Latest innovations for the monitoring of milk hygienic quality, methods automation and standardization

P. Broutin

Speaker: Pierre Broutin

Latest innovations for the monitoring of milk hygienic quality, methods automation and standardization

By Pierre Broutin
Managing Director WEU/Senior Scientist

Presentation outline

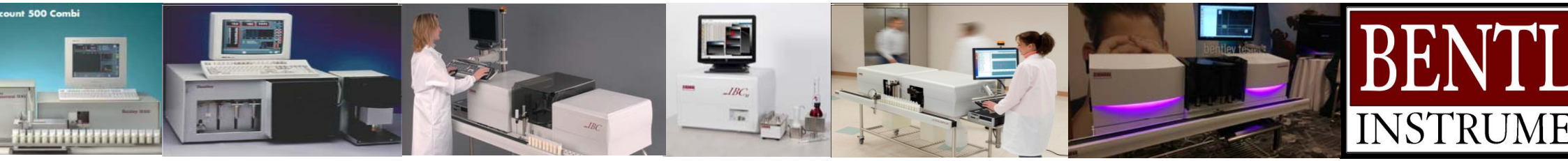
New BactoCount IBC, a unique multiplex solution for the real time monitoring of mil hygienic quality (total flora, somatic cells, bacteria & somatic cells ≠…)

BactoCount/Somacount Worldwide standardization

Milk Amyloid A (MAA) Assay – a better marker for subclinical mastitis diagnostic

New automation solutions for optimum laboratory & methods standardization

Worldwide infrared spectra standardization (patent pending)



Over 25 years of experience in flow cytometry (FC) and monitoring of milk hygienic quality

1991 **Somacount 300/500** (1st somatic cells counter using flow cytometry)

1995 **Bactocount 70** (1st bacteria counter using flow cytometry)

2001 **BactoCount IBC 50-150** (1st ISO 16140 certified bacteria counter (FC))

2002 **BactoCount IBC M** (1st integrated bacteria (ISO 16140) & somatic cells counter (FC))

2008 **Somacount FCM 500/600 –** somatic cells counter (FC)

2017 Bactocount IBC 200 (total bacteria, total somatic cells, bacteria & somatic cells ≠ ..

BactoCount IBC

A new analytical revolution in the monitoring of milk hygienic quality (total bacteria, somatic cells, ≠ …)

BactoCount IBC

A new analytical revolution in the monitoring of milk hygienic quality (total bacteria, somatic cells, ≠ …)

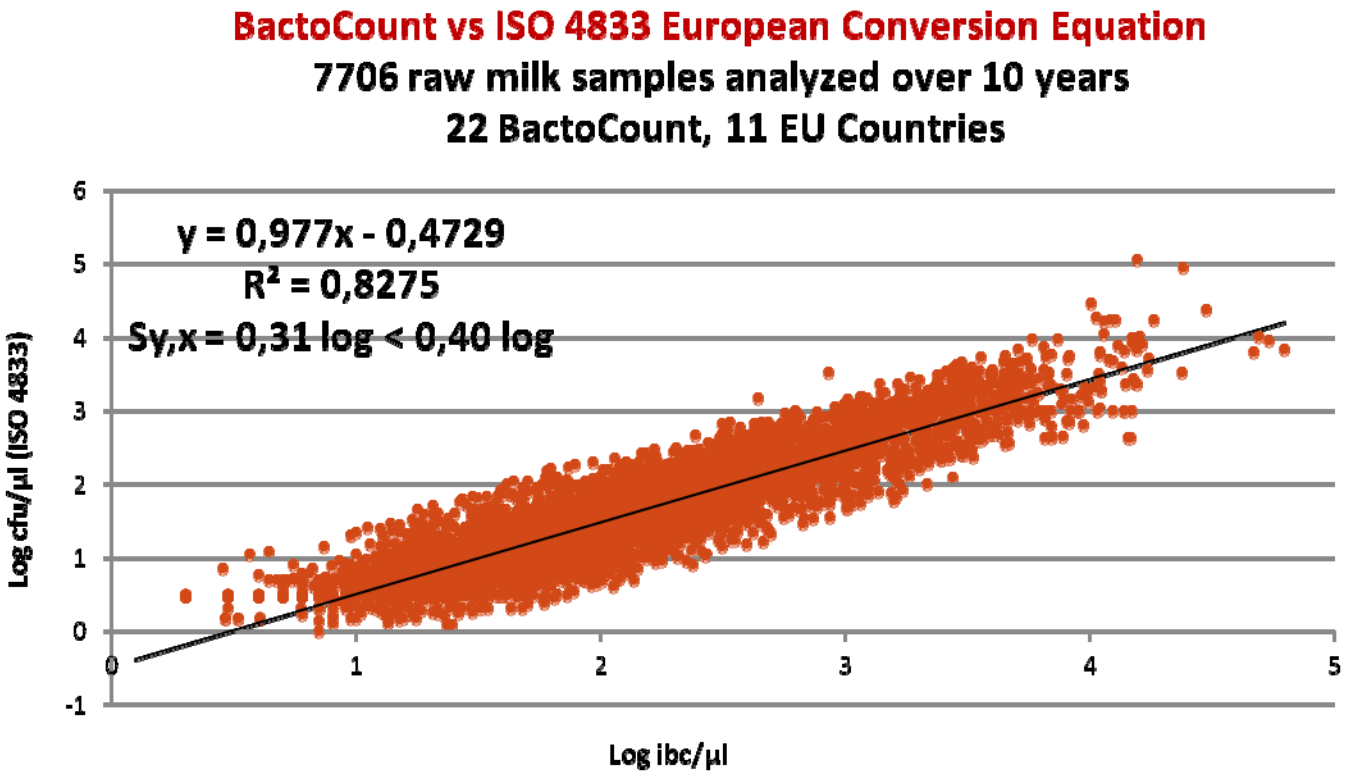
- **A unique multiplex solution for the real time monitoring of milk hygienic quality (total flora, somatic cells, total flora & scc ≠…)**
- Flow cytometer equipped with multiple lasers and detectors
- Automatic cytometer standardization
- Based on proven technologies and over 25 years of experience in flow cytometry
- Same software platform for all our Nexgen instruments
- Full compatible with the new Intellitech ILAS 4000 robot for complete method standardization and automation
- Fully compliant with ISO/IDF international standards
- Up to 200 samples/hour

MICROVAL

European validation and certification organisation

BENTL INSTRUME

BactoCount IBC:
A highly standardized method for international Results Equivalence



BactoCount IBC: A highly standardized method

ISO 17043 Accredited International Proficiency Test (IBC)

September 2016 RT:

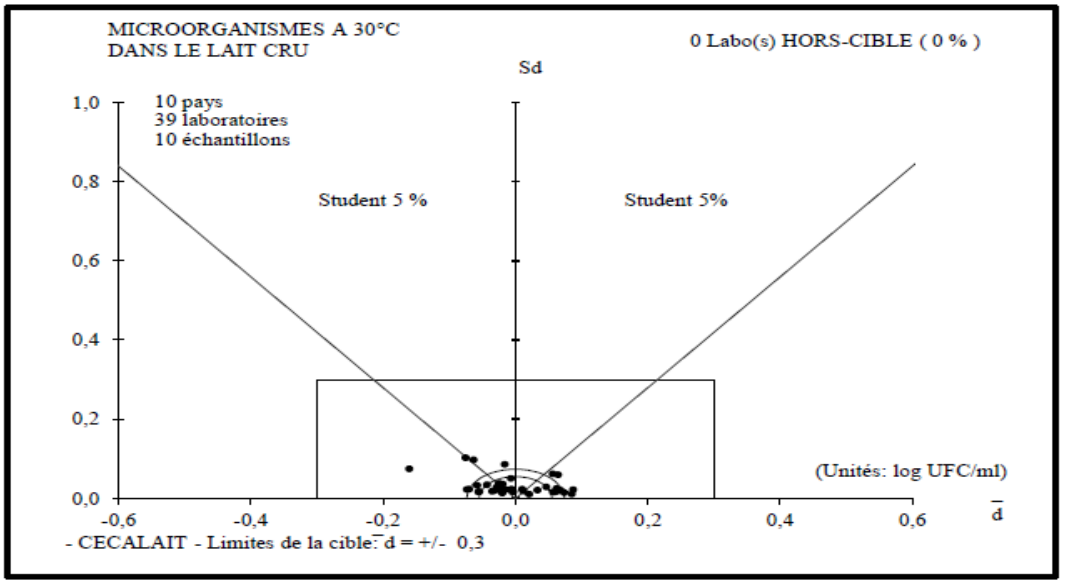
- 39 BactoCount
- 10 countries
- 0% lab. outside target

Sr = 0,017<< 0,09 log* (5x)
SR = 0,064 << 0,16 log* (2,5x)

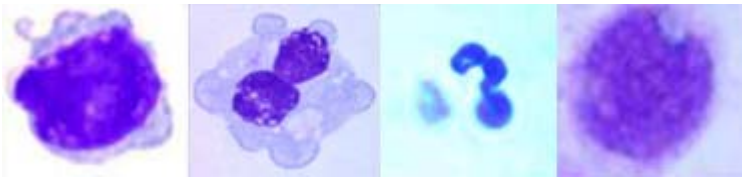
⟹ BactoCount fully complies with ISO 16140*
⟹ BactoCount Reproducibility within ISO 16140* Repeatability

Over 350 participating laboratories in 2016

Figure 2 : **JUSTESSE - Evaluation des performances individuelles (voir le tableau I).**
ACCURACY - Evaluation of the individual performances (to see table I).



Number	1	2	3	4	5	6	7	8	9	10	Mean
N	39	39	38	39	38	39	39	39	38	39	
Mean	5,29	5,75	5,06	4,83	5,27	5,56	5,13	5,46	4,24	4,95	
Sr	0,01	0,02	0,01	0,02	0,01	0,01	0,01	0,01	0,03	0,02	0,017
SR	0,07	0,06	0,05	0,05	0,05	0,07	0,06	0,07	0,09	0,06	0,064



WORLDWIDE EQUIVALENCE OF SOMATIC CELLS COUNT

Bentley Lyophilized Somatic Cells Standards

BENTLEY INSTRUMENTS

DOM 05/2015

CERTIFICATE OF ANALYSIS

Somacount SCC Lyophilized Control Sample

Description	Lot N° D00M05Y15	SCC Value (/µL)
SCC Control Sample	A	126 ± 10% (113-139)
SCC Control Sample	B	313 ± 10%(282-344)
SCC Control Sample	C	465 ± 10%(418-511)
SCC Control Sample	D	778 ± 10%(700-856)
SCC Control Sample	E	1019 ± 10%(917-1121)

Pierre Broutin
Bentley Instruments

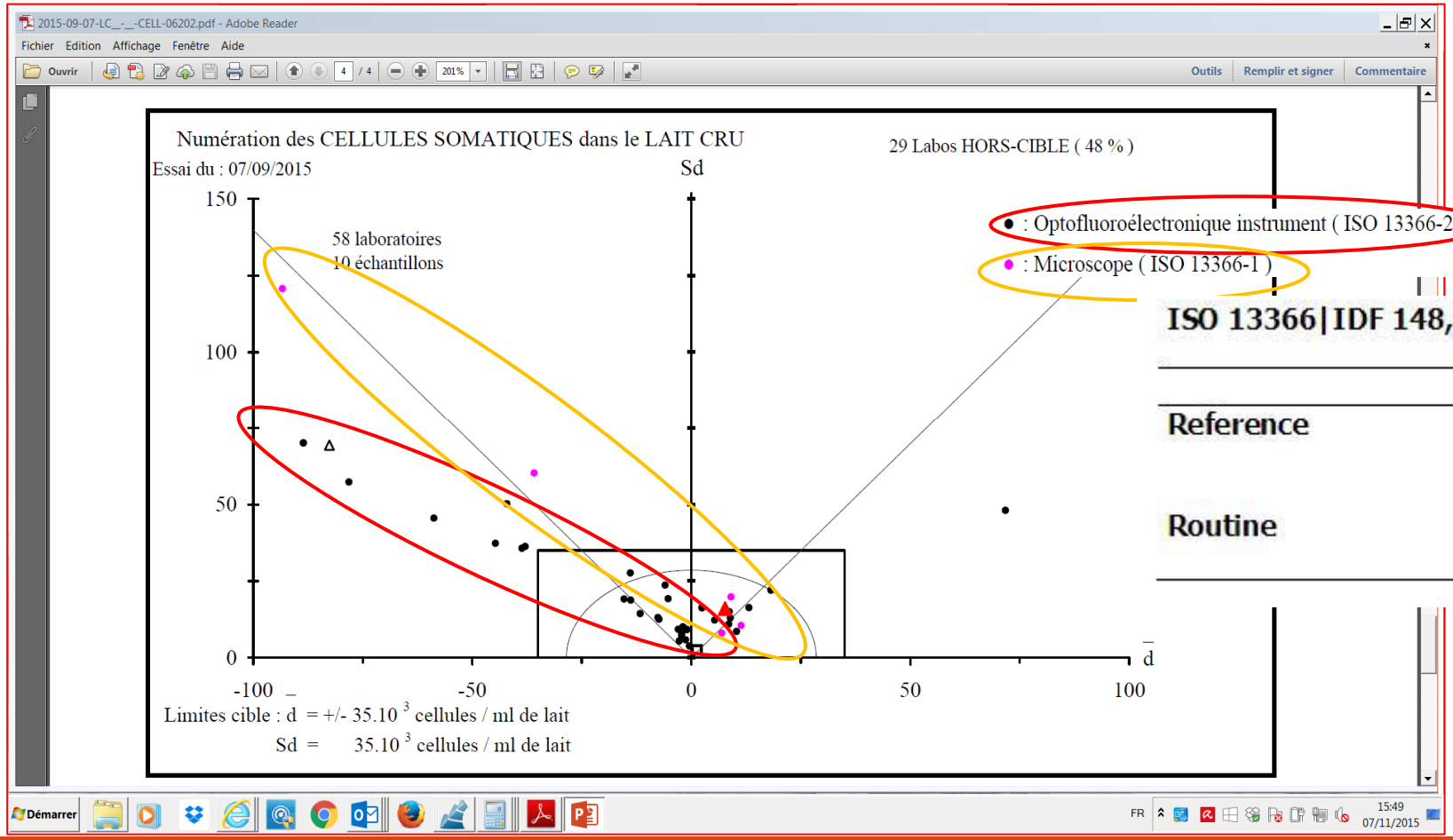
Reconstitution Procedure

- *Heat sterile water to 40°C ± 2°C in a water bath*
- *Remove the aluminium seal and stopper from the vial*
- ***Add 14.0 g ± 0.2g of sterile water into the vial of lyophilized somatic cells***
- *Mix vigorously until lyophilisate is completely re-suspended and place it in a water bath at 40°C±2°C for 10 minutes*
- *Mix vigorously the sample until it looks like regular milk*
- *Analyze the reconstituted sample in duplicates within 30' after reconstitution*

Bentley Instruments – ZA Brumehaut 140 rue Curie 62161 Maroeuil – France

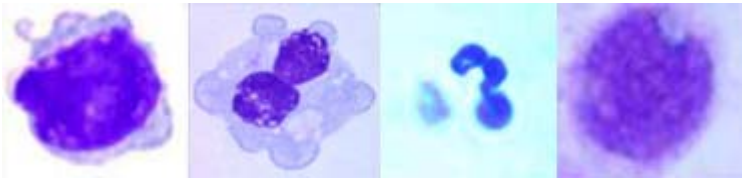
WORLDWIDE EQUIVALENCE OF SOMATIC CELLS COUNTS

Current situation (international RT with Reference & alternative methods)



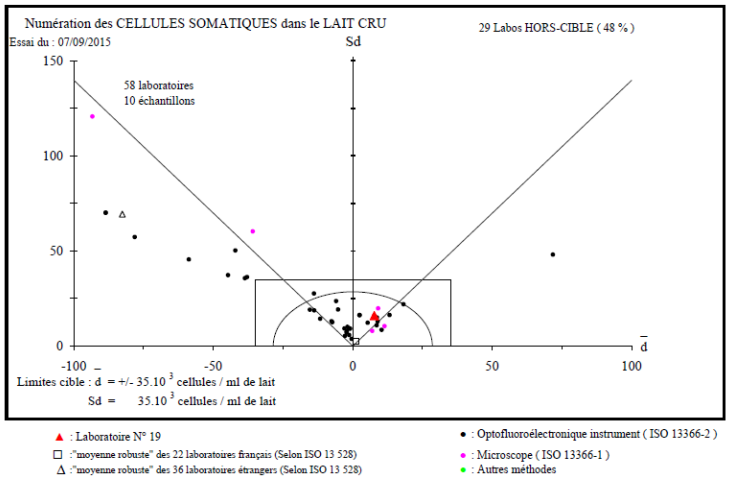
ISO 13366|IDF 148, part 1 vs. part 2 (all values in '000 cells

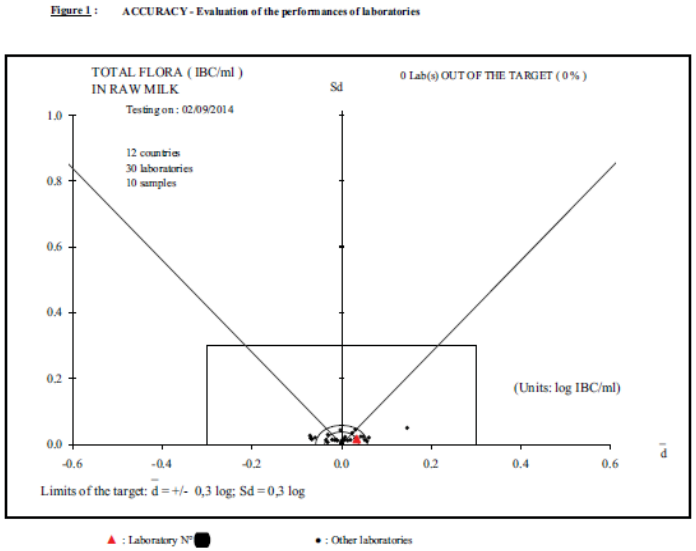
	Mean	sr	sR	r	
Reference	245	38	41	107	1
	679	69	79	192	2
Routine	245	13	20	36	
	679	21	40	59	1



Somacount & BactoCount

ISO 17043 Accredited International Proficiency RT (pending)





BactoCount RT

http://www.fil-idf.org/idficar-project-group-reference-system-somatic-cell-counting/

Milk Amyloid A (MAA) assay to improve subclinical mastitis diagnostic and reduce the use of intramammary antibiotics at drying off

- Milk Amyloid A (MAA) is one of the first Acute Phase Protein produced by the epithelial cells following infection of the mammary gland
- MAA Concentration may increase up to 1000-fold following localized inflammation and has been shown decline rapidly following recovery.
- It provides a useful, more specific marker than somatic cells for the detection of both clinical and sub-clinical mastitis at a quarter level
- MAA can be used for selective dry cow therapy at quarter level with confidence and enables dairy farme to significantly reduce the use of antibiotics at drying off

29% reduction in the use of intramammary antibiotic therapy at quarter level at drying off (Biotek lait)

Tridelta Developmen

Amyloid A (MAA) biomarker to improve subclinical titis diagnostic and reduce the use of intramammary biotics at drying off

Table 2. Proposed cutoff values and resulting sensitivity (Se), specificity (Sp), and area under the curve (AUC) for the California mastitis test (CMT), somatic cell count (SCC), milk haptoglobin (MHp), milk amyloid A (MAA), serum haptoglobin (SHp), and serum amyloid A (SAA) for the detection of subclinical mastitis based on bacterial culture results.

Analyte	Cutoff	Se (95% CI)	Sp (95% CI)	AUC (95% CI)
CMT	>1	82.1 (73.4–88.8)	94.1 (88.2–97.6)	0.965 (0.915–0.989)[a,b]
SCC (× 1000 cells/mL)	>130	89.6 (82.2–94.7)	72.0 (63.0–79.9)	0.948 (0.894–0.980)[a,c]
MHp (mg/L)	>3.9	90.6 (83.3–95.4)	68.6 (59.5–76.9)	0.886 (0.817–0.935)[c,d]
MAA (mg/L)	>16.4	90.6 (83.3–95.4	98.3 (94.0–99.7)	0.998 (0.967–1.000)[b]
SHp (g/L)	>0.05	90.0 (76.3–97.1)	64.43 (54.7–71.6)	0.782 (0.713–0.841)
SAA (mg/L)	>159.1	90.0 (76.3–97.1)	72.1 (61.4–81.2)	0.836 (0.759–0.896)[d]

Analytes with common superscript letters are not significant differently from each other. CI, confidence interval.

Acute phase proteins in the diagnosis of bovine subclinical mastitis

Shahabeddin Safi[1], Ameneh Khoshvaghti[2], Seyed Reza Jafarzadeh[3], Mahmoud Bolourchi[4], Iradj Nov

[1]Department of Clinical Pathology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehra
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University of Kazeroon, Kazeroon, Iran; [3]Department of Me
Epidemiology, University of California, Davis, CA, USA; and [4]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of
Tehran, Iran

Natural Clinical Mastitis		
Sensitivity	Specificity	Reference
93.0%	100%	Eckersall et al. 2001

Natural Subclinical Mastitis		
Sensitivity	Specificity	Reference
90.6%	98.3%	Safi et al. 2009
92.3%	92.1%	Shirazi-Beheshtiha et

nbiFTS complete automation & dardisation with the ILAS3000

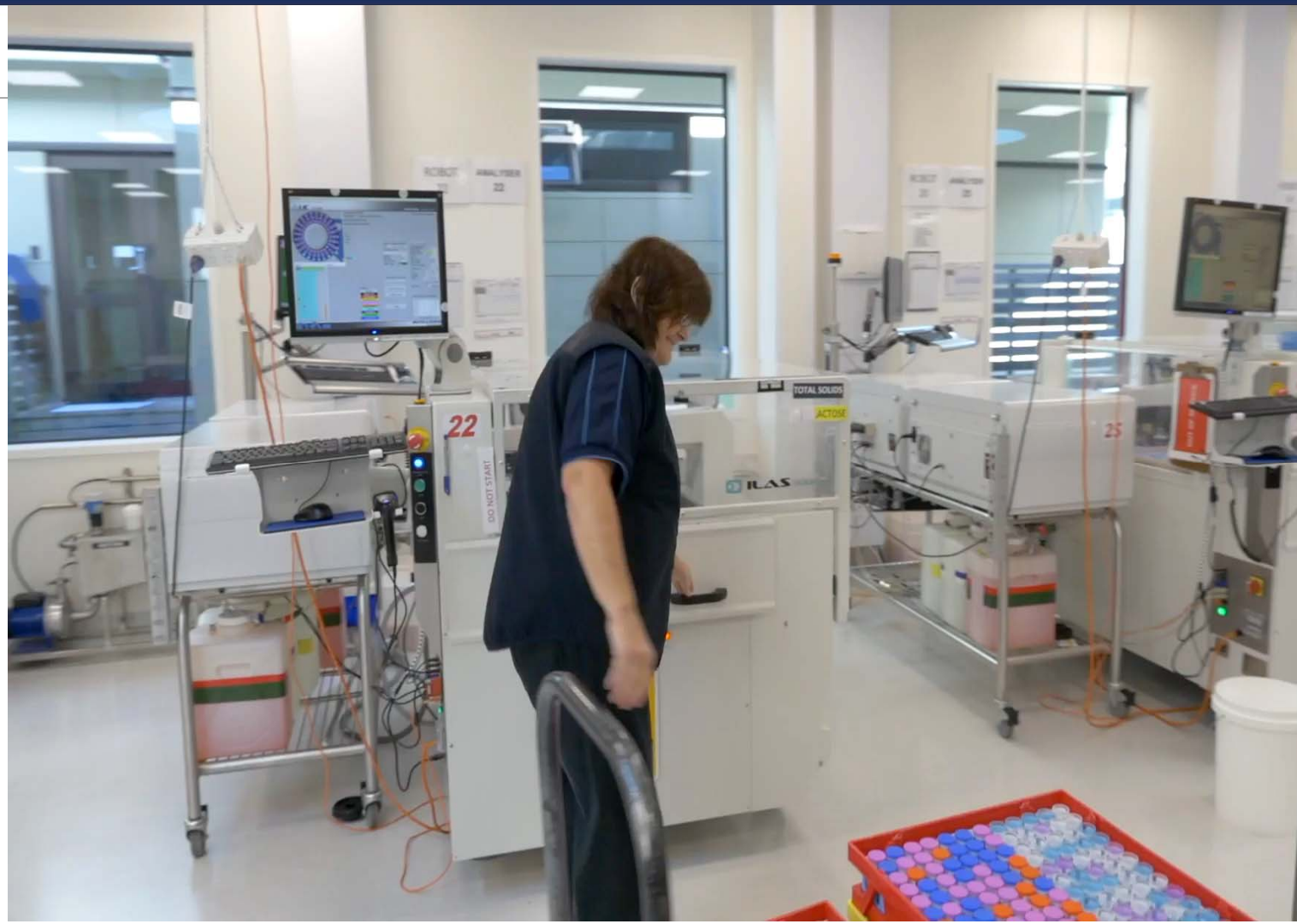
INTELLITECH AUTOMATION

BENTL INSTRUME

ey CombiFTS ILAS 600
IZ

tandardization & automation
mples handling:

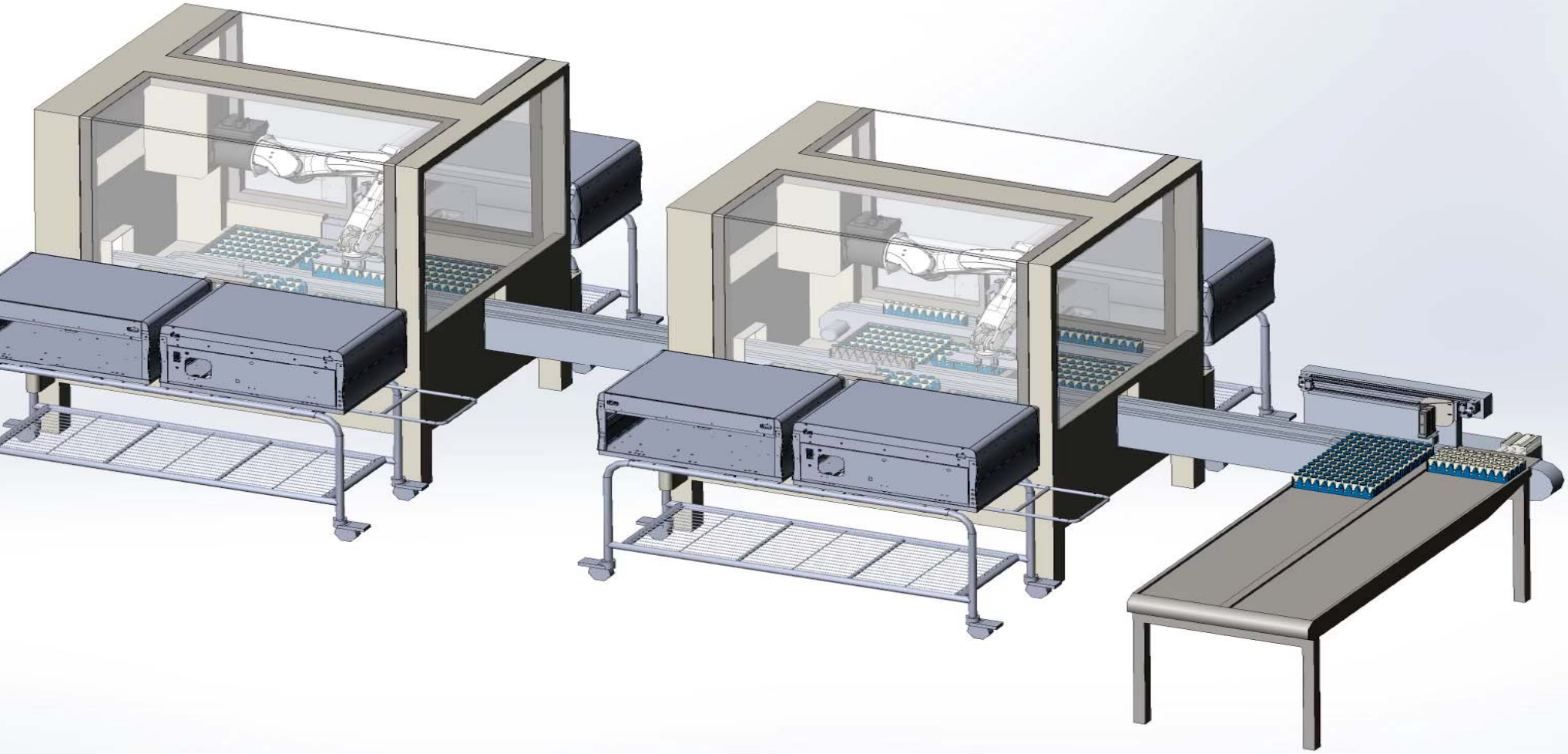
- tification (RFID, Barcode…)
- ples heated up to 40°C
- en
- apped
- ed under instrument pipette
- aced in their original position
- ibility to sort the samples



S3500 Combi Automation (rack based)

INTELLITECH AUTOMATION

BENTL INSTRUME

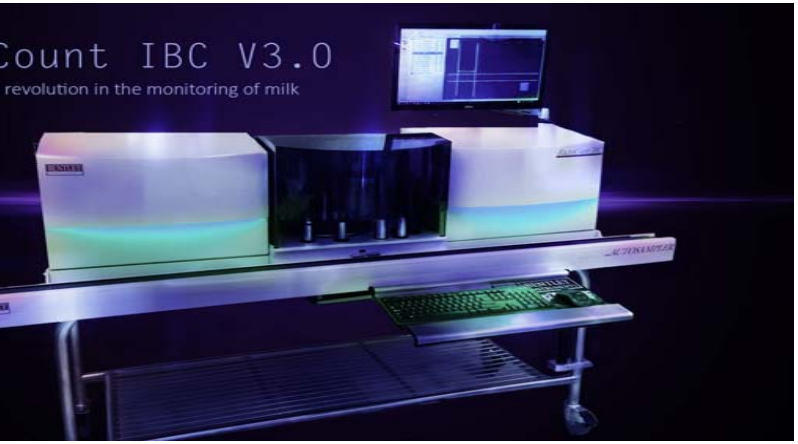


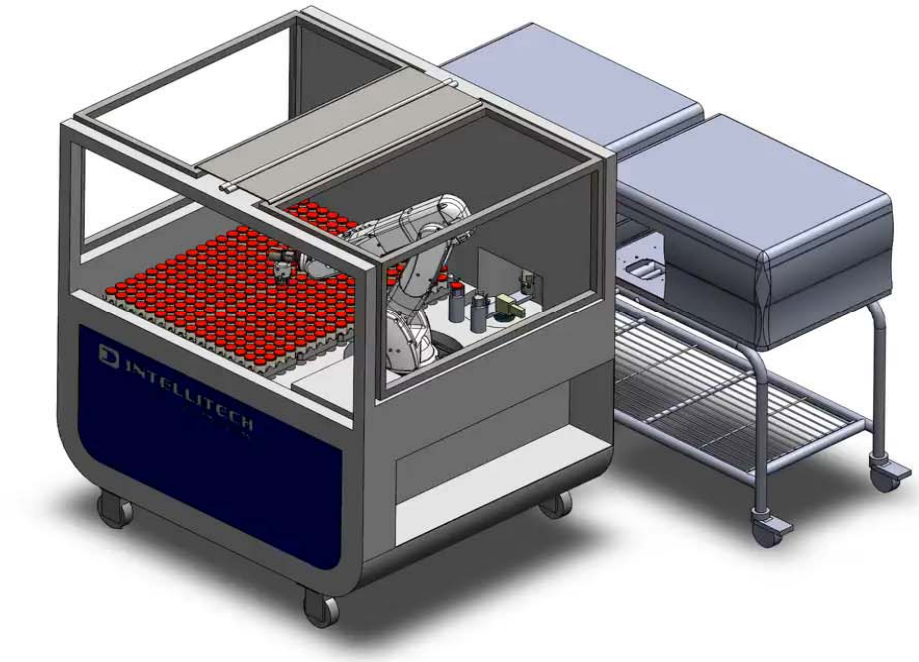
oCount automation & standardisation the ILAS4000

tley BactoCount ILAS 4000 (refrigerated)

standardization & automation
amples handling:

ntification (RFID, Barcode…)
aken
capped/pierced
ced under instrument pipette
placed in their original position





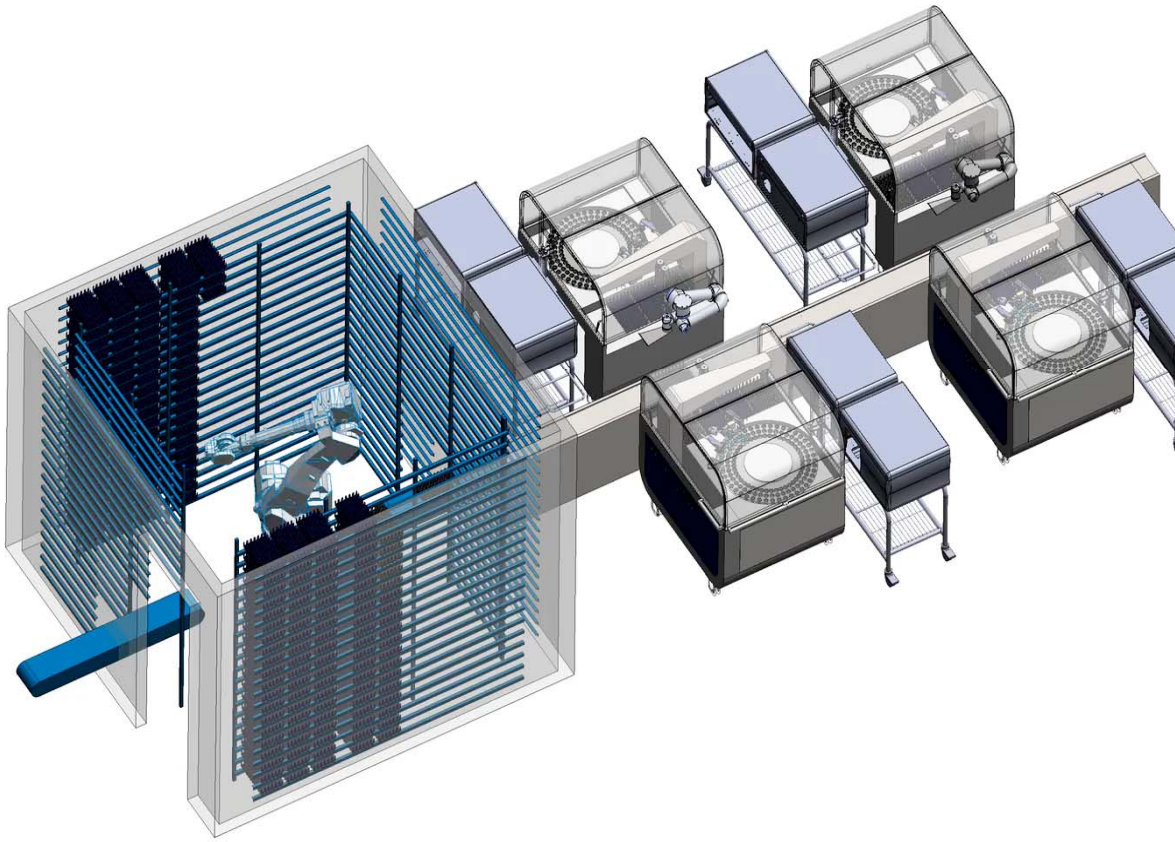
plete Laboratory Automation
andardisation

INTELLITECH AUTOMATION

BENTL INSTRUME

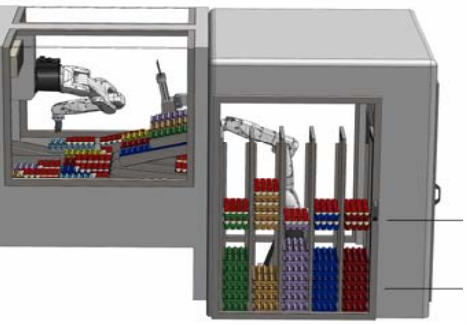
nples trays placed on conveyor system
ys sorted out and stored at 4°C
ys transferred to available BactoCount/CombiFTS
ys loaded/unload automatically on instruments

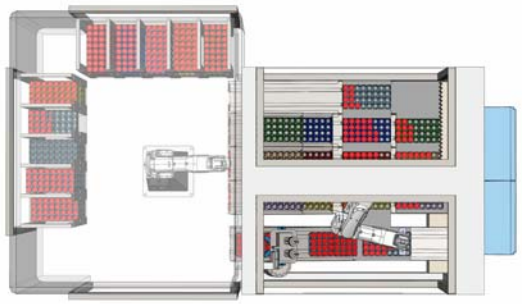
nplete standardization of samples handling
nplete standardization of operating conditions
imized laboratory throughput

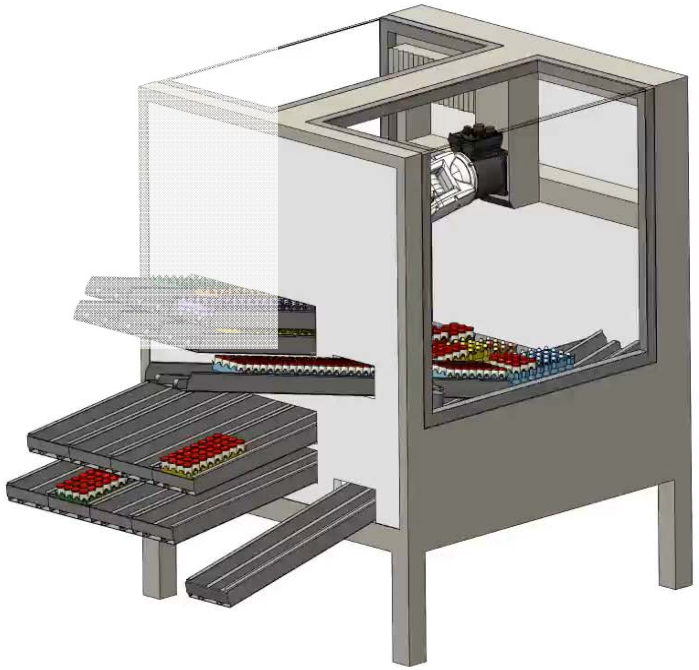


nples Sorting automation

nples automatically sorted out for secondary testing
to 6 parameters
to 8000 vials sorted in 3 hours
nples maintained at 4°C during the process
y color used to identify tests type







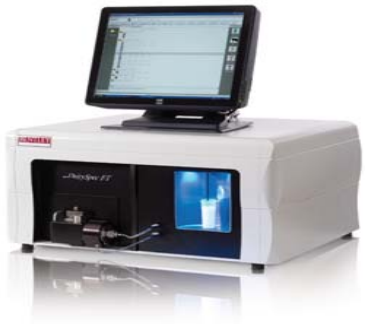
Worldwide MIR Spectra Standardization

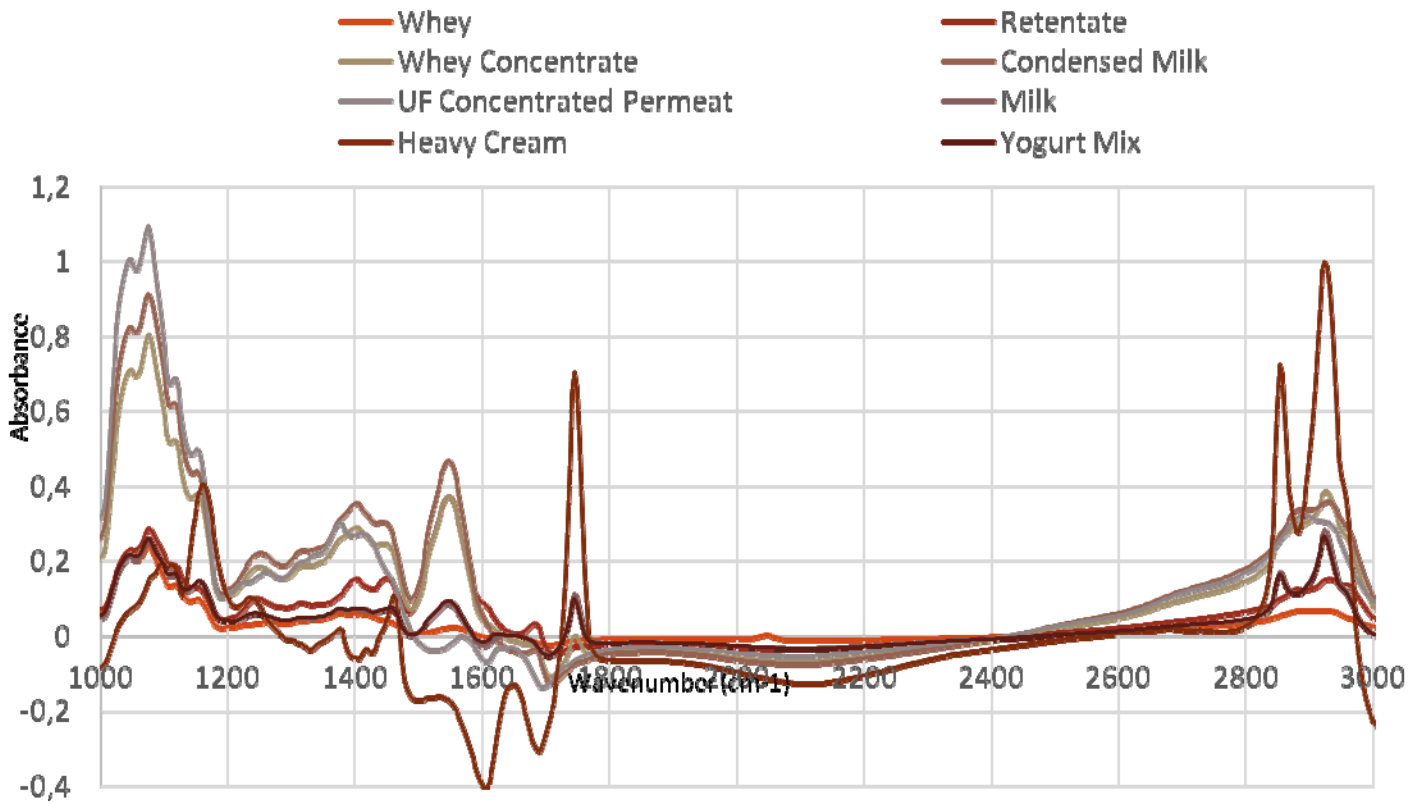
Bentley IR Cell and Spectra standardization
A new highly effective and simple approach (patent pending)

By Pierre Broutin
Managing Director/Senior Scientist

Bentley FTS / DairySpec

Milk Components Absorption wavebands





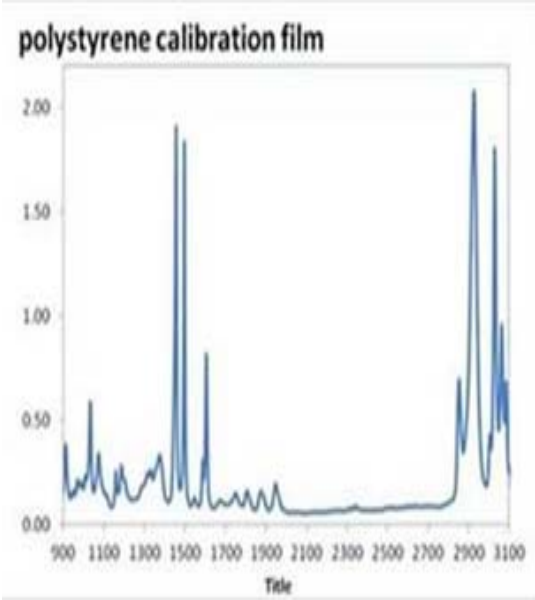
Bentley IR Cell and Spectra standardization
Why?

- Interferometer laser frequency can vary over time ➡ spectrum x axis shift
- Flow cell path length can increase over time ➡ spectrum y axis shift

Thus, spectra standardization is very important:

- For optimum calibration transfer between instruments
- For worldwide results equivalence
- For results/calibration stability (Slope/Bias)
- To reduce calibration development cost (centralized calibrations)
- For implementation of qualitative spectral analysis

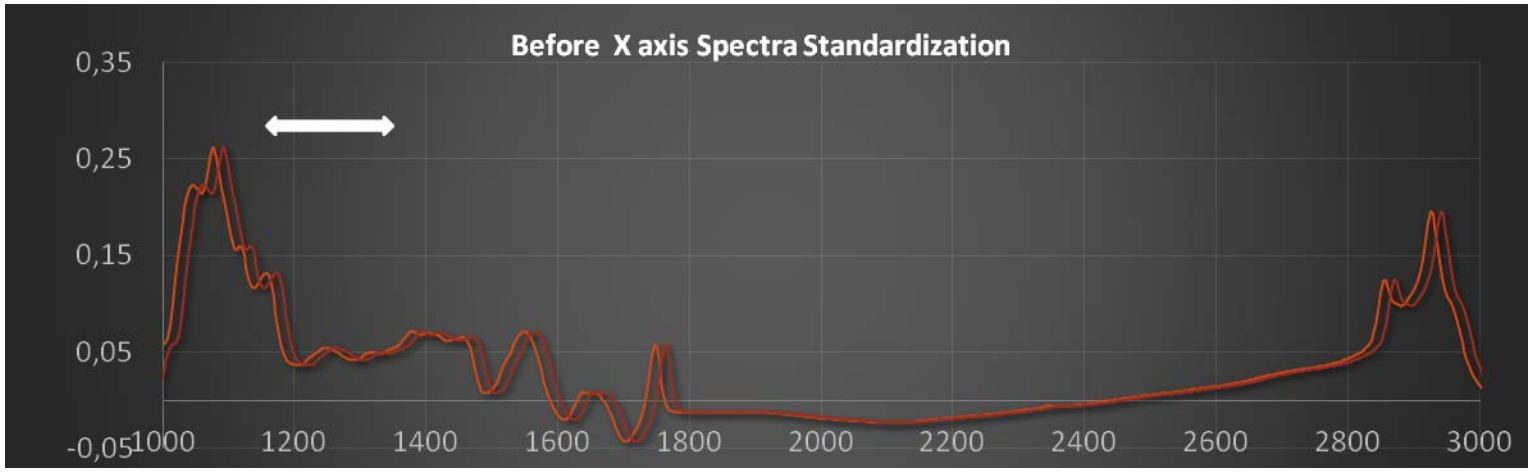
1 - Standardization of spectrum x axis with a polystyrene film to calibrate optimally interferometer laser frequency

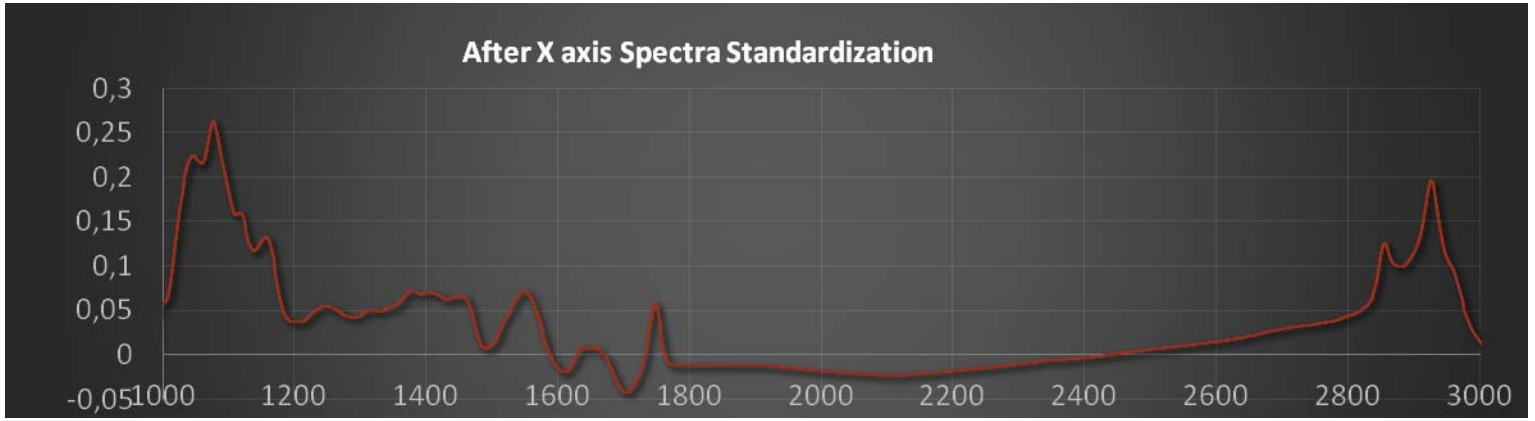


- **Example: Polystyrene Test**
- [4:30 PM Central Daylight Time] Polystyrene Test: STARTED
- Background Scan Completed
- Polystyrene Scan Completed
- **Peak 3082.22 @:3082.18 cm-1**
- **Peak 3060.14 @:3060.12 cm-1**
- **Peak 1601.38 @:1601.37 cm-1**
- **Peak 1583.04 @:1583.24 cm-1**
- **Peak 1028.42 @:1028.59 cm-1**
- [4:31 PM Central Daylight Time] Polystyrene Test: PASSED
-

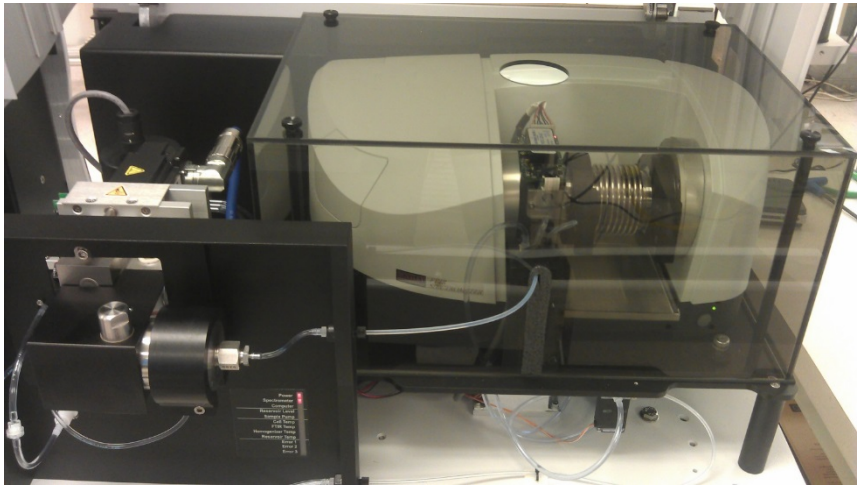
(internationally recognized NIST standard)

1 - Standardization of spectrum x axis with a polystyrene film to calibrate optimally interferometer laser frequency

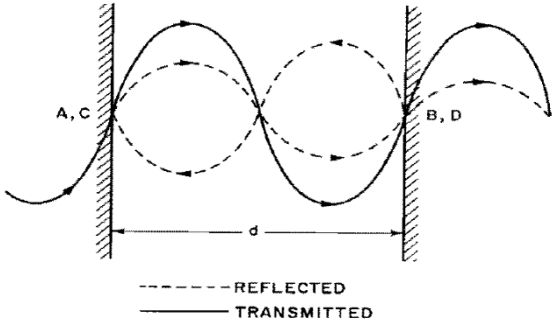


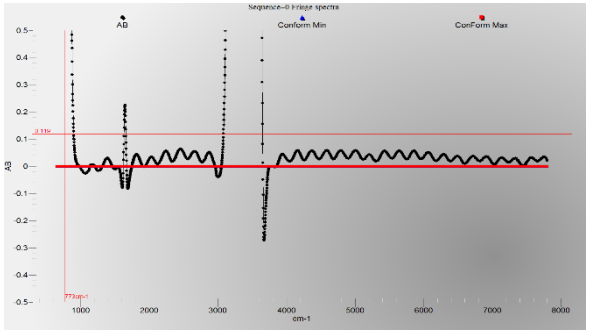


2 - Standardization of spectrum y axis (absorbance) by measuring very accurately and in real time the IR flow cell path length (patent pending)



2 - Standardization of spectrum y axis (absorbance) by measuring very accurately and in real time the IR flow cell thickness (patent pending)



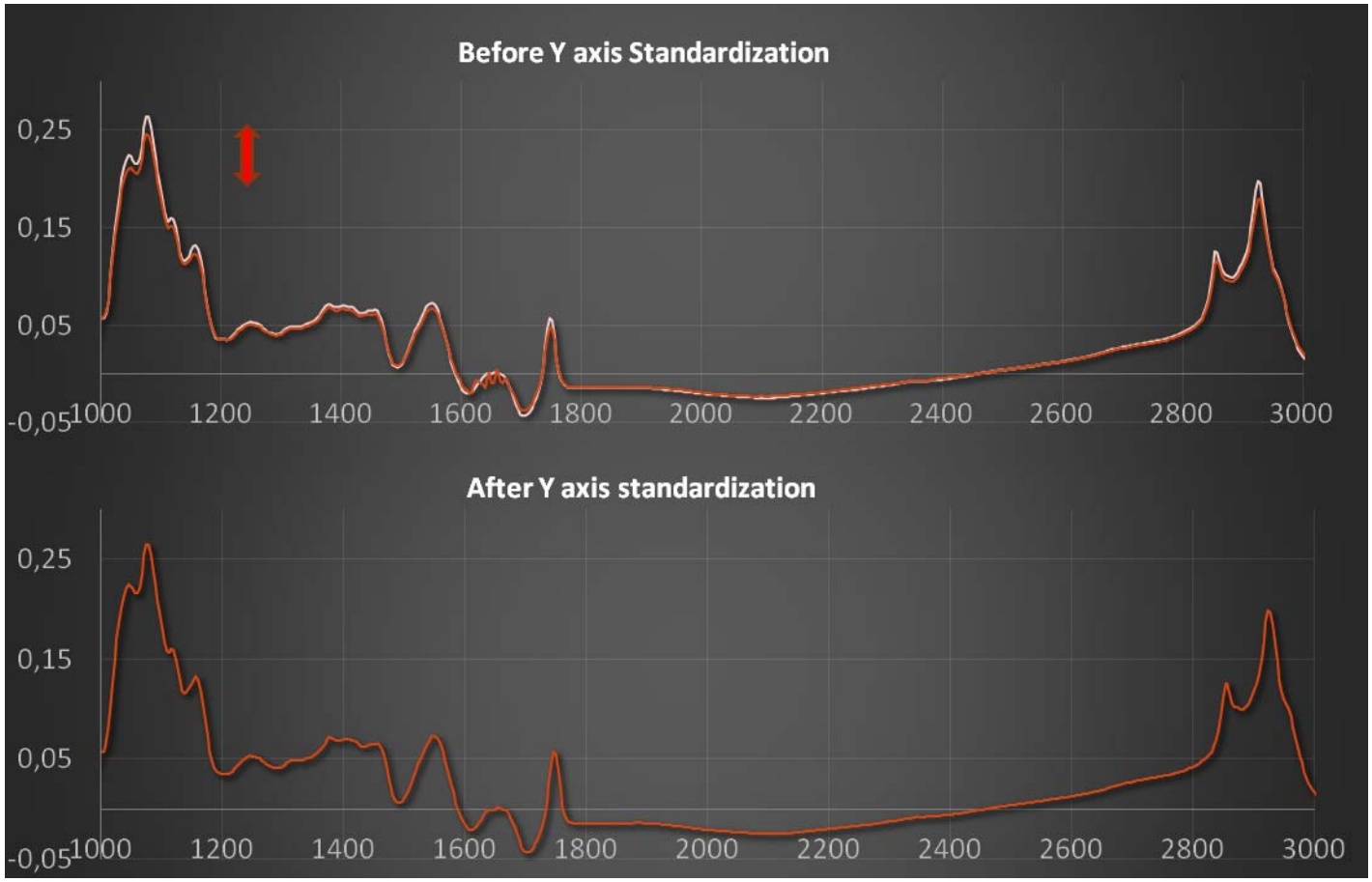


Summary Report

- Repeat 1 = 36.08468
- Repeat 2 = 36.08663
- Repeat 3 = 36.08227
- Repeat 4 = 36.08309
- Repeat 5 = 36.08712
- Repeat 6 = 36.08846
- Repeat 7 = 36.08889
- Repeat 8 = 36.09445
- Repeat 9 = 36.08202
- Repeat 10 = 36.09503

Average Cell Space calculated = 36.0873u
Standard deviation = 0.0046um

2 - Standardization of spectrum y axis (absorbance) by measuring very accurately and in real time the IR flow cell thickness (patent pending)



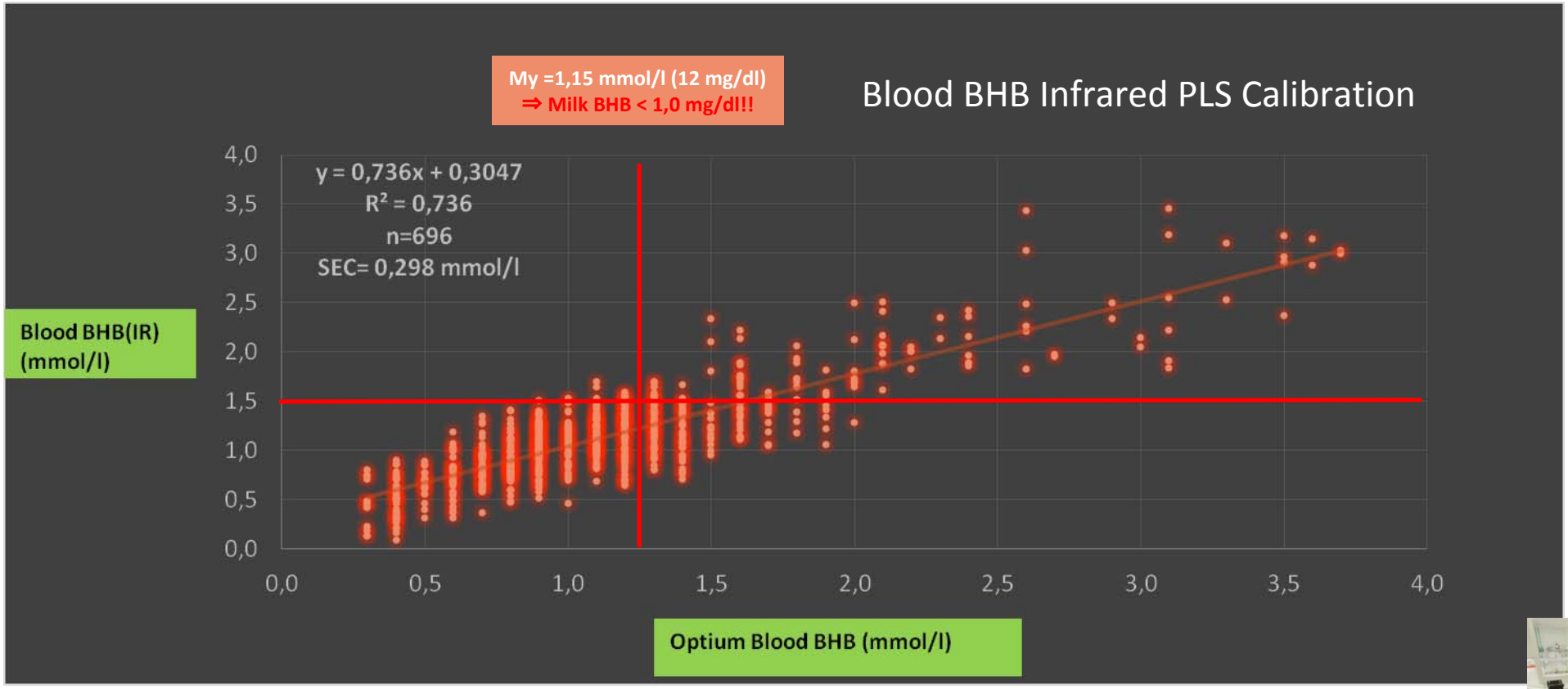
2 - Standardization of spectrum y axis (absorbance) by measuring very accurately and in real time the IR flow cell thickness (patent pending)

Why this way?

- Laser calibrated with NIST traceable films (polystyrene)
- Does not require any highly accurate liquid mixtures
- No consumable to package, store, maintain or process
- Automatic execution, no user dependent operations
- Can be performed as often as necessary
- **A very reliable, easy to implement, and cost effect effective solution**

Ketosis detection in DHIA testing

A new global metabolic infrared spectral approach to predict Cows Blood BHB from their Milk Spectra (patent pending)



Thank you for your attention!

pbroutin@bentleyinstruments.com

www.bentleyinstruments.com