TERMS OF REFERENCE FOR R&D PROJECT

Food and Agriculture Department

Slaughterhouse and Meat Industry Sectional Committee, FAD 18

1 Title of the Project

Development of lateral flow immunoassay integrated smartphone device-based test method for in-situ meat authentication

2 Background

2.1 With technological advances in meat processing industry, economically motivated adulteration or meat fraud has become a common problem. Traditional analytical methods for identifying meat fraud require specially equipped stationary laboratories, high-tech equipment, and qualified personnel. All the existing molecular methods viz, DNA, proteomic and metabolomics methods for meat authentication requires state-of-the art laboratories and skilled manpower. There are few manufacturers for ELISA-based meat authentication kits in India and these kits require either mini-centrifuge, ELISA readers and cold storage conditions. None of the existing assays both in India and abroad are 100% field deployable and cannot be performed within 10-20 minutes. Further, the existing assays fail to detect the closely related meat species and are not amenable under cooked conditions. There is a strong and urgent need for simple, accurate, quick and cheaper detection kits that can be performed on-site during confiscation of meat transporting vehicles, by customs department at ports, to check fraud at restaurant or retailer level, etc.

2.2 Lateral flow immunoassay-based point-of-care tools are emerging techniques for rapid initial screening of meat fraud incidents in a resource-limited environment. Given the universal use of smartphones as portable and affordable devices, it is envisaged to develop smartphone-based immunoassay for rapid and sensitive real time monitoring of meat adulteration. However, there are no standards both in India and globally regarding lateral flow immunoassay-based quick detection methods for meat adulteration.

2.3 In order to address the above concern, it has been decided to conduct a detailed study for optimizing the lateral flow immunoassay methods and validate the assay for authentication of pork, chicken and their tissues and integration with smartphone device for the purpose of framing Indian standard.

More information on rationale of the project is provided at Annex A.

3 Objective of the Project

To develop/optimize lateral flow immunoassay integrated smartphone device-based test method for in-situ meat authentication and validation of the test method.

4 Scope

4.1 Study of existing literature related to published research conducted, international/ regional guidelines & standards related to meat species identification and any other relevant national/ international documents.

4.2 Visit to laboratories/research institute working on development of analytical methodology related to meat species detection, if any.

4.3 Development/optimization of rapid, accurate and highly specific lateral flow immunoassay-based techniques for onsite detection of pork and chicken.

4.4 Development of integrated smartphone-based immunoassay for in-situ quantification of meat adulteration.

4.5 Collection of field samples (meat samples) from different retail meat shops/meat processors/online e-commerce platforms and screening for required validation study.

4.6 Intra and inter-laboratory validation of developed assay and comparative analysis of existing test methods, if any with the new proposed method.

5 Research Methodology

5.1 Conduct secondary survey through study of existing literatures related to published research conducted, international/ regional guidelines & standards related to meat species identification and any other relevant national/ international documents.

5.2 Conduct primary survey through structured interview/ structured questionnaires with laboratories/research institute working on development of analytical methodology related to meat species identification, if any to understand the existing testing protocols or ongoing research related to lateral flow immunoassay integrated smartphone device-based test method for in-situ meat authentication.

5.3 Validate sample extraction, gold nano particle conjugation, and other lateral flow immunoassay protocols.

5.4 Establish the limit of detection, sensitivity, specificity, reproducibility and linearity of the developed LFIA strips for validation of pork (n=100) and chicken (n=100) (AOAC, 2013). Validation of test method shall be done as per relevant parts of ISO 5725 'Accuracy (trueness and precision) of measurement methods and results'.

5.5 Conduct stability and shelf-life studies of developed extraction buffers and assay under different field conditions.

5.6 Conduct screening of field samples (meat samples) (n=100) collected from different retail meat shops/meat processors/online e-commerce platforms etc.

5.7 Ensure data acquisition using a smartphone camera, image segmentation via optimal thresholding and approximation of analyte quantity using linear regression analysis.

6 Deliverables

Detailed project report of the work done, in hard copy and digital formats, as per the scope specified under **4**, with the following as appendices:

a) Novel, rapid, and cost-effective lateral flow immunoassay test method for authentication of pork and chicken in meat mixes or commercial products under both raw and cooked conditions;
b) Validation report including data generated, test results, repeatability, its limit of detection and quantification;
c) Integrated smart phone device-based application for in situ quantification of adulteration
d) Available novel methodology/procedures for meat species identification and the comparative analysis with the proposed method, if any
e) Response/information collected during primary survey.

7 Timeline and Method of Progress Review

7.1 Timeline for the project is 6 months from the date of award of the project.

7.2 Stages for Progress Review

Stage	Timeline
Stage I : Review of the literatures and existing stipulations, sampling plan and validation plan	First month
Stage II : Optimization/development and validation of test method(s) and testing of validated method(s) on the field samples. Submission of interim report to Sectional Committee at the end of third month for review.	Second to Fifth month
Stage III : Draft report submission – Sectional Committee will evaluate the draft report and provide feedback/recommend changes, if required.	End of Fifth month

At the end of 6^{th} month, project allottee to submit final project report incorporating recommendations/feedback of Committee.

Note*: The timelines given above are indicative and calculation of time will start from the date of award of sanction letter for the project to the Project leader.*

8 Support from BIS

8.1 Access to Indian and International Standards

8.2 Letters from BIS to concerned stakeholders, wherever required for support in research project.

9 Nodal Officer

Shri Debasish Mahalik, Scientist-B/ Assistant Director, FAD, BIS may be contacted at fad18@bis.gov.in for any queries on the research project.

Annex A
Rationale /Need of Project

A-1 India has around 11,100 Veterinary hospitals and polyclinics, >22,000 Veterinary dispensaries, 80 export meat plants, 15 Veterinary Universities and 55 Veterinary colleges besides having several State Veterinary Biologicals and quality control labs. Among these only few Institutions have the competence to address the regular meat adulteration issues mainly using DNA-based techniques. Couple of States are using ELISA-kits for detection of meat species in a very primitive way without proper authentication, not being possible to differentiate between closely-related species. There is a strong and urgent need for simple, accurate, quick and cheaper detection kits that can be performed on-site during confiscation of meat transporting vehicles, customs department at ports, fraud at restaurant or retailer level, importing agencies etc.
There are very few assays available in India that can address the quick detection of meat adulteration at field level. Therefore, the developed technology also has huge export potential among the countries where there is a restriction on consumption of meat from certain species due to religious issues or countries where food fraud or meat adulteration is very rampant.

A-2 The rationale for the project "Lateral flow immunoassay integrated smartphone device for in-situ meat authentication" is to optimize and validate the lateral flow immunoassay-based method for onsite screening of samples and to develop integrated mobile device for authentication of chicken and pork. This project is essential for several reasons:

a) Counterfeiting and Misrepresentation: Disruptions in the supply chain, expanding consumption outpacing the production and competition to produce cheaper products to gain easier market access are leading to economically motivated adulteration or misrepresentation (mislabeling) of meat and meat products.
b) Halal authentication: Muslim community do not consume pork and pig derived substances and therefore screening methods for in-situ halal authentication is very much needed.
c) Absence of 100% field deployable assays: Existing techniques like ELISA or LAMP assays have the limitations like not 100% field deployable, requires amplification, requires refrigeration, sample preparation requires centrifugation, requires ELISA readers, costlier and not widely available. Lack of assay which is completely amenable under field conditions starting from extraction to interpretation of results, portable, specific and sensitive without cross-reactivity and which can be completed within 15 minutes without skilled manpower, specially equipped stationery laboratory, cost effective and quantitative/visual detection of results.
d) Currently there are no standardized procedures and validated techniques for 100% field level detection of pork and chicken.

A-3 In summary, the project seeks to optimize lateral flow immunoassay procedures for routine screening of pork and chicken and to develop integrated smart-phone device. By optimizing and validating the robust methodology, the project must aim to propose Regulatory Standards for further harmonization with ISO standards.