Report on Training Program on Method of Analysis of Fortificants in Milk
11th June – 15th June 2018
Anand, Gujarat

A five days training program was organised by Food Safety & Standards Authority of India (FSSAI) and Food Fortification Resource Centre (FFRC) in collaboration with National Dairy Development Board (NDDB) for Food Analysts and laboratory personnel of FSSAI notified National Accreditation Board for Testing and Calibration Laboratories (NABL) on method of analysis of fortificants in milk (Vitamin A and D) from 11th – 15th June 2018 at Centre of Analysis and Learning in Livestock and Food (CALF), NDDB, Anand, Gujarat.

On 15th May, FSSAI issued a letter seeking nomination from laboratories across the country. A total of eleven FSSAI notified NABL labs from across India including the private and state food labs were shortlisted for the training. Participants from eleven labs and one representative each from FFRC and FSSAI attended the training. A list of participants in attached at Annexure 1.

Training Program
In the past, it was reported that there was a considerable variation between the results in testing of fortificants when tested out at different labs. Therefore, need for harmonisation of procedures was generated. In this regard, the training was conducted to equip the lab personnel with requisite testing protocol of Vitamin A and D in milk so that they can adopt a common method and procedure in their labs. The training schedule is attached at Annexure 2.

Training Schedule
Day 1: 11th June

Training started with the inauguration and greeting of all the participants. A brief introduction about the history, strengths, certifications and activities of NDDB and CALF was presented by Dr. Rajesh Nair (Director, CALF). This was followed by tour of their Microbiological, Chemical and Genetic labs where all the tests, equipment and sample analysis was explained.

Lecture session consisting of presentation on Milk fortification and method of analysis of Vitamin A and D was delivered by Ms. Gatha and Ms. Hima respectively. Thereon, FFRC shared with all the participants the re-operationalised FSSAI standards on food fortification and explained in detail the criticality of accurate testing of the micronutrients in fortified foods so that the quality assurance and quality control is checked.

Classroom lecture was followed by laboratory testing and hands on experience on the method of analysis of Vitamin D in milk. Samples of control and fortified milk were prepared as per the AOAC method and passed through various steps including the saponification, digestion and solvent extraction. The day concluded with the completion of sample preparation and setting up of the HPLC instrument.

Day 2: 12th June

The day started with the recap of the day 1, sample preparation and the steps involved in it. Extracted sample from the day 1 was then run through HPLC and fraction of samples were collected. Meanwhile, participants were trained on using the HPLC system including the technical know-how and the reactions involved. All the participants got the hands on training on fraction collection of Vitamin D samples.

A special visit to PARIKRAMA was organised for all the participants by NDDB. The visit was a walk-through of the evolution of NDDB which started with the small group of farmers of Anand in 1946 under Sardar Vallabh Bhai Patel supervision. The walk-through was quite informative including the detailed information on organisational set-up, programmes, departments, brands and subsidiaries of NDDB.

Day 3: 13th June
The day started with the preparation of milk samples for Vitamin A extraction. The step by step procedure was followed. It was assured that each participants have a hands on experience of sample extraction. After sample extraction, standard and extract was run through HPLC. The day closed with all having hands on experience of HPLC.

Day 4: 14th June

The complete day was dedicated to detailed discussion on calculation and precautions to be taken at each step. Every participant was asked to calculate the values of vitamin D2, D3 and vitamin A individually. All the questions arising out of the calculation was resolved by the trainers.

A visit to Amul plant, Anand was organised by NDDB in the evening for all the participants to expose them to milk processing plant. Seven products including the liquid pouch milk, milk powder, butter, ghee, lassi, curd and flavoured milk are being manufactured at this plant. At the time of visit, the plant manager showed the butter manufacturing to the participants. Lastly, the public relation officer met the participants, where FFRC shared the fortification standards, brochure and current status of fortification in India with them.

Day 5: 15th June
The day began with the remarks from Dr. Rajiv explaining about the need of training and importance of sensitivity analysis of micronutrients. The discussion was left open for feedback session and discussion and scope of improvement in the manual. All the participants were asked to give an evaluation test after completion of all the activities of training to judge the effectiveness of training. The training closed with distribution of participation certificates and vote of thanks.

Open House Discussion

The training included question and answer round after each lecture and session which are summarised as follow:

- Limiting factor in adopting the procedure of CALF, NDDB was preparatory HPLC (critical instrument required for testing of fortificants in milk) as every lab is not equipped with the instrument
- Difference in results of testing was discussed where conditions such as transportation, storage, sample handling and temperature conditions play an important role in micronutrient retention. It was concluded by the laboratory participants that as per CODEX, 20-30 % of variation in results is acceptable
- Participants raised the queries on chemicals being used (grade, suppliers, purity, chemical composition), calculation and preparation of standard solution. Lot of queries was also raised on using of HPLC including the solvent, columns, software, graph formation and calculation.
- Precautionary steps to be taken care while doing the analysis of Vitamin A and D were discussed which are as follows:
  a. Single trained analyst should handle a single sample at a time
  b. Step by step addition and mixing of chemicals and water should take place
  c. Accuracy in extraction and transfer of the chemicals/extract should be maintained
  d. High precision in volume making up is required
  e. Sensitivity of testing should be noted and required precautions need to be taken
  f. Correct formula and calculation methods needs to be applied
- The questions/queries raised by participants on calculations, graph interpretation and recovery factor while calculating the values of vitamins was answered by the experts/speakers of CALF

Outcomes

- All the participants from eleven labs agreed to adopt the procedure of Vitamin A and D as demonstrated by CALF, NDDB. FFRC/FSSAI can share the list of laboratories of these participants with the key stakeholders and states, as a reference for getting the fortified samples tested.
- All the participants of labs to perform the tests based on the training at their labs and share any concerns regarding it by end of June with FSSAI and CALF.
- CALF, NDDB to share the revised final SOP with FSSAI after making the necessary modifications.
Way Forward

- FFRC/FSSAI can share the procedures with the stakeholders or upload the procedure of testing (SOP) of Vitamin A and D in milk on the website of FSSAI and FFRC.
- FFRC/FSSAI to conduct more trainings on method of analysis of fortificants in the five commodities. In this regard, FFRC/FSSAI has shared the list of method of analysis currently approved by Scientific Panel with NDDB. A detailed evaluation and validation of these methods will be conducted by NDDB and results will be shared with FSSAI by first week of July. If found suitable, FSSAI can consider them for next trainings on testing of other fortificants.

TRAINING PROGRAM ON “METHOD OF ANALYSIS OF FORTIFICANTS IN MILK”
Duration: 11-15 June 2018
Annexure 1

List of participants
1. Dr. Vijay Pal Singh, QA, FSSAI
2. Ms. Sakshi Jain, FFRC, FSSAI
3. Mr. Chitvendra Singh, Spectro Analytical Labs Ltd., Delhi
4. Mr. Priyesh Amin, Accurate Laboratory, Gujarat
5. Mr. Mohit Sharma, Intertek India Pvt. Ltd., Gurgaon
8. Mr. Sandeep Pawar, Pollucon Laboratory Pvt. Ltd., Surat
9. Ms. Ravneet Kaur, Punjab Biotechnology Incubator, Punjab
10. Mr. Deepak Ashok Temak, Ashwamedh Engineers & Consultants, Nasik
11. Mr. Deepu Joseph, Regional Analytical Laboratory, Kerala
12. Mr. S. Alex, Food Analysis Laboratory, Tamil Nadu
13. Mr. Shashi Shanker, FARE Labs Pvt Ltd, Gurgaon

Experts/Speakers of CALF, NDDB
1. Dr. Rajesh Nair, Director
2. Dr. Rajiv Chawla
3. Ms. Jayashree Srinivasan
4. Ms. Swati Patil
5. Ms. Hima Thakkar
6. Ms. Gadha Raj
7. Mr. Sudharshan Raj
Annexure 2

**Schedule of Training Program on Method of analysis of fortificants of milk**
Food Safety and Standard Authority of India (FSSAI)
in collaboration with
National Dairy Development Board (NDDB) at CALF, NDDB, Anand, Gujarat
*(11th to 15th June, 2018)*

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Program</th>
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<tbody>
<tr>
<td><strong>Day 1 - 11/6/2018</strong></td>
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<tr>
<td>9:00 to 10:45</td>
<td>Registration, Inauguration &amp; Welcome of Delegates</td>
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<tr>
<td>11:00 to 12:30</td>
<td>Presentation on Vitamin A &amp; D methodology</td>
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<td>12:30 to 13:00</td>
<td>Laboratory Safety rules &amp; Laboratory tour</td>
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<td>14:00 to 18:00 or (until complete)</td>
<td>Vitamin D - Sample Extraction &amp; hands on experiment</td>
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<td>Vitamin D - Extraction may continue till late evening &amp;</td>
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<td>Pretreatment of HPLC for Normal phase</td>
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<td><strong>Day 2 - 12/6/2018</strong></td>
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<td>9:00 to 9:15</td>
<td>Recap of day 1 &amp; Planning of Day</td>
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<tr>
<td>9:15 to 18:00 or (until complete)</td>
<td>Purification of sample extract for Vitamin D</td>
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<td></td>
<td>• System setup</td>
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<td>• Fraction collection of samples</td>
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<td>• Hands on training on fraction collection of Vitamin D samples</td>
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<td>14:15 to 15:30</td>
<td>Visit to PARIKRAMA (Show of evolution of NDDB) <em>(Tentative)</em></td>
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<td><strong>Day 3 - 13/6/2018</strong></td>
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<td>9:00 to 9:15</td>
<td>Recap of day 2 &amp; Planning of Day</td>
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<tr>
<td>9:15 to 11:30</td>
<td>Analysis of Vitamin D in milk on Analytical HPLC</td>
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<td>11:30 to 18:00 or (until complete)</td>
<td>Extraction of Vitamin A in Milk</td>
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<td><strong>Day 4 - 14/6/2018</strong></td>
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<td>9:00 to 9:15</td>
<td>Recap of day 3 &amp; Planning of Day</td>
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<tr>
<td>9:15 to 13:00</td>
<td>Analysis of Vitamin A by HPLC</td>
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<tr>
<td>14:00 to 18:00</td>
<td>Vitamin D data processing, calculation &amp; discussion</td>
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<td><strong>Day 5 - 15/6/2018</strong></td>
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<td>9:00 to 9:15</td>
<td>Recap of day 4 &amp; Planning of Day</td>
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<tr>
<td>9:15 to 13:00</td>
<td>Vitamin A in Milk - Data processing, calculation &amp; discussion</td>
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<td>14:00 to 15:00</td>
<td>Q &amp; A session</td>
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<td>15:00 to 15:30</td>
<td>Feedback</td>
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<td>15:30 to 16:30</td>
<td>Conclusion of Program</td>
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* Tea Break 11:00 & 16:00, Lunch Break 13:00 to 14:00