PROTOCOL FOR THE SAMPLING AND ANALYSIS OF FOODS CONTAINING BEEF FOR THE PRESENCE OF UNDECLARED EQUINE MATERIAL

INTRODUCTION

The food industry is responsible for placing safe food on the market and for ensuring that consumers are not misled with respect to the safety or the authenticity of food. Manufacturers of meat-based foods operate to the highest hygiene standards, as required by law. Those that incorporate ingredients from external sources must in turn rely on the integrity of their suppliers to provide ingredients according to specifications. Regular traceability audits enable a customer to check that their product is manufactured to specification. However, it is also necessary in some cases to supplement traceability systems with analytical procedures that can add an extra level of assurance and validate the supply chain.

Recent testing has uncovered an EU-wide issue regarding the undeclared presence of horse meat in some foods containing beef and certain meat ingredients used in their manufacture. For the purposes of establishing the authenticity of such foods it is necessary to establish an industry-wide co-ordinated approach to sampling and analysis, including the reporting of results to the competent authorities.

This protocol has been developed by the FSAI in consultation with the beef industry, retailers and caterers in Ireland and is valid until the 30th April 2013.

LEGAL CONTEXT

General Food Law

All food business operators are required to ensure that foods they produce or supply satisfy the requirements of food law and that they can verify that such requirements are met.

Amongst the requirements are that the labelling, advertising and presentation of food must not mislead consumers. In addition, traceability must be established and detailed records kept.

SAMPLING

Sampling should consist of finished foods containing beef and/or their beef ingredients as appropriate to the point in the food chain where testing is taking place. All types of food containing beef are appropriate for testing and the industry should use its own information and the published
findings of EU-wide testing to focus sampling of those types of beef containing foods with the greatest risk of adulteration.

Samples should be collected hygienically to prevent any possible cross contamination and placed in previously unused containers that are appropriately labelled. Collected samples should be maintained at 4°C or lower in advance of delivery to the testing laboratory. When samples are presented with multiple units, for example a number of burgers from one production batch, the laboratory should ensure that sub-samples from all units are pooled before DNA extraction to ensure optimum representation.

ANALYSIS

Two analytical techniques are currently available to identify and quantify meat species and a number of laboratories in Ireland are accredited to perform these analyses. The Food Safety Authority of Ireland recommends that testing is carried out using DNA-based methods but recognises that immunological methods may be used in certain circumstances.

DNA-Based

PCR (polymerase chain reaction) is a method used to amplify specific sequences of DNA from a relatively low level of starting material. Qualitative PCR analysis generally establishes a presence or absence, while quantitative PCR measures the amount of DNA either in absolute terms or relative to the presence of other DNA. Mitochondrial DNA is sometimes targeted in qualitative analysis as it is more sensitive due to the fact that mitochondria are generally present in greater numbers per cell compared to the single nucleus.

For the purposes of this protocol, if qualitative PCR analysis is used then positive findings must be quantified using quantitative PCR analysis. Alternatively, quantitative PCR analysis may be used without initial screening by qualitative PCR analysis. Quantification of DNA is ideally carried out using single-copy chromosomal DNA segments. FBOs may also wish to do DNA sequencing on positive samples as an additional confirmatory step in the identification of equine DNA.

Immunological

ELISA (Enzyme-Linked Immunosorbent Assay) is a method that takes advantage of the fact that an antibody raised to a particular antigen will recognise and bind specifically to that antigen. This type of assay is in widespread use in many medical and other analytical settings with levels of sensitivity that can vary with a variety of factors.

For the purposes of this protocol, if ELISA analysis is used then the sensitivity of the method must be such that it can detect horse proteins present at less than 1% relative to beef in a beef based product, including uncertainty. Each batch of test kits should be validated by the laboratory with positive and negative controls before use. The ELISA method must be fit for purpose regarding the type of sample being tested, particularly if the beef containing food has been processed by certain
methods e.g. cooking, that could potentially alter the ability of target proteins to bind to the antibodies in the test kit.

LABORATORY
Labs chosen to carry out the analysis should operate quality assurance systems that are accredited to the ISO/IEC 17025 standard or equivalent. It is desirable that the methods used for the purposes of this protocol are covered in the scope of the lab’s accreditation. Labs should be in a position to define limits of detection and/or quantification for the methods used along with the measurement of uncertainty. Laboratories should be designed such that physical separation barriers are in place to limit the risk of cross-contamination.

INTERPRETATION OF RESULTS
At present in EU law, a technical limit allowing for the presence of undeclared meat species in meat-based foods does not exist. However, a European Commission Recommendation (2013/99/EU) has adopted a working limit of 1% undeclared horse meat in a food containing beef as a limit that triggers further investigation of possible adulteration. Food businesses must satisfy themselves that their sampling and analysing regime will ensure compliance with this standard.

For the purpose of this protocol and in the case of certain DNA analysis the Food Safety Authority of Ireland considers that a result reported a 1% equine DNA relative to bovine DNA should be considered equivalent to this working limit.

FBOs should note that this working limit is designed to help with investigations into adulteration of beef containing foods and should not be interpreted as a general ‘tolerable’ limit for horse meat in beef containing foods.

REPORTING RESULTS
The analytical results from all sampling carried out by the industry should be submitted electronically to the FSAI when they become available. Retailers/caterers should clarify whether results have already been submitted by their suppliers to prevent duplication of reporting. The template for recording and submitting analytical results and associated information is attached. (E-mail completed results template to meat@fsai.ie)

FBOs with samples testing positive for horse meat at or above 1% must inform FSAI immediately under the normal protocols for reporting food incidents.

PUBLICATION OF RESULTS
A summary table of results will be published by the FSAI on a periodic basis. Generally, reporting of results will be on an anonymised basis. However, in the case of products found to contain horse
material at or above the working limit of 1%, full details of these products will be published and may be subject to notification under the Rapid Alert System for Food and Feed (RASFF)
**AUTHENTICITY REPORTING TEMPLATE** (E-mail completed results template to meat@fsai.ie)

<table>
<thead>
<tr>
<th>Name of Reporting Business:</th>
<th>Submission Date:</th>
<th>Period Covered By Submission:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Generic description of material tested | Specify if material is end product (EP) or a meat ingredient (MI) | Number of products or ingredients tested | For Meat Ingredients briefly list the types of products made from the ingredient | Sampling date(s) | Number of Samples tested | Test date(s) | Test method used (e.g. ELISA, PCR, qPCR etc.) | Limit of Detection or Quantification (specify) | Measurement of uncertainty | No. of samples negative (confirmed not present) | No. of samples positives (confirmed to be present below 1%) | No. of samples positives (confirmed present at or above 1%) | Level (if known) for positives above 1% |
|---------------------------------------|--------------------------------------------------|-----------------------------------|------------------------------------------|------------------|-------------------------|-----------------|-----------------------------------------------|---------------------------------------------|-----------------------------------|-----------------------------------------------|---------------------------------------------|----------------------------------------|
|                                       |                                                  |                                   |                                          |                  |                         |                 |                                               |                                             |                                   |                                               |                                             |                                        |
|                                       |                                                  |                                   |                                          |                  |                         |                 |                                               |                                             |                                   |                                               |                                             |                                        |
|                                       |                                                  |                                   |                                          |                  |                         |                 |                                               |                                             |                                   |                                               |                                             |                                        |
|                                       |                                                  |                                   |                                          |                  |                         |                 |                                               |                                             |                                   |                                               |                                             |                                        |
|                                       |                                                  |                                   |                                          |                  |                         |                 |                                               |                                             |                                   |                                               |                                             |                                        |
|                                       |                                                  |                                   |                                          |                  |                         |                 |                                               |                                             |                                   |                                               |                                             |                                        |

Please enter N/A if a particular column is not applicable