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牛乳檢測之全球趨勢
Global Trends in Milk Analysis

TLRI, Tainan, Taiwan, 25 May 2016

孫丕忠 Terence Sun

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個人簡介
Personal Profile

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  M. Sc., Food Science, National Ping Tung University of Science & Technology
- 今日儀器股份有限公司 (1990)
  Today's Instruments Co., Ltd. , 1990
- 乳品檢測分析 (1995)
  RMT & Dairy Business, 1995

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議題 Agenda

- 現代乳業面臨之挑戰
  Challenges in Modern Dairy Farming
- FTIR 技術於乳質分析之新應用
  New Applications of FTIR in Milk Analysis
  - 脂肪酸組成 Fatty Acids Profile
  - 游離脂肪酸 Free Fatty Acids, FFA
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  - 乳中摻偽篩檢 Abnormal Milk Screening
- 體細胞數檢測之未來趨勢
  New Application of SCC in Milk
- 快速總生菌數檢測之趨勢與應用
  New Applications of Total Bacteria Counts in Milk

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台灣乳質乳量之演進
Evolution of Milk Yield, DHI, Taiwan

Year	305-2X-ME Milk Yield (Kg)	305-2X-ME Fat Yield (Kg)	Breeding Value of 305-2X-ME Milk Yield (Kg)	Breeding Value of 305-2X-ME Milk Fat (Kg)
2015	7877	321	37	1
2014	7738	314	35	1
2013	7728	311	20	0
2012	7641	305	-9	0
2011	7998	309	49	1
2010	7709	288	268	10
2009	7497	278	253	9
2008	7415	278	238	8
2007	7488	285	240	9
2006	7461	285	237	9
2005	7276	276	211	8
2004	7166	275	198	8
2003	7130	273	203	8
2002	6975	265	188	7
2001	6643	254	179	7

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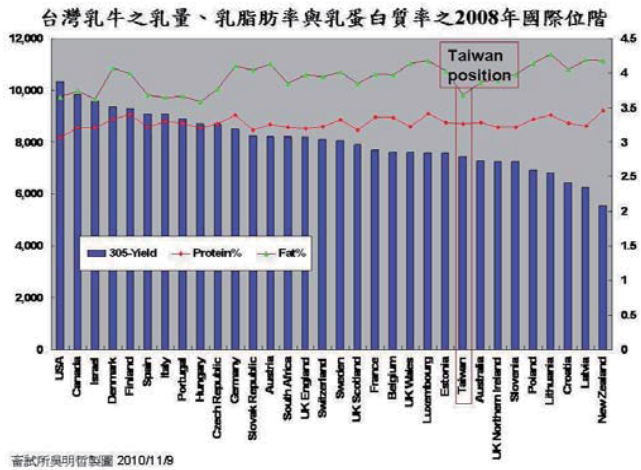
台灣乳質乳量之國際位階, 2008
Ranking of Milk Yield, DHI, 2008



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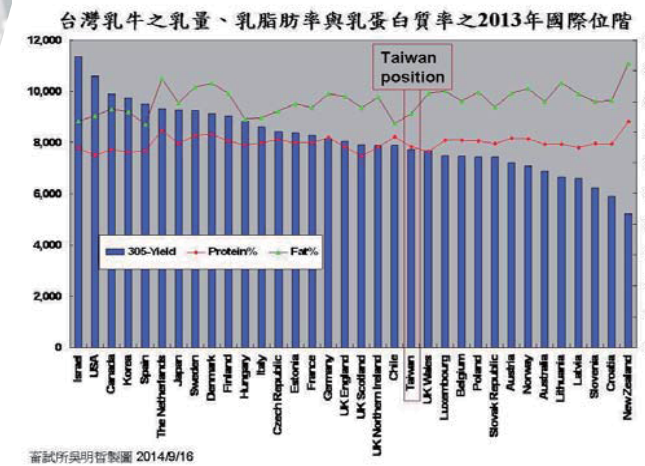
Ranking of Milk Yield, DHI, 2013



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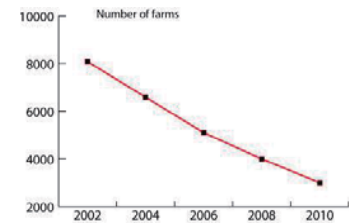
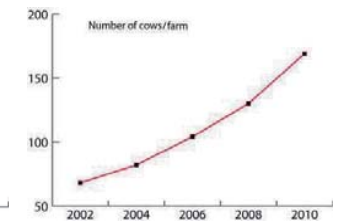
現代乳業面臨之挑戰
Challenges in Modern Dairy Farming

- 穩定獲利 Staying profitable
  - in spite of declining milk prices
- 專業且具效益之牧場經營 Ensuring professional and cost-effective farm operation
  - in spite of shortage of qualified labor
- 提昇產量 Increasing productivity
  - in spite of stricter demands concerning animal welfare & environmental issues
- 牧場整合 Intense consolidation: fewer and much larger farms





Source: Danish Cattle Federation

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現代乳業面臨之挑戰
Challenges in Modern Dairy Farming

- 確保產品品質，維持客戶對品牌之忠誠度
  Maintaining consumer loyalty and global brand protection through consistent product quality
- 通過嚴密的生產控制和節約成本獲得優於競爭對手的利潤率
  Obtaining better margins than competitors through tight production control and cost savings
- 開發具競爭力之新產品
  Development of new products with competitive edge
- 低價之牛乳與乳製品
  Low prices on milk and milk products
- 企業整併
  Consolidation




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乳製品生產流程
Production of milk products

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牛乳之價值鏈
The Milk Value Chain - Overview

生乳計價 Milk Payment - value chain
(CMT – Dairy – Consumer):

- Composition (Fat, Protein, Solids)
- Health improving parameters
  (e.g. Fatty Acids)
- Hygienic quality *(total bacteria count)*
- Milk quality *(e.g. Fatty Acids, Freezing Point Depression)*
- Safety & quality
  (e.g. Abnormal Spectrum Screening)

CMT

牛群性能改良 Herd Improvement - value chain
(CMT – DHIA – Farmer/Cow):

- Productivity *(Fat, Protein, Yield)*
- Mastitis *(Somatic Cells, Microorganisms)*
- Feeding *(Fat, Protein, Urea, Fatty Acids)*
- Cattle diseases *(Acetone/BHB/ Ketosis)*
- Breeding *(all parameters, Yield)*
- Quality *(Free Fatty Acids)*

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議題 Agenda

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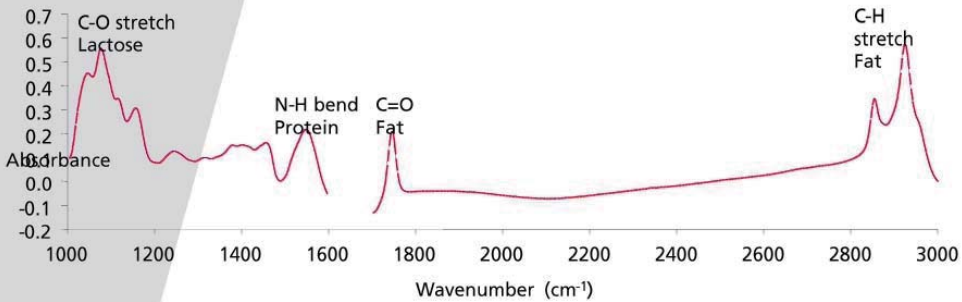
FTIR 檢測之原理
Common Band Absorption Areas

Molecules absorbs energy from the different wavelengths



The spectrum gives information on chemical composition

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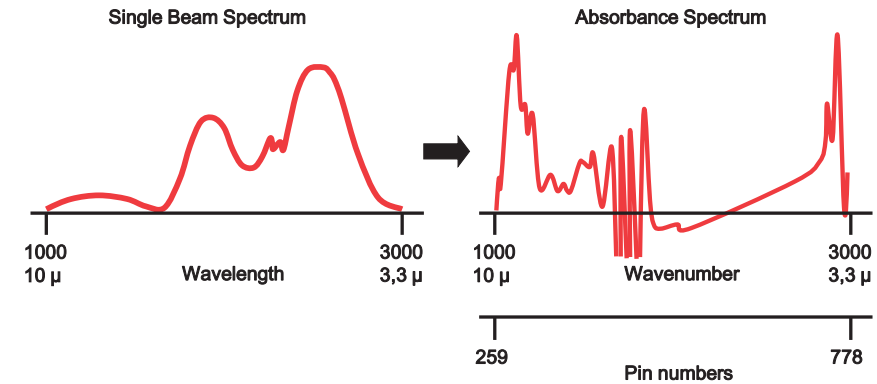
FTIR 之原理
FTIR Interferometer - Theory



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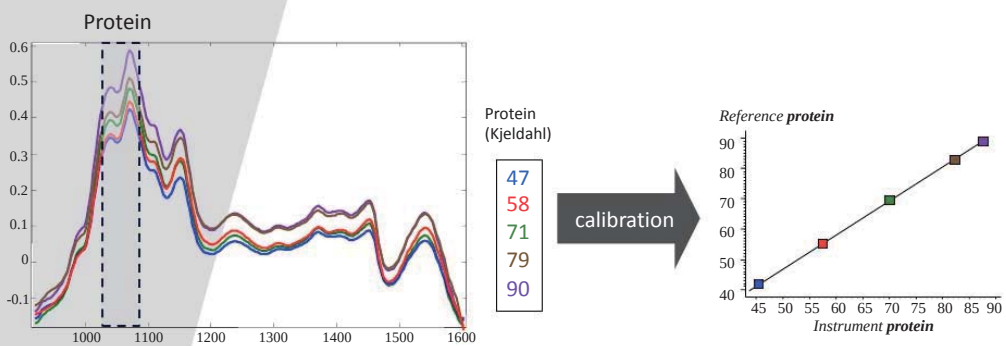
FTIR 檢測之原理-檢量線建立
Calibration Development



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FTIR 檢測技術之應用
What Can FTIR Offer Us Today?

- Mid-Infrared (MIR) spectroscopy has been available since the 1970's as a rapid method for routine measurements of the main constituents in milk (fat, protein, carbohydrates)
- Fourier Transform Infrared (FT-IR) spectroscopy is the state-of-the-art method for acquiring MIR spectra analyzing:
  - Conventional parameters
    - Fat, Protein (true & crude), Casein, Lactose, Solids, Urea, Citric Acid, Free Fatty Acids, PH, Freezing Point Depression
  - New parameters
    - Fatty Acids
    - Ketosis Screening (BHB and Acetone)
    - Abnormal Milk Screening
    - Others

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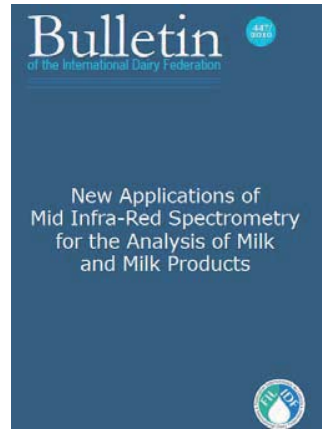
國際乳業聯盟(IDF) 公告
Bulletin-International Dairy Federation

- Published December 2010
- Analysis of results gained using different equipment in 11 different laboratories across Europe
- Assessment of accuracy, reproducibility and repeatability



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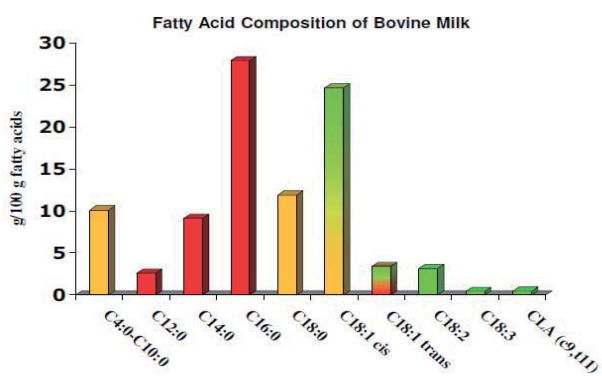
脂肪酸組成
Fatty Acids Profile



Source: Professor Andy Salter, University of Nottingham

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脂肪酸組成
Fatty Acids Profile

Why is Fatty Acid profile interesting:

- Consumers want to get the right fat from their diet
  - The fat should be unsaturated rather than saturated
  - High cholesterol levels causes heart diseases
- The **dairies** need to offer **value added products** to stay profitable
- Improved nutritional image of milk

The Fatty Acid Profile can be used for:

- Payment analysis of milk according to:
  - Unsaturated fatty acids
  - Saturated fatty acids
- The farmers can use the results to optimize the feed of the dairy herd regarding target amounts of unsaturated fatty acids in the raw milk
  - Short term by changes in the feeding
  - Long term by breeding in order to favor a specific fatty acid profile in milk

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FTIR 檢測脂肪酸組成檢量線之項目
MilkoScan FT+ Fatty Acids Calibrations

- Chain length
  - Short Chain Fatty Acids (SCFA): C4 + C6 + C8 + C10
  - Medium Chain Fatty Acids (MCFA): C12 + C14 + C16
  - Long Chain Fatty Acids (LCFA): C18
- Degree of unsaturation
  - Saturated Fatty Acids (SFA)
  - Mono Unsaturated Fatty Acids (MUFA)
  - Poly Unsaturated Fatty Acids (PUFA)

- Major Fatty Acids
  - C14:0
  - C16:0
  - C18:0
  - C18:1 total

Based on samples from 4 countries and validated on samples from 5 countries Ring trial including 7 laboratories for GC analysis.

Type of Fatty Acids		Features
Short Chain Fatty Acids (SCFA) (saturated only)	C4:0 – C10:0	**13-15%** of total fatty acids in milk. Present in milk products only Positive properties only found , - no negative reported Positive influence on body weight, cholesterol in blood and cancer
Medium Chain Fatty Acids (MCFA) (saturated only)	C12:0 –C16:0	**35-45%** of total fatty acids in milk (many plant products are higher May lead to increased risk of cardiovascular diseases. Only malicious if consumed in excess amounts
Long Chain Fatty Acids (LCFA) (saturated only)	> C16:0	**10 – 15%** of total fatty acids in milk Neutral influence on cardiovascular diseases No negative impact on human nutrition >C18 used in nervous structures

Table 1: Features and benefits of the short, medium and long chained saturated fatty acid in human diet, according to presentation from Professor Philippe Legrand Un. Of Rennes.

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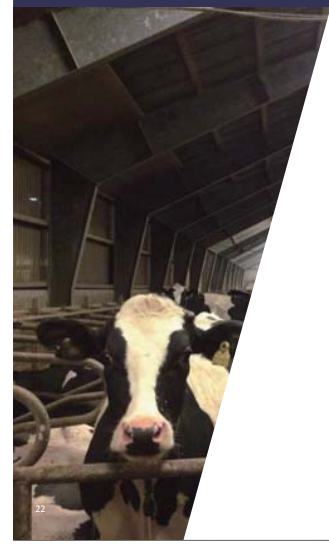
英國實例
Great Britain has Taken the Lead



MARKS & SPENCER

Launched in July 2011

Delivering:
- 6% less saturated fat
- 84 tonnes of saturated fat removed from its customers diets each year
- Improved cow health and welfare (?)

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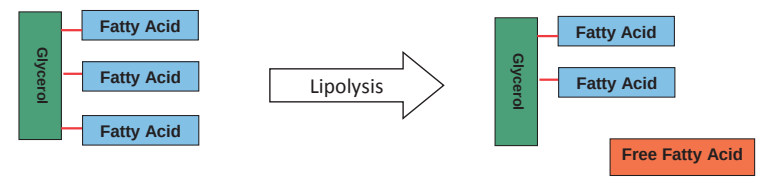
游離脂肪酸
Free Fatty Acids



- Free Fatty Acids is the result of “Lipolysis”, which is an enzymatic process in the milk
- Lipolysis is accelerated by mechanical mal-treatment of milk (defective pumps etc.), thereby causing higher amount of FFA.
- Normal concentration of FFA in raw milk “ab farm” is < 0.7 mmol FFA / 100g Fat
- Sound cow’s milk contains ~0.5 mmol FFA/100 g Fat
- FFA > 1.0 mmol FFA / 100g Fat is considered abnormally high / ‘defective’

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游離脂肪酸含量對乳品品質之影響
FFA in Dairy Products and Suppliers Milk

Problems caused by higher FFA levels:

- Rancidity in high-fat dairy products i.e. butter, cream (FFA has a rancid and soapy taste and smell)
- High levels of FFA influences the shelf life (i.e. reduced shelf life of milk powder products)
- Affects/reduce cheese yield (FFA is not included in the coagulum)
- Adversely affect the flavour and quality of milk dairy products

Why test suppliers milk:

- Farmers, who deliver milk with high FFA, can be helped to correct the problem
- Raw milk with high FFA levels cannot be “repaired”, the damage is done, and the enzymatic process continues
- The milk industry faces increasing competition, and must have more and more knowledge and control over the raw material (“Chain Management”)

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影響游離脂肪酸含量之因素
FFA Influenced and Maintained by

Free Fatty Acids are influenced by:

- The mechanic treatment of the milk (milking machine design)
- Installation and operation
- The general hygiene/bacterial status of the milk
- Physiological status of the cow (stage of lactation, diseases, composition of fodder, seasonality etc.
- Milk handling on the farm and in the factory

Low FFA can be maintained:

- by well designed and monitored milking machines and bulk tanks
- by consistent balanced cow diets
- and by proper drying-off procedures

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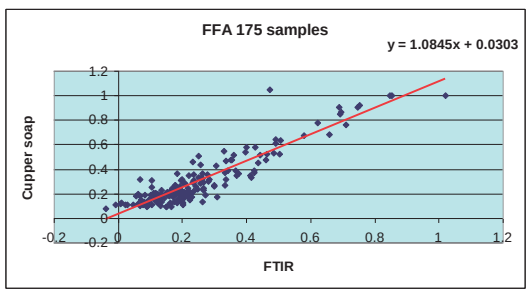
以FTIR技術檢測游離脂肪酸之優點
FFA Calibration and Benefits with MilkoScan FT+

MilkoScan FT+ can be an excellent method for FFA-screening by:

- Reducing analysis costs
- Giving high test frequency and thereby a much better overall picture of FFA levels
- Saved time and costs if using BDI, FIA or copper soap method today
- Quick follow up tests to solve the problem if a high FFA result detected, ex. Errors in milking equipment, unbalanced cow diets
- Improve the shelf life and quality of dairy products



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檢測游離脂肪酸之國家
Countries Measuring Free Fatty Acid

FFA is today measured in:

- **The Netherlands**
- **Norway**
- **France**
- **Belgium**

} **FFA included in Payment Scheme**

- Japan
- Italy
- UK
- Canada
- USA
- Denmark
- Spain
- Brazil
- The Czech Republic
- Taiwan

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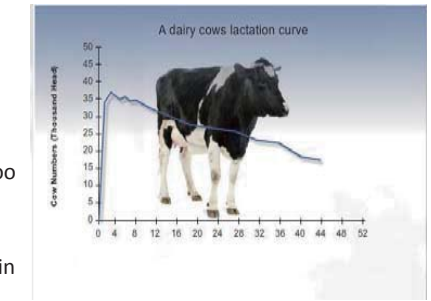
酮症–代謝性疾病
Ketosis, a Metabolic Disease

- Ketosis occurs when energy output (milk production) is too high compared to energy input (feed intake+ uptake from fat deposits)
- Primary ketosis occurs when too little feed (or too low energy concentration in feed) is offered to the cow.
- Secondary ketosis occurs if the cow stops eating (acidosis or other diseases) while still producing (too much) milk.
- In both cases energy uptake from fat deposits becomes too big, the turnover from fat to glucose in the liver too high, - and hence **Acetone** and **Beta Hydroxy Butyrate (BHB)** is excreted as residues.



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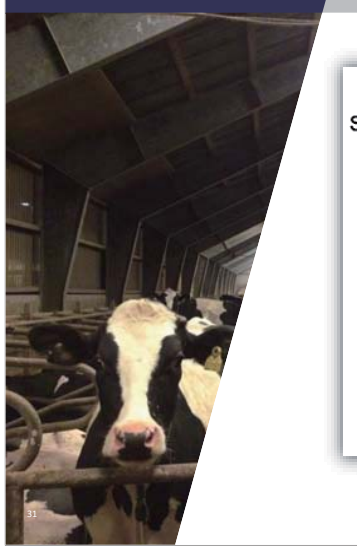
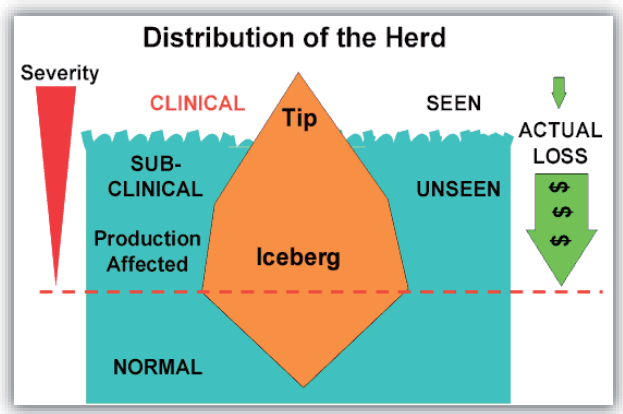
酮症的真實情況
The Real Situation of Ketosis





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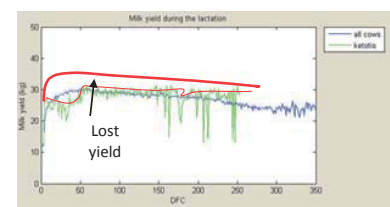
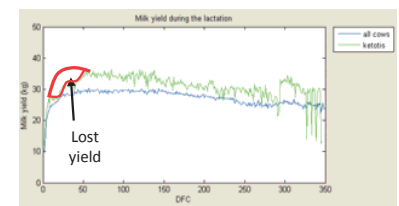
發生酮症之損失
How Costly is Ketosis?

- Once a ketosis problem is detected in the herd it must be solved on herd level
- Strong requests from the DHI associations has urged FOSS to develop this calibration for MilkoScan FT+.
- Below examples are from two Herd Navigator farms (Lattec).
- We here learned that losses due to subclinical cases losses are bigger than earlier anticipated



Problem solved on cow level



Problem solved on herd level

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以FTIR技術篩檢酮症
Screening for Ketosis by FTIR

- An indication of levels of the acetone and beta hydroxy butyrate (BHB) residues can be measured by the FTIR as used in MilkoScan™ FT+ allowing screening for ketosis as part of routine milk testing.
- As ketosis often is a herd problem, a single alert gives the herd manager a timely warning to examine all early lactating cows for problems and to take proactive action as necessary.
- The laboratory can give the herd manager a monthly screening of all cows for ketosis, and individual cows can be singled out for treatment.

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酮症篩檢－半定量法
Semi-quantitative Method

- **Limit for subclinical ketosis is**
  - 0.15 mmol/L for Acetone
  - 0.1 mmol/L for BHB

  They may not increase simultaneously
- **Screening model used for classifying the animals as either ”Low risk” or as ”High risk” of ketosis**

Low risk **High risk of ketosis**

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DHI實驗室-酮症等級分類及應用-加拿大等國
DHI Laboratory: Classification and Application of Results

valacta

ACETONE/BHB CONCENTRATION		BHB (mM/l)
HIGH	- Treat cow	≥0.2
MEDIUM	- Observe cow	≥0.15-<0.20
LOW	- No action	<0.15

RISK OF KETOSIS

As ketosis can often be a herd problem, a single alert gives the herd manager a timely warning to examine all early lactation cows.

Qlip CRV

Combination of Ac and BHB values with:
- fat : protein ratio
- parity
- month of milk recording

→ binary (yes/no) score for ketosis for cows with DIM <60 only

Clasel

Decision tree including Ac and BHB

→ Presented for individual animals

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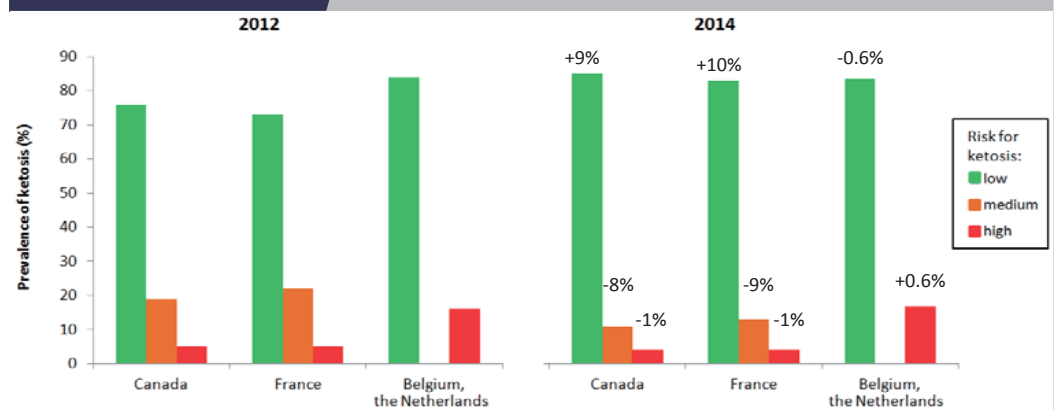
酮症發生率－加拿大、法國、比利時及荷蘭
Development of Ketosis Prevalence Over Time



Prevalence of ketosis (low, medium, high risk) in Canada (Valacta), France (CLASEL) and Belgium (region Flanders) and the Netherlands (Qlip) in 2012 and 2014, respectively. Data for Belgium and the Netherlands are expressed as ketosis yes (high risk) or no (low risk).

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酮症-DHI數據報表及應用 – 美國
From DHI Laboratory to FARM – USA

A Ketosis Prevalences Using 1 Test Day

Overall (for cows 5 to 20 DIM)

Group	Cows Tested	Predicted Ketosis	Ketosis Prevalence	Target
1st Lact	9	1	11%	<5%
2nd+ Lact	25	7	28%	<15%
All Lactations	34	8	24%	<10%

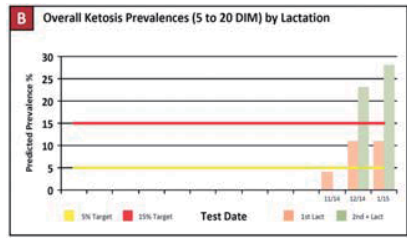
Cows fresh >= 5 days since last test: 71 — 48% of fresh cows were tested 5 - 20 DIM

Early Fresh (for cows 5 to 11 DIM)

Group	Cows Tested	Predicted Ketosis	Ketosis Prevalence	Target
1st Lact	4	1	25%	<5%
2nd+ Lact	8	3	38%	<15%
All Lactations	12	4	33%	<10%

17% of fresh cows were tested 5 - 11 DIM

B Overall Ketosis Prevalences (5 to 20 DIM) by Lactation



AgSource Cooperative Services

D Fresh Cows Predicted to Have Ketosis (5 - 20 DIM)

Barn Name	Vis ID	Pen	Lact Num	DIM	Days Dry	Age at 1st Calving
4667		7	3	5	139	
4758		7	3	9	79	
5919		8	1	9		22
4308		7	4	10	50	
3422		6	6	15	73	
4815		6	3	16	58	
4627		6	3	18	147	
4197		6	4	19	69	

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酮症-DHI數據報表及應用 – 丹麥
From DHI Laboratory to FARM – Denmark

Danish Cattle Federation, Denmark

SEGES

Overview 1: Number of freshening cows with elevated BHB values

	Number of freshening cows (5-35 DIM)	Proportion of cows with elevated BHB values (>0.15 mmol/l)	Status
1st calving	Too few animals**		
2+ calvings	11*	27%	

*Calculation includes the last freshening cows from last 2 DHI testings
**Minimum of 10 animals required for calculations

Threshold for alert: 15%

Recommended interventions:

0 - 15%:	Uncritical
15 – 25%:	Observation of further development
Over 25%:	Adjustment of dry cow management

Overview 2: BHB value for individual cows

Dato	Mælk kg	Fedt %	Fedt Gram	Protein %	Protein Gram	EKM kg	Dage fra kælvn	Celletal 1000	Urea	BHB
12/12/2013	35.1	4.03	1415	3.20	1123	34.7	56	58		0.076
27/11/2014	0.0	0.00	0	0.00	0	0.0	406		0.0	
21/11/2013	34.5	4.19	1446	3.15	1087	34.6	35	45		0.014
04/03/2014	34.5	3.04	1049	3.32	1145	30.2	138	60		0.015
07/04/2015	57.0	2.52	1436	2.90	1653	44.5	39	47		0.096
30/01/2014	30.9	2.76	853	3.28	1014	25.9	105	74		0.042
09/10/2014	19.3	3.04	587	3.52	679	17.2	357	215		0.091
13/05/2014	30.1	3.17	954	3.26	981	26.7	208	710		0.100
12/08/2014	35.2	3.15	1109	3.39	1193	31.5	299	842		0.063

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議題 Agenda

- 現代乳業面臨之挑戰
  Challenges in Modern Dairy Farming
- FTIR 技術於乳質分析之新應用
  New Applications of FTIR in Milk Analysis
  - 脂肪酸組成 Fatty Acids Profile
  - 游離脂肪酸 Free Fatty Acids, FFA
  - 酮症篩檢 Ketosis Screening
  - 乳中摻偽篩檢 Abnormal Milk Screening
- 體細胞數檢測之未來趨勢
  New Application of SCC in Milk
- 快速總生菌數檢測之趨勢與應用
  New Applications of Total Bacteria Counts in Milk

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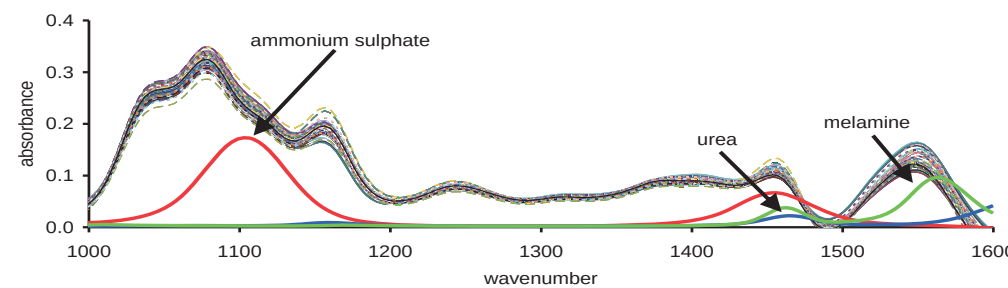
牛乳中摻假檢測
Milk Adulteration



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已知及未知掺假物質篩檢
The Use of Targeted & Untargeted Calibration Models

- Melamine crisis (2008) with contaminated milk powder
- Liquid milk deliberately adulterated for economic gain
- Collaborative project between Fonterra, Arla and FOSS
- Resulted in development of targeted and untargeted models for detecting milk adulteration at economic levels
- In 2014 we have released FTIR models using abnormal spectrum module (ASM) as well as targeted models for specific adulterants

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牛乳中掺假
Milk Adulteration

- Milk quality is tested before unloading. But milk adulteration is rarely tested at the platform.
- Milk Adulteration is a growing problem.
- Adulteration can be deliberate or caused by accident.

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發生乳中掺假之可能原因
Screening for milk adulteration

Unintentional deviations

Caused by accidents or failures.

- Agent in the cows feed that is transported on to the milk
- Cleaning agent
- Water

Intentional deviations

Driven by economic gains:

- amount (weight and/or volume)
- milk fat content
- milk protein content
- dry matter content
- the total bacterial counts and somatic cell counts

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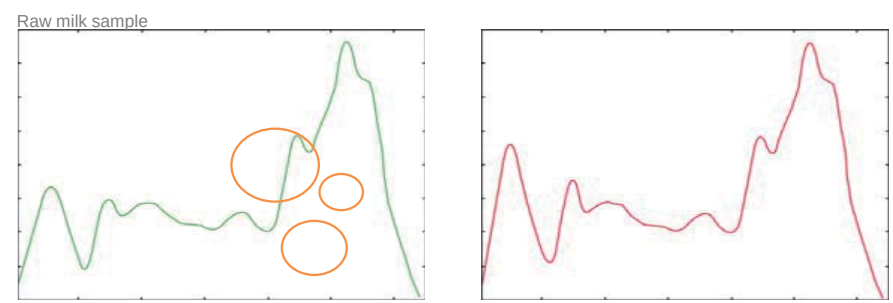
正常 vs 不正常 牛乳光譜
Normal vs Abnormal Spectrum

- FTIR spectra from natural raw milk samples is a unique finger print of normal milk



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未知摻假物質篩檢
Untargeted Screening Model

- You can screen all milk samples against abnormalities. It will not tell you what is in the milk only if it is normal milk or not.
- Advantages untargeted model allows you screening for an unlimited number of unknown potential adulterants within 10 - 30 sec.

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摻假物質篩檢
What Can Be Screened Against with The Untargeted Model ?

- The table shows the Limit of Detection for the adulterants which could be detected as abnormal by the model.
- The LoD´s correspond to a threshold of 3.

Adulterant	LoD	LoD Adulterant	LoD	LoD Adulterant	LoD
Allantoin	300 ppm	Cyanuric acid	170 ppm	Sodium bicarbonate	400 ppm
Amidourea	500 ppm	Cyromazine	300 ppm	Sodium hydroxide	0.06 %
Aminotriazine	800 ppm	Dicyandiamide	300 ppm	Sodium nitrite	200 ppm
Ammonium nitrate	200 ppm	Formaldehyde	400 ppm	Thiourea	500 ppm
Ammonium sulphate	300 ppm	Hydroxyproline	900 ppm	Triuret	800 ppm
Biuret	600 ppm	Maltodextrine	1100 ppm	3-aminotriazole	1100 ppm
CIP agent	1%	Melamine	300 ppm	4-aminotriazole	1400 ppm

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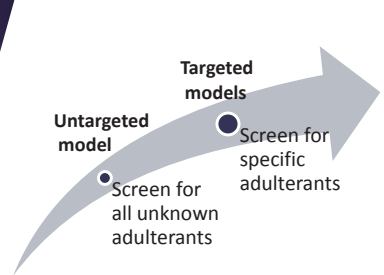
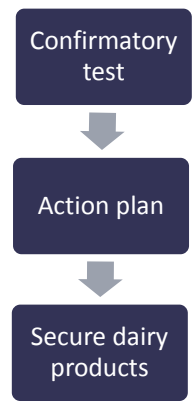
快速完成摻假篩檢
Security Screening in 30 seconds



Rapid inexpensive screening of your milk



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研發中的FTIR新檢測項目
Potential New Parameters

- Many research projects are actively researching new parameters from FT-IR:
  - CRA-W, Agramir, Phénofinlait, OptiMIR, Robust Milk, University Padova and many more
  - FOSS are involved in some of above projects and in several other projects with key customers
- Numerous publications are available
- Some of the new potential parameters are
  - Milk coagulation properties
  - Pregnancy confirmation
  - Protein composition
  - Reconstituted milk screening
  - But there are many more!

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  New Applications of Total Bacteria Counts in Milk

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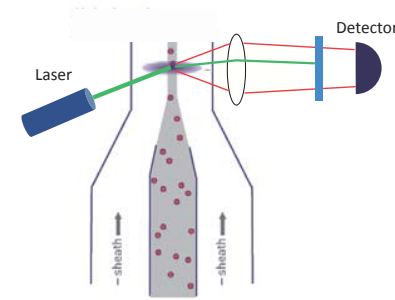
流式細胞儀之原理
Principle of Flow Cytometry

Flow cytometry is a technology designed to count cells in suspension.

The basic steps are the following:

- The cells are stained with a fluorescent dye.
- The sample is stretched to a very thin string (20 µm).
- The sample passes a focused light beam which excites fluorescence from the dye.
- The cells are seen as individual light pulses The fluorescence is collected, filtered and detected.



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何謂乳房炎
What is Mastitis?

- Is an infection of the udder caused by pathogenic bacteria
- In Denmark approx 50% of cows are treated every year
- Many factors are influencing mastitis
- Yield and composition are negatively affected
- Mastitis diagnosis: somatic cell counts (SCC) and bacteriology are standard **(Viguer et al., 2009)**

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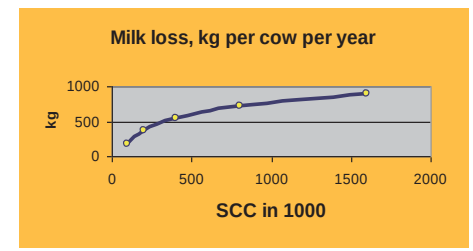
乳房炎造成之影響
Mastitis Consequences

Lower compositional value

Factor	Increase	Decrease	Effect for the Dairy
Fat		⇩	Economy
Free Fatty Acids	⇧		Taste problem
Total casein		⇩	Less cheese yield
Lactose		⇩	Economy
Sodium	⇧		Taste problem
Chloride	⇧		Taste problem
Calcium		⇩	Quality of the product
Lipase	⇧		Taste problem
Bacteria	⇧		Low quality and economy

Lost milk production



Worldwide, mastitis is associated with economic losses of $35 billion annually **(Wellenberg et al., 2002)**

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牛乳中細胞之類別
Mastitis

Leukocytes
- White blood cells (WBC)

Lymphocytes

Monocytes

Macrophages

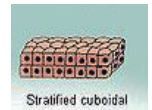
Granulocytes
Polymorphpnuclear (PMN)

Neutrophil

Eosinophil

Basophil

Epithelial cells
(skin cells)



- ~85% of somatic cells under normal conditions
- Relative share **decreases** when cow is infected with Mastetis

- ~10% of somatic cells under normal conditions
- Relative share **increases** to 30-90% when the cow is infected with Mastetis (PMN's are attracted to fight the bacteria)

- ~5% of somatic cells under normal conditions

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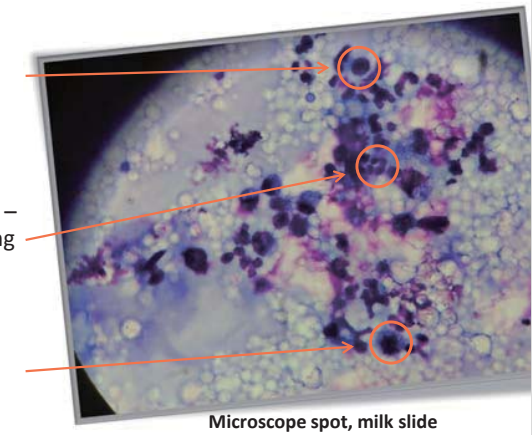
牛乳中細胞之類別
Cells in milk

Consist mainly of three populations:

1. **Lymphocytes** – Initiation and regulation of the immune response, production of antibodies **(Nickerson, 1989; Oviedo-Boyso et al., 2007)**
2. **Polymorphonuclear neutrophils (PMN)** – Phagocytosis of bacteria at the beginning of an inflammation **(Paape et al., 2002; Oviedo-Boyso et al., 2007)**
3. **Macrophages** – Regulation of immune response, phagocytosis of bacteria and cell debris **(Sordillo and Nickerson, 1988)**



Microscope spot, milk slide

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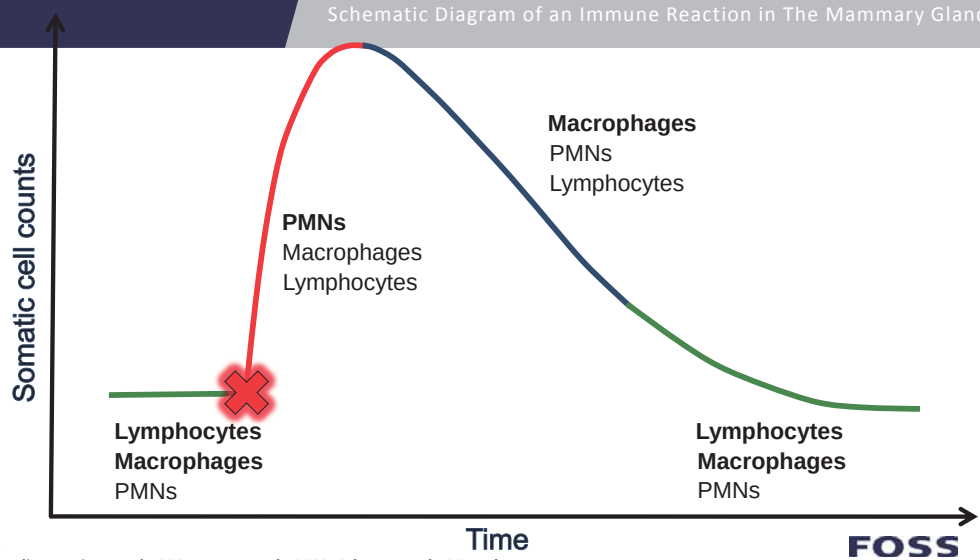
乳房炎免疫反應
Schematic Diagram of an Immune Reaction in The Mammary Gland



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According to Rivas et al., 2001; Paape et al., 2002; Schwarz et al., 2011a,b

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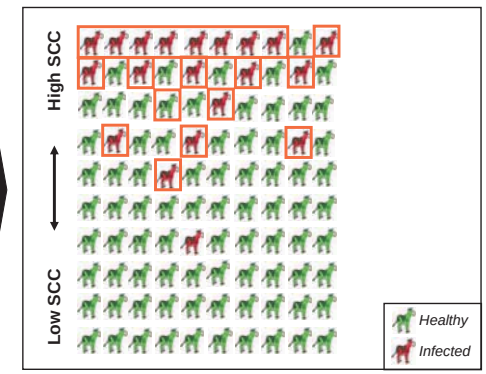
乳房炎監測工具
Enhanced Tool for Mastitis Monitoring

Current state, SCC:

Prospective state, SCC and DSCC:



→Targeted selection of suspicious cows

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DSCC 數據之應用
Application of DSCC Results

Application of DSCC results in daily farm management

Better management of subclinical mastitis

- Identification of mastitis in its early stage

  → Information on time point of infection; segregation/treatment accordingly (e.g. proinflammatory drugs)

- Identifications of cows with bacterial infection (selection for further analysis (e.g. PCR))

  → Segregation/special treatment on farm in order to avoid the spread of infection

- Information about probability of cure

  → Prudent use of antibiotics: treatment worthwhile vs. non-treatable chronic infection

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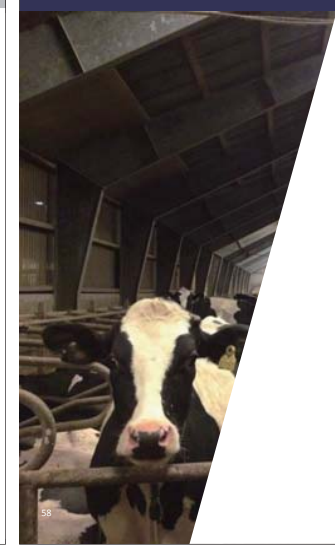
DSCC 新研究計畫
New Research Project



- FOSS has joint forces with the Veterinary Institute of Technical University of Denmark and SEGES
- Financed by Danish Ministry for Environment and Food
- The project:
  - 3-year duration
  - Detailed investigation of the udder health status of 1,000 cows in 5 modern dairy herds
  - Main objective: Investigation of new parameters for mastitis monitoring and development of guidelines for using these new parameters on dairy farms

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未來目標
The Future Goals

- Literature: SCC is an undisputed and well established criterion, but DSCC is well appropriated for more detailed characterization of udder health
- Promising new applications enabling a better management of subclinical mastitis:
  - Identification of mastitis in its early stage
  - Identification of cows with bacterial infection
  - Information about probability of cure
- A lot of research necessary in order to develop the actual application of DSCC in the frame of regular DHI testing

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議題 Agenda

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生乳中微生物之種類
Types of Bacteria

Bacteria present in milk and growth optimum **Temperature < 10 ˚C**

No mesophilic bacteria can reproduce

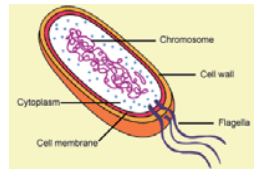
- Mesophilic prefer middle temperature (10°C - 35°C)
- Many are pathogenic bacteria and many are found in the earth, the optimal growth temperature is 25°C - 40°C.
- Stop growing at 40° C - 45°C

No thermophilic bacteria can reproduce

- Thermophilic prefer high temperature, optimum grow 40° C - 60°C . Stop at 60°C - 70°C when in liquid media- when dry media stop at 100°C
- Many bacteria to be found in compost and in manure

The psychrotrophic/-phillic can reproduce

- Prefer cold environment (down to under 0˚C),
- Grow best at 20 ˚C, they stop at 30 ˚C
- Many of these micro-organisms live in water and in the soil
- High temperatures 60˚C - 70˚C kill the micro-organisms
- The psychrotrophic/-phillic bacteria:
  - **Achromobacter**
  - **Pseudomonas**
  - **Micrococcus**



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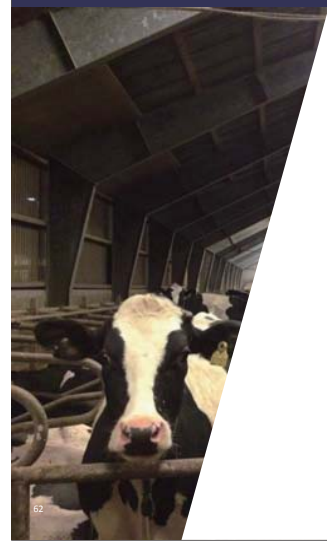
牛乳中微生物含量–丹麥
Standard Methods



Typical content of bacteria in milk (DK):

Raw milk picked-up directly from the farm tank:
< 30,000 CFU/ml

Raw milk from a tanker when it arrives at the dairy:
approx. 100,000 CFU/ml

In pasteurized milk right off the plate heat exchanger:
< 100 CFU/ml

In pasteurized milk bought in a supermarket
< 10,000 CFU/ml

In UHT milk:
0 CFU

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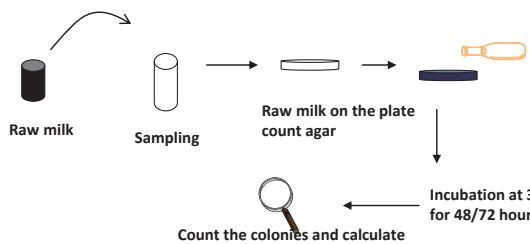
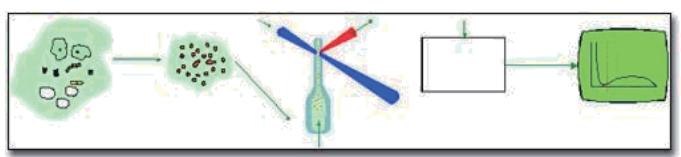
流式細胞法 vs 平板法
Flow Cytometry vs Standard Plate Count



Standard Plate Count



BactoScan FC+
Flow cytometry

- Less than 10 min instead of 2 - 3 days
- Fully automated – reduced human factor
- Total bacteria count - Individual Bacteria (IBC)-9min in first sample, 65, 130, 200 samples/hr

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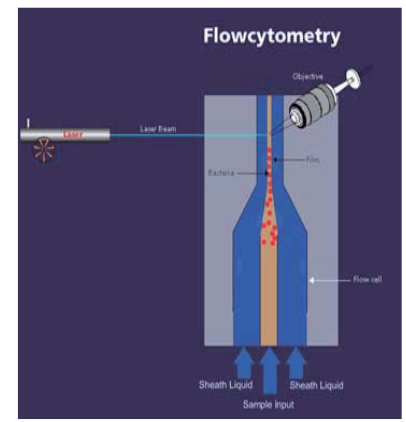
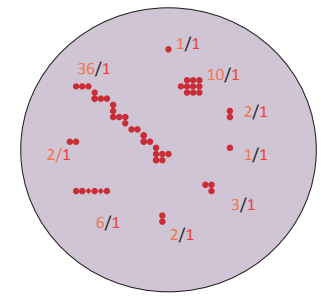
流式細胞法 vs 平板法
Flow Cytometry vs Standard Plate Count

Flow Cytometry results based on individual cell count





Standard plate count method based on colony forming units (CFU)

Black numbers = individual count, grey numbers = CFU. Individual cell count will generally give higher bacteria count that gives a truer indication of quality – see notes.

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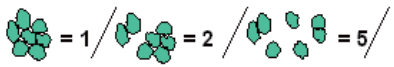
流式細胞法 vs 平板法
Flow Cytometry vs Standard Plate Count

Standard Plate Count / CFU



BactoScan FC / IBC

☺ **The BactoScan FC counts the single bacteria and all bacteria present in the raw milk =>**

- **Giving a very realistic measurement of hygienic quality**
- **This method offers a uniform and high performance in repeatability, reproducibility and accuracy => High reliability**

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嗜熱菌與嗜冷菌
Standard Methods

Why are psychrotrophic/-phillic, thermophilous and coliform bacteria of special interest?

Psychrotrophic/-phillic bacteria

- **Multiply despite chilling**
- **Often produce enzymes (lipases and proteases) = deteriorate end product**

Thermophilous bacteria

- **Survive very high temperature f.inst. 90°C for 30 secs. (pasteurization).**
- **Some are spore forming species. (Spores are the form of certain type of bacteria that survive extreme conditions)**
- **Some can be pathogenic and some can be capable of producing undesirable gas e.g. in cheese and canned food**

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生乳總菌數監測之目的
Monitoring of Tanker Raw Milk

- High bacteria content in the raw milk means low quality products with poor shelf-life and an increased risk of food borne diseases
- Monitoring allows serious contamination to be detected instantly and poor quality milk can be rejected
- Improvement of raw milk quality
- Monitoring of incoming tanker milk
- Payment for raw milk

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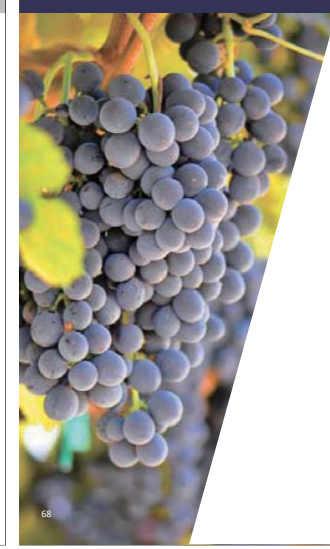
基準方法與轉換曲線-ISO 21187 / IDF196
Reference Methods and Conversion Tables



INTERNATIONAL STANDARD

ISO 21187

IDF 196

First edition
2004-12-01

Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results

Lait — Mesure quantitative de la qualité bactériologique — Lignes directrices pour établir et vérifier une relation de conversion entre les résultats de la méthode de routine et les résultats de la méthode d'ancrage

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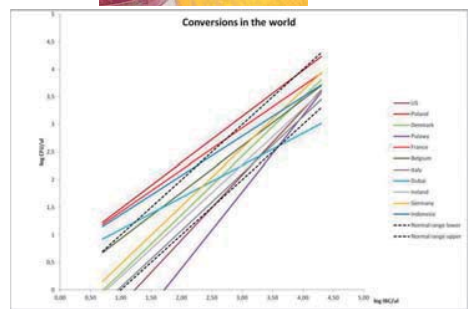
基準方法與轉換曲線-ISO 21187 / IDF196
Reference Methods and Conversion Tables

- The reference method is standard plate count (SPC), resulting a bacteria count in CFU/ml (Colony Forming Units).
- SPC is carried out by manually spreading a small subsample on agar in a petri dish and incubating for 2 days(!) at 37° C.
- Counts how many bacteria colonies that can grow
  - Only counts living bacteria.
  - One cluster of living bacteria may end up forming a single colony.
- Take a sample a send it to various labs: the Bactoscans will agree while the CFU count can vary wildly!
- In many countries legislation and the payment system is based on CFU counts and a conversion table is thus used to convert between the two numbers.
- The correlation between IBC and CFU is poor a varies from country to country (note log scale on the figure!)



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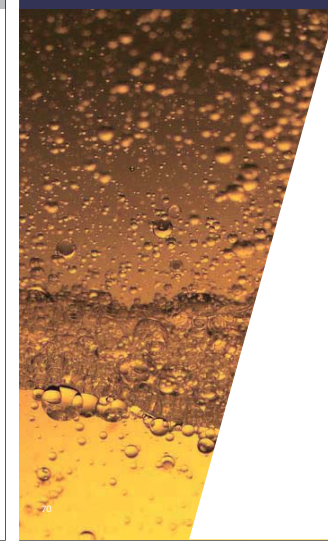
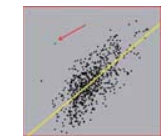
不同國家之IBC vs CFU 轉換曲線
To Convert or Not to Convert



- One fixed **national conversion** table for many years:
  - Germany, U.S., New Zealand, Ireland, Poland, Belgium
- **Several conversion** tables: Portugal
- One conversion table **per instrument**:
  - Holland (rolling conversion table)
- **No conversion**:
  - Norway. Ring test counting IBC. Payment limits in A+, B, C etc.
  - Canada.
- **International** conversion table - working group for developing one conversion table at the level of European Union



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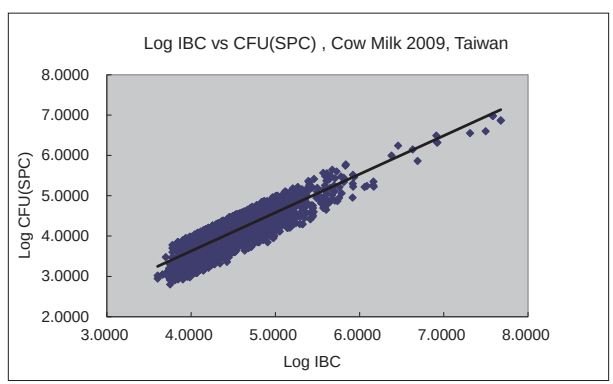
台灣 IBC vs CFU 轉換曲線
IBC vs CFU Conversion Table Cow milk, Taiwan

BactoScan FC IBC/CFU Conversion Table, Cow Milk, Taiwan, 2009
International Standard IDF 196 / ISO 21187



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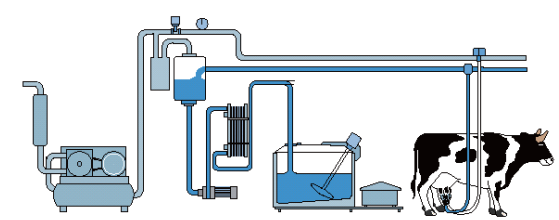
榨乳設備清潔度之監測
Microbial Contamination Sources

- Within the udder (mastitis bacteria)
- Exterior of udder
- Milking equipment
- Bulk tank (growth during storage)



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乳車、收乳站、儲乳槽之監控
Collection of Milk

- Every 24 or 48 hours
- A sample is taken for analyses once a week

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優良產品及食品安全之願景
Goodproduct- A Strong Vision

GoodProduct™

Tools to secure an effective and safe supply chain

Screen the feed for abnormalities

Screen the milk & dairy product for abnormalities

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