



# Methods for the Detection of Bovine Milk in Milk of Minor Species

Rajan Sharma

**NDRI - Karnal**

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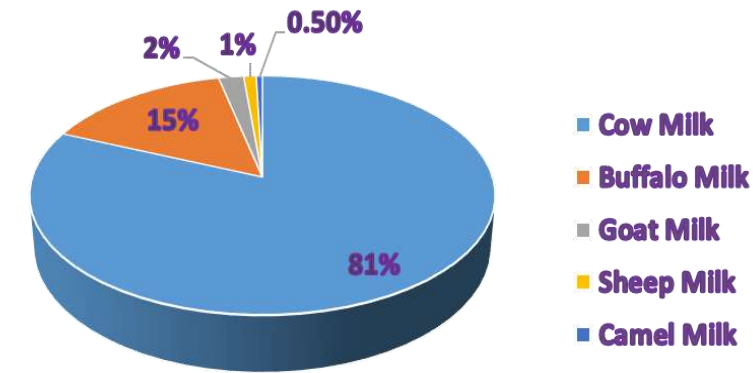


# Introduction

- ✓ Species authentication of milk is a requirement of **regulatory bodies of many countries**
- ✓ FSSAI has defined standards of milk of different species such as *cow, buffalo, goat, sheep and camel*
- ✓ Technological reasons & consumers' demand – driving the market of milk of a particular species
- ✓ **EU Regulations (2018/150)** – verification of cheese made from milk of minor species for presence of cow milk components

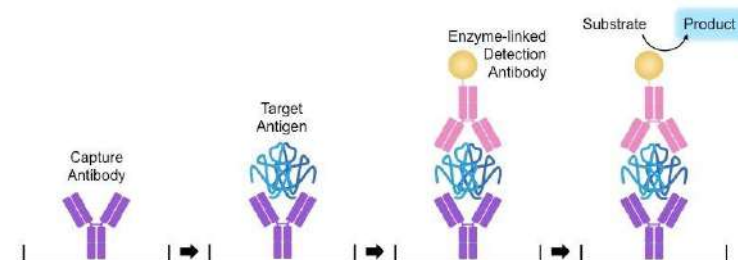
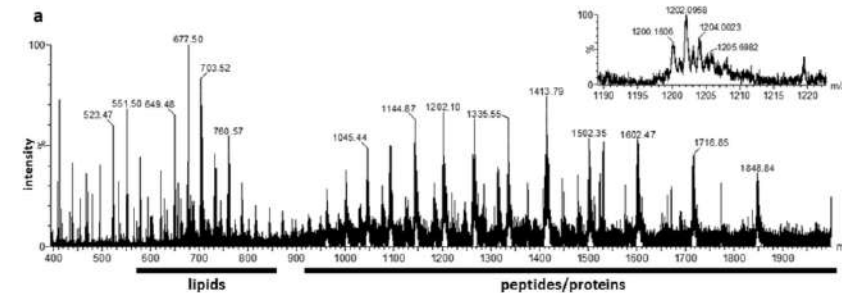
<https://www.fao.org/dairy-production-products/production/milk-production/en>

Global Milk Production (%) Breakdown (FAO, 2022)

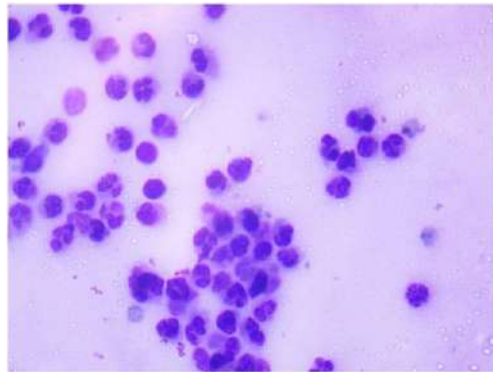


# Basis for the Authentication

- ✓ Species specific identification of DNA – PCR
- ✓ Protein-based detection methods
  - Immunological methods
  - Chromatographic
- ✓ Marker compounds in bovine milk



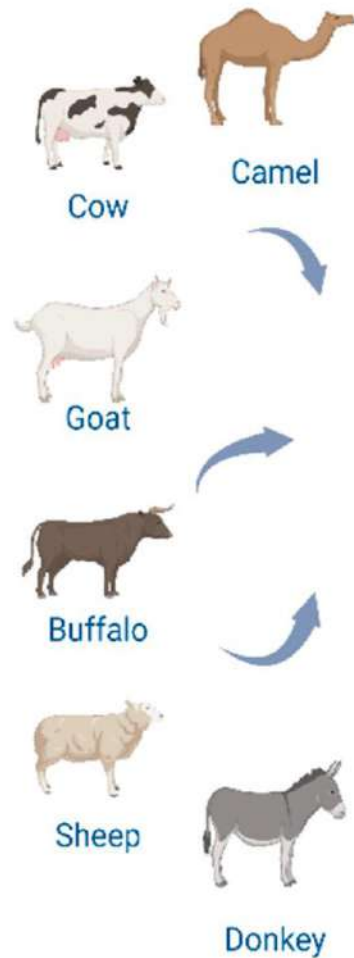
# Species specific identification of DNA



Somatic Cells

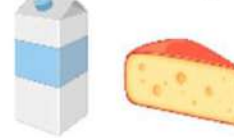
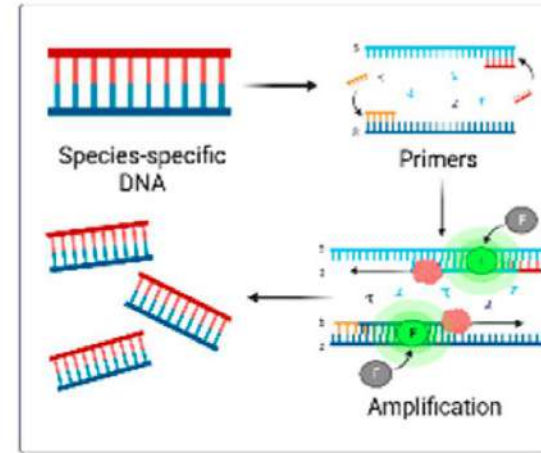
## DNA targets

- ✓ Mitochondrial DNA – high copy number
- 12S and 16S rRNA
- Cytochrome b
- D-loop sequence



Dairy products

DNA in milk originates from somatic cells



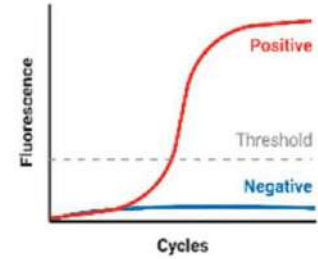
## DNA Extraction

- Organic solvent extraction
- Salting-out extraction
- Silicon centrifugal column extraction
- Magnetic bead extraction

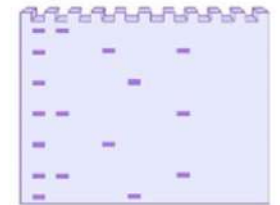


## PCR

- PCR-RFLP
- Multiplex PCR
- Real-time PCR
- Digital PCR
- RAPD-PCR



Amplification curves



Gel electrophoresis

PCR-RFLP: PCR-restriction fragment length polymorphism

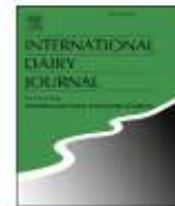
dPCR: digital PCR

RAPD-PCR: random amplified polymorphic DNA-PCR



Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: [www.elsevier.com/locate/idairyj](http://www.elsevier.com/locate/idairyj)

## Multiplex PCR for detection of camel milk adulteration with cattle and goat milk

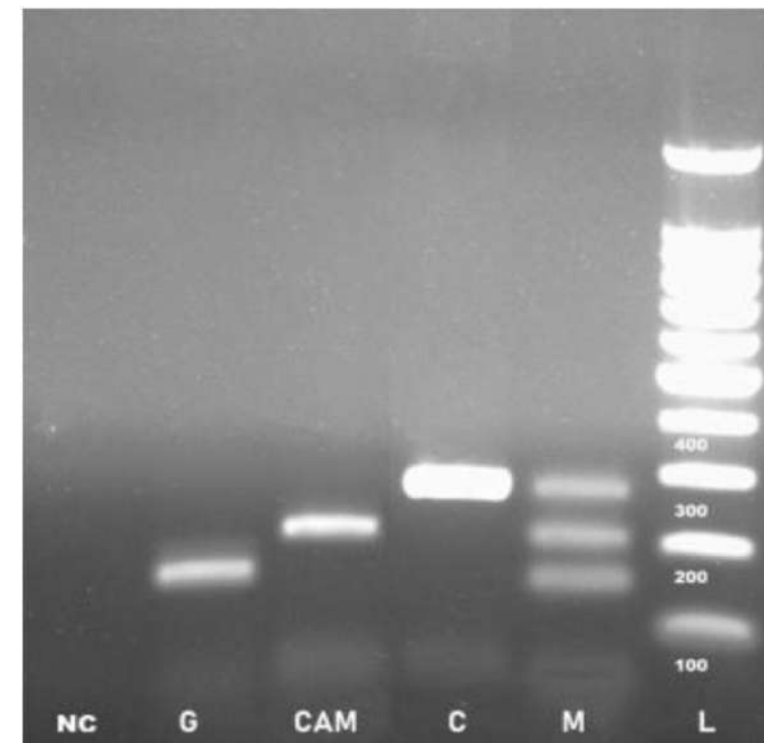
Deepraj Sarkar <sup>a</sup>, Rakesh Ranjan <sup>b</sup>, Sumnil Marwaha <sup>b</sup>, Artabandhu Sahoo <sup>b</sup>,  
Sanay Naha <sup>a, c, \*</sup>

<sup>a</sup> Department of Forensic Science, National Forensic Sciences University-Tripura Campus, Agartala, Tripura, 799001, India

<sup>b</sup> ICAR-National Research Centre on Camel, Bikaner, Rajasthan, 334001, India

<sup>c</sup> School of Advanced Sciences & Languages, VIT University, Bhopal, Madhya Pradesh, 466114, India

- Based on the amplification of the Cytochrome b gene.
- The developed technique successfully amplified the target fragment of 208 bp (Camel), 274 bp (cattle), and 174 bp (goat) of the gene.
- LOD of **cattle and goat milk** in camel milk was found to be **10% and 5%**, respectively.



Agarose gel electrophoresis of multiplex PCR products amplified from goat (G) 174 bp, camel (CAM) 208 bp, cattle (C) 274 bp. The M Lane is for sample containing mixture of DNA of goat, camel and cattle, NC lane is showing negative control and L lane is showing DNA ladder.

## SHORT COMMUNICATION

## Simplex PCR assay for detection of cow milk presence in goat milk

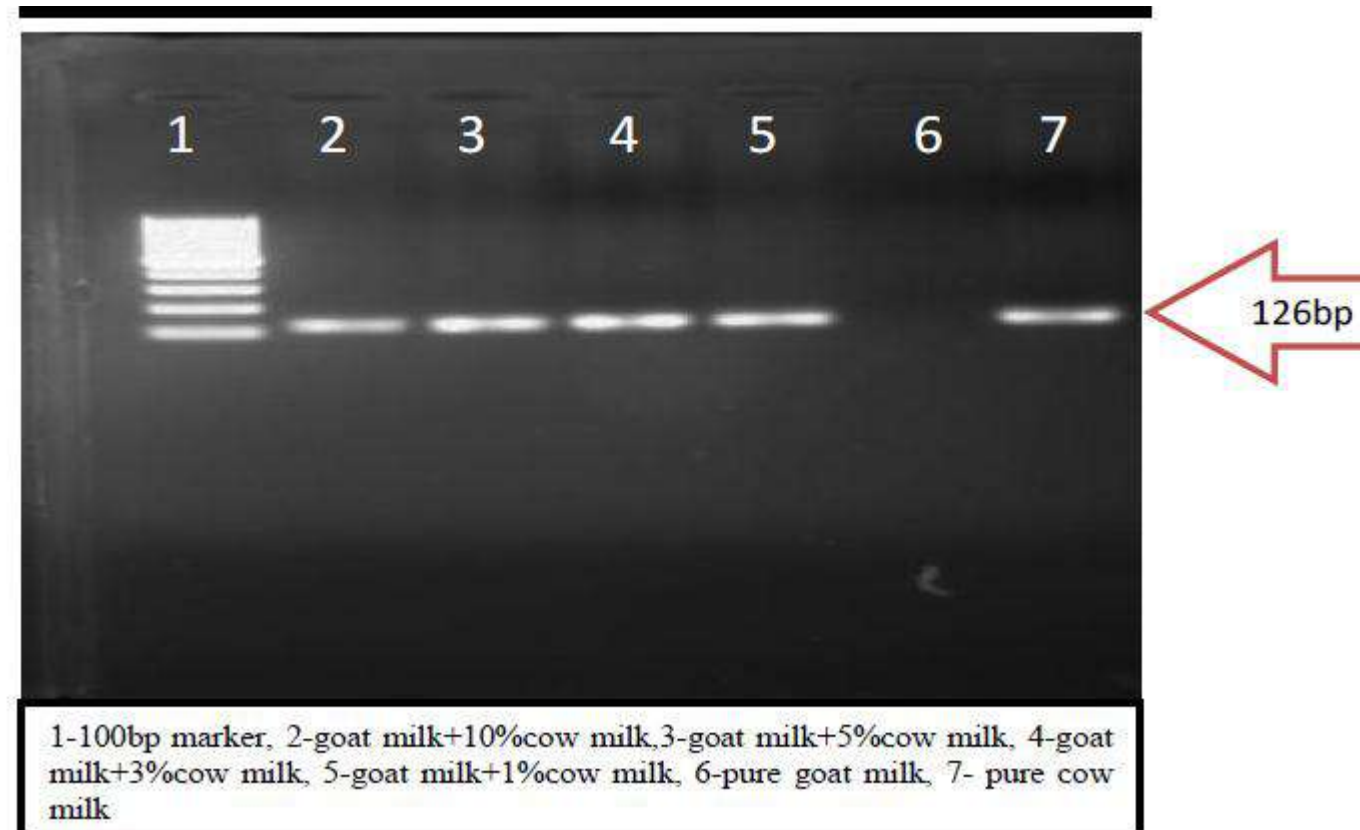
Tanmay Hazra, Vivek Sharma, Rekha Sharma and Sumit Arora

Dairy Chemistry Division

ICAR-National Dairy Research Institute, Karnal

Table 1. PCR oligonucleotide primers

|                     | Primer Sequence (5' to 3')   | PCR product size |
|---------------------|--|------------------|
| Universal(U)        | F: CCATCCAACATCTCAGCATGATGAAA<br>R: GCC CCT CAG AAT GAT ATT TGT CCT CA | 360bp            |
| P-1(cow specific)   | F:CGCCCATACACAGACCACAG<br>R: ATG CCT GGT AAA ATT CAT TAA ATA GCG       | 126bp            |
| P-2 (Goat specific) | F: TTCTTCAGGGCCATCTCATC<br>R: GCGGATGCATGGTGAAAT                       | 294bp            |



Bovine specific primers (P1) targeting D-loop (displacement loop) of mt-DNA (mitochondrial DNA) was selected and standardized to amplify cow DNA resulting into 126 bp amplicon.

# Technology available from NDRI



- ✓ DNA Based Method for Differentiation of Cow, Buffalo, Sheep, Goat and Camel Milk



DNA based method for Detection of  
**Cattle, Buffalo, Sheep,  
Goat and Camel MILK**



Test features:

- # Detection limit less than 5% mix
- # DNA based precise methodology
- # Result in less than 5 hours

Isolation and disruption of the milk somatic cells, protecting the nucleic acids and finally purifying them



DNA is processed further for tracking their origin of species by PCR

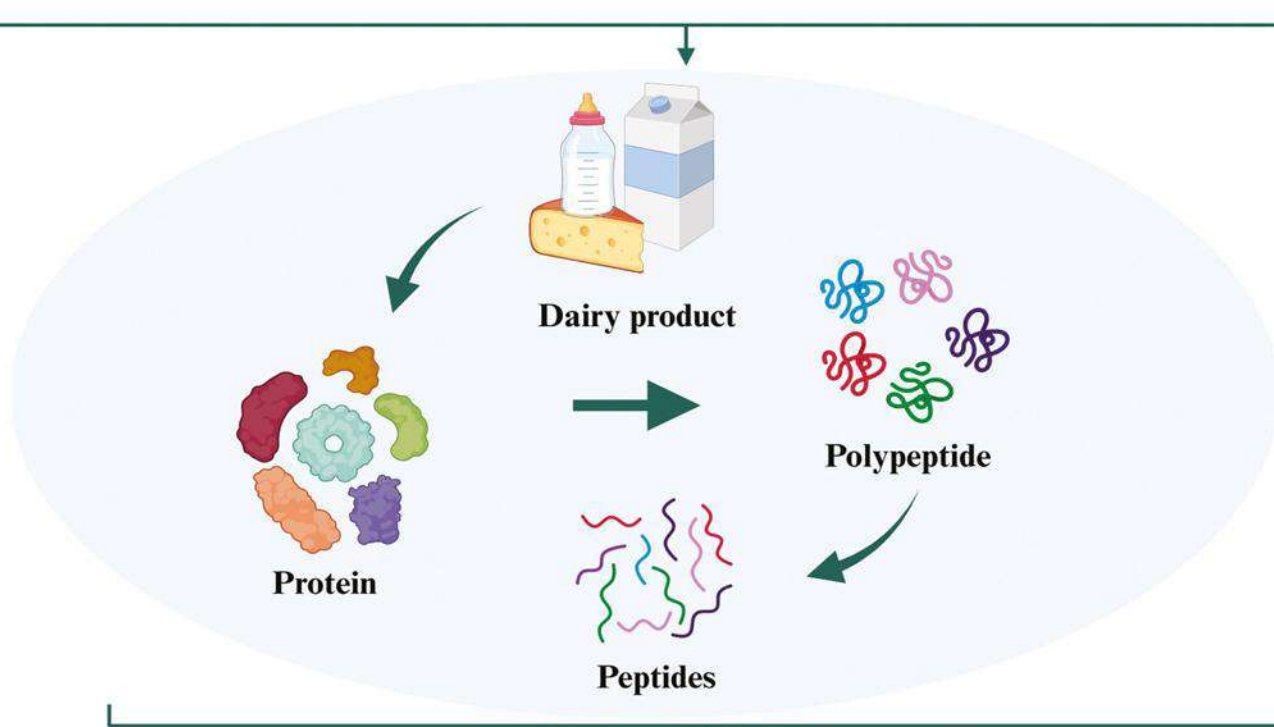
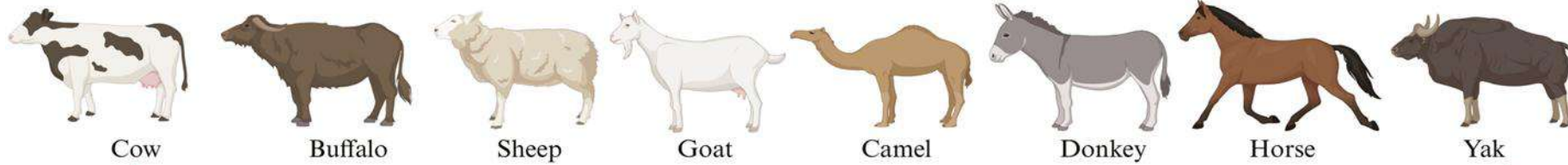
**FSQA-20. DNA BASED METHOD FOR DIFFERENTIATION OF COW, BUFFALO, SHEEP, GOAT AND CAMEL MILK**

*Sachinandan De, Sushil Kumar and Devika Goutam*

Animal Biotechnology Centre

Email: [sachinandan@gmail.com](mailto:sachinandan@gmail.com) | Phone: 9416483670

# Protein-based detection methods



Food Quality and Safety, Volume 8, 2024, fyaee039, <https://doi.org/10.1093/fqsafe/fyae039>

**PAGE**  
LOD: 5%

**CE**  
LOD: 3.1%

**ELISA**  
LOD: 0.01%

**MS**  
LOD: 0.5%

**General rapid test**

- Analyte in sample
- Coat with antigen (Ag) conjugate
- Test Line: an antigen antibody
- Control Line: an antibody control (Ag) antibody

Microfluidic Membrane

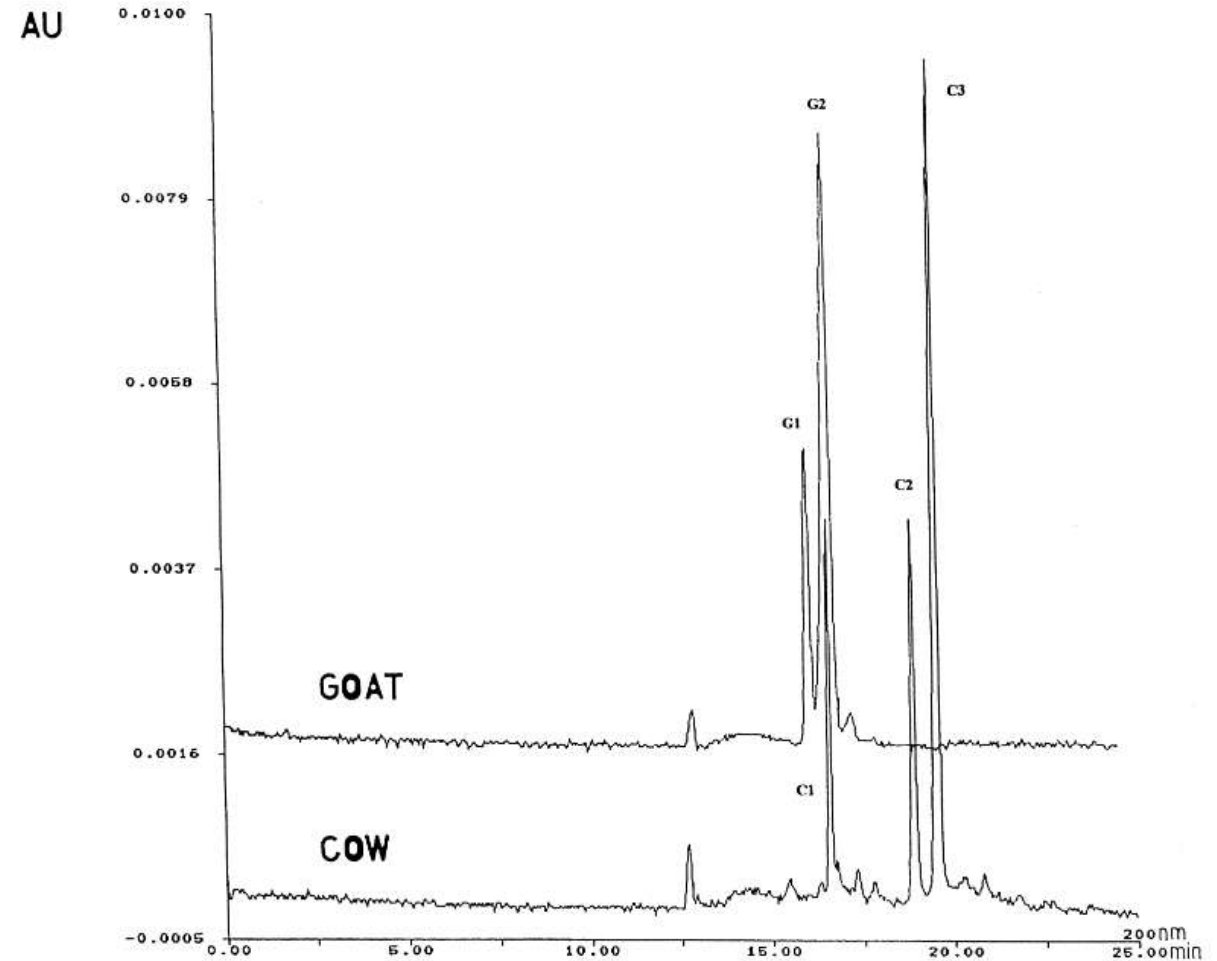
Positive (C, T) / Negative (C)

**LFIA**



# Capillary Electrophoresis

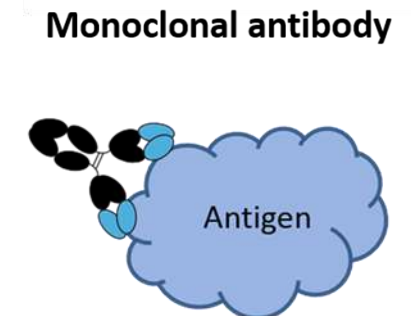
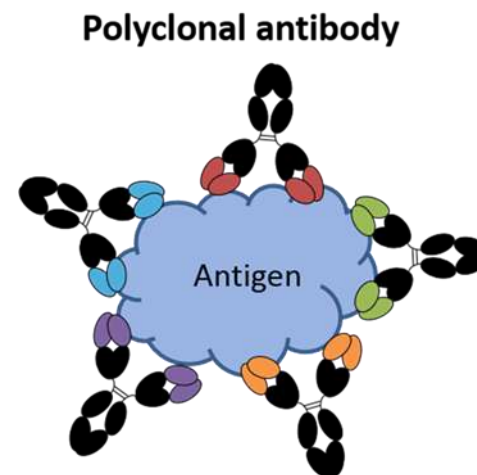
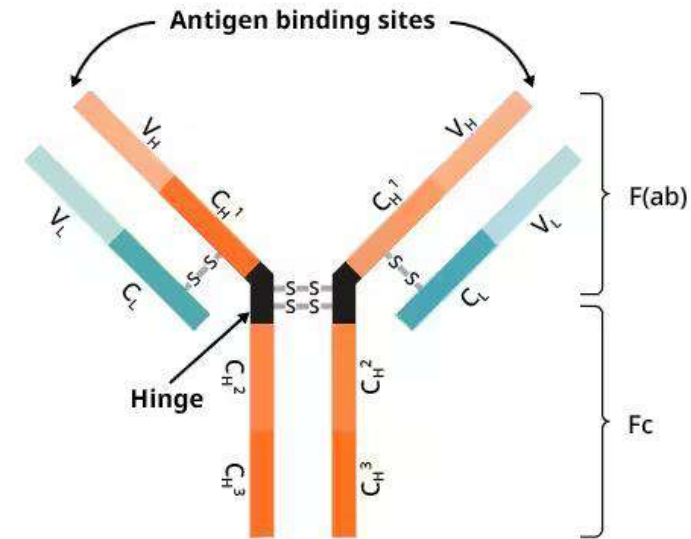
- ✓ The official EU method to detect the presence of cow milk in ewe, goat, and buffalo milks is the **isoelectric focusing of  $\gamma$ -caseins after plasminolysis**.
- ✓ This method is based on comparison with the **protein patterns** of certified reference standards, and it enables qualitative estimation of cow milk in tested samples.



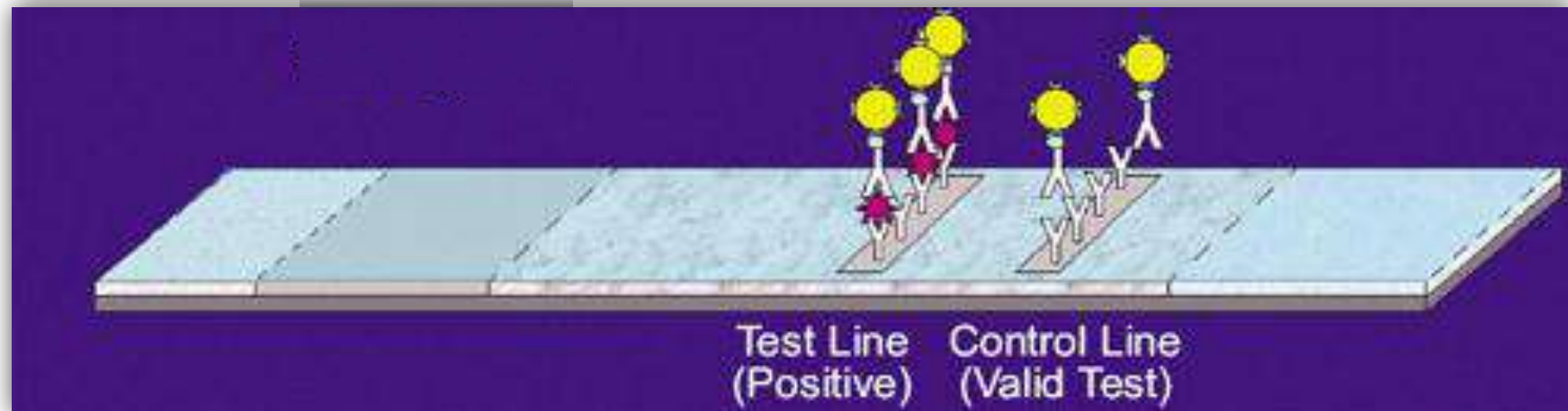
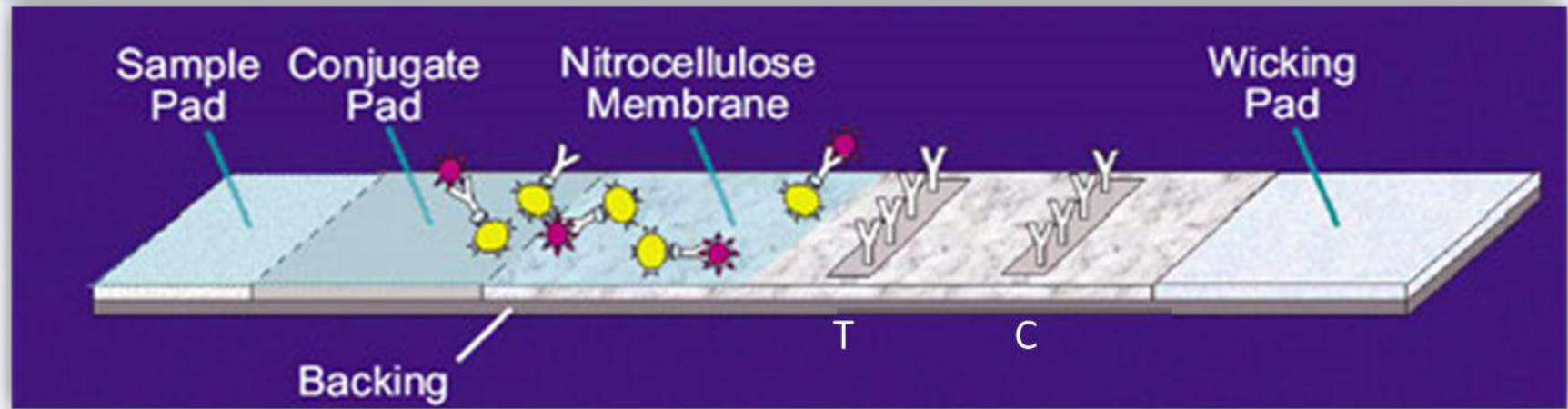
# Lateral flow immunoassay based tests

## Antibodies are required

- ✓ Whole casein
- ✓  $\alpha_{s1}$  – casein
- ✓  $\beta$ -lactoglobulin
- ✓ Immunoglobulin (IgG)

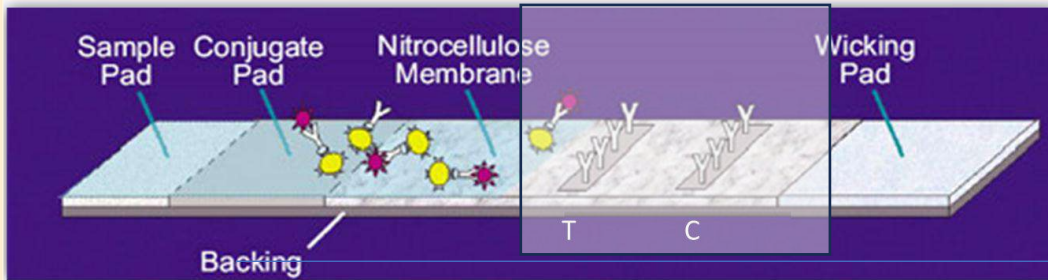


# Lateral Flow Assay Architecture

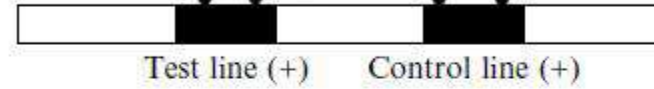


Coated antibody

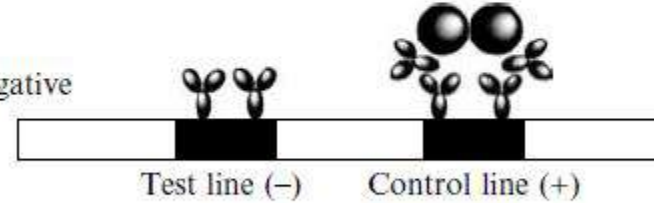
## Direct (Sandwich) Type LFA



Positive



Negative

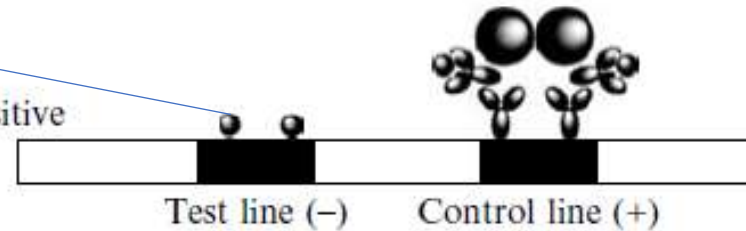


: Conjugated antibody/antigen; : Blotted antibody/SPA; : Analyte

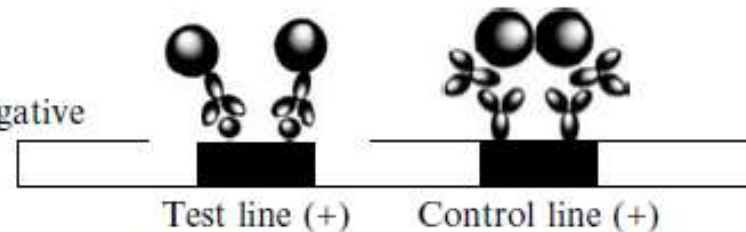
Coated antigen

## Competitive Type LFA

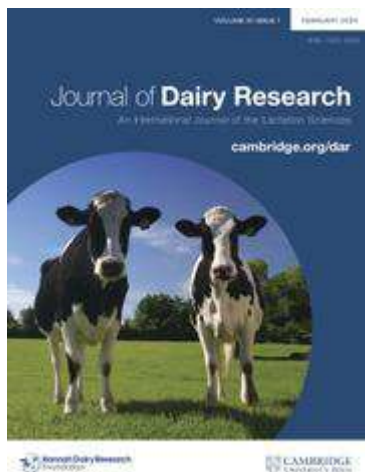
Positive



Negative



: Conjugated antibody; : Blotted antibody; : Blotted or free Hapten



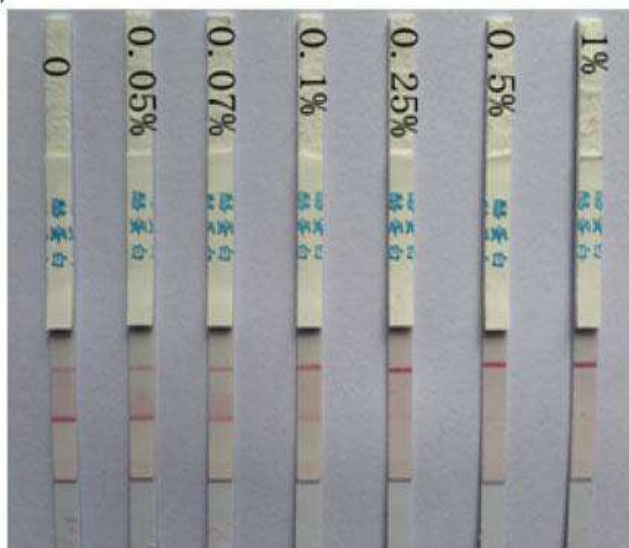
# Rapid detection of cow milk adulteration/contamination in goat milk by a lateral flow colloidal gold immunoassay strip

Bochao Liu<sup>1,†</sup>, Jinhong Si<sup>1,†</sup>, Fang Zhao<sup>2,3</sup>, Qi Wang<sup>1</sup>, Yu Wang<sup>4</sup>, Jinfeng Li<sup>1</sup>, Chengyao Li<sup>1,5</sup> and Tingting Li<sup>1</sup>

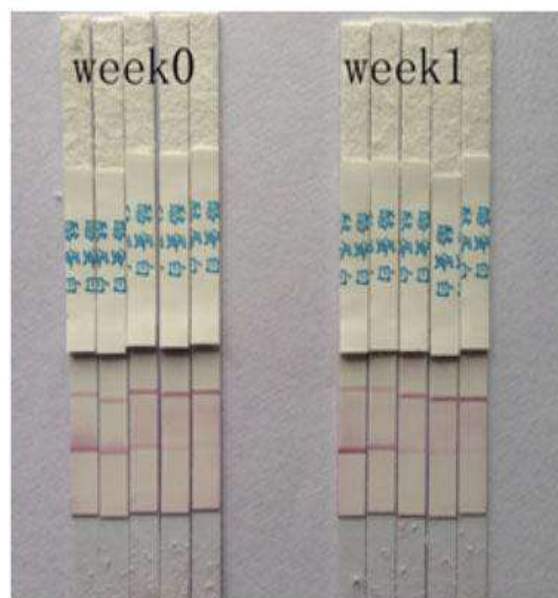
<sup>1</sup>Department of Transfusion Medicine, School of Laboratory Medicine and Biotechnology, Southern Medical University, Guangzhou, China; <sup>2</sup>Shenzhen Key Research Laboratory of Detection Technology R&D on Food Safety, Technical Centre for Food Inspection and Quarantine, Shenzhen Entry-Exit Inspection and Quarantine Bureau, Shenzhen, China; <sup>3</sup>Shenzhen Academy of Inspection and Quarantine, Shenzhen, China; <sup>4</sup>Nanjing Entry-exit Inspection and Quarantine Bureau, Nanjing, China and <sup>5</sup>School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, China

[Journal of Dairy Research](#), [Volume 86](#), [Issue 1](#), February 2019, pp. 94 - 97

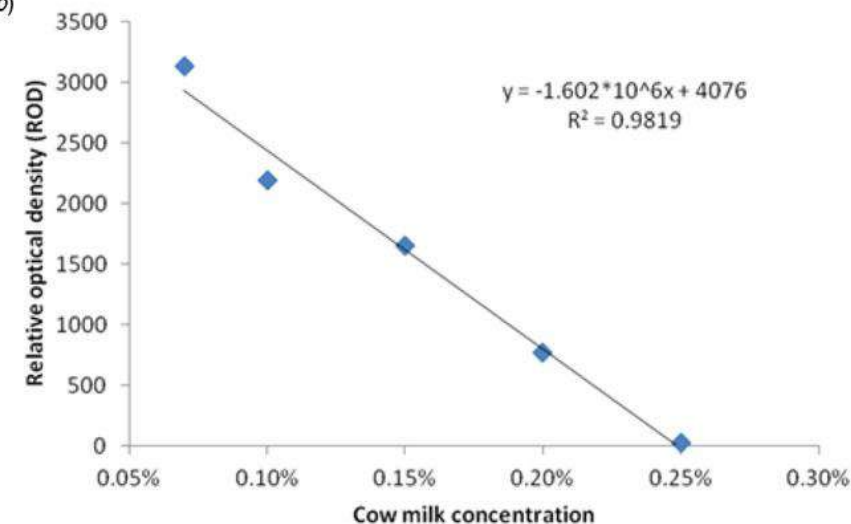
(a)



(c)



(b)



(a) Sensitivity for detection of cow milk in goat milk. (b) Linear regression equation derived using membrane strip reader. (c) Stability for detection of cow milk casein. The strips were tested after 1 week post production, in which cow milk was detected in a concentration of 0, 0.05, 0.1, 0.2 and 0.5%

# Commercial available products....



- Results in 6 mins.
- Detect cow milk.
- Incubate at 40±2 °C.

### HOW TO USE

1. Take 200µ sample and mixed
2. Incubate 3 min at 40°
3. Place Test Strip
4. Visually Interpretation

40° 3 min

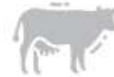
### RESULTS

Visual interpretation of the result by comparing the intensity of the 'TEST' line with the one of the "CONTROL"line.

|   |       |         |          |               |          |
|---|-------|---------|----------|---------------|----------|
| C | Blank | Invalid | Negative | Weak positive | Positive |
| T | Blank | Weak    | Weak     | Weak          | Strong   |

C—Control Line T—Test Line

# Commercial available products....



## RAPID TEST COW

for the quantification of Cow's milk in Sheep's or Goat's milk



## RAPID TEST GOAT

for the quantification of Goat's milk in Sheep's milk



- Fast 3-minute tests
- Highly sensitive - LOD 0.1%
- All disposables included
- Suitable for both field & lab
- Tracing adulteration also in Cheese

LATERAL FLOW TESTING

QUANTITATIVE RAPID TESTS FOR MILK ADULTERATION

VISUAL INTERPRETATION OF RESULTS AVAILABLE

# Commercial available products....

 **BIOPREMIER**

Portugal



[Home](#) > [Food](#) > [Food Adulteration](#) > RAPID TEST COW ADULTERATION

## RAPID TEST COW ADULTERATION

Brand: [Rapid Prognosis](#)

Lateral-flow method of 30 or 120 strips, detecting Cow milk presence in raw Goat and Sheep milk, in 3 minutes.

SKU: R1230

Choose the product variant: \*

R1230: 30 tests

R12120: 120 tests

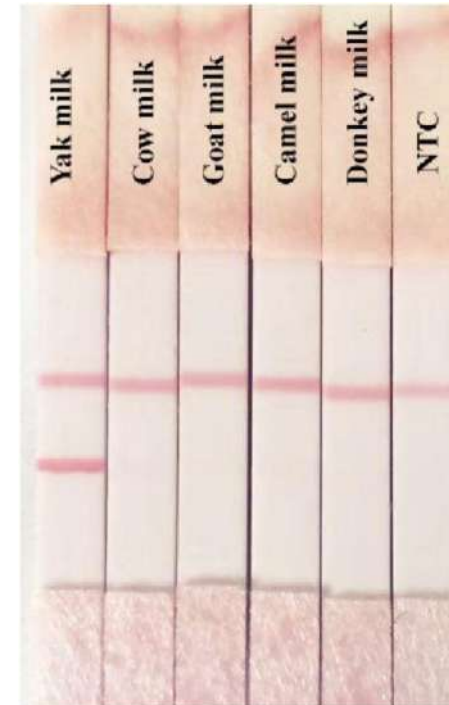
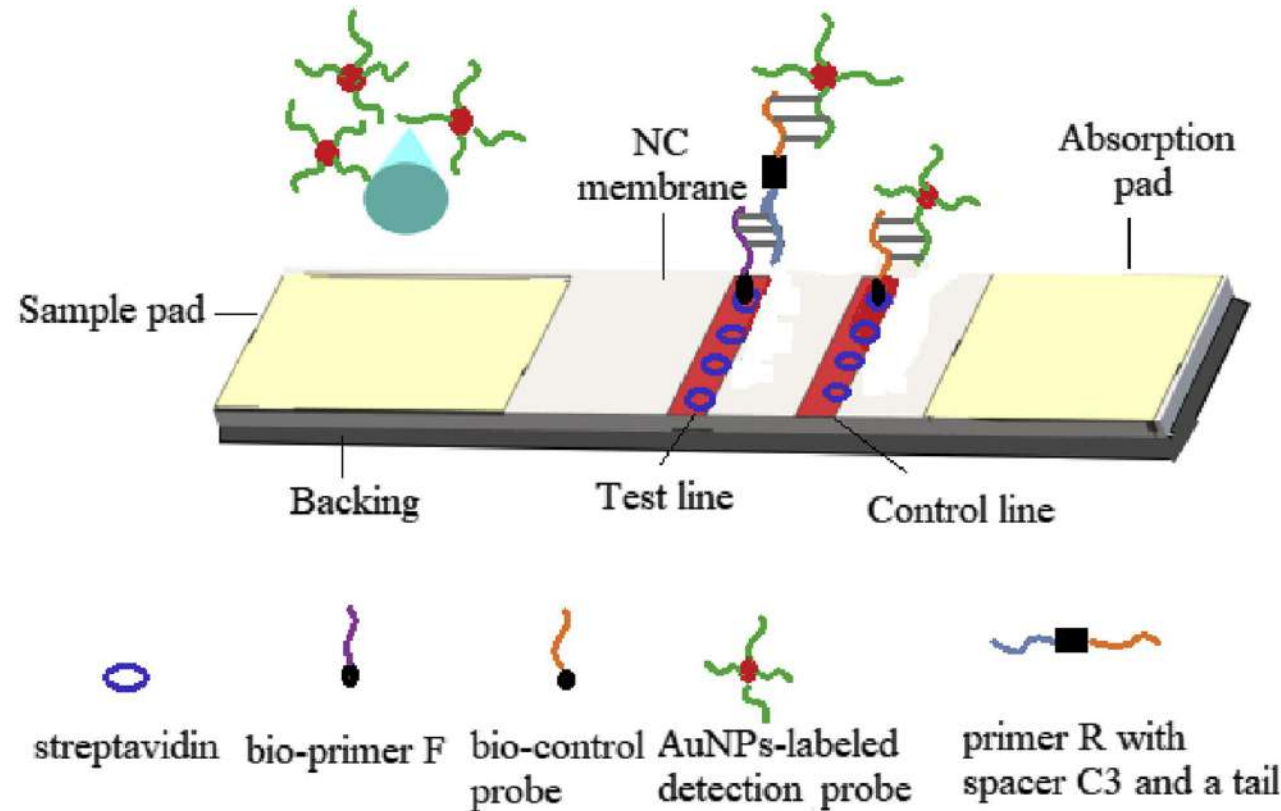
Register or Log In in order to request a quote

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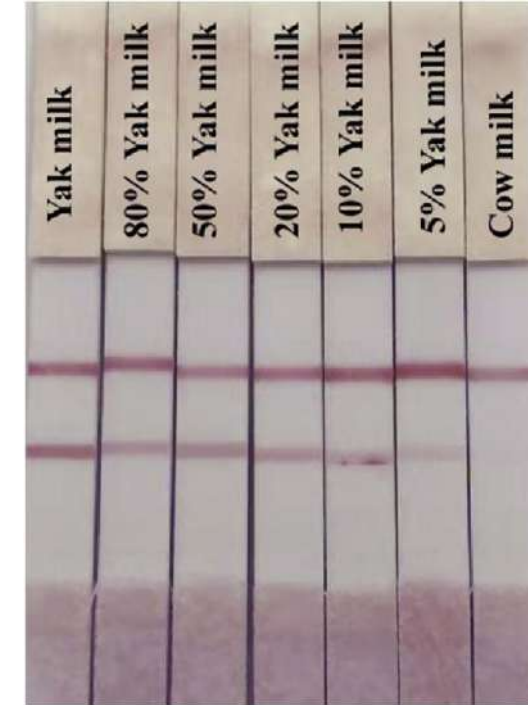
[LOGIN](#)



# Lateral flow nucleic acid assay (LFNAA)



Specificity



Sensitivity

Table 1  
The sequences of the primers and probes used in this paper.

|                  |  |
|------------------|--|
| Primers          | Oligonucleotide primers  |
| Primers          | F: Bio-CACITTTATCCTCCCATTTAATTATTAGAGCA<br>R: TTGGTCGTGGTGGTGGTTT-spacer C3-GAATAGTACCAGAAGTATTAGGGCTAGAAT |
| Detection probes | AAACCACCACGACCAA(T) <sub>15</sub> -HS-SH C6  |
| Control probes   | Bio-TTGGTCGTGGTGGTGGTTT  |

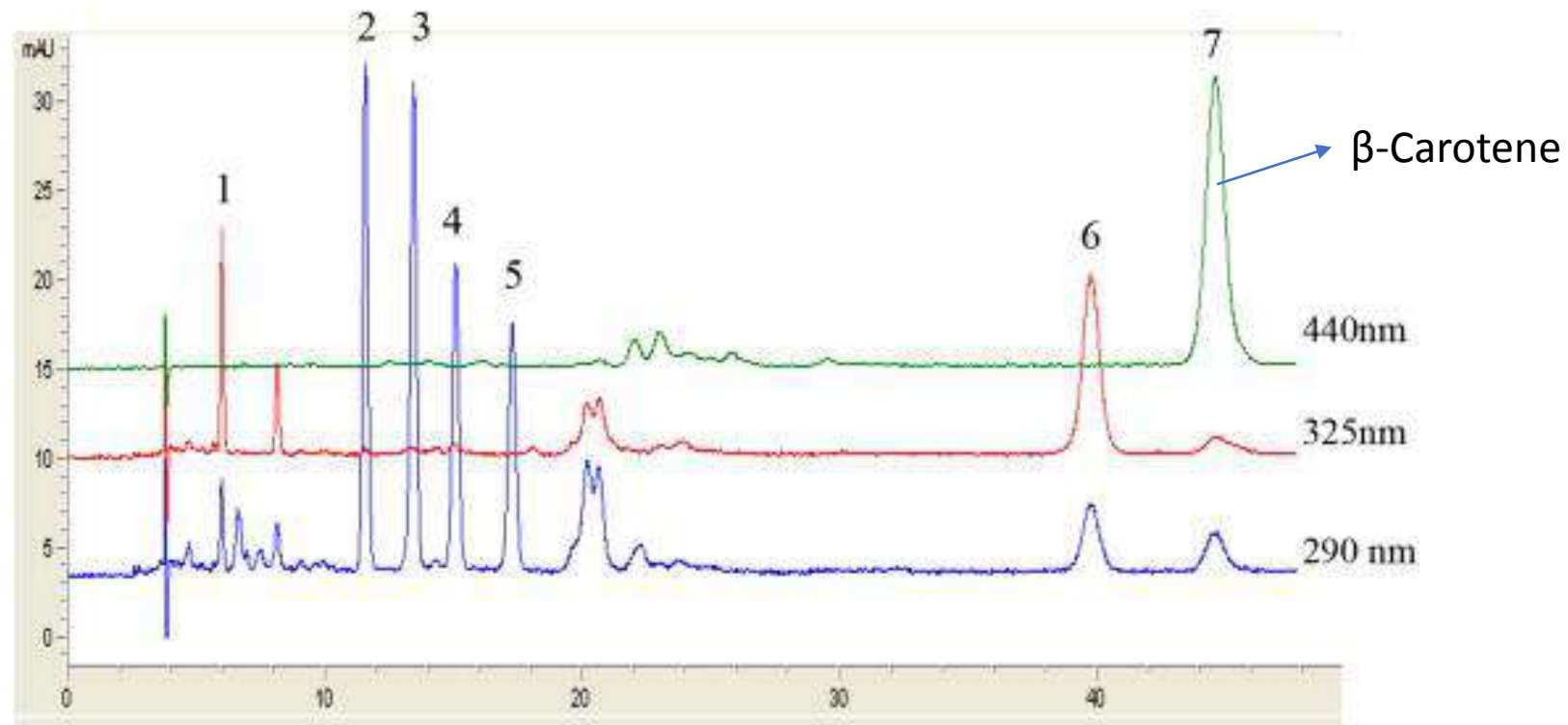
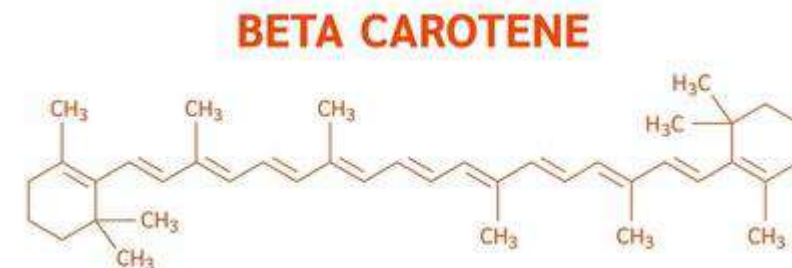


# Absence of $\beta$ -Carotene

- ✓  $\beta$ -Carotene is absent in goat milk & sheep milk
- ✓ It is metabolized into vitamin A\*
- ✓  $\beta$ -carotene could be marker for the authenticity of goat milk
- ✓ HPLC could be method of choice
- ✓ UV detector

Retinol (1),  $\delta$ -tocopherol (2),  $\gamma$ -tocopherol (3),  $\alpha$ -tocopherol (4),  $\alpha$ -tocopheryl acetate (5), retinyl palmitate (6) and  $\beta$ -carotene (7)

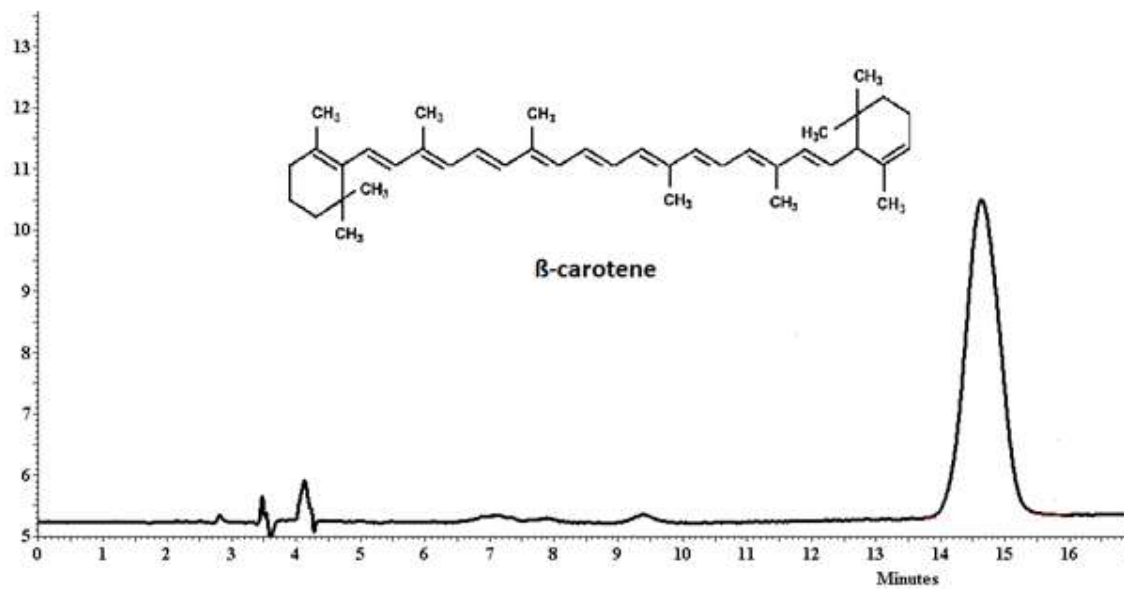
\* $\beta$ -Carotene is cleaved by  $\beta$ -carotene-15,15'-monooxygenase



## Optimization of various steps for RP-HPLC determination of $\beta$ -carotene in milk fat

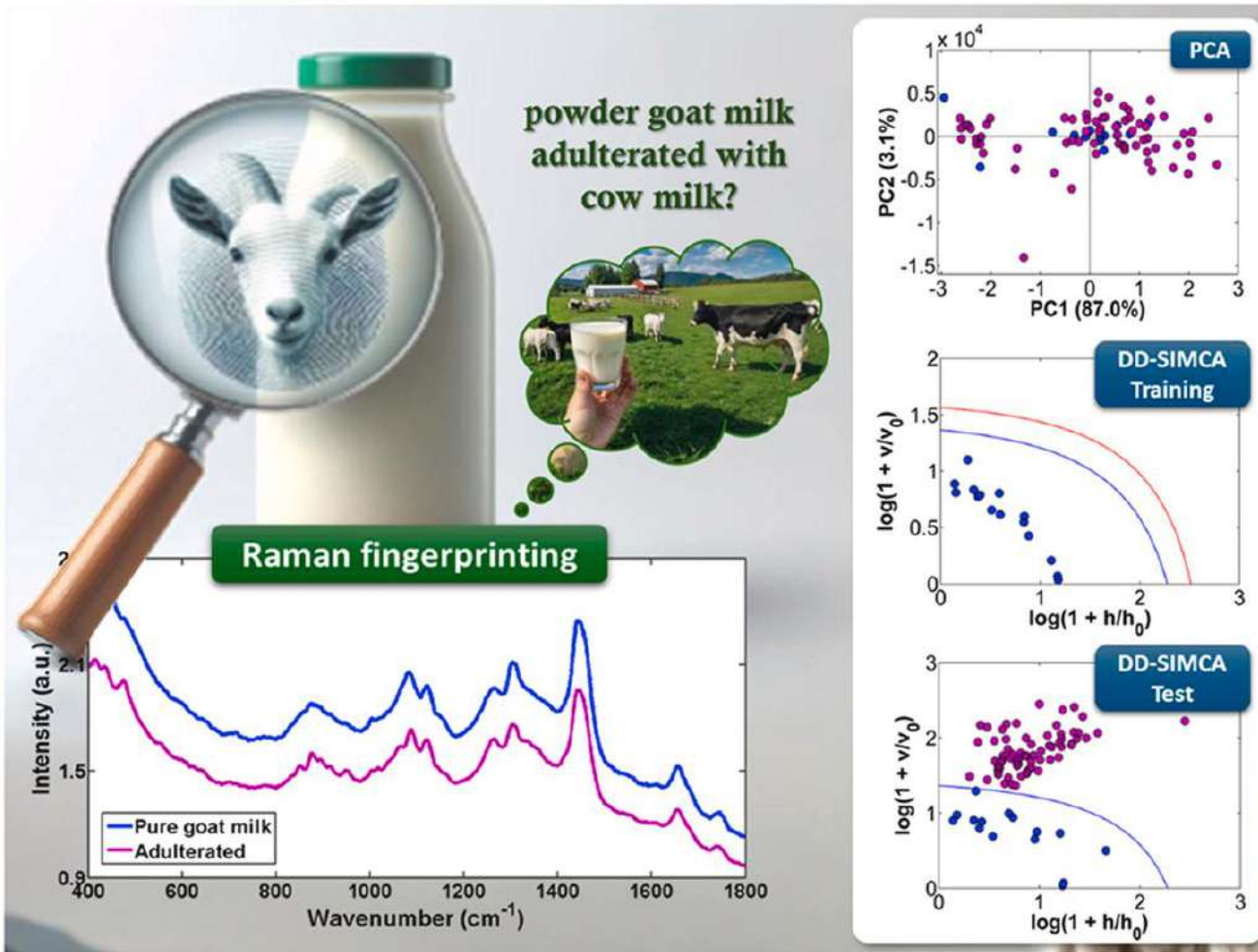
\*Dhankhar, J., Sharma, R. and Mann, B.

*Dairy Chemistry Division, National Dairy Research Institute, Karnal, Haryana, India-132001*



- ✓  $\beta$ -carotene is fat soluble carotenoid
- ✓ The aim of current study was to obtain a rapid, reliable, and effective method to evaluate  $\beta$ -carotene in milk fat.
- ✓ Saponification
- ✓ Ascorbic acid addition

# Absence of $\beta$ -Carotene



J Food Sci Technol (August 2020) 57(8):3091–3098  
<https://doi.org/10.1007/s13197-020-04342-4>



ORIGINAL ARTICLE

## A rapid method for detection adulteration in goat milk by using vibrational spectroscopy in combination with chemometric methods

Hülya Yaman<sup>1,2</sup>

Department of Food Science and Technology, The Ohio State University, Columbus, OH, USA

Food Control 108 (2020) 106808



Contents lists available at ScienceDirect

Food Control

journal homepage: [www.elsevier.com/locate/foodcont](http://www.elsevier.com/locate/foodcont)



## Development of synchronous fluorescence method for identification of cow, goat, ewe and buffalo milk species



Duygu Ozer Genis<sup>a</sup>, Banu Sezer<sup>a</sup>, Gonca Bilge<sup>b</sup>, Sahin Durna<sup>c</sup>, Ismail Hakki Boyaci<sup>a,\*</sup>

<sup>a</sup> Department of Food Engineering, Hacettepe University, Beytepe, 06800, Ankara, Turkey

<sup>b</sup> NANOSENS Industry and Trade Inc., Ankara University Technology Development Zone, 06830, Golbasi, Ankara, Turkey

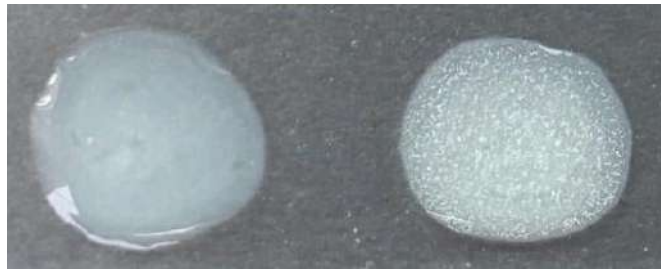
<sup>c</sup> Ataturk Forestry Farm, 06560, Yenimahalle, Ankara, Turkey

Food Control; 2025, vol: 167, 110800



# Classical test for detection of presence of buffalo milk in cow milk

## Hansa Test



1

2

## Agglutination Assay

Incubation of **antiserum** raised in rabbit against the protein fraction isolated from buffalo's milk with

1. Diluted cow milk
2. Diluted buffalo milk



## Immunodiffusion Assay

Interaction between the **antiserum** raised in rabbit against the protein fraction isolated from buffalo milk (A) with

B - Buffalo milk

C- Cow milk

D – Buffalo milk + cow milk (50:50)

E - Buffalo milk + cow milk (25:75)

# Carbon nanoparticles-based lateral flow immunoassay for detection of buffalo milk in cow milk

Food Chemistry 351 (2021) 129311

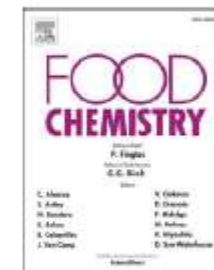


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Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Food Chemistry

journal homepage: [www.elsevier.com/locate/foodchem](http://www.elsevier.com/locate/foodchem)



Adulteration of cow's milk with buffalo's milk detected by an on-site carbon nanoparticles-based lateral flow immunoassay

Rajan Sharma<sup>a,\*</sup>, Archana Verma<sup>b</sup>, Nitin Shinde<sup>a</sup>, Bimlesh Mann<sup>a</sup>, Kamal Gandhi<sup>a</sup>, Jan H. Wichers<sup>c</sup>, Aart van Amerongen<sup>c</sup>

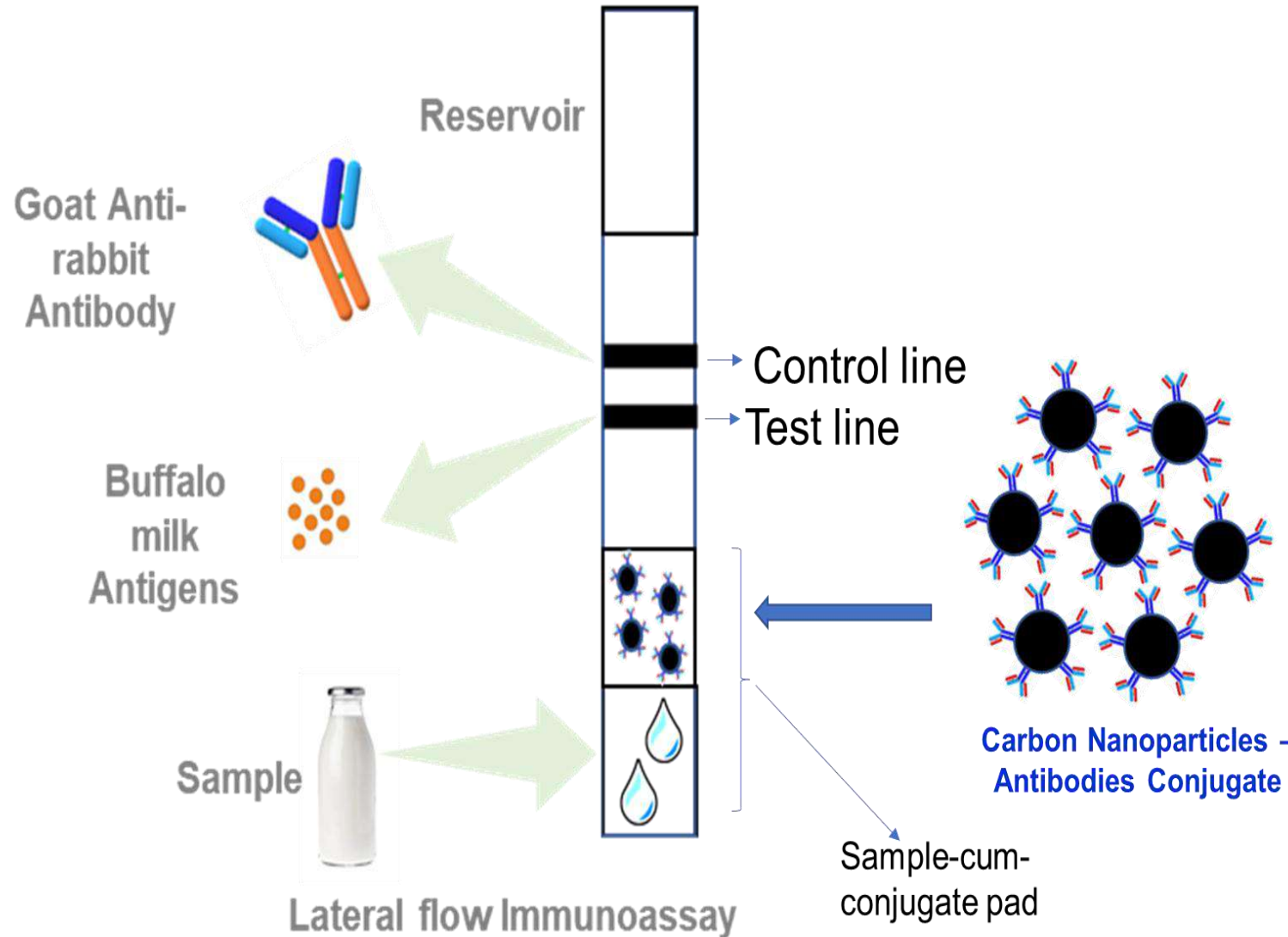
<sup>a</sup> Dairy Chemistry Division, ICAR-National Dairy Research Institute, Karnal 132 001, India

<sup>b</sup> Animal Genetics & Breeding Division, ICAR-National Dairy Research Institute, Karnal 132 001, India

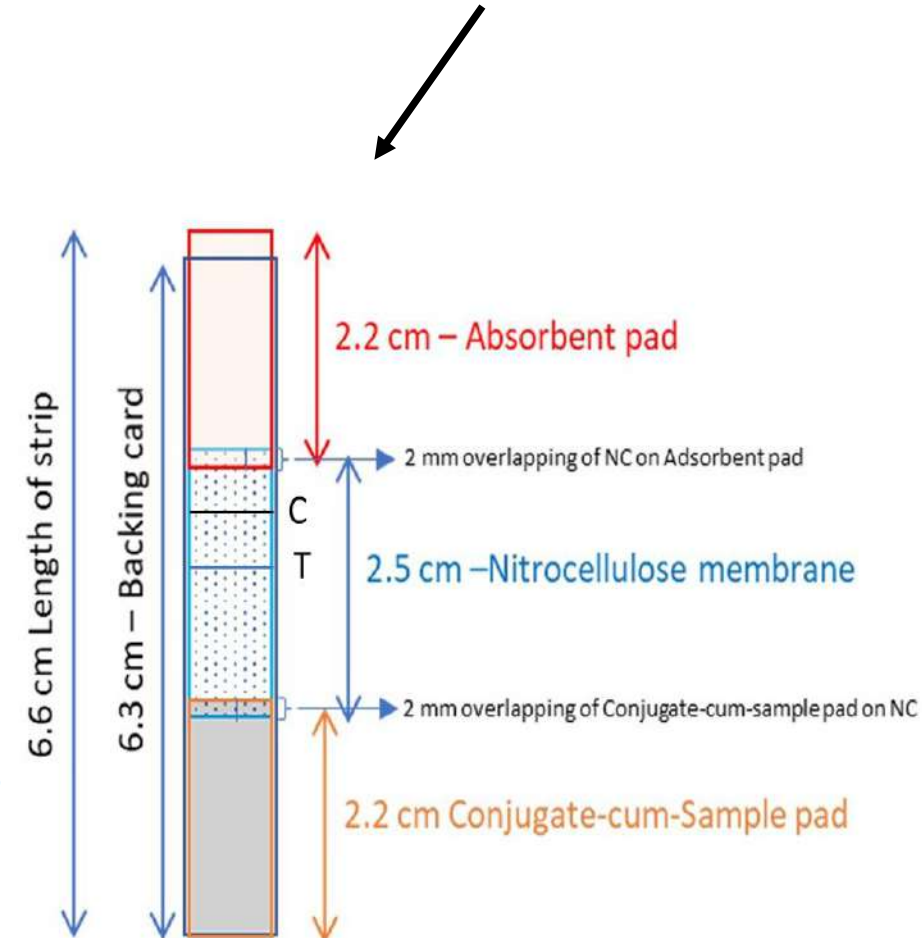
<sup>c</sup> BioSensing & Diagnostics, Wageningen Food & Biobased Research, Wageningen University & Research, Bornse Weiland 9, 6708 WG Wageningen, The Netherlands



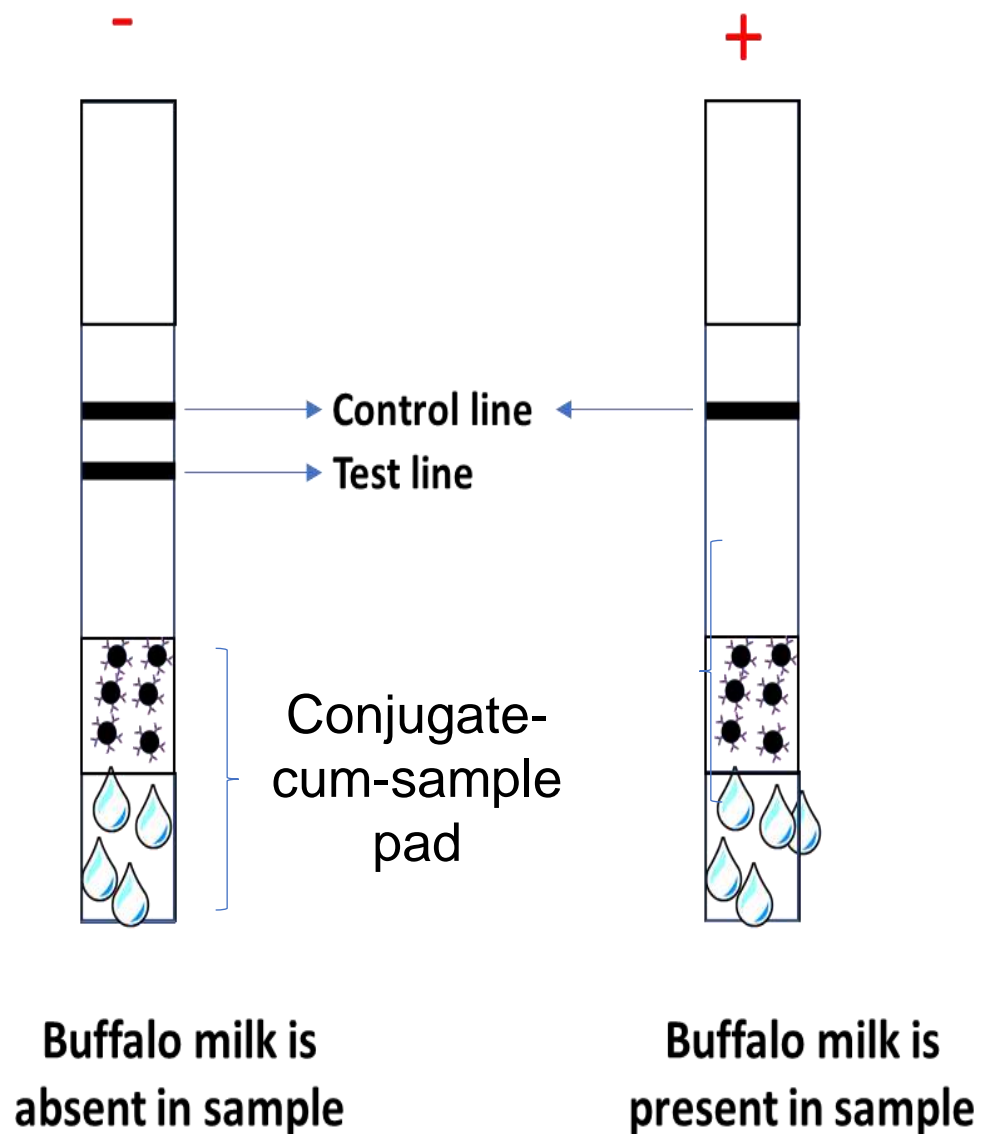
# Lateral flow assay – competitive type



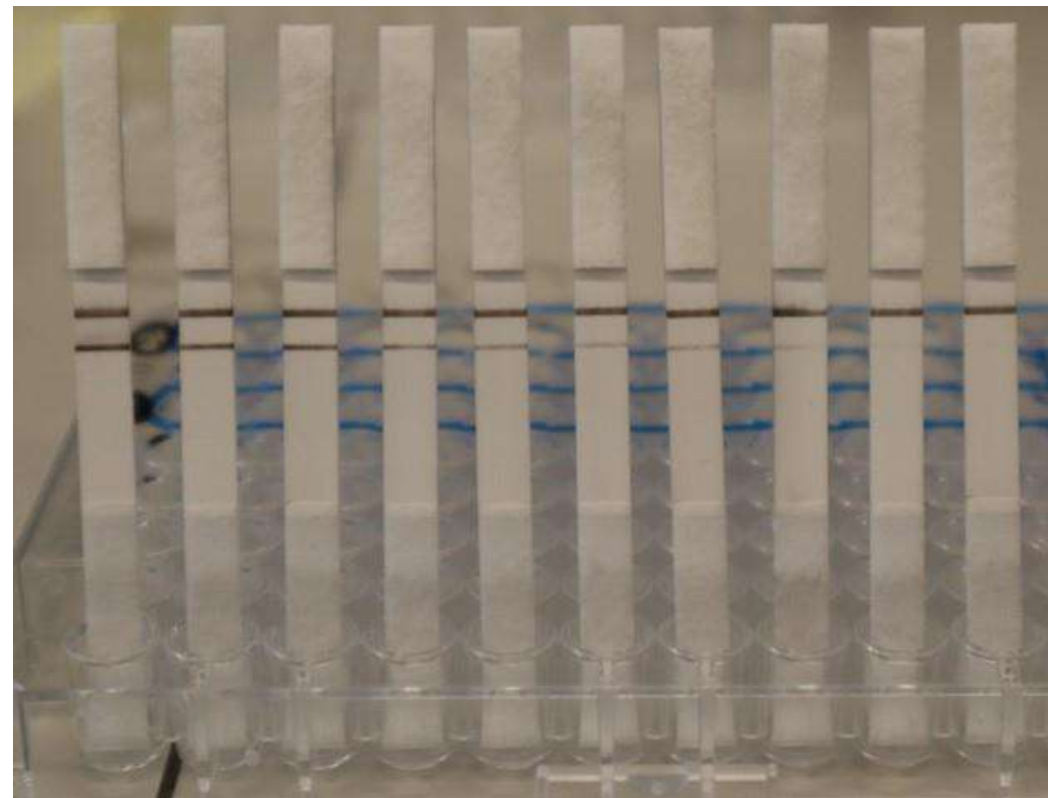
## Architecture of the strip



# Lateral flow assay – competitive type



- ✓ Appearance of two lines indicate that analyte is absent
- ✓ Disappearance of test line indicates, buffalo milk is present







100  $\mu$ l of 10 times diluted sample

0 1 3 5 7 10 20 100

Buffalo's milk in cow's milk (%)

**FSQA-9. LATERAL FLOW ASSAY-BASED METHOD FOR RAPID DETECTION OF PRESENCE OF BUFFALO MILK IN COW MILK**

*Rajan Sharma, Archana Verma, Nitin Shinde, Kamal Gandhi and Bimlesh Mann*

**Dairy Chemistry Division**

Email: [rajan.sharma@icar.gov.in](mailto:rajan.sharma@icar.gov.in) | Phone: 9416120181

Technology is available from NDRI on commercial basis

# Conclusion

- With the rise in popularity of non-bovine milk, fraudulent practices may also increase
- DNA based methods are available
- Field test – requirement
- Lateral flow immunoassay (LFIA) are effective tools to be used in self-control in dairies and to verify the quality of milk prior to collection from farms
- **No indigenous technology is available!!**

2023

# वार्षिक रिपोर्ट ANNUAL REPORT



1923 से नवीनतम डेरी तकनीकियों के विकास के साथ देश की सेवा में समर्पित



भाकृअनुप-राष्ट्रीय डेरी अनुसंधान संस्थान  
(मानद विश्वविद्यालय) करनाल - 132 001 भारत  
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE  
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# TECHNOLOGIES

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