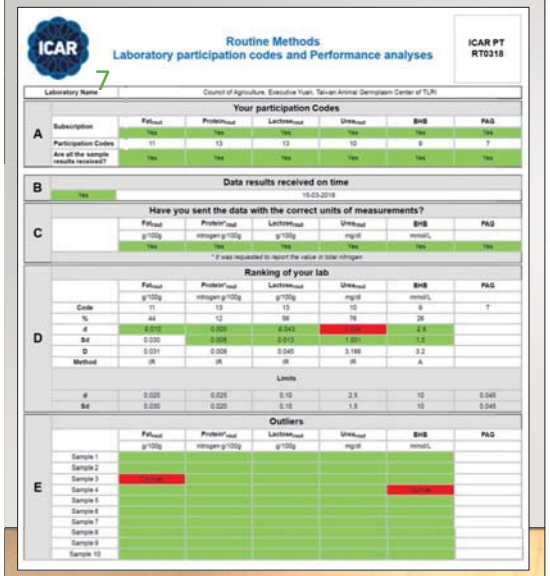

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Domestic PT test in Taiwan

- Our goal was to extend the feasibility of ICAR proficiency testing, analytical method quality assurance system, and production of reference samples for calibration of infrared milk analyzers to achieve a more efficient use of resources and reduce costs while maximizing analytical accuracy within and among milk payment-testing laboratories.
- To achieve this, a multi-laboratory combined proficiency testing is conducted following ICAR analytical method for quality-assurance system to evaluate and improve the analytical performance of domestic milk processors in Taiwan.

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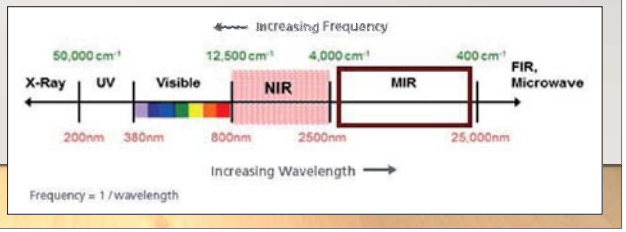
Our Objectives

- To use data from a set of milk samples to monitor the analytical performance to evaluate and improve the analytical proficiency of individual laboratories, and to calculate all-laboratory mean reference values for fat, protein, lactose, TS and SCC for these milks.
- This enables the proficiency test samples to be used as reference materials to calibrate a high-speed FTIR measurement.



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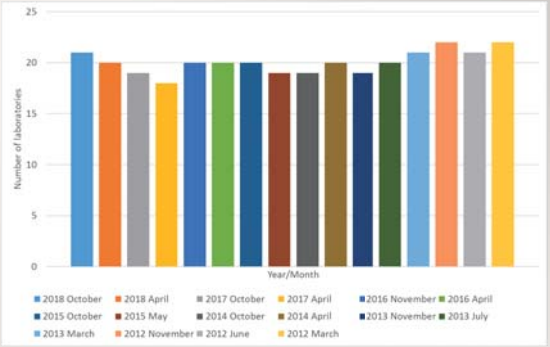
Participant and Test Item Statistics of domestic PT test in Taiwan

Year	Month	Number of laboratories	Fat	Protein	Lactose	SNF	SCC
2018	October	21	●	●	●	●	●
2018	April	20	●	●	●	●	●
2017	October	19	●	●	●	●	●
2017	April	18	●	●	●	●	●
2016	November	20	●	●	●	●	●
2016	April	20	●	●	●	●	●
2015	October	20	●	●	●	●	●
2015	May	19	●	●	●	●	●
2014	October	19	●	●	●	●	●
2014	April	20	●	●	●	●	●
2013	November	19	●	●	●	●	●
2013	July	20	●	●	●	●	●
2013	March	21	●	●	●	●	●
2012	November	22	●	●	●	●	●
2012	June	21	●	●	●	●	●
2012	March	22	●	●	●	●	●



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Rationale

- Milk payment laboratories have a need for quality-assurance control of both chemical reference methods and secondary instrumental methods to ensure accuracy of testing.
- This approach allowed us to characterize analytical deviations from the mean of all laboratories for each laboratory for each method, to determine the causes of deviations, and to recommend possible corrections and improvements that could be made.

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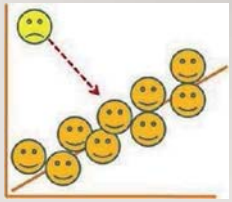
Approach

- A set of milk samples are formulated to produce an orthogonal matrix of fat, protein, lactose, SNF, and SCC concentrations and are analyzed by a group of laboratories.
- Statistical outliers were removed and all-laboratory mean reference values and within- and between-laboratory variation (i.e., S_r and S_R) with outliers removed were calculated for each milk sample for each component (fat, protein, lactose, TS, and SCC) per laboratory.
- The proficiency of the was evaluated utilizing Z-scores and Euclidian distance plots without outliers removed.



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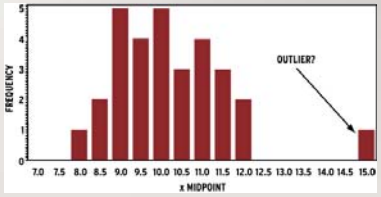
Statistical Methods: Outlier Data Removal Procedures

- In the context of PT performance analysis, one of the primary goals was to produce reference values for each sample that reflect as closely as possible the true value for the concentration of each component in a sample.
- The reference values will be used to calibrate many high-speed electronic milk-testing instruments that will influence the payment for very large volumes of milk.
- The harmonized outlier removal procedure is a sequential statistical process utilizing the Cochran, single Grubbs, or double Grubbs procedures, in that order.



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Statistical Methods: Within- and Between-Laboratory Method Performance

- The statistical metric for within-laboratory variation of a method is the repeatability standard deviation (S_r) and the metric for between-laboratory variation is the reproducibility standard deviation (S_R).
- The data are analyzed by the statistical procedures using the Microsoft Excel to determine the S_r, relative standard deviation of repeatability (RSD_r), r-value, S_R, relative standard deviation of reproducibility (RSD_R), and R-value for each sample.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{mean})^2}{n-1}}$$

$$RSD = \frac{s}{x_{mean}}$$

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DETERMINATION of SCC in RAW (cow) MILK

表二：重複性 - 單一樣品重複測試間之絕對差異

Table II: REPEATABILITY - Absolute difference between replicates in 10^3 cells / ml

Lab code	Sample 1	Sample 2	Sample 3	Sample 4	Sr	NL
A	22	15	8	3	7	12
B	27	21	27	5	11	12
C	64	148 *	47	35	44	12
D	33	6	14	7	9	12
F	37	9	35	14	14	12
G	71	30	29	14	21	12
H	4	5	2	0	2	12
I	52	31	76	21	24	12
J	35	21	19	9	12	12
K	13	32	8	10	9	12
L	13	15	12	12	7	12
M	44	10	5	8	12	12
Q	45	30	65	38 *	24	12
Sr	21	23	17	9		
r	63	50	42	42		
NE	39	36	39	36		
L	36	40	29	16		

- Sr: 重複性標準差限制 repeatability standard deviation of each laboratory limit
- NL: 各實驗室列入統計之測量數目 number of measurements per laboratory
- L: 三重複測量間之差異限制 Limit for difference between triplicates according Cochran test at 5% level.
- SE: 單一樣本重複性標準差 repeatability standard deviation per sample.
- NE: 單一樣本列入統計之測量數目 number of measurements per sample.
- *: 不列入統計之 Cochran 檢定離群值 discarded data using the test of Cochran at 5 %.
- r: 重複測量間之差異依據 ISO 13366-2 | IDF 148-2 規範 limit of repeatability, absolute difference between replicates according ISO 13366-2 / IDF 148-2.

Level 10^3 / mL	Sr %	r
150	6	25
200	5	42
450	4	50
750	3	63
1500	3	126

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DETERMINATION of SCC in RAW (cow) MILK

表三：單一樣品重複測試平均值

Table III: Means of the replicates in 10^3 cells / ml

Lab code	Sample 1	Sample 2	Sample 3	Sample 4
A	988	663	409	226
B	816	532	376	215
C	891	555	374	209
D	945	628	393	215
F	865	607	388	225
G	923	625	402	216
H	782	488 *	349	193
I	828	618	384	209
J	844	534	363	202
K	815	625	391	193
L	905	602	449 *	261 *
M	983	669	415	226
Q	961	616	412	234
M	888	597	393	217
REF.	888	615	389	214
SD	66	52	25	17

M = 單一樣本測量平均值 mean per sample

REF. = 單一樣本參考標準值 reference values

SD = 單一樣本測量標準差 standard deviation per sample

*: 不列入參考標準值統計之 Grubbs 檢定離群值 discarded data using the test of Grubbs at 5 %

表四：離群值

Table IV: Outlier identification

Sample	1	2	3	4
Outliers Cochran	H		K	
Outlier Grubbs	H	H	F	
Sr	9	9	4	5
SR	108	64	19	9
Sr%	2%	2%	2%	3%
SR%	24%	19%	9%	5%

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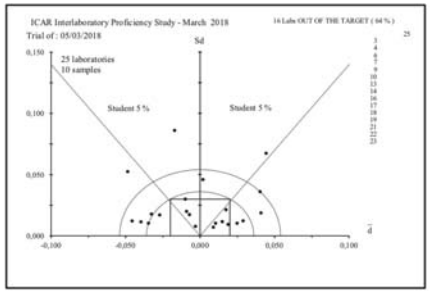
Statistical Methods: Laboratory Proficiency Evaluation

- The mean difference (MD), standard deviation of the difference (SDD), and Euclidean distance (ED) were used to identify laboratories that were having problems with a particular method, for ranking the performance of laboratories over time for each method, and for documenting performance improvement across time.
- The ED plots are statistical measures of similarity that are the distance from an individual data point to the centre point, which is calculated using the MD and SDD from the all-laboratory mean. The diagrams are extremely useful for rapidly determining problems that need attention first.



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DETERMINATION of FAT in RAW (cow) MILK

表一：實驗室總排名

Table I: Ranking of the laboratories

單位：g / 100 g

Unit: g / 100 g

Nb	%	Lab code	d	Sdso	D
1	8%	D	-0.01	0.01	0.012
2	15%	B	-0.01	0.01	0.016
2	15%	R	0.01	0.01	0.016
3	23%	E	0.02	0.01	0.022
4	31%	A	0.03	0.01	0.029
5	38%	C	0.02	0.02	0.032
5	38%	I	-0.01	0.03	0.032
5	38%	G	-0.03	0.01	0.032
5	38%	S	-0.02	0.03	0.032
6	46%	Q	-0.03	0.02	0.037
7	54%	L	-0.04	0.02	0.043
8	62%	P	0.04	0.05	0.061
8	62%	F	0.06	0.01	0.061
9	69%	H	-0.03	0.06	0.065
10	77%	J	0.09	0.03	0.093
11	85%	K	-0.14	0.05	0.145
12	92%	M	-0.10	0.15	0.178
13	100%	O	0.50	0.54	0.737

本表是與第一對照，了解實驗室檢測結果是否位於可接受之限制範圍：

- d: ± 0.020 g / 100 g
- Sd: 0.030 g / 100 g

The table should be studied in parallel with figure 1 where the laboratories are located according to an acceptability area (or target) the limits of which are:

± 0.020 g / 100 g for d and 0.030 g / 100 g for Sd

REF: 參考數值利用 ISO 13528 algorithm A 進行每個樣品之標準值計算，並依 ISO 9622 | IDF 141 標準 Grubbs test 去除 5% 離群值。

REF: Assigned values are robust average values per sample according to algorithm A of standard ISO 13528, using routine method ISO 9622 | IDF 141, after outlier discarding using Grubbs test at 5 % risk level.

(NC: 數據不足不列入排名 OUT of RANKING because of insufficient data number)

(Nb: 實驗室排名 laboratory rank; % 排名相對百分比 relative rank)

(N°: 實驗室代碼 laboratory identification number)

(d et Sd: 各實驗室檢測結果與標準值差異之平均與標準差 mean and standard deviation of the differences (laboratory -reference))

(D: 歐氏距離 Euclidian distance to YX-axis origin = SQUARE ROOT(d2 + Sd2))

- 重複性標準差 Repeatability standard deviation of this proficiency test (after Cochran elimination at 5 %) Sr= 0.011
- 再現性標準差 Reproducibility standard deviation of this proficiency test (after Cochran and Grubbs elimination at 5 %) SR= 0.163

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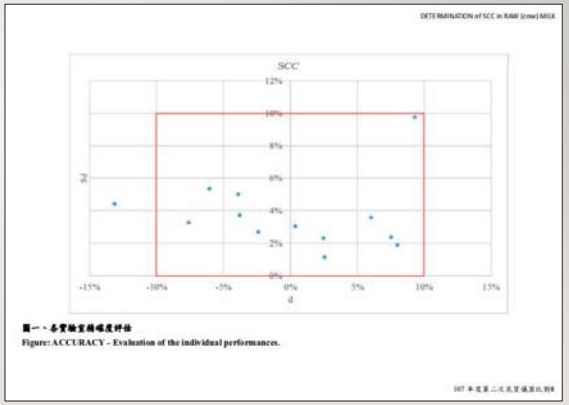

DETERMINATION of SCC in RAW (cow) MILK

SCC

12%
10%
8%
6%
4%
2%
0%
Sd
-15%
-10%
-5%
0%
5%
10%
15%
d

圖一、各實驗室精確度評估

Figure: ACCURACY - Evaluation of the individual performances.

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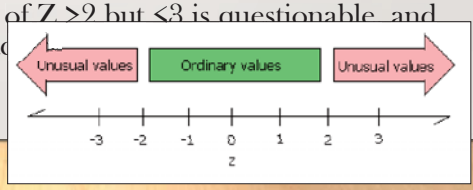
Statistical Methods: Z-score calculations

- The Z-score is a dimensionless quantity derived by subtracting the population mean (with statistical outliers removed) from an individual laboratory's (mean with no outliers removed) and then dividing the difference by the population long-term standard deviation of reproducibility.
- Classifying the Z-scores to determine if a system is well-behaved or not is indicated by the following guidelines: the absolute value of Z ≤ 2 is satisfactory, the absolute value of Z >2 but <3 is questionable, and the absolute Z-value ≥3 is unsatisfac



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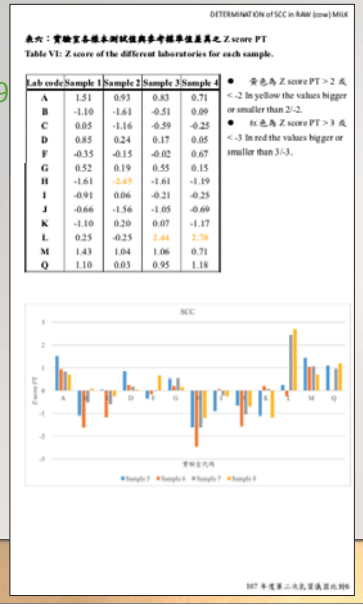

DETERMINATION of SCC in RAW (cow) MILK

表六：實驗室各樣本測試值與參考標準值差異之 Z score PT

Table VI: Z score of the different laboratories for each sample.

Lab code	Sample 1	Sample 2	Sample 3	Sample 4
A	1.51	0.93	0.83	0.71
B	-1.10	-1.61	-0.51	0.09
C	0.05	-1.16	-0.59	-0.25
D	0.85	0.24	0.17	0.05
F	-0.35	-0.15	-0.02	0.67
G	0.52	0.19	0.55	0.15
H	-1.61	-2.45	-1.61	-1.19
I	-0.91	0.06	-0.21	-0.25
J	-0.66	-1.56	-1.05	-0.69
K	-1.10	0.20	0.07	-1.17
L	0.25	-0.25	2.44	2.70
M	1.43	1.04	1.06	0.71
Q	1.10	0.03	0.95	1.18

- 黃色為 Z score PT > 2 或 < -2 In yellow the values bigger or smaller than 2/-2.
- 紅色為 Z score PT > 3 或 < -3 In red the values bigger or smaller than 3/-3.

SCC

Z score PT

A D G Q L M Q

實驗室代碼

Sample 5 Sample 6 Sample 7 Sample 8

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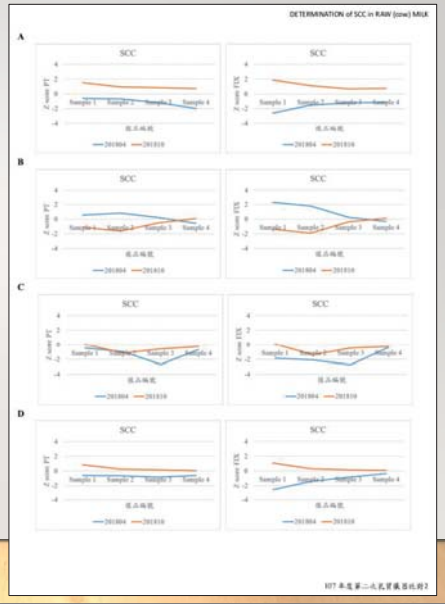

DETERMINATION of SCC in RAW (cow) MILK

A

B

C

D

SCC

Z score PT

Z score FDS

Sample 1 Sample 2 Sample 3 Sample 4

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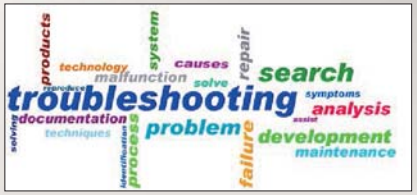
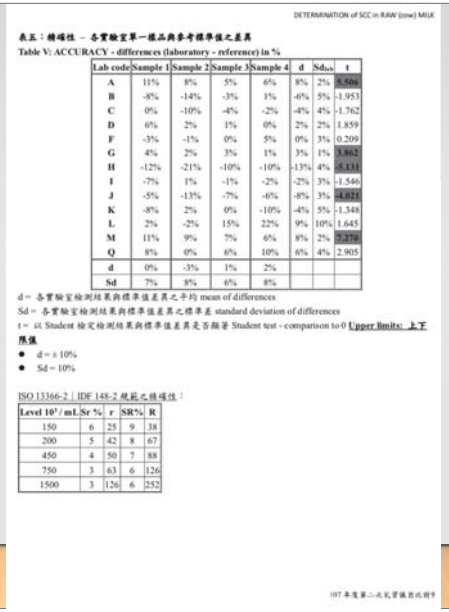
Troubleshooting Problems in Individual Laboratories

- Through the use of ED plot of MD and SDD, individual laboratories within each test item that need the most help to improve performance can be identified.
- Examination of the ED plots for that laboratory help determine if the performance of the laboratory indicates high random error or high systematic bias.
- This information is communicated to the laboratory and troubleshooting is done to achieve improved performance. The goal of the approach is to achieve improve performance of the laboratories (i.e., reduced S_r and S_R).





DETERMINATION of SCC in RAW (cow) MILK

表五：精確性 – 各實驗室第一樣品與參考標準值之差異

Table V: ACCURACY - differences (laboratory - reference) in %

Lab code	Sample 1	Sample 2	Sample 3	Sample 4	d	Sd_{lab}	t
A	11%	8%	-5%	6%	8%	2%	5.506
B	-8%	-14%	-3%	1%	-6%	5%	-1.953
C	0%	-10%	-4%	-2%	-4%	4%	-1.762
D	6%	2%	1%	0%	2%	2%	1.859
F	-3%	-1%	0%	5%	0%	3%	0.209
G	4%	2%	3%	1%	3%	1%	3.862
H	-12%	-21%	-10%	-10%	-13%	4%	-5.131
I	-7%	1%	-1%	-2%	-2%	3%	-1.546
J	-5%	-13%	-7%	-6%	-8%	3%	-4.021
K	-8%	2%	0%	-10%	-4%	5%	-1.348
L	2%	-2%	15%	22%	9%	10%	1.645
M	11%	9%	7%	6%	8%	2%	7.270
Q	8%	0%	6%	10%	6%	4%	2.905
d	0%	-3%	1%	2%			
Sd	7%	8%	6%	8%			

d= 各實驗室檢測結果與標準值差異之平均 mean of differences

Sd= 各實驗室檢測結果與標準值差異之標準差 standard deviation of differences

t= 以 Student 檢定檢測結果與標準值差異是否顯著 Student test - comparison to 0 **Upper limits: 上下限值**

- d = ± 10%
- Sd = 10%

ISO 13366-2 | IDF 148-2 規範之精確性：

Level 10^3 / mL	Sr %	r	SR%	R
150	6	25	9	38
200	5	42	8	67
450	4	50	7	88
750	3	63	6	126
1500	3	126	6	252

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Communication of Experience and Techniques

- Over a period of years, the approach has enabled the group of laboratories to document improved analytical performance (i.e., reduced within- and between-laboratory variation) of high-speed electronic milk-testing equipment.
- An annual meeting of the laboratory technicians allows for review of results and discussion of each method and provides a forum for communication of experience and techniques that are of value to new analysts in the group.

Thanks for your listening