NOVEL TECHNOLOGIES OF QUALITY IN THE SHEEP & GOAT DAIRY SECTOR

By George Psathas and Ouranios Tzamaloukas

HIGHLIGHTS OF PRESENTATION

- THE KEY INDUSTRY TRENDS
- THE FORUM OF SHEEP & GOAT DAIRY SECTOR
- APPLICABILITY AND REFORMATION OF ISO STANDARD METHODS
- THE ROLE OF CENTRAL TESTING LABORATORY IN SECTOR DEVELOPMENT
- NOVEL METHODS IN MILK COMPOSITION – FTIR
- NOVEL METHODS IN MILK HYGIENE – FLOWING CYTOMETRY FOR TBC AND SCC
- SCREENING METHODS FOR ANTIBIOTICS RESIDUES AND AFLATOXIN M1 DETECTION
- QUALITY ASSURANCE IN THE LAB
- APPLICATION OF LIMS
- MILK ADULTERATION AND MILK MIXTURES IDENTIFICATION
- AUTHENTICITY OF SHEEP & GOAT PDO PRODUCTS

THE KEY INDUSTRY TRENDS

- GOVERNMENT SUPPORT OF MILK PRODUCTION WITH REFORMATION OF FARMS AND EDUCATION OF PRODUCERS
- ANIMAL HEALTH AND NUTRITION – ENVIROMENTAL IMPACT
- ADAPTATION TO CONSUMERS DEMANDS FOR ORGANIC PRODUCTS
- BETTER QUALITY – FLAVOR AND TEXTURE BASED ON NATURAL INGREDIENTS OF RAW MILK
- PRODUCT SAFETY – CONTROL OF DESEASES, MICROBIAL CONTAMINATIONS AND CONTROL ON RESIDUES OF ANTIBIOTICS AND AFLATOXIN M1 FROM THE FARM TO THE FOLK OF CONSUMER

THE FORUM OF SHEEP & GOAT DAIRY SECTOR

AN INTERIM OF 30 YEARS 1985 – 2015 WITH 7 SYMPOSIUMS WITH APPROXIMATE 800 SCIENTIFIC WORKS IN RAW MATERIAL, PROCESSING AND PRODUCT, PRODUCTS CHARACTERISTICS, AND MARKETING STRATEGIES

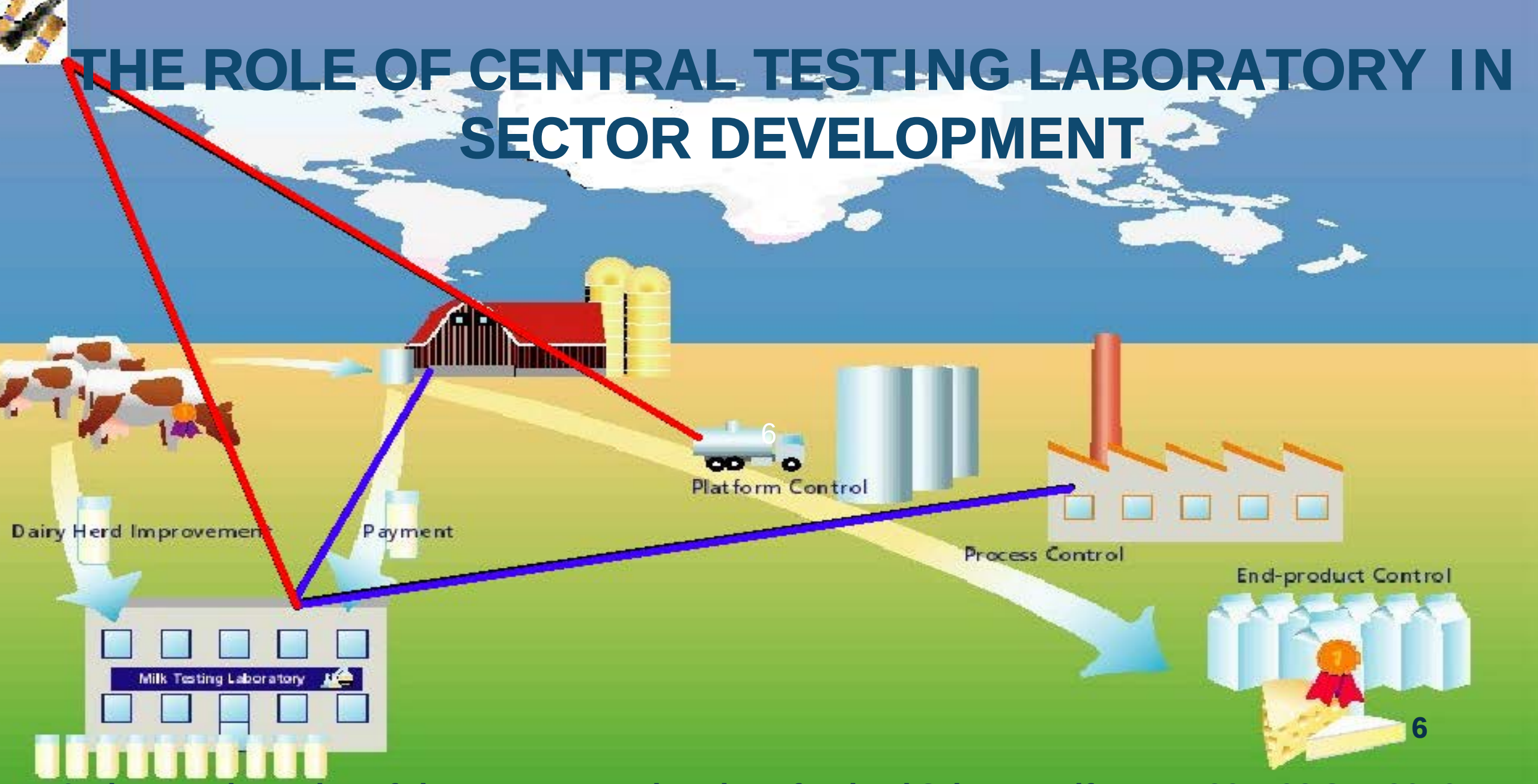
POSITIVE OUTCOMES ON MILK QUALITY:

- *REFORMATION OF MILK TESTING METHODS*
- *REVEAL OF FATTY ACIDS BIOLOGICAL VALUE IN HUMAN NUTRITION*
- *PROTEINS POLYMORFISM AND THE CONNECTION TO MILK PRODUCTION AND CHEESE YIELD*
- *GENETIC CHARACTER OF LACTIC ACID BACTERIA IN FERMENTED PRODUCTS* – **PROBIOTICS AND BACTERIOCINS PRODUCTION**

APPLICABILITY AND REFORMATION OF ISO - IDF STANDARD METHODS

- ✓Fat in milk: - Rose Gottlieb (FIL/IDF 1D) – Reference method
- ✓Nitrogen in milk – Kjeldahl (FIL/IDF 20B: 1993) – Reference method
- ✓Freezing Point in milk (FIL/IDF 108:2001 Draft) – Reference method
- ✓Urea in milk (FIL/IDF- ISO 14637 Draft) – Reference method
- ✓Somatic cells count in milk (FIL/IDF 148A: 1995) – Reference method
- ✓Microbial inhibitor tests in milk (ISO 13969/IDF 183 Draft)
- ✓Determination of Fat, Protein and Lactose content in milk (FIL/IDF 141A:1990- Mid IR guidance) – Routine method
- ✓Milk - Quantitative determination of bacteriological quality - Guidance on evaluation of routine methods (FIL/IDF 161B)

THE ROLE OF CENTRAL TESTING LABORATORY IN SECTOR DEVELOPMENT



THE ROLE OF CENTRAL TESTING LABORATORY IN SECTOR DEVELOPMENT

- PRODUCERS MILK PAYMENTS ACCORDING TO QUALITY – SCHEMES OF COMPOSITION, TBC, SCC, ANTIBIOTICS, AFLATOXIN M1 DETECTION AND MILK ADULTERATION PENALTIES
- MILK RECORDING ACTIVITIES – HERD MANAGEMENT (NUTRITION, BREEDING CONTROL)
- COVERING NEEDS OF EU REG. 853/2004 IN CONCERN OF MILK HYGIENE

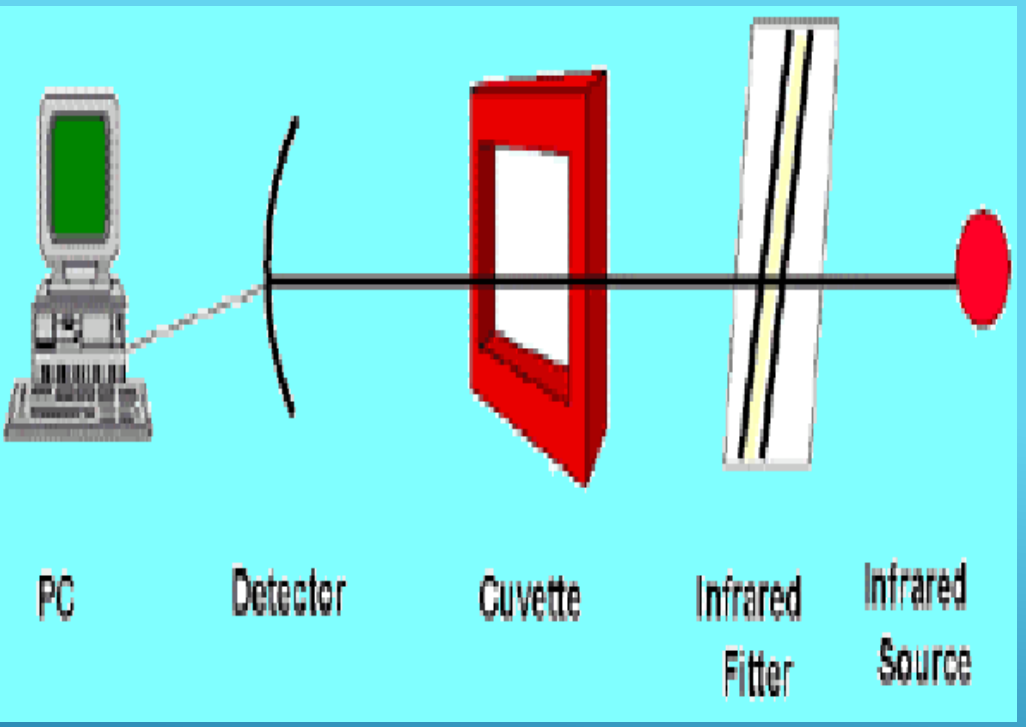
THE ROLE OF CENTRAL TESTING LABORATORY IN SECTOR DEVELOPMENT

CHARACTERISTICS OF THE LABORATORY:

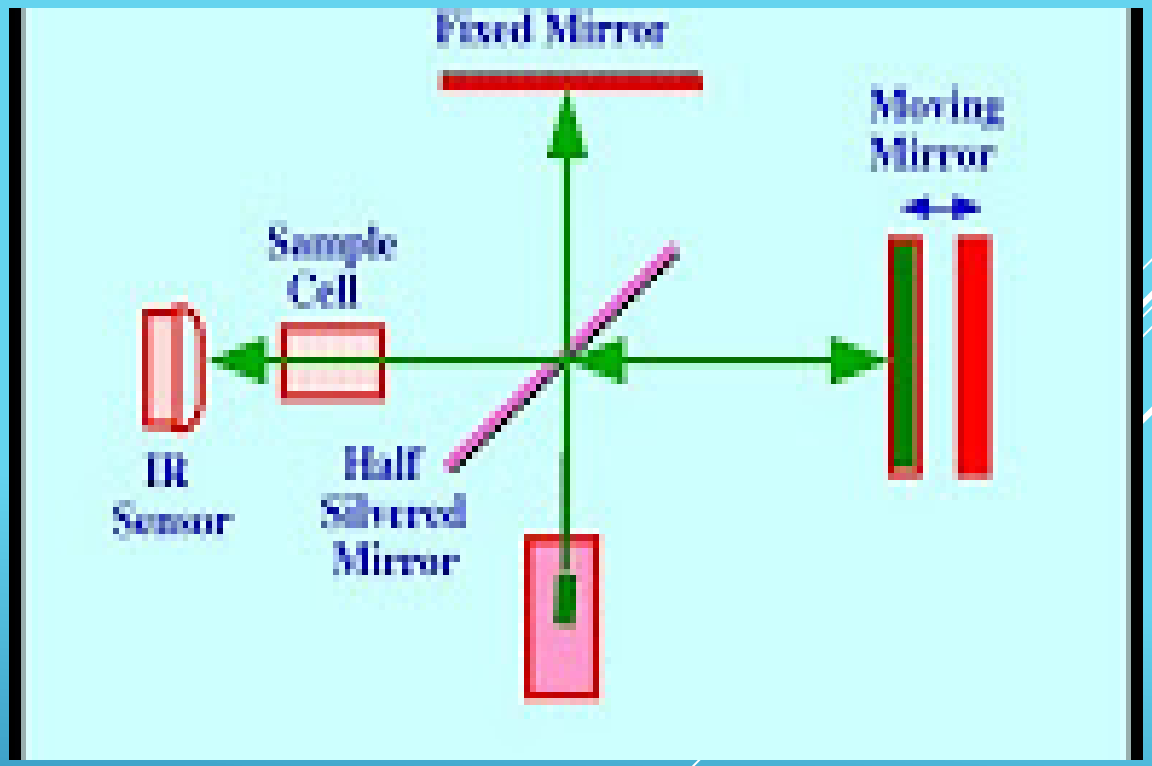
- MASSIVE NUMBER OF MILK SAMPLES
- AUTOMATIC SAMPLING PROCEDURES BY ROAD TANKERS DURING MILK COLLECTION AND DELIVERY TO PROCESSORS
- AUTOMATIC DETECTION OF PRODUCERS AND PROCESSORS AND CAPTURE OF SAMPLING DATA BY THE USE OF GPS AND GSM
- USE OF RFID SYSTEM FOR MILK RECORDING IN HERD MANAGEMENT
- AUTOMATIC SAMPLES REGISTRATION AND PREPARATION OF BATCHES FOR ANALYSIS
- USE OF AUTOMATIC INSTRUMENTS FOR ANALYSIS
- HANDLE OF ANALYTICAL DATA PRODUCED BY THE USE OF LIMS
- RAPID FINAL RESULTS UP TO 48 HRS AND INTERESTED CUSTOMERS BRIEFING THROUGH INTERNET OR CELLULAR PHONE

NOVEL METHODS IN MILK COMPOSITION - FTIR

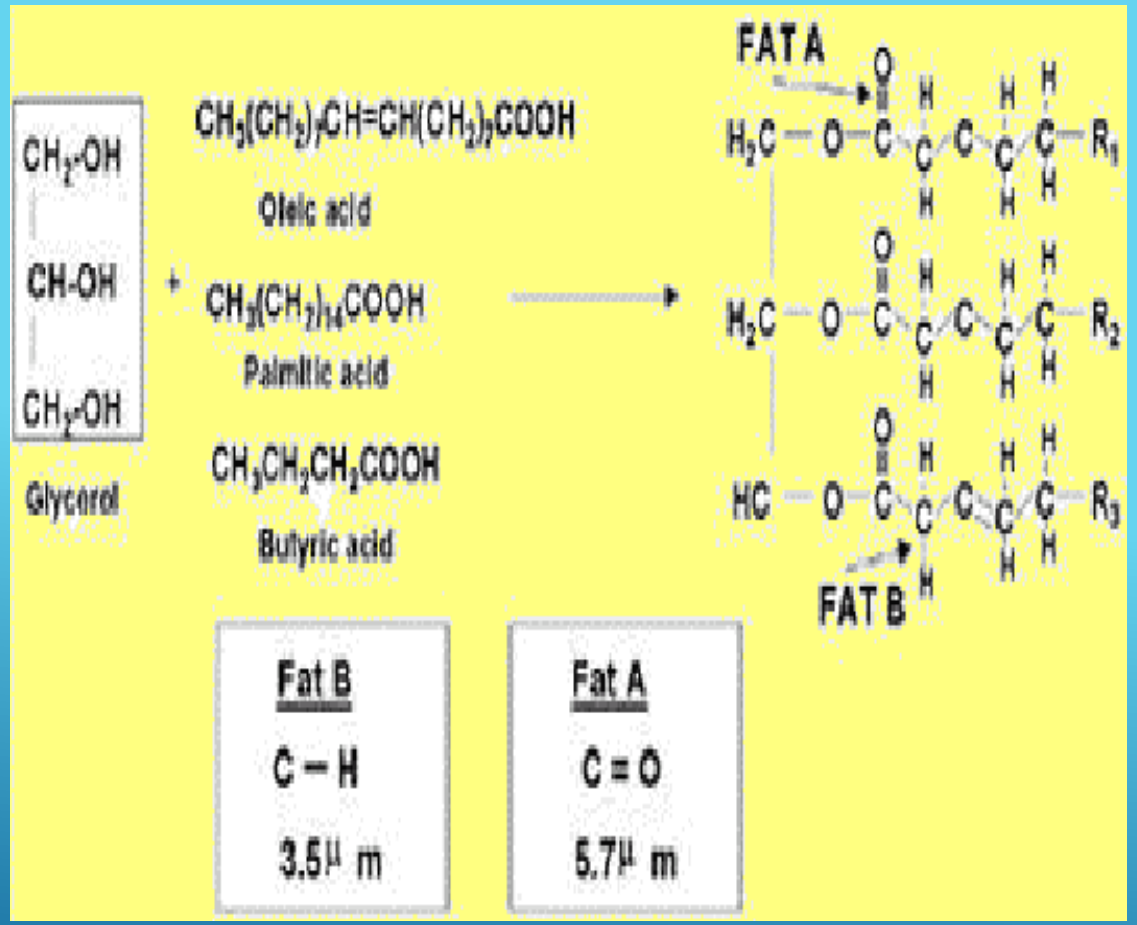
PREVIOUS FILTER TECHNIQUE

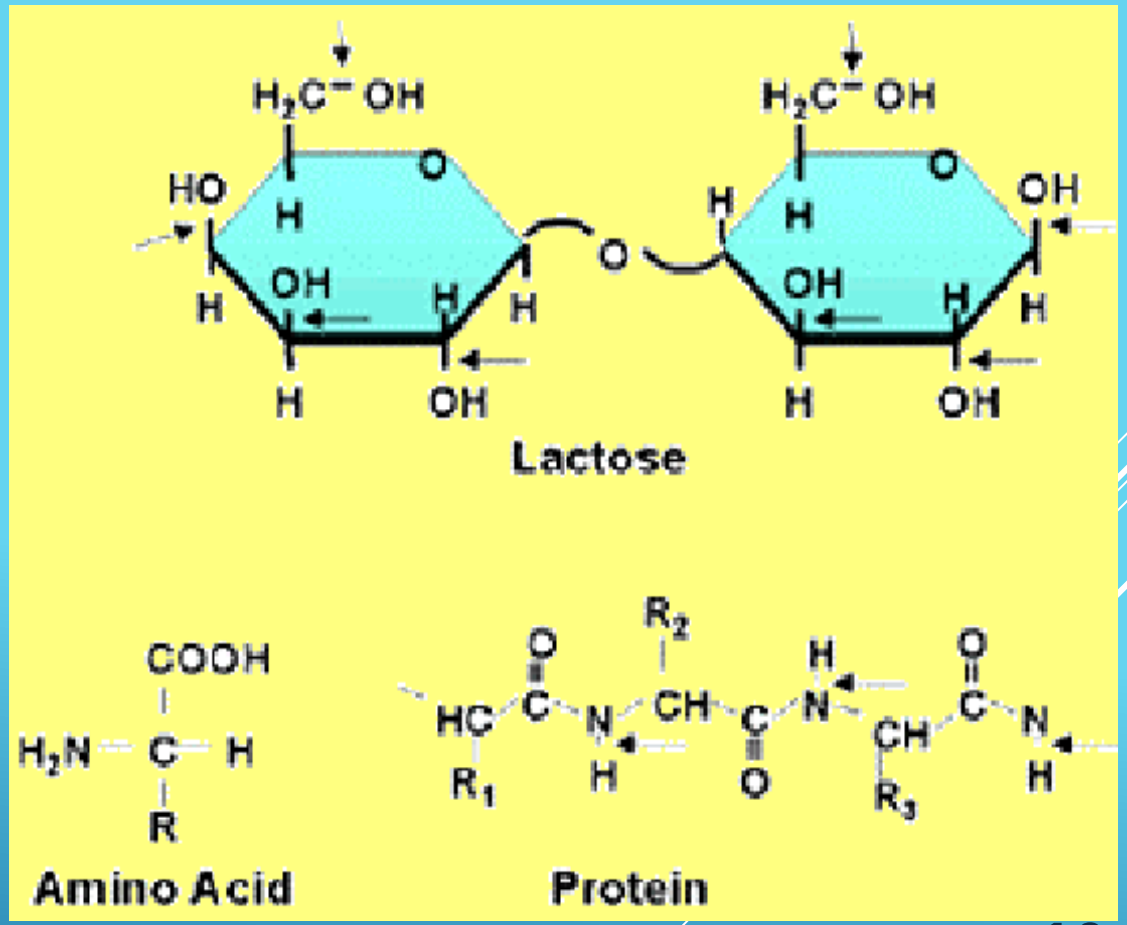


NOVEL INTERFEROGRAM TECHNIQUE



NOVEL METHODS IN MILK COMPOSITION - FTIR





NOVEL METHODS IN MILK COMPOSITION - FTIR

Component	Measuring range	Performance range	Repeatability	Accuracy Bulk	Accuracy Single animal
Fat (A)	0-15%	2-15%	Cv < 0.5%	Cv < 1.0%	Cv < 1.5%
Fat (B)	0-15%	2-15%	Cv < 0.5%	Cv < 1.0%	Cv < 1.5%
Protein	0-10%	2-10%	Cv < 0.5%	Cv < 0.9%	Cv < 1.5%
Lactose	0-10%	2-10%	Cv < 0.5%	Cv < 0.9%	Cv < 1.5%
Solids	0-20%	2-20%	Cv < 0.5%	Cv < 1.0%	Cv < 1.5%
Urea	10-100mg/dl	10-100mg/dl	Sd <1.5mg/dl	Sd < 3mg/dl	Sd < 3.5mg/dl
Citric Acid	0.1-0.5%	0.1-0.5%	Sd< 0.005%	Sd < 0.01%	Sd < 0.015%
FPD(Screening)	400-600 m°C	450-550 m°C	Sd <0.5 m°C	Sd <4 m°C	N/A
Component	Calibration Range	Validation Range			
Casein	2.17 - 3.45%	2.17 - 3.24%	N/A	N/A	N/A
FFA(mmol/10kg)	0.072 - 10.04	0.49 - 6.63	N/A	N/A	N/A
Ketosis Screening (acetone>0.15mmol/lt, acetoacetic acid, and beta-hydroxybutyric acid – BHB>0.1mmol/lt)					

NOVEL METHODS IN MILK COMPOSITION - FTIR

Component	Calibration Range	Validation Range
Saturated Fatty Acids	0.87 - 5.08%	0.87 - 5.08%
Mono Unsaturated Fatty Acids	0.31 - 2.84%	0.31 - 2.84%
Poly Unsaturated Fatty Acids	0.03 - 0.36%	0.03 - 0.36%
Unsaturated Fatty Acids	0.35 - 5.39%	0.35 - 5.39%
Trans Fatty Acids	0.04 - 0.08%	0.04 - 0.08%
Short chain Fatty Acids	0.35 - 0.44%	0.35 - 0.44%
Medium chain Fatty Acids	1.58 - 2.06%	1.58 - 2.06%
Long chain Fatty Acids	0.38 - 3.48%	0.38 - 3.48%
C:14_0*	0.16 - 1.38%	0.16 - 1.38%
C:16_0*	0.37 - 4.48%	0.37 - 4.48%
C:18_0*	0.11 - 1.25%	0.11 - 1.25%
C:18_1*	0.26 - 4.29%	0.26 - 4.29%

** FA g/100g milk (applies to all fatty acid profile components). ** Calculated component.*

NOVEL METHODS IN MILK COMPOSITION - FTIR

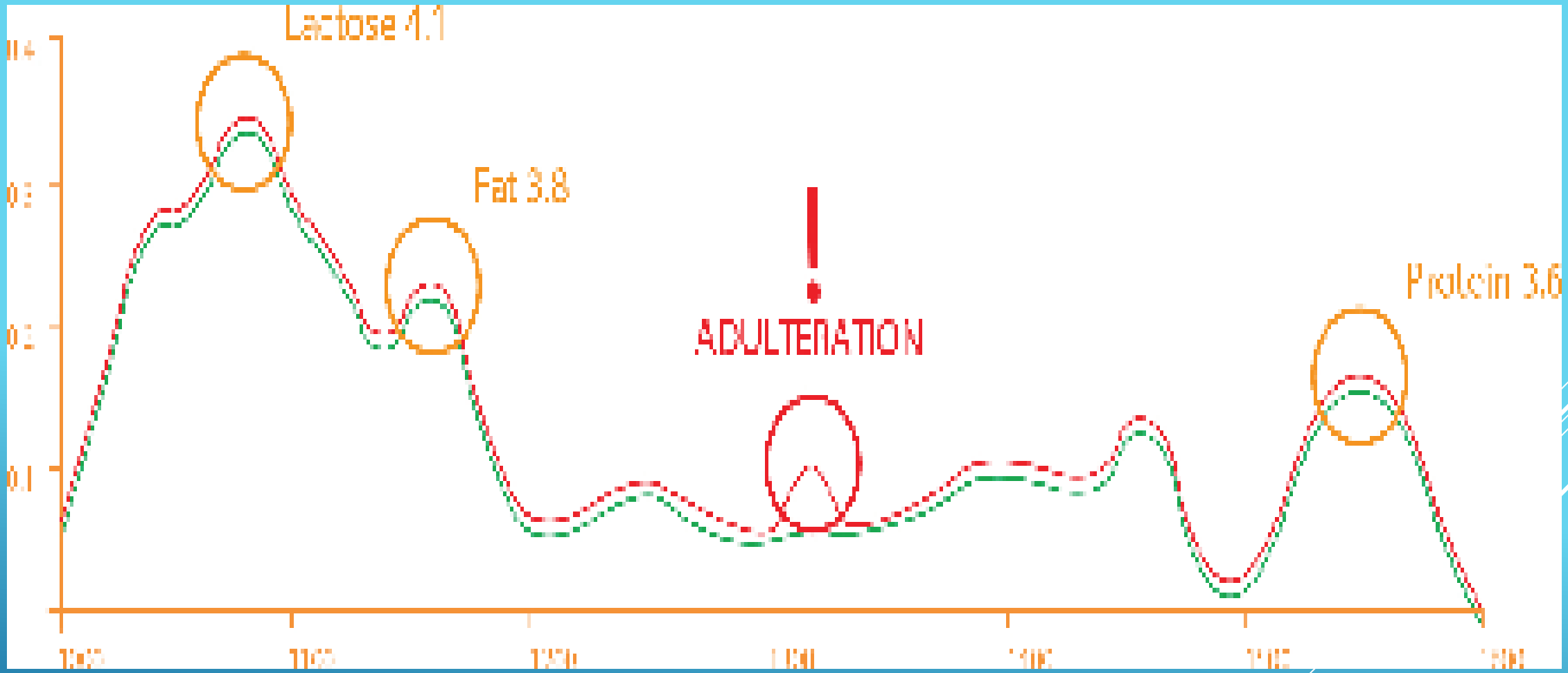
Targeted models

Targeted models can be used to quantify the content of known adulterants such as Hydroxyproline, Sodium Nitrite, Melamine, Maltodextrine and Cyanuric acid.

It can only predict the concentration of the adulterant for which it was developed. If there is a need to monitor other known adulterants, specific targeted models will have to be used for each of these.

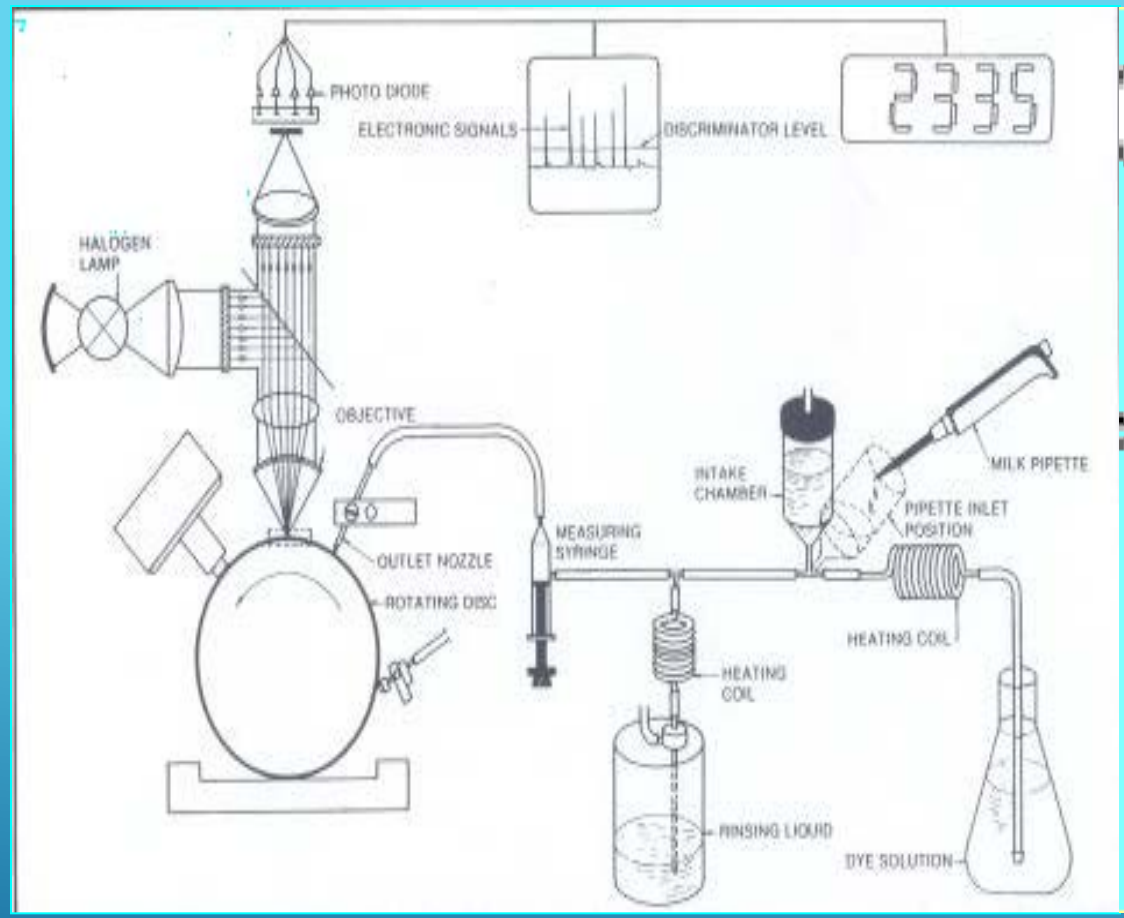
With a targeted model, a sample of milk is tested against a profile for normal milk adulterated with a known substance. A warning is given if there is a match and a measurement of the quantity of adulterant is given.

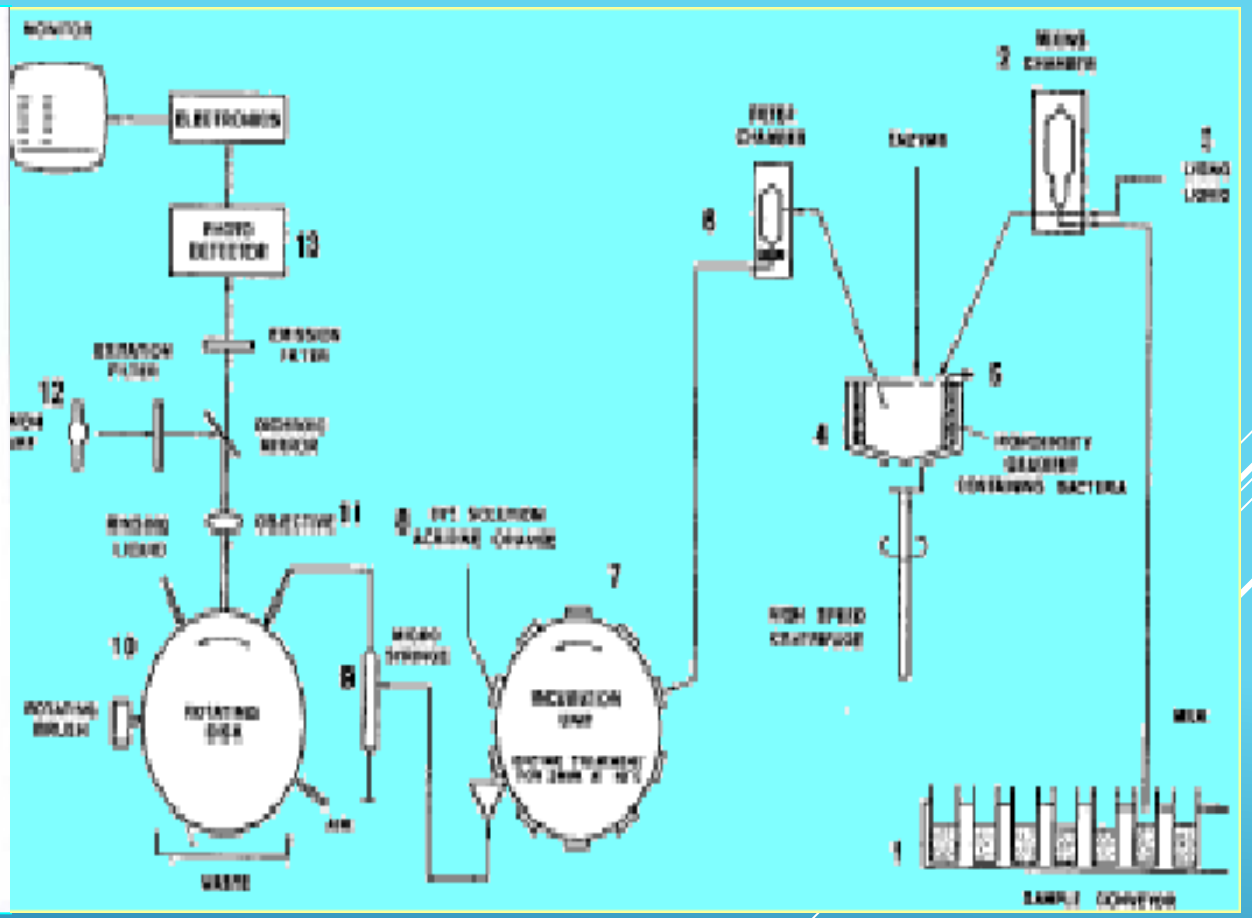
NOVEL METHODS IN MILK COMPOSITION - FTIR



NOVEL METHODS IN MILK HYGIENE – FLOWING CYTOMETRY FOR TBC AND SCC

PREVIOUS TECHNOLOGY OF ROTATING DISC FOR SCC AND BACTERIA COUNTING





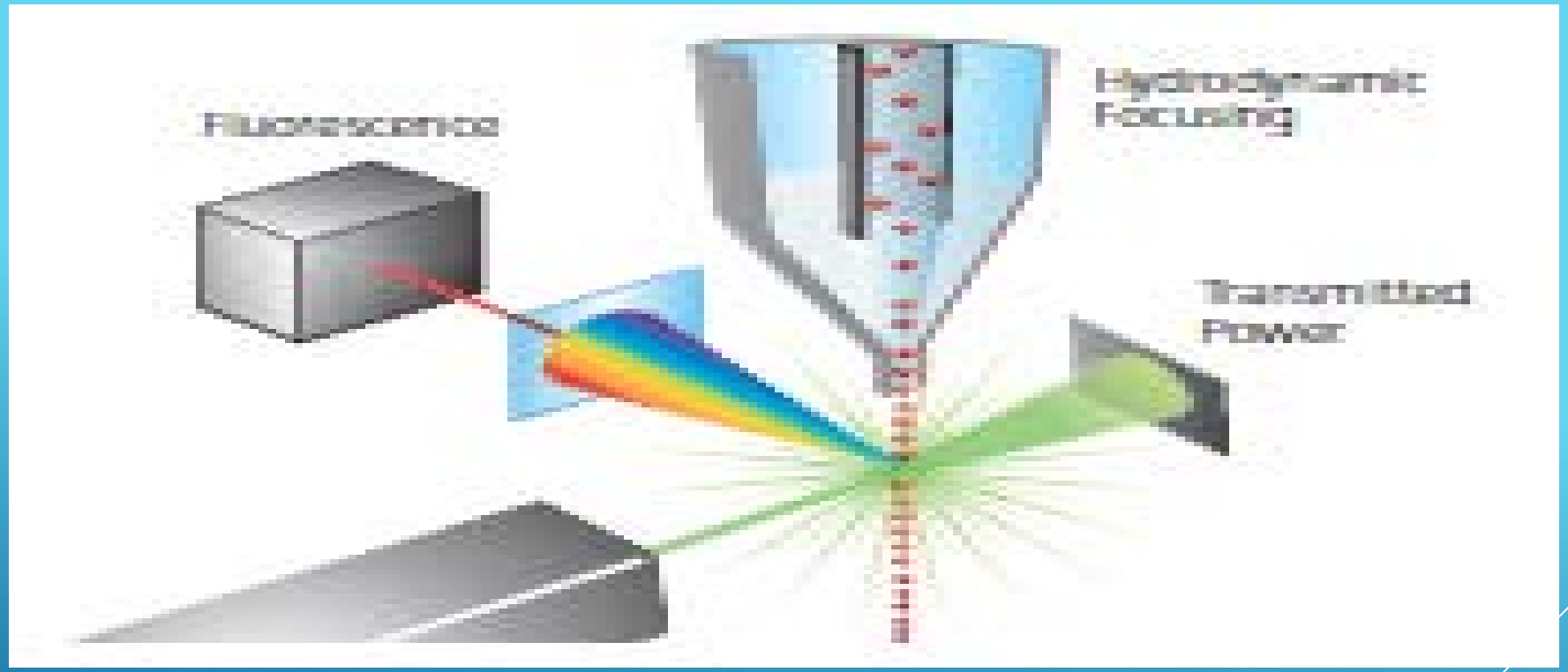
NOVEL METHODS IN MILK HYGIENE FLOWING CYTOMETRY FOR TBC

PRINCIPLE OR THE NOVEL METHOD

- MILK IS SAMPLED AND DISPENSED IN A HEATED CAROUSEL AT 50 °C
- ADDITION OF PROTEOLYTIC ENZYME TO BRAKE DOWN THE MILK MICELLES
- ADDITION OF FLUORESCENT MARKER OF MICROBES DNA
- SONICATION OF SAMPLE IN ORDER TO IMPROVE MICROBIAL CELLS DETECTION
- TRANSFER OF SAMPLE TO FLOW CYTOMETER AND EXPOSURE TO INTENSE LASER BEAM AND FLUORESCENCE
- THE SIGNAL IS COLLECTED BY OPTICS, FILTERED AND DETECTED BY FOTO MULTIPLIER
- PULSES INTENSITY AND HEIGHT ARE RECORDED AND TRANSLATED IN INDIVIDUAL BACTERIA

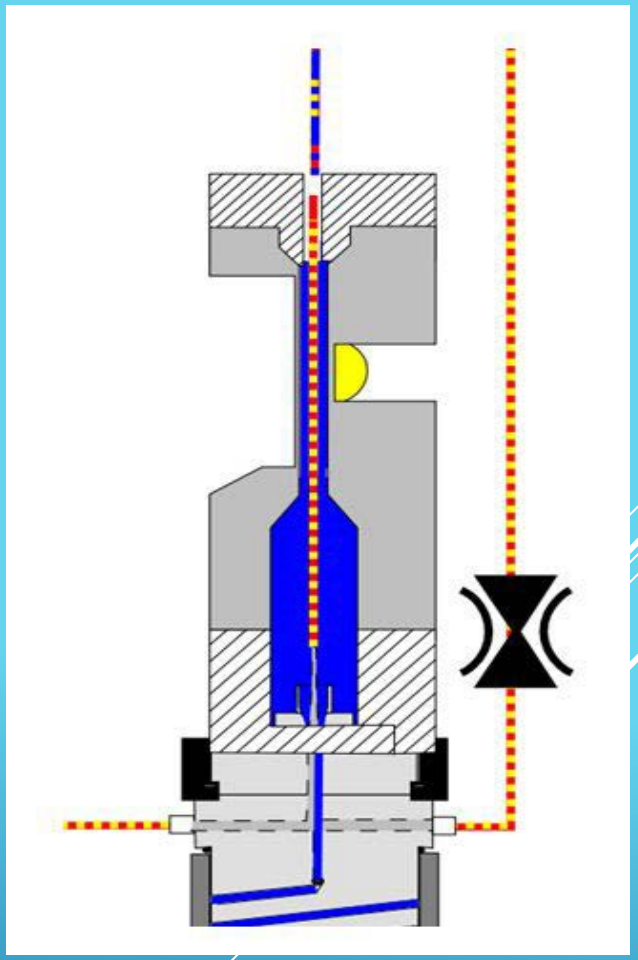
NOVEL METHODS IN MILK HYGIENE FLOWING CYTOMETRY FOR TBC

PRINCIPLE OF THE NOVEL METHOD



NOVEL METHODS IN MILK HYGIENE FLOWING CYTOMETRY FOR SCC

- How does FC works
- The FC counts somatic cells based on recognition of DNA from the cells.
- A mixture of milk and staining solution is surrounded by a sheath liquid and passed through a flow cell. In the flow cell, the stained somatic cells are exposed to light of a specific wavelength. The cells then emit fluorescent light pulses at a different wavelength, the pulses are counted and displayed.
- The design of the flow cell ensures that only one somatic cell is detected at a time. Higher precision can be obtained at grading limits using precision set-up feature.



SCREENING METHODS FOR ANTIBIOTICS RESIDUES DETECTION

DIRECTIVE 96/23 EC - MRLs of Residues of Harmful substances

- **ALL COMMERCIAL METHODS ARE AIMING IN MEETING THESE MRLs**
- **The methods are classified in Qualitative and Quantitative**
- **Choice of method depends on milk quantity and time limits**
- **All methods are applied with same practice for all milk species**
- **Confirmatory testings are required on positive results of 1st screening**

SCREENING METHODS FOR ANTIBIOTICS RESIDUES DETECTION

Actual antibiotic residue test kits available on the market

Manufacturer	Name of test	Detection limits	Analysis time	Test method
AIM, Germany	BRT	Betalactam, 2 ppb	2,5 hrs	Qualitative
	*MCR3	Betalactam, <2 ppb	6 min	Quantitative
DSM, Netherlands	Delvotest®Accelerator	Betalactam, 2 ppb, Tetracycline, 40 ppb	1.5-2 hrs	Quantitative
	Delvotest® BLF	Betalactam, 4 ppb	5 min	Qualitative
Charm Science, USA	Charm II	Betalactam, 4 ppb, Tetracycline, 40 ppb	8-10 min 10-15 min	Qualitative
	Rosa Combo	Betalactam, 4 ppb, Tetracycline, 40 ppb	2 min	Qualitative
IDEXX Laboratories, USA	SNAP®	Betalactam, 4 ppb, Tetracycline, 50 ppb	10 min	Qualitative
Neogen, USA	Betastar®	Betalactam, 4 ppb	5 min	Qualitative
	Betastar® Combo	Betalactam, 4 ppb, Tetracycline, 40 ppb	5 min	Qualitative
Unisensor, Belgium	TwinSensor	Betalactam, 4 ppb, Tetracycline, 40 ppb	6 min	Qualitative

SCREENING METHODS FOR ANTIBIOTICS RESIDUES DETECTION

Commercial instruments available in the market

Fig. 1: AIM, MCR3

Fig. 2: Charm Science, Charm II

Fig. 3: DSM, Delvotest Accelerator

Fig. 4: Neogen, Betastar

QUALITY SYSTEM - QUALITY ASSURANCE

- Methods selection (Decision 2002/657/EC)
- Equipment operation
- Methods validation
- SOPs
- Quality Manual
- Controls (Standard samples, control charts)
- Proficiency testing
- Documentation
- Accreditation(17025)

APPLICATION OF LIMS IN THE CTLAB

- ✓ SAMPLES RECEPTION AND MATCHING PROCESS WITH SAMPLIND DATA
- ✓ BATCHES PREPARATION FOR ANALYSIS
- ✓ ANALYTICAL PROCEDURES
- ✓ HANDLING OF ANALYTICAL DATA WITH SAMPLING DATA
- ✓ STATISTICAL PROCESSING FOR MONTHLY CUSTOMERS REPORTS
- ✓ INDIVIDUALS REPORT FOR EVENTUAL CUSTOMERS
- ✓ HORIZONTAL TRACEABILITY PER SAMPLE IN CASE OF RECALLS OF INDVIDUAL RESULTS
- ✓ VERTICAL TRACEABILITY OF QUALITY ASSURANCE MEASURES OF INDVIDUAL ANALYSIS
- ✓ STATISTICAL REPORTS PER CLAIMING PERIOD
- ✓ APPLICATION OF CUSTOMERS BRIEFING THROUGH INTERNET OR GSM CELLULAR PHONE
- ✓ REAGENTS AND CONSUMABLES REPORTS- EARLY WARNING ON DEFICIENCIES
- ✓ KEEPING RECORDS ON EQUIPMENT MAINTENANCE, SPARE PARTS REPLACE
- ✓ ACCREDITATION PROCEDURES REPORTS ACCORDING TO QUALITY MANUAL
- ✓ MONTHLY/YEARLY REPORT ON LAB EXPENSES
- ✓ ANNUAL REPORT ON COST EFFECTIVENESS PER PARAMETER PER SAMPLE

MILK ADULTERATION AND MILK MIXTURES IDENTIFICATION

Current Methods

- Electrophoresis - gel (WP, C)
- - Capillary electrophoresis
- Chromatographic methods RP-HPLC (WP, C)
- Immunological methods (WP, C)
- Polymerase Chain Reaction (somatic cells DNA)

MILK ADULTERATION AND MILK MIXTURES IDENTIFICATION

Markers of cow's milk and methods used for their detection (~80 papers in last 10 years)

Marker	Methodology	
α_{s1}-CN	PAGE, PAGE-SDS, IEF, CE, para-κ-casein	PAGE, CE, IEF
γ-CN	PAGE-SDS, IEF	
β-Lg	HPLC, FPLC, IEF, CE	
α-La	IEF, CE, MALDI-TOF	
IgG	ELISA	
Casein fractions	CE, HPLC	
CMP	ELISA	

AUTHENTICITY OF SHEEP & GOAT PDO PRODUCTS

- CHARACTERISTICS OF RAW MILK (animals specie, composition of non adulterated raw product, animal breed, local vegetation, specific aromatic compounds, trace elements and metals)
- CLOTTING ENZYMES used for cheese making - Specific cultures used or Eco flora isolated in product
- PROCESSING PRACTICES in product manufacturing
- RIPENING PROCESS for cheese (character of cheese – soft, semi hard, hard – characteristic peptides, amino acids in hard cheese)
- CHARACTERISTIC TEXTURE of cheese, holes in cheese..
- FLAVOR AND AROMA

THANK YOU FOR YOUR ATTENTION