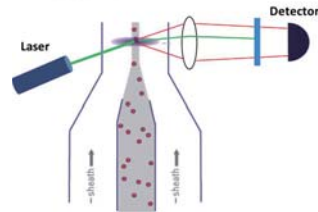
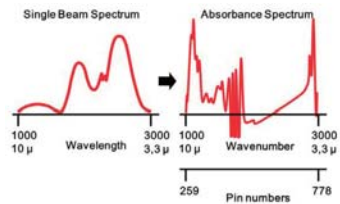




原料乳品管快篩科技
Screening Technology for Quality Assessment of Raw Milk

孫丕忠 Terence Sun
August 2nd 2019, TLRI, Tainan, Taiwan



健康衛生 Health / Hygiene

生乳總生菌數含量快速檢測技術
Rapid Method for Total Bacteria Count in Raw Milk

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



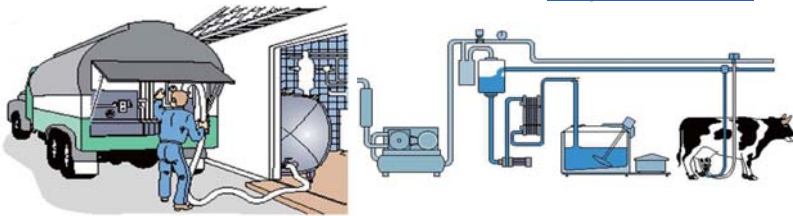
- High bacteria content in the raw milk means low quality products with poor shelf-life and an increased risk of food borne diseases
- Monitoring allows serious contamination to be detected instantly and poor quality milk can be rejected
- Improvement of raw milk quality
- Monitoring of incoming tanker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

生乳中微生物的來源 Source of Bacteria in Raw Milk

FOSS

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

Temperature < 10 ° C



5

生乳中微生物之種類 Types of Bacteria

FOSS

Bacteria present in milk and growth optimum

Temperature < 10 ° C

中溫菌-不繁殖 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

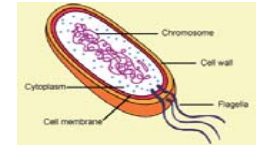
嗜熱菌-不繁殖 No thermophilic bacteria can reproduce

- 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

嗜冷菌-繁殖 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**



6

嗜熱菌與嗜冷菌 Thermophilous and Psychrotrophic/-phillic Bacteria

FOSS

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

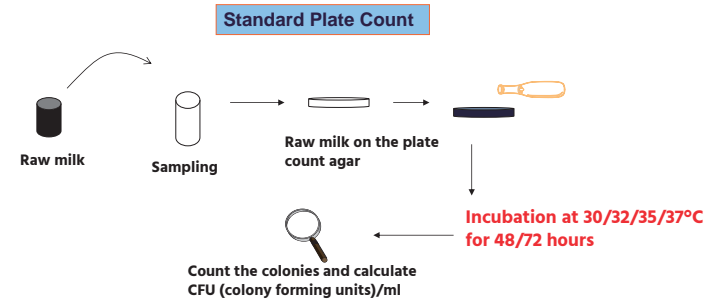
- Survive very high temperature f.inst. 90°C for 30 secs. (pasteurization).
- Some are spore forming species. (Spores are the form of certain type of bacteria that survive extreme conditions)
- Some can be pathogenic and some can be capable of producing undesirable gas e.g. in cheese and canned food



7

平板法 Standard Plate Count Method, SPC

FOSS



8

IDF / ISO 標準方法及ICAR指南 IDF / ISO Standard & ICAR Guidance

FOSS

INTERNATIONAL
STANDARD

ISO
21187
IDF
196

First edition
2004-12-01



SCSA: STANDING COMMITTEE ON STATISTICS AND AUTOMATION

- S01 Statistics of analytical data - Interlaboratory study result of other (Permanent group)
- S09 Reference system for somatic cell counting
- S11 Revision of ISO 21187 | IDF 196 - Quantitative determination of bacteriological quality**
- S12 Guidelines for the use of in-line infrared spectrometry
- S13 Guidance on the application of a conversion equation for quantitative determination of bacteriological quality in milk
- S14 Milk -- Definition and evaluation of the overall accuracy of alternative methods of milk analysis -Part 3
- S15 Improvement of the reference method for somatic cell counting (for discussion of NW1)
- S16 Milk and liquid dairy products -- Guidelines for the application of near infrared spectrometry [extension of ISO 21543|IDF 201: 2006]
- S17 Revision of IDF/ISO standard for Milk and milk products -- Sensory analysis -- Part 3
- S18 Milk - Bacterial count - Protocol for the evaluation of alternative methods

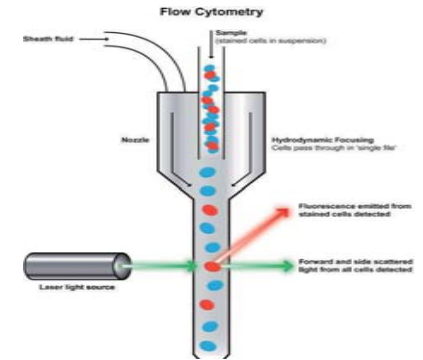
Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results

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

流式細胞儀檢測原理 Principle of Flow Cytometry

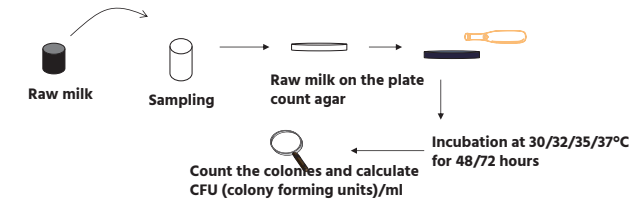
FOSS

- ▶ Based in **Flow Cytometry**
- ▶ Bacteria are stained with a fluorescent dye
- ▶ Injected into the Flow Cell in a single file
- ▶ Laser intercept cell in flow cell
- ▶ Detector converts the light emitted into electronic signal
- ▶ IBC count is shown in the display



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

FOSS



Standard Plate Count

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

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

FOSS



INTERNATIONAL
STANDARD

ISO
21187
IDF
196

First edition
2004-12-01

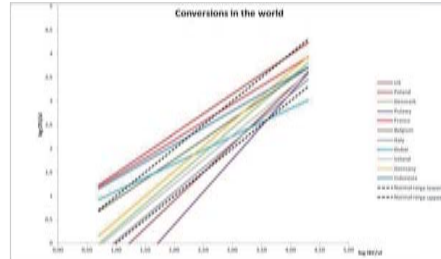
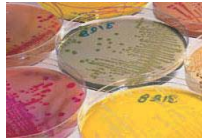
Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results

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

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

FOSS

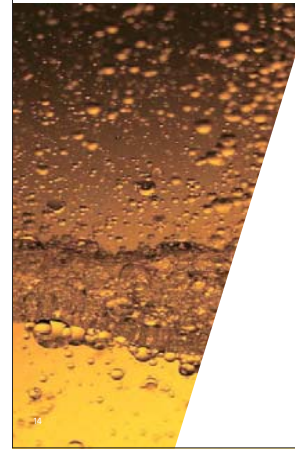
- The reference method is standard plate count (SPC), resulting a bacteria count in CFU/ml (Colony Forming Units).
- SPC is carried out by manually spreading a small subsample on agar in a petri dish and incubating for 2 days(!) at 37° C.
- Counts how many bacteria colonies that can grow
 - Only counts living bacteria.
 - One cluster of living bacteria may end up forming a single colony.
- Take a sample and 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!)



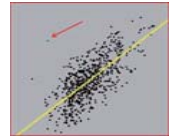
13

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

FOSS



- 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 B, C etc.
 - Canada.
- International** conversion table - working group for developing one conversion table at the level of European Union



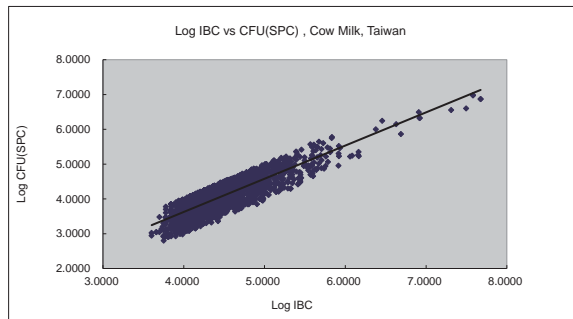
14

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

FOSS



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



15

生乳品質 Raw Milk Quality

FOSS

生乳品質快速檢測技術 Rapid Method for Raw Milk Quality

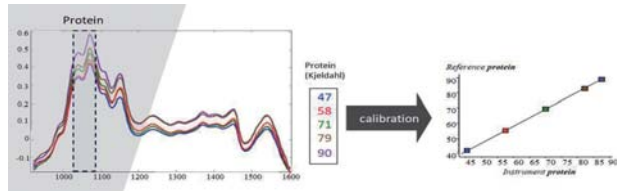
ANALYTICAL SERVICES

16

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

FOSS

- 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 (FTIR) spectroscopy is the state-of-the-art method for acquiring MIR spectra analyzing:
 - Fat, Protein (true & crude), Casein, Lactose, Solids, Urea, Citric Acid, Free Fatty Acids, PH, Freezing Point Depression, Fatty Acids, Ketosis Screening (BHB and Acetone), Abnormal Milk Screening.....



17

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

FOSS



IDF 447 Published 2010



IDF 490 Published 2017

18

ICAR & IDF 2019 會議 – FTIR 新的檢測模式 ICAR 2019 Conference – Focus on New Tools

FOSS

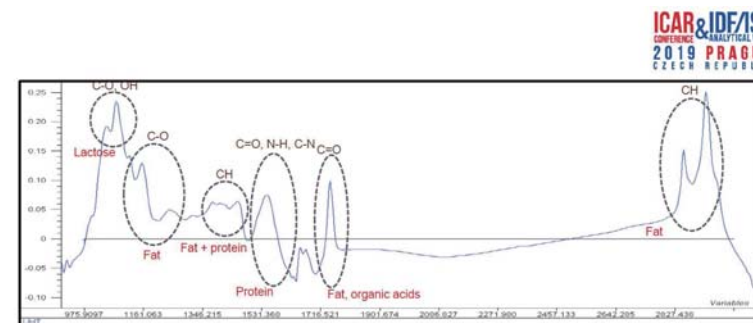
Hall Panorama

Time	Session Title	Chairpersons
8:30-10:30	Technical Session 7 Challenges in Creating Additional Value from Milk Analysis	Silvia Orlandini and Jere High
8:30-8:50	S07(T)-DP-1 Additional value of cell differentiation in the course of DHI testing	Folkert Onken
8:50-9:10	S07(T)-DP-2 Pregnancy testing in dairy cows using a PAG test in milk samples: Different thresholds for different stages of the pregnancy	Daniel M. Lefebvre
9:10-9:30	S07(T)-DP-3 New quality assurance challenges with recent mid-infrared models	Frédéric Dehareng
9:30-9:50	S07(T)-DP-4 Implementation of a routine Fourier-transform infrared procedure for fatty acid analysis in milk	Daniel M. Lefebvre
9:50-10:10	S07(T)-DP-5 Routine infrared phosphorous determination in ex-farm milk giving better insight in the phosphorous cycle on dairy farms	Harrie van den Bijgaart
10:10-10:30	Question and Discussion	

19

生乳 FTIR 光譜 Mid IR Spectra of Liquid Milk

FOSS



- Position of the peaks → Qualitative analysis
- Intensity of the peaks → Quantitative analysis

20

FTIR 可檢測項目 A Broader Range of Robust Calibrations

FOSS

Mass Balance/Standardization/Payment

Fat,
Protein (true & crude),
Casein
Lactose,
Total Solids
Solids non Fat
Urea
Ketosis Screening (BHB and Acetone)

End Product Control

Low Lactose
Glucose
Galactose

Quality Components

pH
Total Acids
Citric acids
Density
Fatty Acids Profiling (SFA, MUFA, PUFA, SCFA, MCFA, LCFA.....etc.)
Free Fatty Acids
Freezing Point Depression

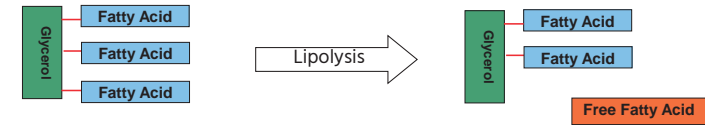
Milk Parameters

20+ standard milk components
All included with the FTIR
+ ASM Module (Abnormal Milk Screening, Adulterant Screening)

21

游離脂肪酸 Free Fatty Acids

FOSS



影響游離脂肪酸含量之可能因素 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

22

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

FOSS

游離脂肪酸太高導致之問題 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



檢測游離脂肪酸之應用 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
- ◆ by well designed and monitored milking machines and bulk tanks
- ◆ by consistent balanced cow diets
- ◆ by proper drying-off procedures

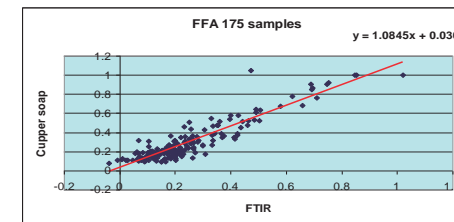
23

以FTIR技術檢測游離脂肪酸之優點 FFA Calibration and Benefits with FTIR Tech.

FOSS

FTIR Milk Analyzers 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



- ◆ 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 mmol FFA / 100g Fat is considered abnormally high / 'defective'

24

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

FOSS



FFA is today measured in:

- The Netherlands
- Norway
- France
- Belgium
- Japan
- Italy
- UK
- Canada
- USA
- Denmark
- Spain
- Brazil
- The Czech Republic
- Taiwan

FFA included in Payment Scheme



25

冰點檢測 Freezing Point Depression (FPD)

FOSS

Has someone added water to the milk?

Freezing point of authentic ab farm milk is quite constant and lower than **-0.520°C degrees Celsius (ca -0.538 "Hortvet")**, and has therefore for a long time been used as a milk quality parameter.

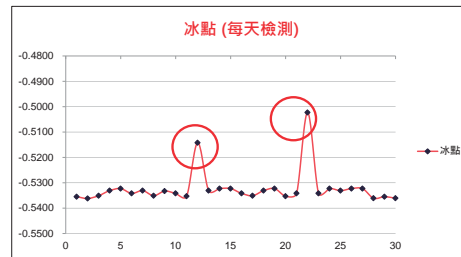
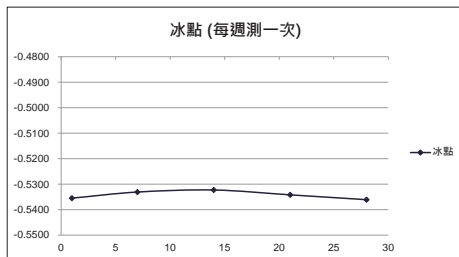
In 1989, J. Koops et al proved that combining data from **Infra Red analyzer** with that of **electrical conductivity** can give a good prediction of the actual freezing point of a milk sample.

FTIR technology is the third generation IR analyzers used for this purpose, with which we can now provide an **accuracy of < 4 m°C**, which ensures an extremely **efficient screening** and very **low cost** per positive sample.

26

冰點檢測之應用 Application of Freezing Point Depression (FPD)

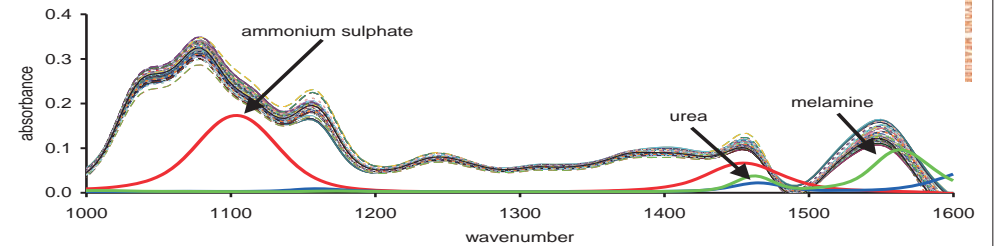
FOSS



27

牛乳中摻假檢測 Milk Adulteration

FOSS

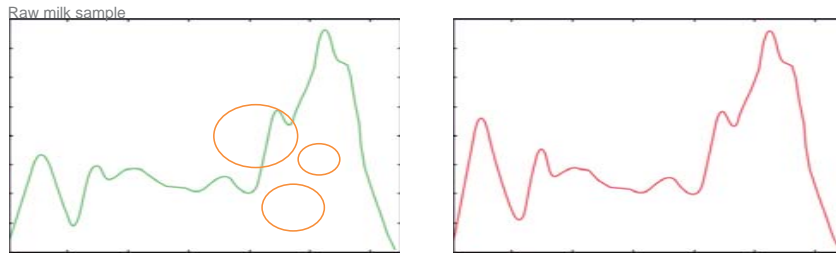


28

正常 vs 不正常 牛乳光譜 Normal vs Abnormal Spectrum

FOSS

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



29

摻假物質篩檢

FOSS

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

30

發生乳中摻假之可能原因 Screening for Milk Adulteration

FOSS

非故意的 Unintentional deviations

Caused by accidents or failures.

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

刻意的 Intentional deviations

Driven by economic gains:

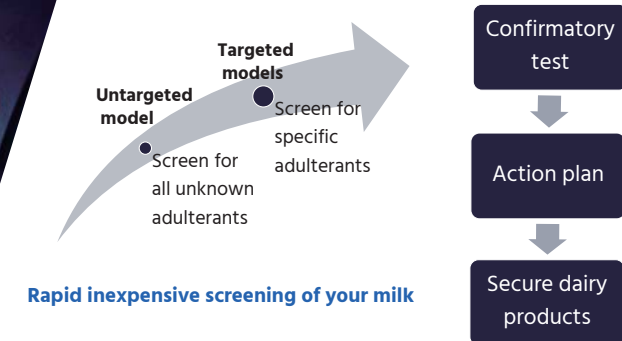
- amount (weight and/or volume)
- milk fat content
- milk protein content
- dry matter content
- the total bacterial counts and somatic cell counts



31

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

FOSS



32

生乳藥物殘留快速篩檢技術

Screening Methods for Residues of Veterinary Medicines



IDF/ISO Welcome reception

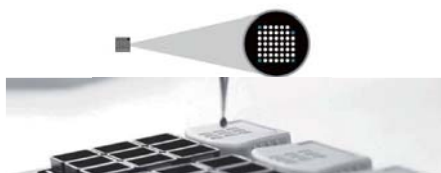
SCAMAC: STANDING COMMITTEE ON ANALYTICAL METHODS FOR ADDITIVES AND CONTAMINANTS

- A05 Milk, milk products and infant formulae – guideline for the quantitative determination of melamine and cyanuric acid by LC-MS/MS
- A10 Guidelines for the validation of screening methods for residues of veterinary medicines

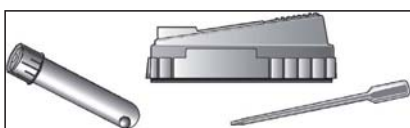
SCAMC: STANDING COMMITTEE ON ANALYTICAL METHODS FOR COMPOSITION

- C19 Milk – Determination of acetone content by continuous flow analyzer/Milk – Determination of beta-Hydroxybutyric acid (BHB) content by continuous flow analyzer
- C27 Dried milk – Determination of titratable acidity and extension of its scope to raw milk
- C28 Titratable acidity of milk fat
- C32 Milk, dried milk and cream – Determination of fat content – Rose Gottlieb method
- C33 Determination of individual proteins
- C34 Cheeses and processed cheeses, caseins and caseinates – Determination of fat content – SBR method
- C35 Determination of urea in milk by continuous flow analyzer

Biochip Array Technology



ELISA Technology



Fluorescence Technology

PAY AS YOU NEED



健康 衛生

Health / Hygiene

- Flow Cytometry
- 總生菌數快速檢測 IBC

生乳品質

Raw Milk Quality

- FTIR Technology
- 游離脂肪酸、冰點及摻假 FFA, FPD & ASM

藥物殘留

Residues of Veterinary Medicines

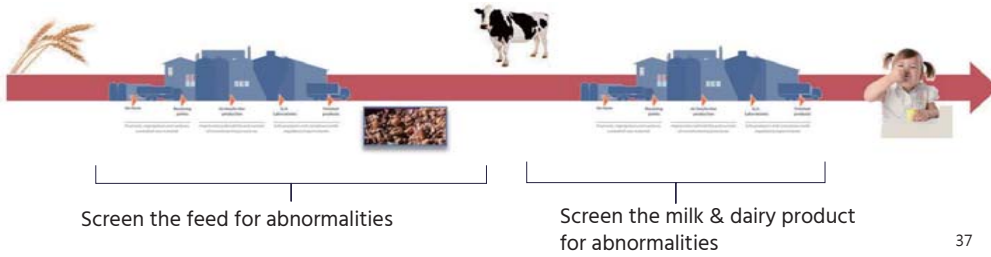
- ELISA, Fluorescence, Biochip Array Technology
- 藥物殘留快速篩檢

優良食品安全之願景
Goodproduct – A Strong Vision

FOSS



Tools to secure an effective and safe supply chain



FOSS

THANK YOU

ANALYTICS BEYOND MEASUREMENT