Latest analytical and automation solutions for the dairy industry

A new holistic model for the multiplex analysis and optimal valorization of milk and dairy products composition

By Pierre Broutin
Managing Director/Senior Scientist
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Overview

- New biomarkers to improve herd management and milk quality
- Prerequisites to implement complex calibration models
- How to rationalize mass multiplex analyses to extract samples essential information

New biomarkers necessary to keep improving :

- Early mastitis detection (Milk Amyloid A (MAA), Somatic Cells, Lactoferrin, citrate, lactose…)
- Early detection of metabolic disorders (Blood BHB *, Blood Glucose *, Blood NEFA *, citrate, Fatty Acids Profile (FAP) …)
- Feeding optimization (FAP, urea, protein, …)
- Mitigation of milk production environmental impact (methane, nitrogen, phosphorus…)
- Milk/Dairy Products composition & quality to meet consumers expectations

New biomarkers for early mastitis diagnosis:

Limits of current markers

Total Somatic Cells	Bacteria	Somatic Cells ≠
Affected by numerous physiological factors (age, lactation period, parity, stress…)	No bacteria growth is detected between: **- 23,7% à 43,9%** of milk samples from quarters infected by clinical mastitis (false -) **- 28,7% à 38,6%** of milk samples from quarters infected with sub-clinical mastitis (false -)	- Limits of the total somatic cells count AND - No accuracy specifications for the different cells fractions - No calibrations standards available - No cells differentiation on low somatic cells samples - Not applicable to all types of milk (only cow)
Remains elevated several weeks after curing the infection and mammary gland recovery (false +)	Rapid bacteria growth and risk of potential contamination	
Somatic cells present even in the absence of infection (false +)	Risk of potential contamination	

New biomarkers for early mastitis diagnosis:

A new potent biomarker: Milk Amyloid A (MAA) advantages

Somatic Cells	Bacteria	MAA (Patented)
Affected by numerous physiological factors (age, lactation period, parity, stress…)	No bacteria growth is detected between: - **23,7% à 43,9%** of milk samples from quarters infected by clinical mastitis (false -) - **28,7% à 38,6%** of milk samples from quarters infected with sub-clinical mastitis (false -)	- **MAA: Only Acute Phase Protein produced directly by udder epithelium in response to bacterial infection in the udder** - Immediate & direct marker of infection, irrespective of the microorganism inducing the disease - Not affected by extramammary inflammatory disorders - Declines rapidly following recovery - Low or undetectable MAA level in healthy quarters - increase rapidly in infected quarters
Remains elevated several weeks after curing the infection and mammary gland recovery (false +)	Rapid bacteria growth and risk of potential contamination	
Somatic cells present even in the absence of infection (false +)	Risk of potential contamination	

New biomarkers for early mastitis diagnosis:

A new potent biomarker: Milk Amyloid A (MAA) advantages

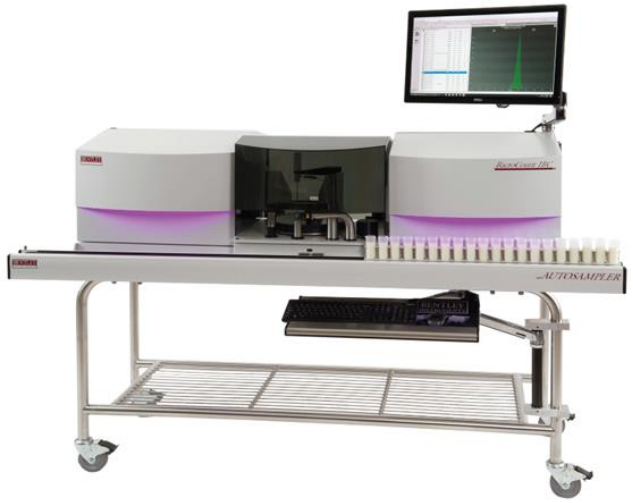
Milk Type	MAA Threshold	SCC (k) Threshold	Sensitivity	Specificity	Mastitis type	Reference
Quarter	> 16,4 mg/l		90,6%	98,3%	Subclinical	Safi et al. 2009
Quarter		> 130	89,6%	72,0%	Subclinical	Safi et al. 2009
Composite		100	96,6%	57,9%	Major pathogen	Jaeger et al. 2016
Composite	3,9µg/ml		84,7%	96,6%	Major pathogen	Jaeger et al. 2016
Composite	3,9µg/ml (2)	100 (1)	82,0%	98,0%	Major pathogen	Jaeger et al. 2016
Tank	20,78 ng/ml	<200>	97,3%	46,7%	Subclinical	Taghdiri, 2018

MAA recommended as a simple & precise marker for early mastitis detection, milk quality monitoring and reduction antibiotic use

New biomarkers for early mastitis diagnosis & milk quality monitoring:

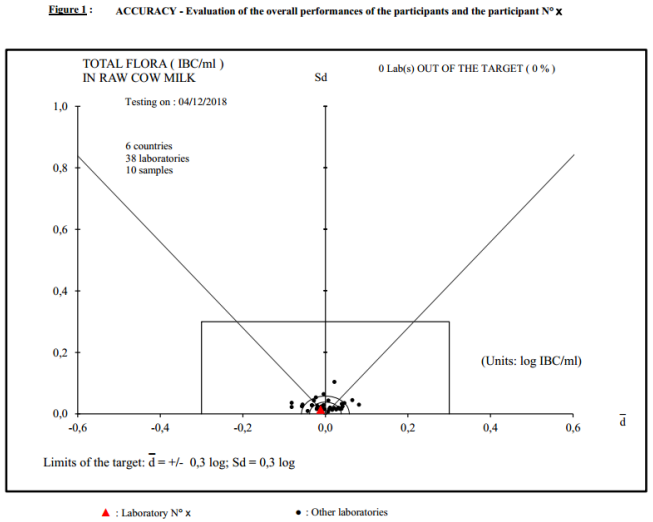
New BactoCount 3.0 Ilas 4000 Multiplex

Total Flora, Somatic Cells, Bacteria & Somatic cells ≠, +++ …



BactoCount IBC 3.0

Ilas 4000



Bentley International ISO 17043 IBC Proficiency Test

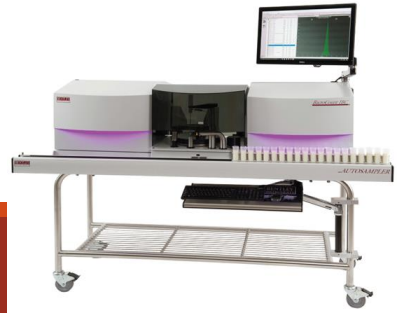
New biomarkers for early mastitis diagnosis & milk quality monitoring:

New BactoCount 3.0 Ilas 4000 Multiplex

Bacteria differentiation, why?

Raw milk bacteriological composition affects the quality, shelf life, safety of raw and processed dairy products (Barbano et al, 2006)

- Raw milk bacteriological can be affected by several factors including:
  - Milk storage temperature and time
  - Season, geography
  - Herd size, Farm practices
- High bacteriological count reflects herd's general health status
- High bacteriological count can generate significant economic losses for milk producers in terms of loss in milk production and potential penalties

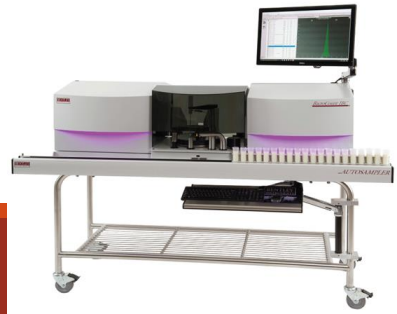


New biomarkers for early mastitis diagnosis & milk quality monitoring:

New BactoCount 3.0 Ilas 4000 Multiplex

At the farm, pathogenic bacteria identification is essential for mastitis diagnostic/treatment and animal welfare

- G+/G- bacteria *(Langerhuus et al, 2013)*
- Mastitis pathogens (*Gey et al. , 2013):*
  - *Staphylococcus aureus*
  - *Streptococcus agalactiae, Streptococcus uberis*
  - *Enterococcus faecalis, Enterococcus faecium*
  - *Escherichia coli*
  - *Trueperella pyogenes*



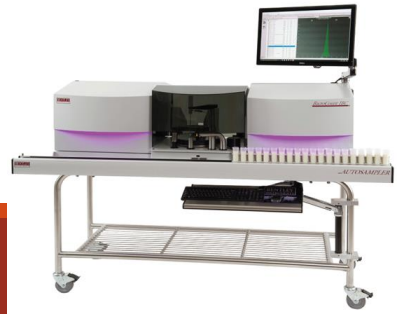
New biomarkers for early mastitis diagnosis & milk quality monitoring:

New BactoCount 3.0 Ilas 4000 Multiplex

For milk and dairy products safety and quality monitoring

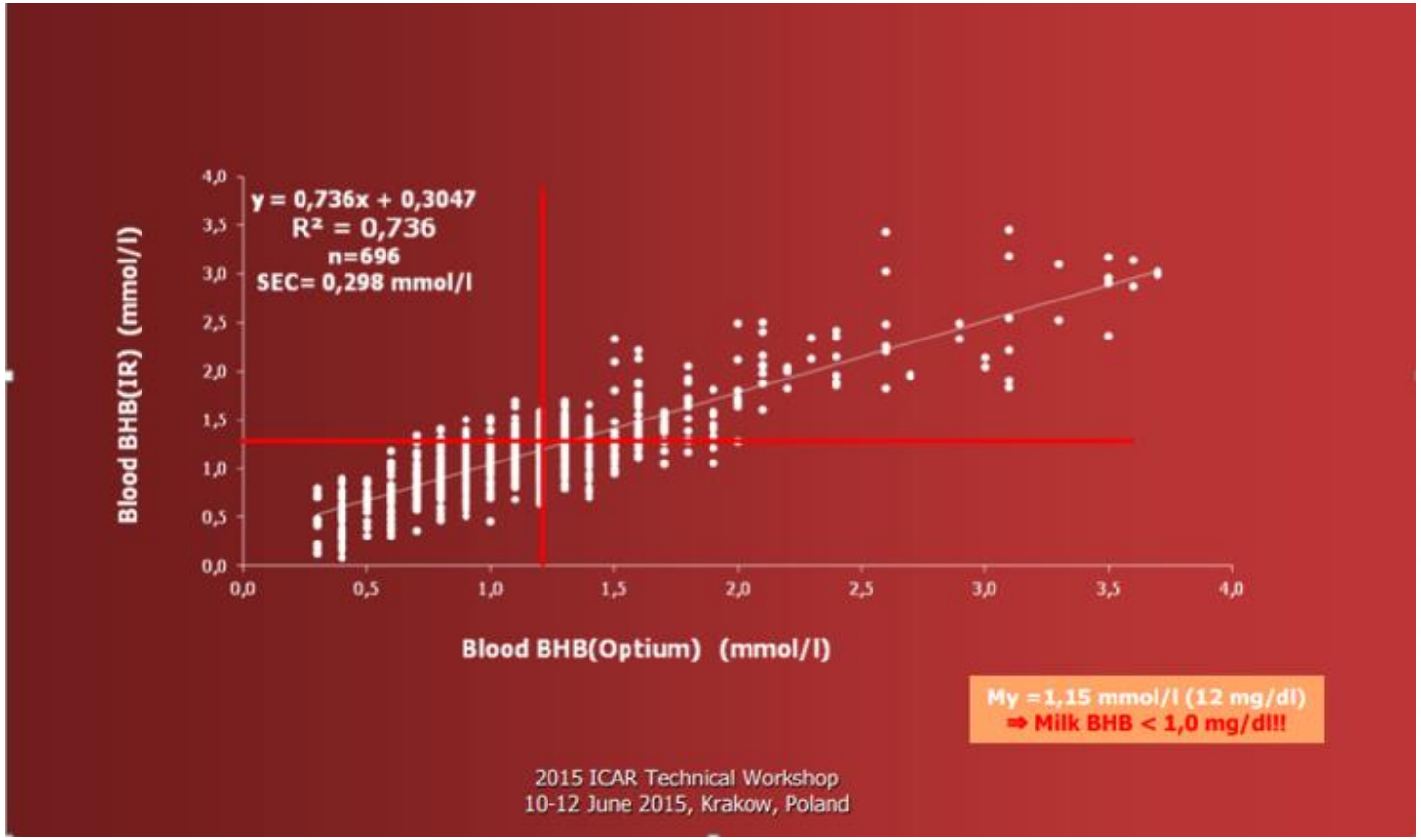
- Viable culturable, viable nonculturable, nonviable bacteria
- Thermoduric bacteria
- G- psychrotroph bacteria – prevalent bacteria in refrigerated raw milk (Ramsahoi et al., 2011)
- Lactic acid (LAB), probiotic bacteria (Bunthof et al., 2001)
- Listeria monocytogenes in raw milk (Donelly et al., 1986)
- Specific Salmonella spp. in raw and processed milk (Clark et al, 1998; McClellan et al, 1994; Pinder et al., 1994)
- Specific detection of Pseudomonas spp. (Gunasekera, 2003)

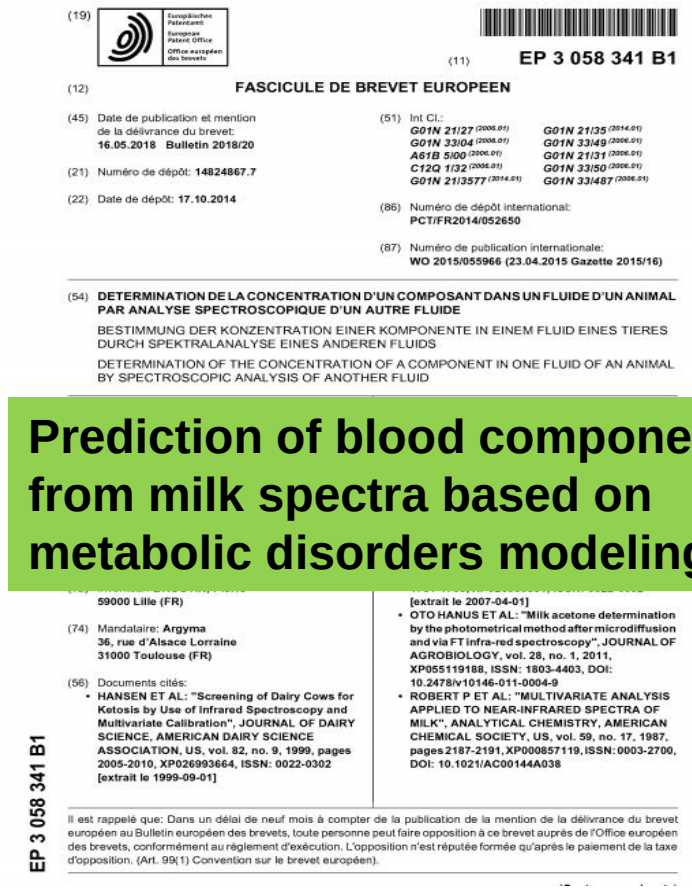
Many potential applications for the in-depth scrutiny of milk hygienic quality



New biomarkers for ketosis detection

Blood BHB*(from milk spectrum) – Patented





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New biomarkers for ketosis detection

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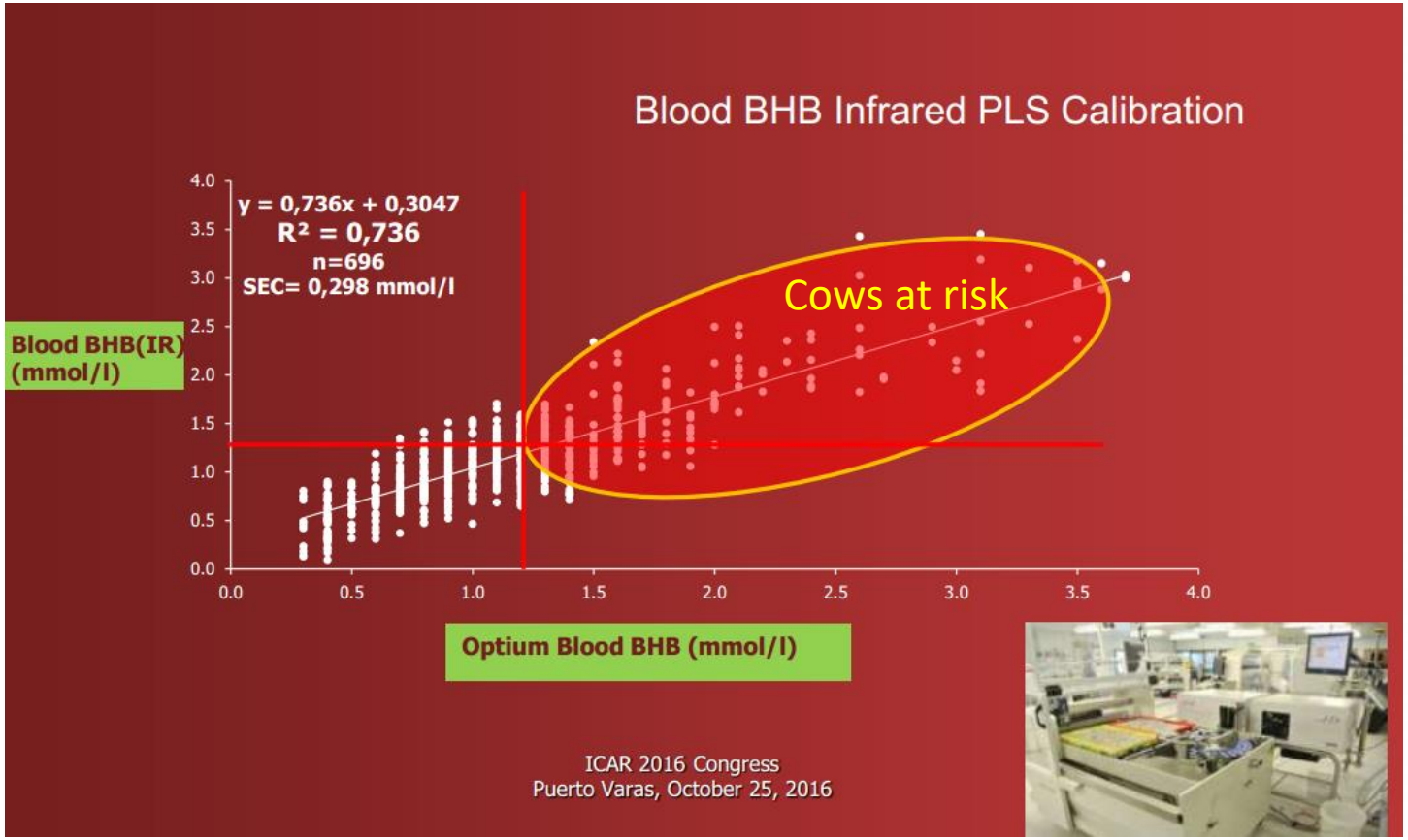

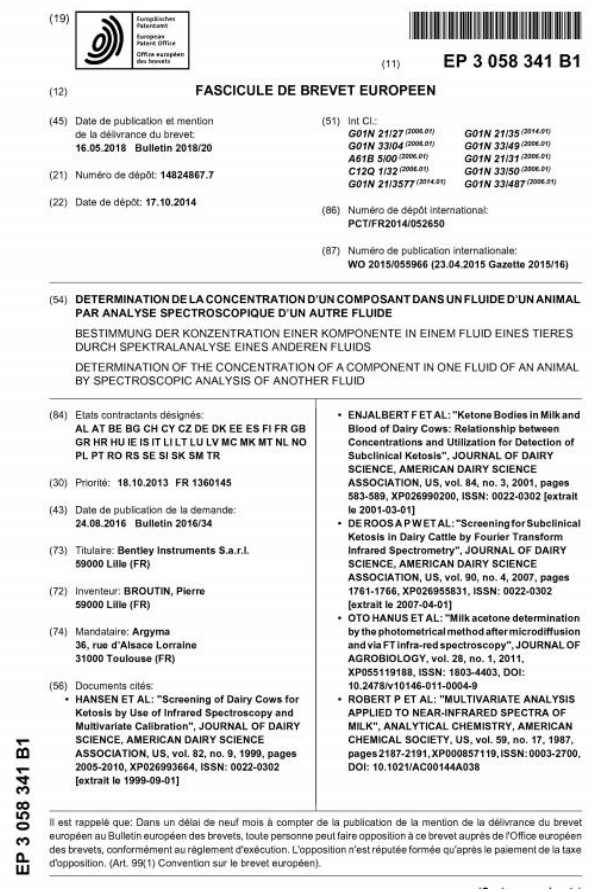

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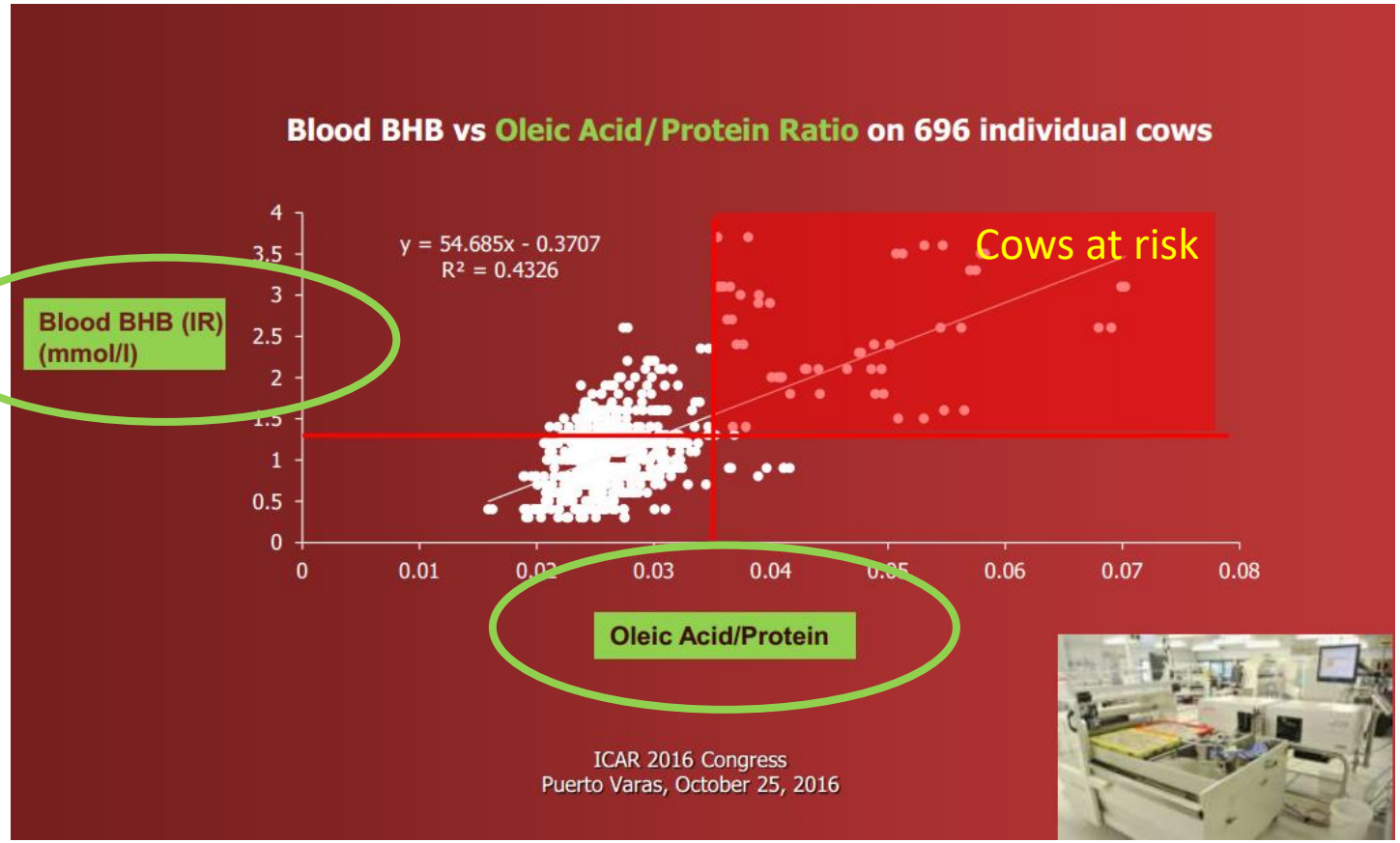

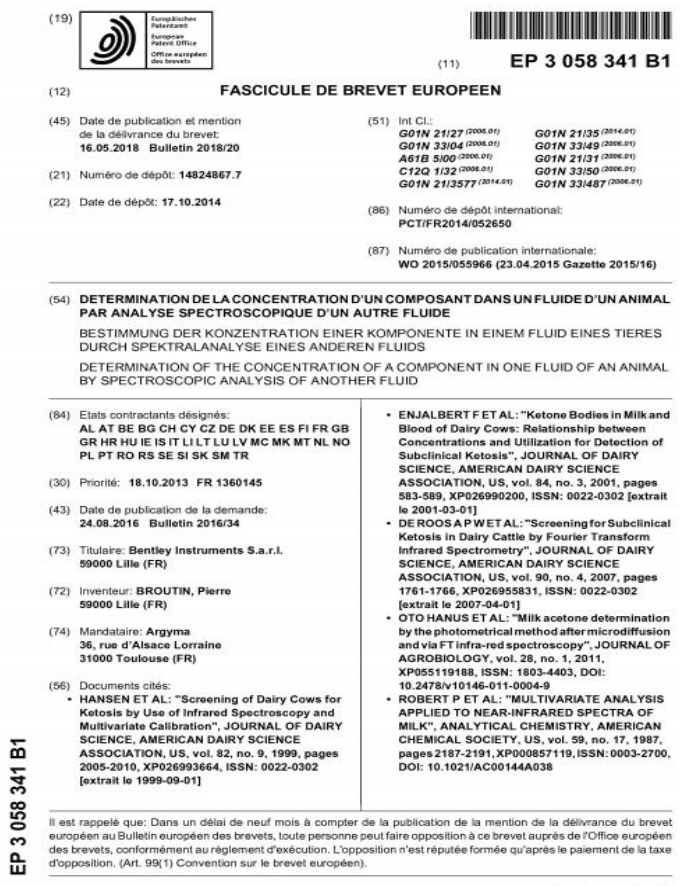

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New biomarkers for metabolic disorder detection

Other Blood components (from milk spectrum) - Patented

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- Blood Hormones

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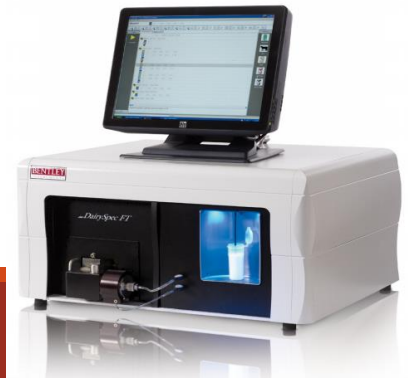


New biomarkers for ketosis detection

A new probabilistic approach to monitor individual cows status

BENTLEY INSTRUMENTS

Ketosis Detection
A New Probabilistic Approach

Ketosis Detection - Probabilistic Approach

Main Goals

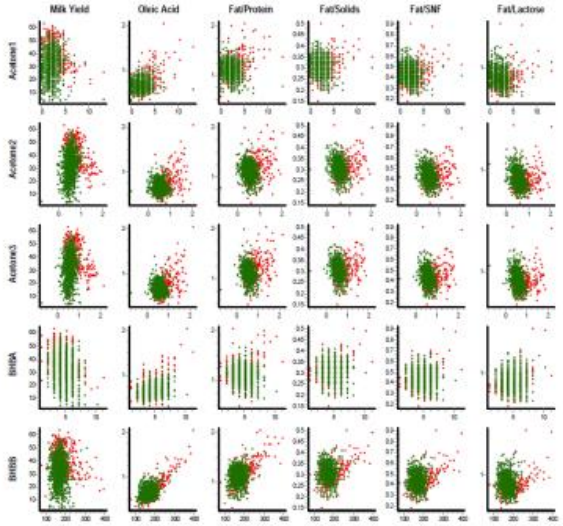
- usage of milk recording samples & automated SW solution
  - overview of herd situation in time, monthly based report, animal recording data, no additional sampling&costs, to give a new tool for farm management
- to combine different group of parameters to increase probability of detection
  - animal recording data, ketosis related results, milk components ratios
- minor milk components calibrations related to ketosis
  - Aceton, BHB, BBHB, OA, CA, sampling frequency related calibrations

Unique solution done by Bentley Instruments

BENTLEY INSTRUMENTS

Ketosis Detection
A New Probabilistic Approach

Detailed Farm Results - Correlations



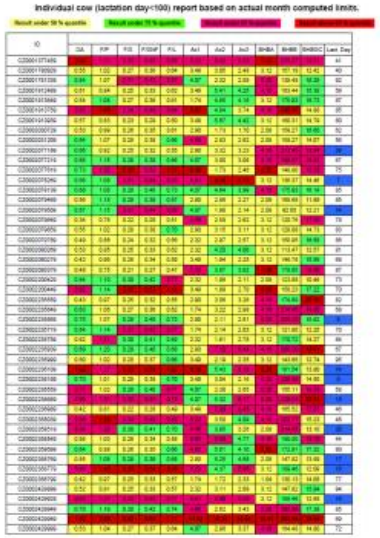
New biomarkers for ketosis detection

A new probabilistic approach to monitor individual cows status

BENTLEY INSTRUMENTS

Ketosis Detection
A New Probabilistic Approach

Farm Report - II - Individual Report



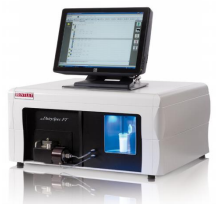
BENTLEY INSTRUMENTS

Ketosis Detection
A New Probabilistic Approach

Ketosis Detection - Farm Feedback

- 0 % of False Negative Animals Identified
- less than 1 % of False Positive Animals Indentified
- 100 % of True Positive Animals Identified

Unique solution done by Bentley Instruments



BENTLEY INSTRUMENTS

New potential biomarkers

Milk Fatty Acids profile (FAP)

- > 400 fatty acids in milk
- 26 major studied (C4:0 to C22:6)

Conventional Fatty Acids
Saturated, Unsaturated, Mono & Poly Unsaturated (PUFA/MUFA)
Palmitic (C16:0*), Stearic (C18:0*), Oleic (C18:1 9c*)

De novo (18-30%)
C4:0, C6:0, C8:0, C10:0, C12:0, C14:0*, C14:1

Mixed (35%)
C16:0*, C16:1

Preformed (30-45%)
C18:0*, C18:1, C18:2, C18:3

* main milk fatty acids

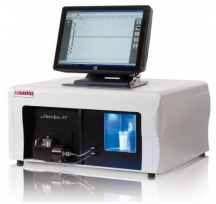
MOATE ET AL.

Table 2. The proportions of 26 individual fatty acids (mg/g) in the total milk fatty acids from milks described in 28 publications

Fatty acid[1]	n[2]	Mean ± SD	Median	Minimum	Maximum
4:0	95	31.3 ± 6.8	29.9	18.4	49.2
6:0	111	19.4 ± 5.2	19.2	6.3	32.3
8:0	111	11.7 ± 3.5	12.0	4.8	20.9
10:0	111	24.8 ± 7.3	25.0	10.3	39.4
12:0	111	29.9 ± 8.5	29.0	15.0	52.2
14:0	111	103.8 ± 17.1	101.7	63.3	135.0
14:1c9	101	10.8 ± 3.6	10.9	3.5	21.3
15:0	88	10.5 ± 3.3	10.2	4	22.6
16:0	120	285.1 ± 49.8	281.5	147.1	462.1
16:1c9	109	17.3 ± 6.3	17.3	4	36.5
C_{17}	78	7.3 ± 3.5	5.9	3.3	16.7
18:0	120	105.1 ± 35.9	99.7	30.6	268.7
18:1*t*6–8	33	4.6 ± 2.1	4.9	1.2	9.6
18:1*t*9	37	4.4 ± 2.0	4.4	1.4	11.4
18:1*t*10	30	13.1 ± 15.2	8.1	0.3	64.7
18:1*t*11	90	33.3 ± 21.8	32.6	5.8	99.5
18:1*t*12	19	6.5 ± 3.6	6.3	0.9	12.7
18:1c9	120	205.0 ± 53.5	199.6	70.3	371.4
18:2c9,*c*12	120	31.3 ± 21.1	26.6	5.1	133.0
18:2c9,*t*11	76	10.2 ± 6.0	8.4	2.8	24.5
18:2*t*10,*c*12	35	0.4 ± 0.3	.3	0	1.4
18:2c11,*t*13	6	0.4 ± 0.3	0.3	0.2	0.9
OCLA	25	1.5 ± 1.4	1.1	0	4.2
18:3	114	5.9 ± 3.6	4.9	0.2	19.0
20:0	30	1.5 ± 0.6	1.5	0.4	3.0
20:5	39	1.0 ± 1.1	0.5	0	4.8
22:6	31	0.7 ± 0.7	0.4	0	2.6
Others	120	75.1 ± 56.2	76.4	0	237.3
Total CLA	82	10.3 ± 6.6	8.3	3	28.4
Total 18:1 *trans*	94	42.5 ± 26.3	39.5	8.6	145.1
Total de novo	120	232.6 ± 42.4	237.9	136.6	300.6
Total C_{16}	120	300.9 ± 52.7	303.0	154.2	462.1
Total preformed	120	466.5 ± 75.8	477.4	315.6	641.3

[1]c = *cis*; *t* = *trans*; OCLA = other conjugated linoleic acids; CLA = conjugated linoleic acid; total 18:1 *trans* = sum of 18:1*t*6–8, 18:1*t*9, 18:1*t*10, 18:1*t*11, and 18:1*t*12 isomers; total de novo = sum of 4:0 to 15:0 fatty acids; total C_{16} = sum of 16:0 and 16:1; total preformed = sum of all milk fatty acids with more than 17 carbon atoms.

[2]n = total number of dietary treatments contributing to each mean.



BENTLEY INSTRUMENTS

New potential biomarkers

Fatty Acids Biomarkers & Properties

De novo FA	**C4 to C14:00**	**Can be used to monitor cows' energy status and optimize the feeding to increase De novo FA concentration. Higher de novo concentration has been associated with higher fat and protein content in the bulk tank**	**De novo are saturated FA, Increasing De novo concentration will increase milk saturated FA concentration. SFA have been associated with potential detrimental health effects. Individual SFA contribution to human health needs to be considered. All saturated fatty acids from C8 to C16, especially C14, main de novo FA, raise the serum LDL cholesterol concentration when they are consumed in the diet**
Oleic FA	C18:1 c9	Can be used as a marker of ketosis. Elevated proportions of C18:1 c9 are visible in milk fat 2 wk before SCK diagnosis	**linked with a reduction in the risk of coronary heart disease (CHD)**
Linolenic Acid (EFA)		Bioactive fatty acids could be used to enhance feeding and concentration of this bioactive fatty acids in the milk	**typically present in low percentages in milk (< 5%) but exert a significant positive impact on human health**
Linoleic Acid (EFA)			
CLA			
Vaccenic Acid (VA)	C18:1 t11		
Rumenic Acid (RA)	C18:2, c9, t11		
Branched-Chain Fatty Acids (BCFA)	Iso-13:0 to iso-18:0 and anteiso-13:0 to anteiso 17:0)	Potential marker for rumen function – positively related to rumen concentration of acetate.	**BCFA also possess anti carcinogenic properties**

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Milk Fatty Acids. I. Variation in the Concentration of Individual Fatty Acids in Bovine Milk

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†School of Veterinary Science, University of Sydney, New South Wales 2006, Australia

Table 4. Pairwise correlation coefficients between concentrations (g/kg of milk) of individual milk fatty acids or groupings of fatty acids

Fatty acid[1]	4:0	6:0	8:0	10:0	12:0	14:0	14:1	15:0	16:0	16:1	C_{17}
4:0	1										
6:0	0.80*	1									
8:0	0.67*	0.95*	1								
10:0	0.56*	0.87*	0.93*	1							
12:0	0.49*	0.85*	0.91*	0.97*	1						
14:0	0.56*	0.86*	0.89*	0.93*	0.95*	1					
14:1*c*9	0.28	0.55*	0.54*	0.63*	0.65*	0.68*	1				
15:0	0.75*	0.71*	0.64*	0.68*	0.64*	0.70*	0.39	1			
16:0	0.58*	0.74*	0.72*	0.70*	0.76*	0.85*	0.59*	0.58*	1		
16:1*c*9	−0.04	0.31	0.36	0.38*	0.45*	0.45*	0.63*	0.16	0.58*	1	
C_{17}	0.60*	0.51*	0.50*	0.56*	0.46*	0.61*	0.26	0.71*	0.51*	0.13	1
18:0	0.56*	0.55*	0.46*	0.32	0.25	0.36	−0.05	0.58*	0.33	−0.29	0.51*
18:1*t*6–8	−0.59	−0.59	−0.62	−0.65	−0.69*	−0.59	−0.61	−0.88*	−0.54*	−0.59*	−0.67*
18:1*t*9	−0.30	−0.51	−0.68*	−0.71*	−0.75*	−0.53	−0.29	−0.74*	−0.27	−0.29	−0.47
18:1*t*10	−0.43	−0.43	−0.37	−0.28	−0.24	−0.31	−0.13	−0.48	−0.35	0.00	−0.45
18:1*t*11	−0.22	−0.30	−0.26	−0.22	−0.27	−0.17	−0.43	−0.01	−0.16	−0.13	0.71*
18:1*t*12	−0.22	−0.39	−0.59	−0.63	−0.67	0.10	0.37	−0.41	0.48	0.10	−0.37
18:1*c*9	0.31	0.30	0.26	0.07	0.06	0.11	0.04	−0.02	0.26	0.01	−0.03
18:2*c*9,*c*12	−0.04	−0.22	−0.16	−0.13	−0.14	−0.24	−0.17	−0.46	−0.25	−0.24	−0.41
18:2*c*9,*t*11	−0.24	−0.35	−0.36	−0.52*	−0.54*	−0.49*	−0.32	−0.44	−0.44*	−0.34	−0.32
18:2*t*10,*c*12	−0.56	−0.69*	−0.69*	−0.68*	−0.66*	−0.58	−0.56	−0.48	−0.26	−0.46	−0.60
OCLA	−0.68	0.06	−0.02	−0.09	−0.18	−0.17	−0.29	−0.65	−0.46	−0.53	−0.44
18:3	0.25	0.25	0.24	0.18	0.12	0.11	−0.18	−0.06	0.06	−0.28	−0.04
20:0	0.57	0.49	0.45	0.45	0.45	0.55	0.12	0.49	0.50	−0.09	0.59
20:5	−0.48	−0.36	−0.41	−0.41	−0.40	−0.41	−0.03	−0.42	−0.41	−0.02	−0.44
22:6	−0.49	−0.37	−0.47	−0.53	−0.54	−0.50	−0.19	−0.60	−0.41	−0.16	−0.41
Total 18:1 *trans*	−0.15	−0.14	−0.18	−0.15	−0.19	−0.06	−0.25	0.21	−0.16	−0.28	0.37
Total de novo	0.65*	0.89*	0.90*	0.95*	0.95*	0.97*	0.69*	0.75*	0.79*	0.33	0.66*
Total C_{16}	0.55*	0.74*	0.72*	0.71*	0.76*	0.85*	0.60*	0.57*	0.99*	0.62*	0.50*
Total preformed											
Total milk fat	0.72*	0.85*	0.83*	0.78*	0.77*	0.86*	0.51*	0.69*	0.85*		

[1]*c* = *cis*; *t* = *trans*; OCLA = other conjugated linoleic acids; total 18:1 *trans* = sum of 18:1*t*6–8, 18:1*t*9, 18:1*t*10, 18:1*t*11, and 18:1*t*12 isomers; total de novo = sum of 4:0 to 15:0 fatty acids; total C_{16} = sum of 16:0 and 16:1; total preformed = sum of all milk fatty acids with more than 17 carbon atoms.
**P* < 0.05 (multiple correlations with Bonferroni adjustment).

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Quantification of individual fatty acids in bovine milk by infrared spectroscopy and chemometrics: Understanding predictions of highly collinear reference variables

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FA don't have specific IR absorption signal on milk spectrum

High covariation between Fatty Acids and Total Fat

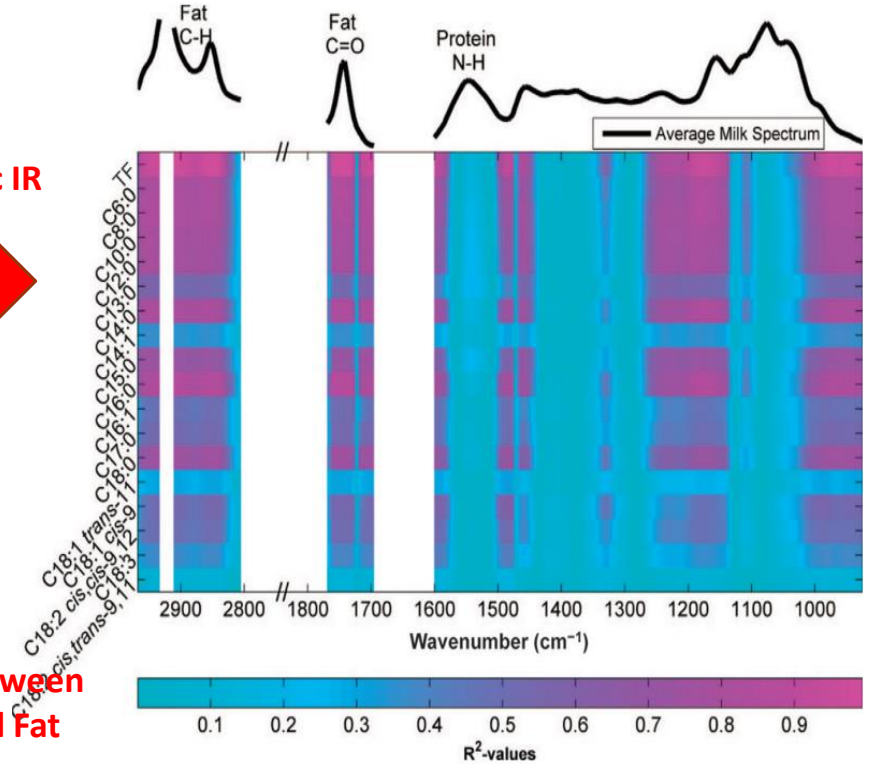

Figure 8. Coefficient of determination (R^2) between measured fatty acids (g/100 g of milk) and raw Fourier transform infrared measurements. TF = total fat content. Color version available in the online PDF.

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Milk Fatty Acids. I. Variation in the Concentration of Individual Fatty Acids in Bovine Milk

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†School of Veterinary Science, University of Sydney, New South Wales 2006, Australia

Table 4. Pairwise correlation coefficients between concentrations (g/kg of milk) of individual milk fatty acids or groupings of fatty acids

Fatty acid[1]	4:0	6:0	8:0	10:0	12:0	14:0	14:1	15:0	16:0	16:1	C_{17}
4:0	1										
6:0	0.80*	1									
8:0	0.67*	0.95*	1								
10:0	0.56*	0.87*	0.93*	1							
12:0	0.49*	0.85*	0.91*	0.97*	1						
14:0	0.56*	0.86*	0.89*	0.93*	0.95*	1					
14:1*c*9	0.28	0.55*	0.54*	0.63*	0.65*	0.68*	1				
15:0	0.75*	0.71*	0.64*	0.68*	0.64*	0.70*	0.39	1			
16:0	0.58*	0.74*	0.72*	0.70*	0.76*	0.85*	0.59*	0.58*	1		
16:1*c*9	−0.04	0.31	0.36	0.38*	0.45*	0.45*	0.63*	0.16	0.58*	1	
C_{17}	0.60*	0.51*	0.50*	0.56*	0.46*	0.61*	0.26	0.71*	0.51*	0.13	1
18:0	0.56*	0.55*	0.46*	0.32	0.25	0.36	−0.05	0.58*	0.33	−0.29	0.51*
18:1*t*6–8	−0.59	−0.59	−0.62	−0.65	−0.69*	−0.59	−0.61	−0.88*	−0.54*	−0.59*	−0.67*
18:1*t*9	−0.30	−0.51	−0.68*	−0.71*	−0.75*	−0.53	−0.29	−0.74*	−0.27	−0.29	−0.47
18:1*t*10	−0.43	−0.43	−0.37	−0.28	−0.24	−0.31	−0.13	−0.48	−0.35	0.00	−0.45
18:1*t*11	−0.22	−0.30	−0.26	−0.22	−0.27	−0.17	−0.43	−0.01	−0.16	−0.13	0.71*
18:1*t*12	−0.22	−0.39	−0.59	−0.63	−0.67	0.10	0.37	−0.41	0.48	0.10	−0.37
18:1*c*9	0.31	0.30	0.26	0.07	0.06	0.11	0.04	−0.02	0.26	0.01	−0.03
18:2*c*9,*c*12	−0.04	−0.22	−0.16	−0.13	−0.14	−0.24	−0.17	−0.46	−0.25	−0.24	−0.41
18:2*c*9,*t*11	−0.24	−0.35	−0.36	−0.52*	−0.54*	−0.49*	−0.32	−0.44	−0.44*	−0.34	−0.32
18:2*t*10,*c*12	−0.56	−0.69*	−0.69*	−0.68*	−0.66*	−0.58	−0.56	−0.48	−0.26	−0.46	−0.60
OCLA	−0.68	0.06	−0.02	−0.09	−0.18	−0.17	−0.29	−0.65	−0.46	−0.53	−0.44
18:3	0.25	0.25	0.24	0.18	0.12	0.11	−0.18	−0.06	0.06	−0.28	−0.04
20:0	0.57	0.49	0.45	0.45	0.45	0.55	0.12	0.49	0.50	−0.09	0.59
20:5	−0.48	−0.36	−0.41	−0.41	−0.40	−0.41	−0.03	−0.42	−0.41	−0.02	−0.44
22:6	−0.49	−0.37	−0.47	−0.53	−0.54	−0.50	−0.19	−0.60	−0.41	−0.16	−0.41
Total 18:1 *trans*	0.15	0.14	0.18	0.15	0.19	0.06	0.25	0.21	0.16	−0.28	0.37
Total de novo	0.65*	0.89*	0.90*	0.95*	0.95*	0.97*	0.69*	0.75*	0.79*	0.3	
Total C_{16}	0.55*	0.74*	0.72*	0.71*	0.76*	0.85*	0.60*	0.57*	0.99*	0.62*	0.56*
Total preformed	0.43*	0.29	0.26	0.09	0.01	0.10	−0.20	0.12	0.10	−0.35	0.19
Total milk fat	0.72*	0.85*	0.83*	0.78*	0.77*	0.86*	0.51*	0.69*	0.85*	0.27	0.60*

[1]*c* = *cis*; *t* = *trans*; OCLA = other conjugated linoleic acids; total 18:1 *trans* = sum of 18:1*t*6–8, 18:1*t*9, 18:1*t*10, 18:1*t*11, and 18:1*t*12 isomers; total de novo = sum of 4:0 to 15:0 fatty acids; total C_{16} = sum of 16:0 and 16:1; total preformed = sum of all milk fatty acids with more than 17 carbon atoms.
*$P < 0.05$ (multiple correlations with Bonferroni adjustment).

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Quantification of individual fatty acids in bovine milk by infrared spectroscopy and chemometrics: Understanding predictions of highly collinear reference variables

C. E. Eskildsen,*[1] M. A. Rasmussen,* S. B. Engelsen,* L. B. Larsen,† N. A. Poulsen,† and T. Skov*
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†Department of Food Science, Aarhus University, DK-8830 Tjele, Denmark

FA don't have specific IR absorption signal on milk spectrum

High covariation between De novo Fatty Acids

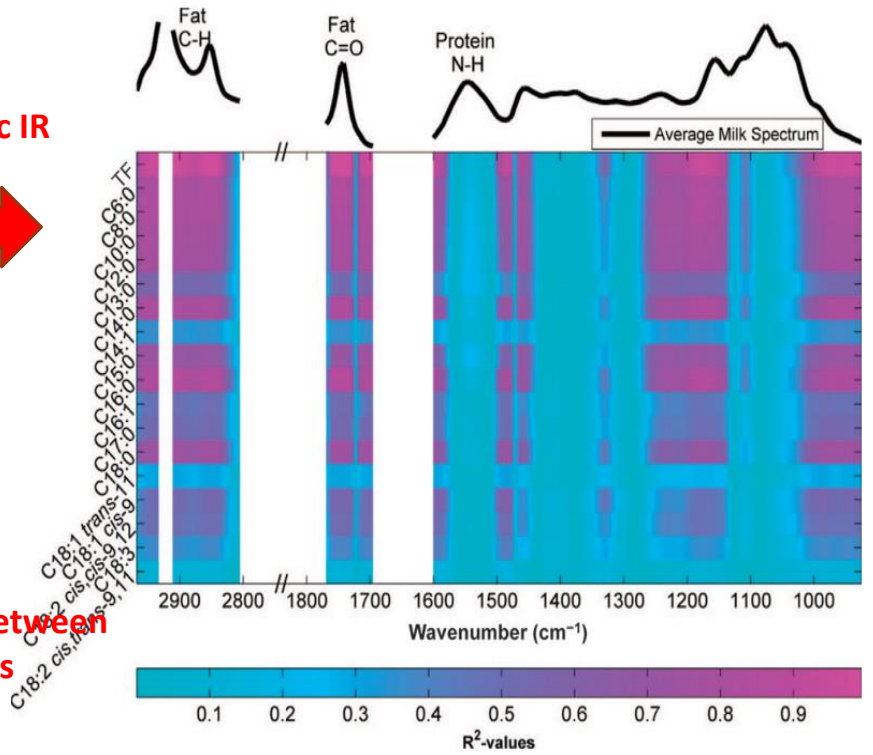

Figure 8. Coefficient of determination (R^2) between measured fatty acids (g/100 g of milk) and raw Fourier transform infrared measurements. TF = total fat content. Color version available in the online PDF.

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Table 4. Pairwise correlation coefficients between concentrations (g/kg of milk) of individual milk fatty acids or groupings of fatty acids

Fatty acid[1]	4:0	6:0	8:0	10:0	12:0	14:0	14:1	15:0	16:0	16:1	C_{17}
4:0	1										
6:0	0.80*	1									
8:0	0.67*	0.95*	1								
10:0	0.56*	0.87*	0.93*	1							
12:0	0.49*	0.85*	0.91*	0.97*	1						
14:0	0.56*	0.86*	0.89*	0.93*	0.95*	1					
14:1*c*9	0.28	0.55*	0.54*	0.63*	0.65*	0.68*	1				
15:0	0.75*	0.71*	0.64*	0.68*	0.64*	0.70*	0.39	1			
16:0	0.58*	0.74*	0.72*	0.70*	0.76*	0.85*	0.59*	0.58*	1		
16:1*c*9	−0.04	0.31	0.36	0.38*	0.45*	0.45*	0.63*	0.16	0.58*	1	
C_{17}	0.60*	0.51*	0.50*	0.56*	0.46*	0.61*	0.26	0.71*	0.51*	0.13	1
18:0	0.56*	0.55*	0.46*	0.32	0.25	0.36	0.05	0.58*	0.33	−0.29	0.51*
18:1*t*6–8	−0.59	−0.59	−0.62	−0.65	−0.69*	−0.59	−0.61	−0.88*	−0.54*	−0.59*	−0.67*
18:1*t*9	−0.30	−0.51	−0.68*	−0.71*	−0.75*	−0.53	−0.29	−0.74*	−0.27	−0.29	−0.47
18:1*t*10	−0.43	−0.43	−0.37	−0.28	−0.24	−0.31	−0.13	−0.48	−0.35	0.00	−0.45
18:1*t*11	−0.22	−0.30	−0.26	−0.22	−0.27	−0.17	−0.43	−0.01	−0.16	−0.13	0.71*
18:1*t*12	−0.22	−0.39	−0.59	−0.63	−0.67	0.10	−0.37	−0.41	0.48	0.10	−0.37
18:1*c*9	0.31	0.30	0.26	0.07	0.06	0.11	0.04	−0.02	0.26	0.01	−0.03
18:2*c*9,*c*12	−0.04	−0.22	−0.16	−0.13	−0.14	−0.24	−0.17	−0.46	−0.25	−0.24	−0.41
18:2*c*9,*t*11	−0.24	−0.35	−0.36	−0.52*	−0.54*	−0.49*	−0.32	−0.44	−0.44*	−0.34	−0.32
18:2*t*10,*c*12	−0.56	−0.69*	−0.69*	−0.68*	−0.66*	−0.58	−0.56	−0.48	−0.26	−0.46	−0.60
OCLA	−0.68	0.06	−0.02	−0.09	−0.18	−0.17	−0.29	−0.65	−0.46	−0.53	−0.44
18:3	0.25	0.25	0.24	0.18	0.12	0.11	−0.18	−0.06	0.06	−0.28	−0.04
20:0	0.57	0.49	0.45	0.45	0.45	0.55	0.12	0.49	0.50	−0.09	0.59
20:5	−0.48	−0.36	−0.41	−0.41	−0.40	−0.41	−0.03	−0.42	−0.41	−0.02	−0.44
22:6	−0.49	−0.37	−0.47	−0.53	−0.54	−0.50	−0.19	−0.60	−0.41	−0.16	−0.41
Total 18:1 *trans*	−0.15	−0.14	−0.18	−0.15	−0.19	−0.06	−0.25	0.21	−0.16	−0.28	0.37
Total de novo	0.65*	0.89*	0.90*	0.95*	0.95*	0.97*	0.69*	0.75*	0.79*	0.33	0.66*
Total C_{16}	0.55*	0.74*	0.72*	0.71*	0.76*	0.85*	0.60*	0.57*	0.99*	0.62*	0.50*
Total preformed	0.43*	0.29	0.26	0.09	0.01	0.10	−0.20	0.12	0.10	−0.35	0.19
Total milk fat	0.72*	0.85*	0.83*	0.78*	0.77*	0.86*	0.51*	0.69*	0.85*	0.27	0.60*

[1]*c* = *cis*; *t* = *trans*; OCLA = other conjugated linoleic acids; total 18:1 *trans* = sum of 18:1*t*6–8, 18:1*t*9, 18:1*t*10, 18:1*t*11, and 18:1*t*12 isomers; total de novo = sum of 4:0 to 15:0 fatty acids; total C_{16} = sum of 16:0 and 16:1; total preformed = sum of all milk fatty acids with more than 17 carbon atoms.
*$P < 0.05$ (multiple correlations with Bonferroni adjustment).

High De Novo covariation with other FAs and Total Fat

J. Dairy Sci. 97:7940–7951
http://dx.doi.org/10.3168/jds.2014-8337

Quantification of individual fatty acids in bovine milk by infrared spectroscopy and chemometrics: Understanding predictions of highly collinear reference variables

C. E. Eskildsen,*[1] M. A. Rasmussen,* S. B. Engelsen,* L. B. Larsen,† N. A. Poulsen,† and T. Skov*
*Department of Food Science, University of Copenhagen, DK-1958 Frederiksberg, Denmark
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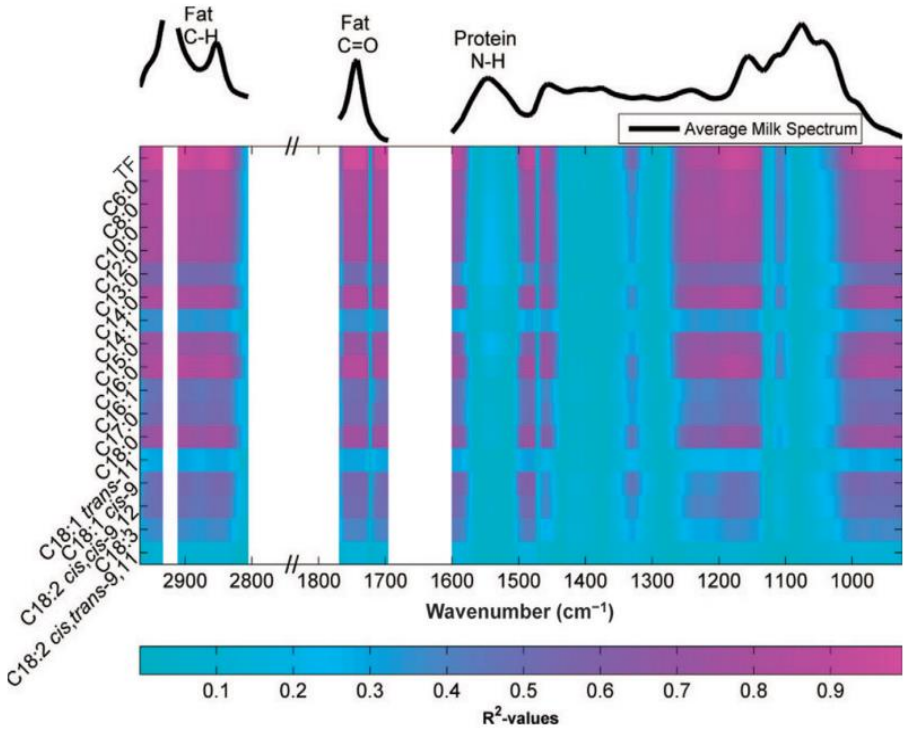
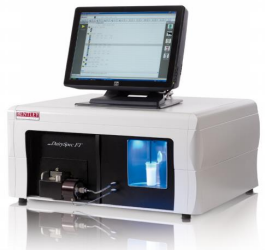


Figure 8. Coefficient of determination (R^2) between measured fatty acids (g/100 g of milk) and raw Fourier transform infrared measurements. TF = total fat content. Color version available in the online PDF.

Bentley CombiFTS/DairySpec Calibrations

Up to 64 components predicted simultaneously

Standard Calibrations	
Fat	x
Protein	x
Lactose	x
Urea	
BHB	x
Acetone	x
Citric Acid	x
FFA	x
Somatic cells	x

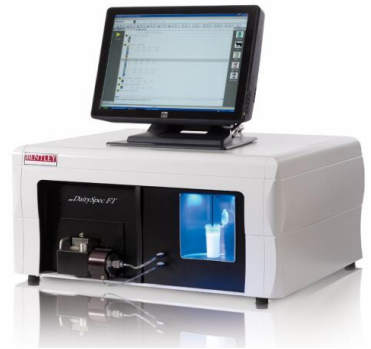


FA Calibrations	
C4:0	x
C6:0	x
C8:0	x
C10:0	x
C12:0	x
C14:0	x
C15:0	UD
C16:0	x
C16:1	x
C17:0	UD
C18:0	x
C18:1	x
C18:2	x
C18:3	x

FA Calibrations	
De Novo	x
Mixed	x
Preformed	x
Saturated Fatty Acids (SFA)	x
Unsaturated Fatty Acids (UFA)	x
Mono Unsaturated Fatty Acids (MUFA)	x
Poly Unsaturated Fatty Acids (PUFA)	x
Trans Fatty Acids (TFA)	UD
Lactoferrin	x
X: Available; UD: Under Development	

Bentley CombiFTS/DairySpec Calibrations

Up to 64 components predicted simultaneously



SCFA	C 18:1 12t
MCFA	C 18:1 13c
LCFA	C 18:1 15c
Isoanteiso	C 18:1 16t
Omega 6	C 18:1 6t+8t
Total 18 trans	C 18:2 9t12c
C 11:0	C 18:3 n-6
C 13:0	C 20:0
C 14:0 iso	C 20:1 11c
C 14:1 9c	C 20:1 9c
C 15:0 anteiso	C 20:2 n-6
C 15:0 iso	C 20:4 n-6
C 16:0 iso	C 21:0
C 17:0 anteiso	C 23:0
C 17:0 iso	C 24:0
C 17:1 10c	**Sodium**
C 18:0 iso	**Calcium**
C 18:1 11c	**Phosphorus**
C 18:1 11t+10t	**Magnesium**
C 18:1 12c	**Potassium**

Other potential parameters

Prerequisites to optimize new models implementation & precision

New FT-MIR models are complex, require access to a lot of data & expertise, and are costly to develop

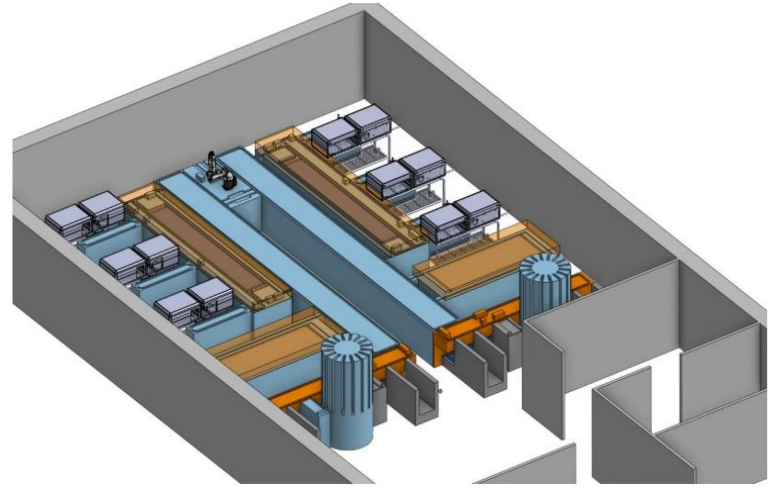
Thus, methods need to be optimally standardized to capitalize on models potential:

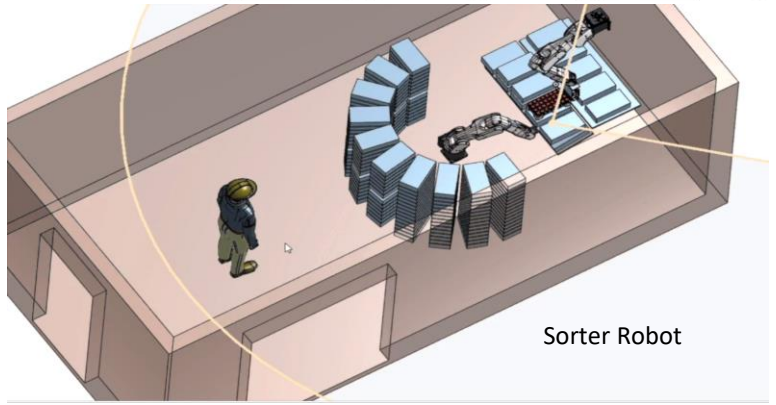
- PR 1: Samples collection (no carry-over), type of preservative, storage conditions
- PR 2: **Samples preparation Automation & Standardization**
- PR 3: **Spectra Standardization (x, y)**

Methods Standardization - Prerequisite 2

Laboratory Automation & Standardization

- Samples ID, heated, mixed, uncapped & analyzed
- Samples sorted out (3/6/9 parameters) depending on:
  - RFID/LIMS data
  - Analysis results
  - Samples history…
- Samples aliquoted before/after testing (tubes or µplates)
- Diverted to next testing stations

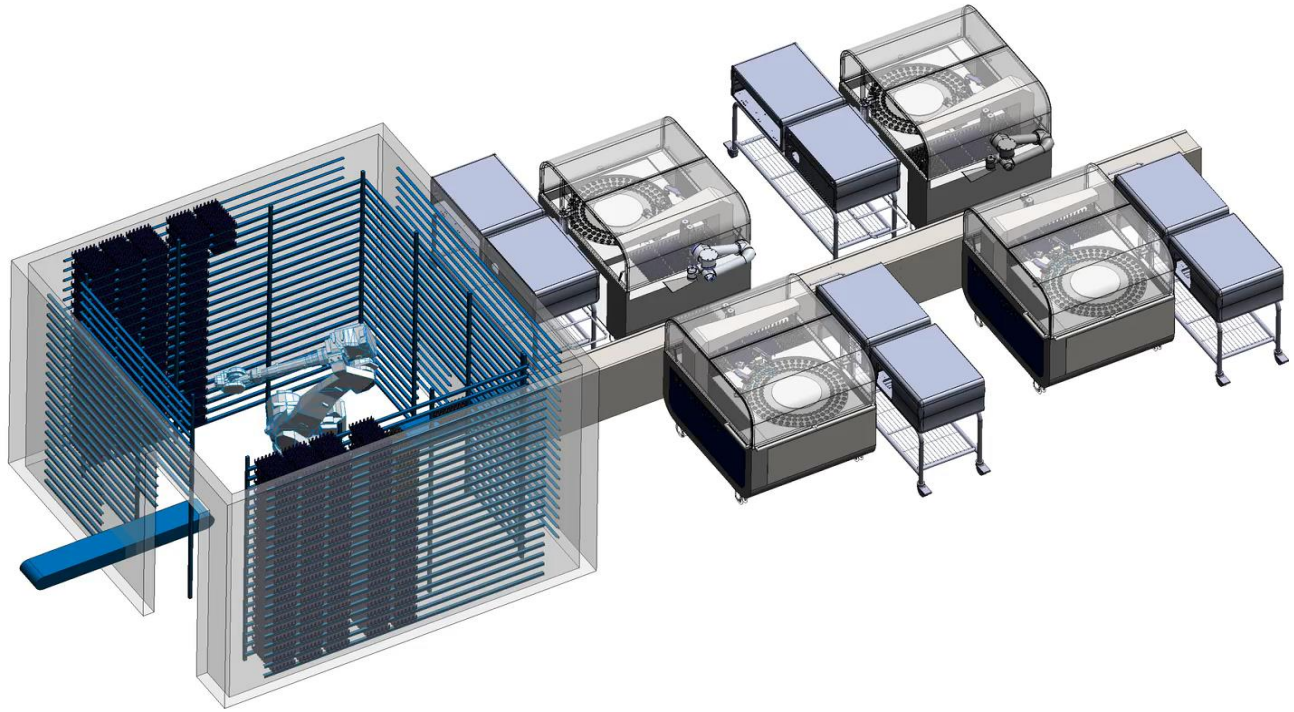




Automation enables methods standardization & mass multiplex testing at sample level to optimize the value of every samples

Methods Standardization - Prerequisite 2

Laboratory Automation & Standardization



Ilas 3000

Ilas 4000

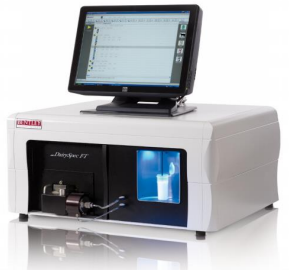
Methods Standardization - Prerequisite 3

Real-time Spectra standardization (x,y)

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

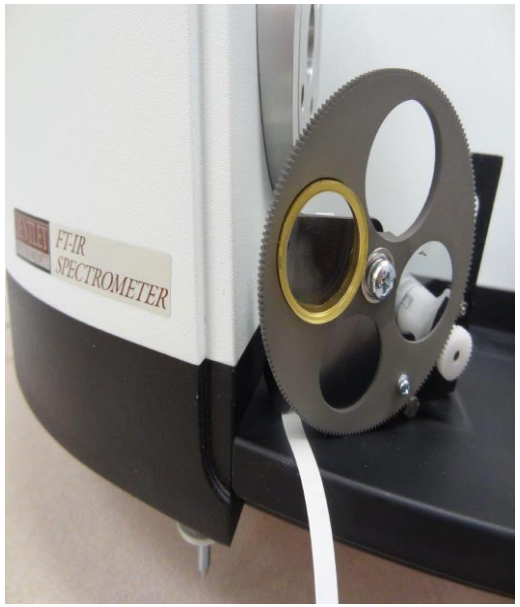
Thus, spectra standardization is very important:

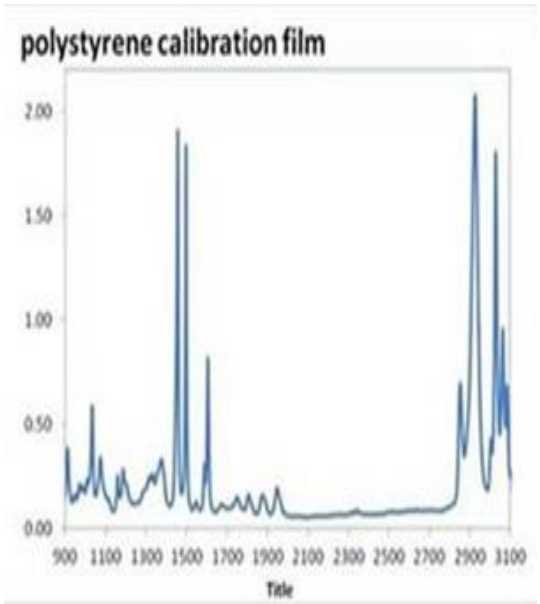
- For optimum calibration transfer between instruments
- For worldwide spectra & results equivalence
- For results/calibration stability (Slope/Bias)
- To reduce calibration development cost (centralized calibrations)
- For implementation of qualitative spectral analysis



Method Standardization - Prerequisite 3

Real-time Spectra standardization (x)



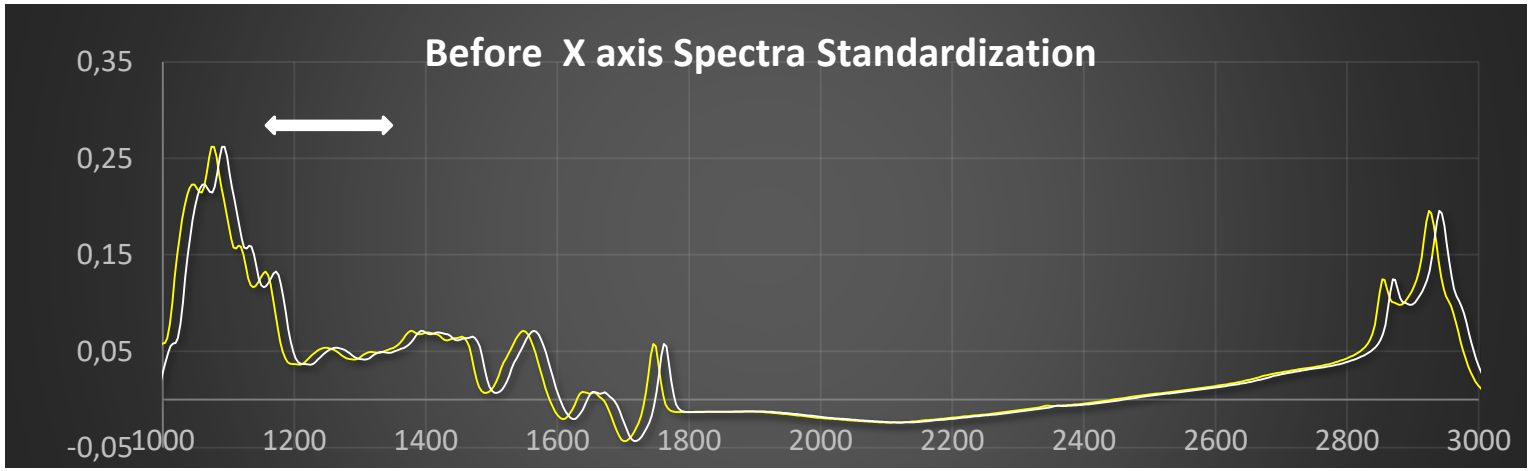


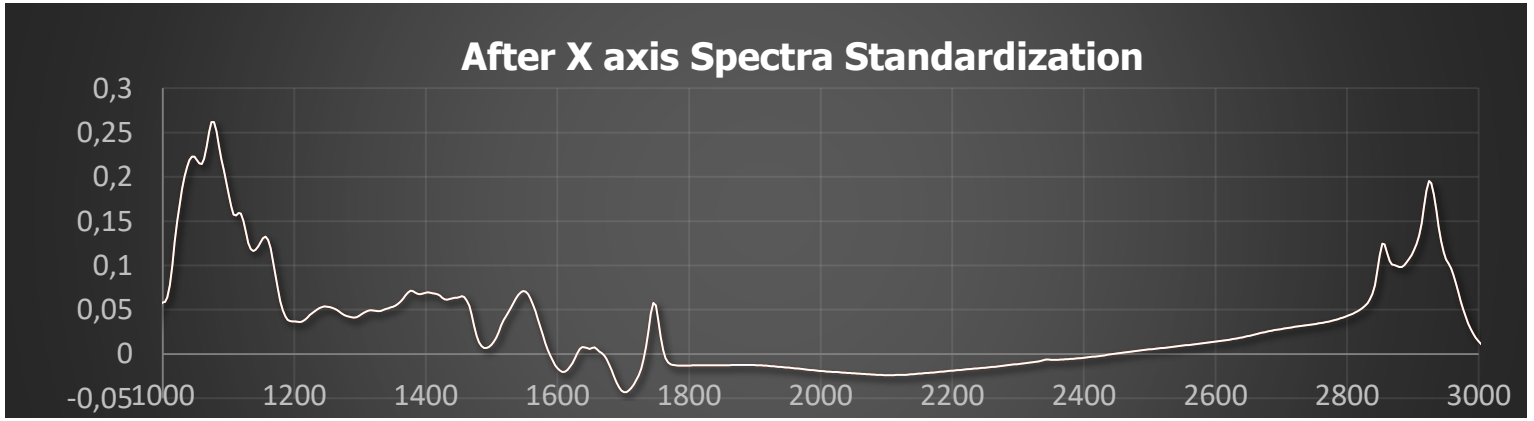
- **Example: Polystyrene Test**
- [4:30 PM Central Daylight Time] Polystyrene Test: STARTED
- Background Scan Completed
- Polystyrene Scan Completed
- **Peak 3082.22 @:3082.18 cm-1**
- **Peak 3060.14 @:3060.12 cm-1**
- **Peak 1601.38 @:1601.37 cm-1**
- **Peak 1583.04 @:1583.24 cm-1**
- **Peak 1028.42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

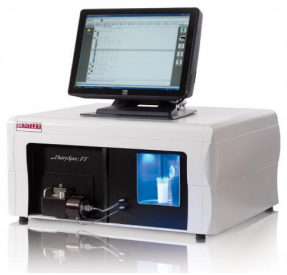
Standardization of spectrum x-axis with a polystyrene film (internationally recognized NIST standard) to calibrate optimally interferometer laser frequency

Method Standardization - Prerequisite 3

Real-time Spectra standardization (x) without reagent

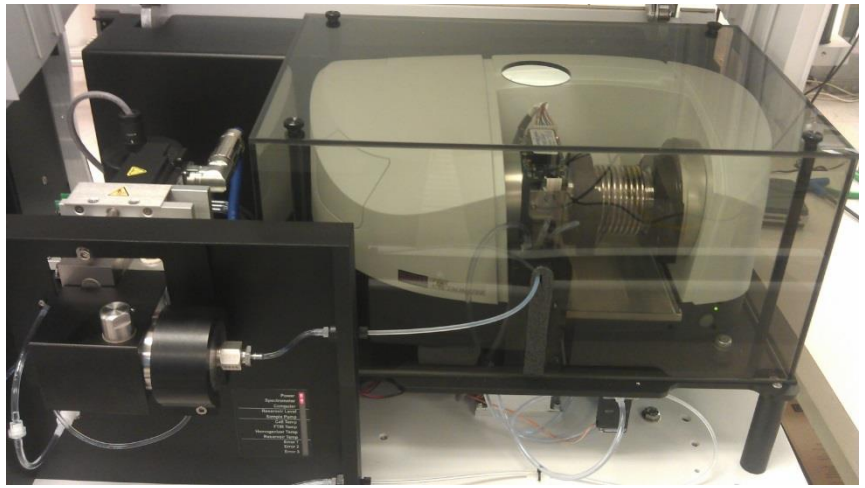


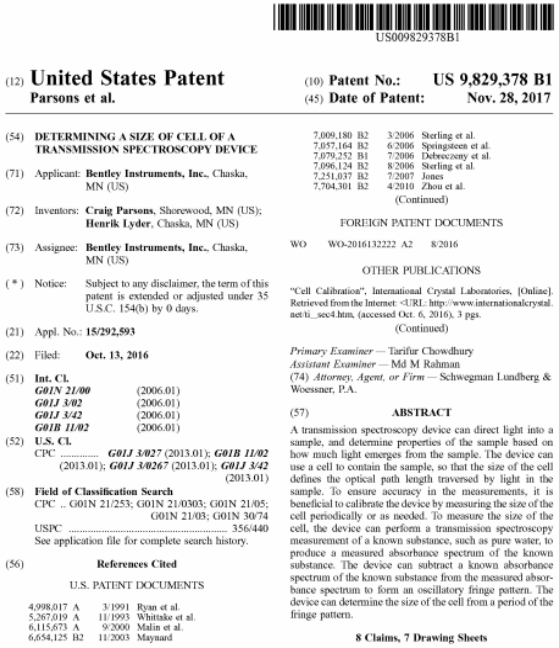




Method Standardization - Prerequisite 3

Real-time Spectra standardization (y) without reagent- Patented





US009829378B1

(12) **United States Patent**
Parsons et al.

(10) **Patent No.: US 9,829,378 B1**
(45) **Date of Patent: Nov. 28, 2017**

(54) **DETERMINING A SIZE OF CELL OF A TRANSMISSION SPECTROSCOPY DEVICE**

(71) Applicant: **Bentley Instruments, Inc.**, Chaska, MN (US)

(72) Inventors: **Craig Parsons**, Shorewood, MN (US); **Henrik Lyder**, Chaska, MN (US)

(73) Assignee: **Bentley Instruments, Inc.**, Chaska, MN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/292,593**

(22) Filed: **Oct. 13, 2016**

(51) **Int. Cl.**
G01N 21/00 (2006.01)
G01J 3/02 (2006.01)
G01J 3/42 (2006.01)
G01B 11/02 (2006.01)

(52) **U.S. Cl.**
CPC ***G01J 3/027*** (2013.01); ***G01B 11/02*** (2013.01); ***G01J 3/0267*** (2013.01); ***G01J 3/42*** (2013.01)

(58) **Field of Classification Search**
CPC .. G01N 21/253; G01N 21/0303; G01N 21/05; G01N 21/03; G01N 30/74
USPC 356/440
See application file for complete search history.

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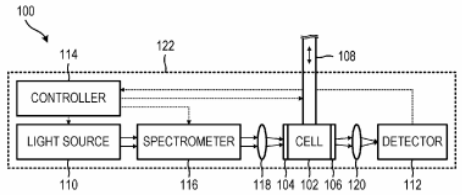
Primary Examiner — Tarifur Chowdhury
Assistant Examiner — Md M Rahman
(74) *Attorney, Agent, or Firm* — Schwegman Lundberg & Woessner, P.A.

(57) **ABSTRACT**

A transmission spectroscopy device can direct light into a sample, and determine properties of the sample based on how much light emerges from the sample. The device can use a cell to contain the sample, so that the size of the cell defines the optical path length traversed by light in the sample. To ensure accuracy in the measurements, it is beneficial to calibrate the device by measuring the size of the cell periodically or as needed. To measure the size of the cell, the device can perform a transmission spectroscopy measurement of a known substance, such as pure water, to produce a measured absorbance spectrum of the known substance. The device can subtract a known absorbance spectrum of the known substance from the measured absorbance spectrum to form an oscillatory fringe pattern. The device can determine the size of the cell from a period of the fringe pattern.

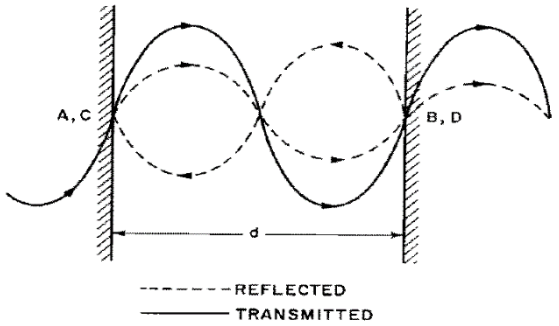
8 Claims, 7 Drawing Sheets

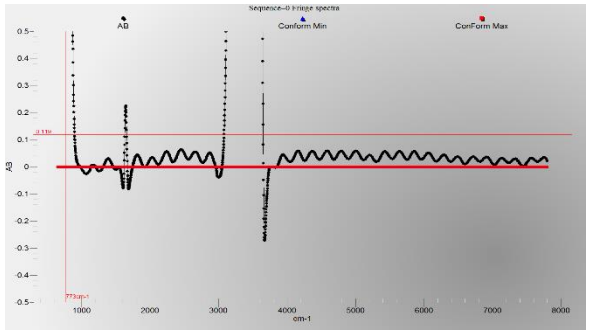


Standardization of spectrum y axis (absorbance) by measuring very accurately and in real time the IR flow cell thickness

Method Standardization - Prerequisite 3

Real-time Spectra standardization (y) without reagent- Patented





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 = 36.0873um
Standard deviation = 0.0046um

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

Method Standardization - Prerequisite 3

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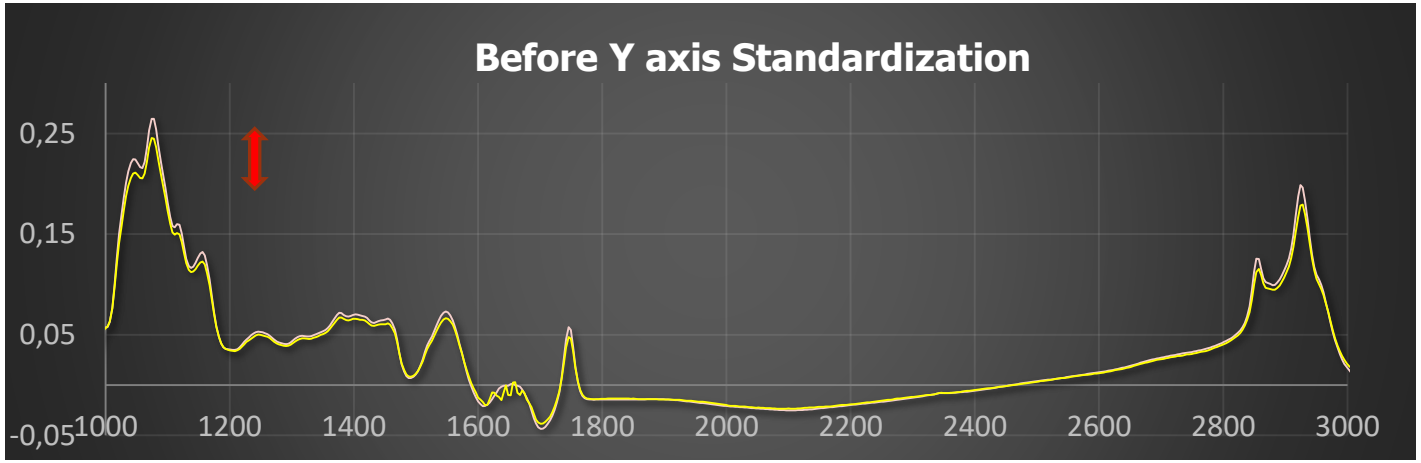

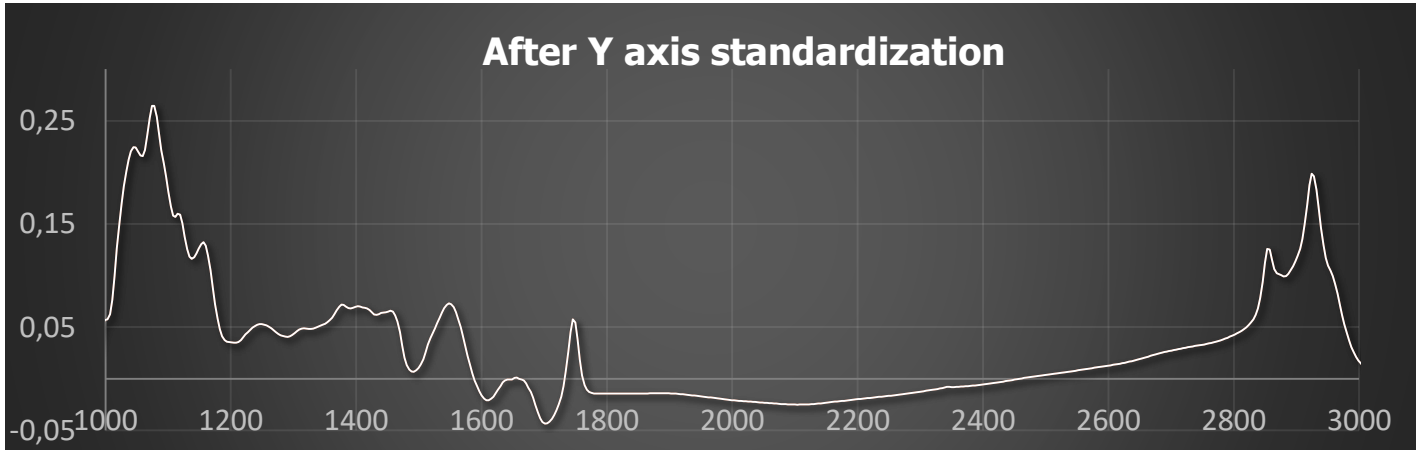

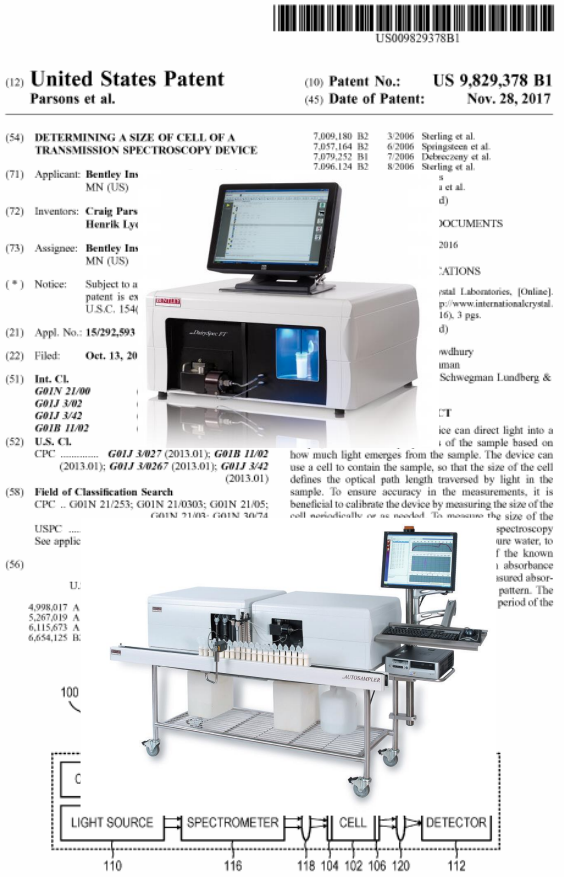

US009829378B1

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(72) Inventors: **Craig Pars** **Henrik Ly**

(73) Assignee: **Bentley Ins** MN (US)

(*) Notice: Subject to a patent is ex U.S.C. 154(

(21) Appl. No.: **15/292,593**

(22) Filed: **Oct. 13, 20**

(51) **Int. Cl.**
G01N 21/00
G01J 3/02
G01J 3/42
G01B 11/02

(52) **U.S. Cl.**
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(58) **Field of Classification Search**
CPC .. G01N 21/253; G01N 21/0303; G01N 21/05;
USPC .....
See applic

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Summary/Conclusions

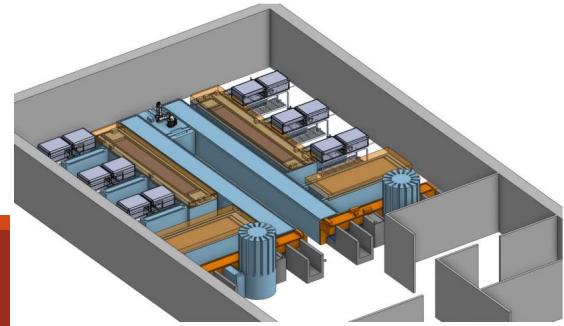
Milk is a very complex and rich source of functional groups. Over the years, hundreds of compounds from a variety of molecular classes have been identified.

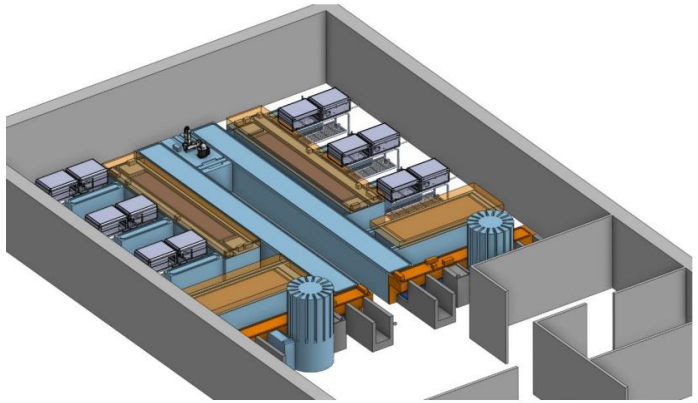
New biomarkers are needed to keep improving herd management, milk nutritional & economic value, and mitigate milk production impact on the environment.

Milk's compositional complexity precludes the use of a single analytical method to test all components

Analytical methods need to be fully standardized to capitalize on new models potential

The development of an holistic model combining latest high throughput standardized methods in combination with complete laboratory automation makes it possible to streamline mass & multiplex testing at the sample level for the benefit of the dairy industry.





Thank you for your attention!

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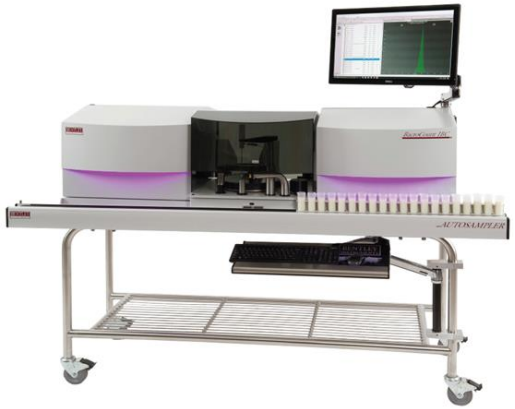
Solutions for the Central Milk Testing Laboratories



BactoCount IBC
Total Bacteria/SCC

DairySpec Combi 100-300
FTIR + FC (up to 64 components)

CombiFTS 400-600
FTIR+FC (up to 64 components)

Solutions for the Dairy Plants

DairySpec FT
Milk/Dairy Products
Up to 64 components

Somacount FC
Somatic Cells

BactoCount IBCM
Total Bacteria/SCC