Establishment of National Referral Centre for Milk Quality and Safety at ICAR-NDRI, Karnal

National Referral Centre for milk Quality and Safety (NRCMQS) has been set up at ICAR-National Dairy Research Institute, Karnal with funding support from National Agricultural Innovation Project Agriculture (NAIP) under project entitled on" Development of Biosensors and Micro-techniques for Analysis of Pesticide residues, Aflatoxin M1, Heavy Metals & Bacterial Contamination in Milk with an amount of **Rs. 17.43 crores**.

Inauguration of NRC on Milk Quality and Safety-19th Feb. 2016

Objectives/Activities of National Referral Centre for Food Quality and Safety

1. **Risk Profiling / Scientific investigation related to outbreaks associated with adulteration/ contamination of milk**
   - Risk profiling of Environmental Pollutants and Contaminants -Antibiotic /Pesticides /Aflatoxin M1 /Heavy Metal in milk and standard setting with FSSAI
   - Surveillance on Zoonotic, Mastitis and AMR pathogens in milk
   - Profiling of selected major and minor mineral in milk and milk products
   - Profiling of milk fat of different species with respect to fatty acids, sterols &vitamins
2. **Rapid Biosensor Based Tools / Assay / Kits for monitoring adulterants & potential chemical & bacterial contaminants in milk**
   - Spores /Enzymes /Polymer based sensors for chemical contaminants/pathogens & hygiene indicators in milk

- Paper strip based assay based on Enzymes / new protein bio-markers for detection of sub-clinical mastitis in milk
- Development of analytical tools to assess the quality of UHT milk
- Development of LC-MS based method for identification and quantitation of veterinary drugs in milk.

3. **Licensing / patenting / commercialization of developed IPs to end user through establishing linkage with regulatory agencies (FSSAI) for field application**
    - Evaluation /Validation of Kits and their approval from regulatory agency and their transfer for field application
    - Impact assessment of technologies in collaboration with Extension functions
4. **NABL accreditation and Analytical services to end users for revenue generation , Standard development / capacity building program in specialized field of food safety and Quality Assurance**
    - NABL accreditation of the National Referral Center for Milk Quality and Safety
    - Imparting Analytical services to industry , farmers and other stake holders as per FSSAI /regulatory standards
    - Contribution in standard development programme to FSSAI through expert panel members
    - Training to Farmers/ industry personnel in the field of clean milk and Quality Assurance and Food safety

Infrastructure Facility at <u>National Referral Centre:</u>The centre is equipped with state of the art equipmentsand has capability to do testing in the following areas:

- ✓ Microbiological testing of milk and milk products
- ✓ Chemical testing of milk and milk products
- ✓ LC-MS/MS analysis of milk and milk products

Unit-I (Microbiological Section) of NRCMQS is having two section namely Microbiological Section (BSL-3) and Biosensor lab. BSL-3 is equipped with media preparation, autoclaving, inoculation chamber, incubation room, de-contamination room and pathogen detection section which complies WHO biosafety standards guideline. BSL-3 facility is equipped with ante room, Biosafety cabinet etc.

BSL-3 Containment

Bio-sensor Lab

Unit-I is equipped with following AOAC approved system for testing of pathogens, hygiene indicators and antibiotics residues in milk.

- VIDAS for rapid testing of *L.monocytogenes*, *E. coli* O157:H7, *Salmonella*, *Staph. aureus*Enterotoxin and *Campylobacter* in 24 hrs.
- D- Count for testing of Total Viable Count (TVC) in 2 h and Yeast& Mold Count (YMC) in 10 hrs.
- Charm 6602 / ROSA for testing different groups of antibiotics within 30 min as per codex standards.

Unit-I (Microbiological section) is offering analytical testing for following parameters (89 no's) for different categories of milk and milk products as per FSSAI standard requirements:

1. **Hygiene indicators** :Aerobic plate count / coliform / *Staph aureus, E. coli* and Yeast and mold counts)
2. **Pathogens testing**-*Salmonella / Shigella, L. monocytogenes, B. cereus, Enterobactersakazakii*, Sulphite reducing clostridia (SRC) as per requirement of FSSAI.

Unit–I has developed kits for rapid detection of bacteria (L. monocytogenes, E. coli, coliform, enterococci), antibiotics including β-lactam group, pesticide residues, heavy metals and mastitis in milk.

Unit-II (Chemical Section) of NRCMQS has two major sections which includes Wet Chemical Laboratory having facilities for routine chemical analytical work, adulteration testing, protein estimation, electrophoresis and Lyophilizer facilities. The other area consists of

Chromatographic Laboratory, Protein Laboratory, sample preparation laboratory, hot area etc. Following equipments are housed in Chemical Section:

- HPLC,GCMS,Atomic Absorption Spectroscopy ,FTIR,Particle Size Analyzer,Digital Refractometer ,PCR,Electrochemical Station,Automatic protein analyzer, Nanodrop etc.

Wet Chemistry Laboratory

LC-MS/MS facility

National Referral Centre for Food Quality and Safety (NRCQS) has been accorded NABL (National Accreditation Board for Testing and Calibration Laboratories) accreditation as per ISO **17025: 2017in Feb. 2020**. The Analytical Services of Chemical Section are open for Dairy as well as Food Industry for following 187 test parameters.

1. **Chemical testing of milk and milk products**
2. **Mineral profile of dairy products**
3. **Aflatoxin M1 estimation in dairy products**
4. **Fatty acid profile of dairy products**

Unit-II has developed technologies on strip based tests for checking adulteration in milk and have been commercialised in india

Unit – III (MS Section): The LC-MS/MS section has been established with an aim to identify and quantify the proteins, peptides and veterinary drugs in milk and milk products. The LC-MS/MS facility contains four standalone equipment namely;

- Ultra-High pressure liquid chromatography (UHPLC) system
- Nano-liquid chromatography system (Nano-LC)
- Electrospray Ionization Quadruple Time of Flight (ESI qTof) Mass Spectrometer
- Liquid Chromatography-Triple quadruple system (LC-TQD)

Following testing facilities are offered by LC-MS/MS facility:

- Fractionation of proteins/peptides
- Determination of exact mass of proteins and peptides
- Identification and screening of proteins / peptides in biological samples (Individual/complex)
- Relative quantitation of proteins / peptides using high throughput proteomic approaches likeTMT, Label free quantitation
- LC-MS/MS based screening and quantitation of melamine , veterinary drugsetc

New methods developed by MS Section:

- UHPLC-coupled MS based method for **A1A1, A1A2**, and A2A2 type milk
- LC-MS/MS based method for Progesterone β-hydroxy butyrate, melamine, Sulphadimidine, Sulphamethoxazole, Ciprofloxacin, Enrofloxacin, Ampicillin in milk

REVENUE GENERATIONat NRCMQS through Analytical services, Supply of testing kits Training programme, evaluation /validation of kits and Technology Development/Licensing are as follows:

Year	Various components	NRCMQS		
		Net amount received	GST component	Total amount
2020-21 (April 20-March 21)	**Sample analysis**	369375.00	66487.50	**435862.5**
	Consultancy	1156245.00	208122.00	**1364367.00**
	Equipment Usage	13720.00	2470.00	**16190.00**
	Training	25000.00	4500.00	**29500.00**

	Technology transfer	-	-	-
	Total	**1564340.00**	**281579.50**	**1845919.50**
2019-20 (April 19-March 20)	**Sample analysis**	394051.26	74669.85	**468721.11**
	Consultancy	419982.00	81634.50	**501616.50**
	Equipment Usage	18175.00	3271.50	**21446.50**
	Training	513597.60	101282.40	**614880.00**
	Technology transfer	1726100.00	378900.00	**2105000.00**
	Total	**3071905.86**	**639758.25**	**3711664.11**
2018-19 (April 18 - March 19)	**Sample analysis**	233849.86	43603.14	**277453.00**
	Consultancy	662071.70	130439.30	**792511.00**
	Equipment Usage	29400.00	5292.00	**34692.00**
	Training	496452.74	106287.25	**602739.99**
	Technology transfer	2401780.00	527220.00	**2929000.00**
	Total	**3823554.30**	**812841.69**	**4636395.99**

Total Revenue Generated during the period from 2018-2021: Rs. 10193979.60

Utilization of National Referral Centre facility as Central facility by the institute:

1. **Microbiological Section** : Equipment Facility on BSL-2 containment, Incubator; Real Time PCR; Multimode Plate reader are used by the scientists, Researchers &students for research purpose from different divisions like ABTC, DT,ABC, AN ,DM and DC for research work on AMR, Bacteriophage, analysis of dried milk, Absorption /OD/Fluorescence / DNA quantification and molecular biology work etc..

2. **Chemistry section** : RT-PCR ,PCR, FTIR , ELISA ,AAS, Particle size analyser , GCMS, high speed centrifuge, Magnetic stirrer, Refrectometer, Sonicator,Refrigerator, Lyophilizer , Precision balance, HPLC facility are availed for research purpose by the Scientists, Students,Researchers from different divisions like DC, DM, DT, AN, ABTC, LPM and ABC etc.

3. **MSSection:** MS facility has been extensively utilized by the different divisions like ABTC, LPM, DM, DC, ABC and SRS Bangalore. NBAGR & CIRB has also used MS Facility for research purpose.

Scientific group working at National Referral Centre for Milk Quality and Safety at ICAR-NDRI, Karnal

1. Dr Naresh Kumar , PS & I/C NRCMQS
2. Dr Rajan Sharma , PS & Quality Manager
3. Dr Raghu , H V ,Scientist & Technical Manager (Microbiology Section)
4. Dr Richa Singh , Scientist & Dy. Quality Manager (Chemistry Section)
5. Dr Kamal Gandhi , Scientist & Technical Manager (Chemistry Section)
6. Dr Sudharshan Kumar, Scientist & Technical Manager (MS Sections)