# GMO & Food Authenticity

## Meat Authenticity Verification – By DNA Analysis

**Speciation of meats used for adulteration**

As a result of our on-going research programme Premier Analytical Services can now offer a range of diagnostic PCR tests to detect the adulteration of meat based ingredients and meat products with undeclared or cheaper meats. DNA is extracted from the sample and subjected to intensive purification prior to 'real-time' PCR to detect the major meat species (beef, lamb, pork, chicken, turkey and horse) that may be present. The financial aspect of fraudulent activity is clear, as is the need to maintain reputation and quality by both manufacturer and retailer. Surveillance to detect adventitious or fraudulent contamination in meat products serves to reassure all parties within the food supply chain. This is particularly true of ethnic groups whose religious beliefs forbid the consumption of particular species and who require assurance that they are purchasing food that conforms to their beliefs.

**Detection of meat as a vegetarian food contaminant**

As part of the suite of analyses, Premier Analytical Services can also offer a 'universal' meat detection assay, also based upon DNA and which is ideally suited to the detection of meat contamination in vegetarian products. Meat is readily detected above the 0.05% level even in the presence of both milk and cheese, ingredients that traditionally render analyses ineffective. Due diligence by manufactures and retailers to detect such adventitious or fraudulent contamination in these products serves to reassure vegetarians and ethnic groups whose religious beliefs forbid consumption of particular species that they are indeed purchasing food of true vegetarian origin.

## Meat Authenticity Verification – By Immunological Methods

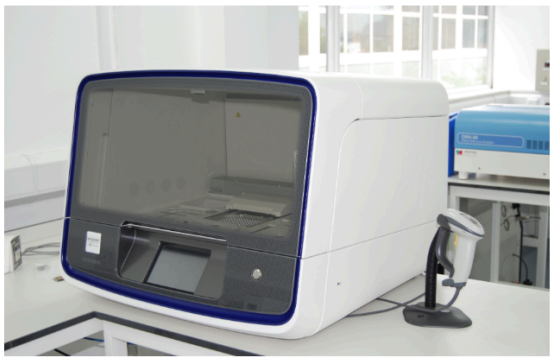
Adulterants that may be found in meat might include proteins from non-meat sources such a soya protein, whey proteins for example casein or wheat protein like gluten. These can all be detected, as meat adulterants, by Premier Analytical Services using ELISA techniques.

## Next Generation Sequencing

At PAS we are continuously striving to offer the most innovative and industry-shaping technologies on the market, the latest of which in food testing is Next Generation Sequencing (NGS).

NGS allows the simultaneous sequencing of targeted DNA present in food samples. This novel approach allows the sensitive and specific detection of the component species present in food samples. Presently, the analytical methods utilised by the food industry are reactive – "please analyse my sample for X " OR "do we have X below this legislative LOD?" NGS will however ensure that we move the industry to a more proactive stance on testing, enabling us to ask "WHAT is in my sample?" and will generate a more complete breakdown of the components present within a given sample.

NGS is capable of sequencing the many different variants of specific genes, that are known to show considerable variation between species, and which are present in the test sample. The DNA sequences obtained can then be compared to a reference database to establish the exact species present – forming the basis of the customer's analytical report.



The possibilities of testing using NGS are vast, especially in the areas of Food Authenticity and Food Fraud which is where we have shaped our testing in order to analyse –

- Meat
- Plants (including Herbs & Spices)
- Fish
- Crustacea

For more information about Next Generation Sequencing and how this could revolutionise your current testing regimes get in touch with us

PAS
PREMIER ANALYTICAL SERVICES

# Authenticity Testing

## Meat Detection

| | | Turnaround Time (Working Days) | UKAS Accredited | Minimum Sample Size |
|---|---|---|---|---|
| **Meat Detection / Speciation** | | | | |
| **Non Specific Meat DNA detection by Polymerase Chain Reaction (PCR)** | | | | |
| **Meat Detection** | PCR | 10 | No | 50g |
| **Specific Meat DNA detection / speciation by Polymerase Chain Reaction (PCR)** | | | | |
| **Tested individually e.g. Is this Chicken?:** | | | | |
| **Chicken** | PCR | 10 | No | 50g |
| **Turkey** | PCR | 10 | No | 50g |
| **Pork** | PCR | 10 | No | 50g |
| **Beef** | PCR | 10 | No | 50g |
| **Lamb** | PCR | 10 | No | 50g |
| **Goat** | PCR | 10 | No | 50g |
| **Horse** | PCR | 10 | No | 50g |
| **Tested in groups e.g. Is this Chicken and/or Pork** | | | | |
| **2 of above meats** | PCR | 10 | No | 50g |
| **3 of above meats** | PCR | 10 | No | 50g |
| **4 of above meats** | PCR | 10 | No | 50g |
| **5 of above meats** | PCR | 10 | No | 50g |
| **6 of above meats** | PCR | 10 | No | 50g |
| **Screen to detect/speciate all of the above meats** | PCR | 10 | No | 50g |

## Protein Fortification

| | | Turnaround Time (Working Days) | UKAS Accredited | Minimum Sample Size |
|---|---|---|---|---|
| **Protein Fortification** | | | | |
| **Soya as a fortifier of protein content** | ELISA | 10 | No | 50g |

## Yeast DNA Detection

| | | Turnaround Time (Working Days) | UKAS Accredited | Minimum Sample Size |
|---|---|---|---|---|
| **Yeast DNA Detection** | | | | |
| **Yeast DNA detection by PCR** | PCR | 10 | No | 50g |

## Pasta, Cous Cous & Semolina Authenticity Service

| | | Turnaround Time (Working Days) | UKAS Accredited | Minimum Sample Size |
|---|---|---|---|---|
| **Pasta, Cous Cous & Semolina Authenticity Service** | | | | |
| **Detection of non Durum wheat in Pasta, Cous Cous and Semolina using Polyacrylamide Gel Electrophoresis (PAGE).**<br><br>**The screening method for determining the presence of adulterating T.aestivum in T.durum pasta is carried out using Acid Polyacrylamide Gel Electrophoresis (Acid PAGE). The procedure fractionates wheat proteins extracted from the pasta product and determines the presence of a low mobility group of proteins known as omega gliadins. These proteins are present only in T. aestivum. The amount of omega gliadin present is compared to reference standards containing known levels of T. aestivum adulteration that are co-analysed with the test samples.** | Dry products | 10 | Yes | 50g |
| | Fresh or frozen products and "ready meals" | 10 | Yes | 50g |
| **DNA confirmation by PCR of results of concern obtained by PAGE** | | 10 | No | |

Please refer to the UKAS Schedule of Accreditation for the specific matrices.

## Vegetarian Products

| Analysis | LOD | UKAS | Comments | |
|---|---|---|---|---|
| **A. Processed foods with "suitable for vegetarians" label but no mention of dairy content in ingredients** | | | | |
| **Potentially 3 phased:** | | | | |
| **Phase 1:** | | | | |
| Non Specific Meat DNA detection by PCR | 0.05% | No | Non Specific PCR Negative = Product suitable for vegetarians. | Non Specific PCR Positive = A 5 Event Specific DNA Meat detection by PCR would be performed as Phase 2 |
| **Phase 2:** | | | | |
| 5 Event Specific DNA Meat detection by PCR viz Chicken; Turkey; Pork; Bovine Y; Ovine Y; | 0.05% | No | Specific PCR Negative = Indicates contamination detected by Non Specific Meat DNA assay is by either a different meat type to that included in specific assay or dairy. Dairy contamination to be confirmed by casein test. | Specific PCR Positive = Product NOT suitable for vegetarians. |
| **Phase 3:** | | | | |
| Casein Test | | | Casein Negative = Confirms contamination by a different meat type to that included in specific assay. Product NOT suitable for Vegetarians. | Casein Positive = Confirms dairy contamination. Differentiation between bovine/ovine X (female) DNA from meat and bovine/ovine X DNA from dairy content is not possible. Assuming however that any bovine/ovine meat contamination should be made up of a proportion of each gender the absence of bovine and ovine Y DNA (as proven in the specific assay) should be an indicator of suitability for vegetarians. |
| **B. Processed foods with "suitable for vegetarians" label + dairy content declared in ingredients** | | | | |
| **Potentially 2 phased:** | | | | |
| **Phase 1:** | | | | |
| Non Specific Meat DNA detection by PCR | 0.05% | No | Non Specific PCR Negative = Product suitable for vegetarians. | Non Specific PCR Positive = A 5 Event Specific DNA Meat detection by PCR would be performed as Phase 2 |
| **Phase 2:** | | | | |
| 5 event Specific DNA Meat detection by PCR viz Chicken Turkey Pork Bovine Y Ovine Y | 0.05% | No | Specific PCR Negative = Differentiation between bovine/ovine X (female) DNA from meat and bovine/ovine X DNA from dairy content is not possible. Assuming however that any bovine/ovine meat contamination should be made up of a proportion of each gender the absence of bovine/ovine Y DNA, as well as the other specified meats, should be an indicator of suitability for vegetarians. | Specific PCR Positive = Confirms DNA is from a specific meat source. Product NOT suitable for vegetarians. |

# GMO Determination

The presence of Genetically Modified Organisms (GMOs) in foods remains a major issue for consumers. European Union (EU) regulations have been designed to ensure that the consumers' right to choose what they eat is facilitated by full and frank ingredient disclosure. In order to achieve this all ingredients that contain or consist of GMOs, or contain ingredients produced from GMOs must be labelled as such.

Harmonised EU systems regarding documentation make it easier to trace identity preserved (IP), non-GM, ingredients throughout the supply chain. Legislation also permits a certain amount of adventitious contamination of I.P. ingredients:

A threshold of 0.9% of the ingredient applies for the accidental presence of approved GM material below which labelling of food or feed is not required.
However there is no threshold for the presence of GM material that has not been approved for use in the EU.
Routine analysis of IP ingredients and products, for due diligence demonstration of GMO threshold compliance, remains an important part of any HACCP control. Now more than ever before this GMO analysis must feature accurate and reliable quantification.

Premier Analytical Services's GMO Detection and Quantification Service assures confidence in results you can trust with the first method accredited by UKAS, to the ISO 17025 standard, for the detection and QUANTIFICATION of GM materials in both PROCESSED FOODS AND INGREDIENTS.

Our dedicated team utilise four separate laboratories to avoid sample cross contamination. They employ the real time polymerase chain reaction (PCR) technique using TaqMan chemistry with custom designed primers and probes.

# Authenticity Testing

## Dectection

| | Analysis | Targets | UKAS Accredited | Level of Detection |
|---|---|---|---|---|
| General Screen | Screen to detect most common components of GMOs coupled with the detection of endogenous marker genes | • CaMV35S promoter sequence | YES | The limit of detection for both maize and soya in processed and raw materials can be calculated for each sample analysed. These are typically 0.01% for processed foods and 0.001% for raw materials. |
| | | • NOS terminator sequence | | |
| | | • Soya lectin & maize zein endogenous marker genes | | |
| General screen for samples with known or expected Soya component | General Screen plus: Detection of MON 89788<br>AND<br>DP-356043-5<br>which do not contain CaMv35S or NOS | General Screen plus:<br>• MON 89788 specific sequence<br>• DP-356043-5 specific sequence | YES | |
| GM Rice Screen | Screen to detect components of GM Rice and Rice endogenous marker gene | • P35S::Bar (for LL Rice 62 & LL Rice 601) | NO | |
| | | • Bt 63 | YES | |
| | | • Rice endogenous marker gene | | |
| GM Tomato Screen | Screen to detect components of GM Tomatoes and endogenous marker gene | • CaMV35S promoter sequence | YES | |
| | | • Npt II | NO | |
| | | • Tomato endogenous marker gene | NO | |
| GM Honey Screen | Screen to detect most common components of GMOs coupled with the detection of endogenous marker genes | • CaMV35S promoter sequence | NO | |
| | | • NOS terminator sequence | | |
| | | • Npt II | | |
| | | • EPSPS (Round UP Gene) | | |
| | | • Soya lectin, maize zein, FatA canola and eukaryotic endogenous marker genes | | |

## Identification

| | Analysis | Targets | UKAS Accredited | Level of Detection |
|---|---|---|---|---|
| SOYA | Samples positive for CaMV35S and NOS and the soya marker gene or permutations of the three | Subject to expert interpretation of Phase 1 result, establish the presence of one or more of the following:<br>• Establish the presence of Roundup Readyä soya<br>• Establish the presence of Bayer A2704<br>• Establish the presence of Bayer 5547-127 | YES | The limit of detection for both maize and soya in processed and raw materials can be calculated for each sample analysed. These are typically 0.01% for processed foods and 0.001% for raw materials. |
| MAIZE | Samples positive for CaMV35S and NOS and the maize marker gene | • Establish the presence of:<br>Bt 176 ; MON 810; T25; DAS 1507; DAS 59122;Bt 11; NK 603; Starlink (CBH 351); MON 863; GA21; Mir 604; MON 88017; SYN3272 | YES | |
| | **OR** Samples positive for just CaMV35S and the maize marker gene | • Establish the presence of:<br>Bt 176 ; MON 810; T25; DAS 1507; DAS 59122; | YES | |
| | **OR** samples positive for just NOS and the maize marker gene | • Establish the presence of :<br>GA21; Mir 604; SYN3272 | YES | |
| RICE | Samples positive for P35S::Bar | • Establish the presence of LL Rice 62 | YES | |
| CANOLA + | None of the targets give positive results except for the FatA canola and eukaryotic endogenou<br>s marker genes. | • Establish the presence of EPSPS<br>• Establish the presence of NptII | NO | |

Please refer to the UKAS Schedule of Accreditation for the specific matrices.

## Quantification

| | Analysis | Targets | UKAS Accredited | Level of Detection |
|---|---|---|---|---|
| SOYA: Samples identified as containing Roundup Ready Soya | Semi-quantification of Roundup Ready soya | • Roundup Readyä soya; | YES | The limit of detection for both maize and soya in processed and raw materials can be calculated for each sample analysed. These are typically 0.01% for processed foods and 0.001% for raw materials. |
| MAIZE: Samples identified as containing one or more of the following GM Maize events:<br>Bt 176 ;<br>MON 810;<br>T25;<br>DAS 1507;<br>DAS 59122;<br>Bt11; (SEE Bt10 BELOW)*<br>NK 603;<br>MON 863;<br>GA21;<br>Mir 604 ;<br>MON 88017;<br>SYN3272 | Semi-quantification of each event as required | • Bt 176 ;<br>• MON 810;<br>• T25;<br>• DAS 1507;<br>• DAS 59122;<br>• Bt 11;<br>• NK 603;<br>• MON 863;<br>• GA21;<br>• Mir 604 ;<br>• MON 88017;<br>• SYN3272 | YES | |
| *Bt10<br>Samples testing positive with significant quantities of Bt11 should be subsequently tested for Bt10 | Semi-quantification of Bt10 | • Bt 10 | YES | |

## Specific Detection

| | Analysis | Targets | UKAS Accredited | Level of Detection |
|---|---|---|---|---|
| MAIZE : Following on from Event Specific Identification | Quantification of GM Maize | • Bt 176;<br>• MON 810;<br>• Bt 11;<br>• GA21;<br>• DAS1507;<br>• MON863;<br>• NK603 | YES | The limit of detection for both maize and soya in processed and raw materials can be calculated for each sample analysed. These are typically 0.01% for processed foods and 0.001% for raw materials. |
| RICE: Following on from Event Specific Identification | Quantification of LL Rice 62 | • LL Rice 62 | NO | |
| **GMO DETECTION – SPECIFIC ASSAYS** | | | | |
| **GM Potato specific detection** | | | | |
| Specific detection of Amylopectin potato | | Event EH92-527-1 | YES | |
| **GM Papaya specific detection** | | | | |
| Specific detection of 2 GM Papayas coupled to the detection of endogenous marker gene. | | Papaya 55-1 and Papaya 66-1 | NO | The limit of detection for both maize and soya in processed and raw materials can be calculated for each sample analysed. These are typically 0.01% for processed foods and 0.001% for raw materials. |

Please refer to the UKAS Schedule of Accreditation for the specific matrices.

## Enzyme Assays

| | | Limit of Detection |
|---|---|---|
| **Alpha amylase** | Megazyme Ceralpha method<br><br>For cereal based products only | 0.003 Ceralpha units (CU)<br><br>CU = amylase activity req'd to release 1 micromole of p-nitrophenol from asssay substrate under the defined assay conditions (time, temp, pH & dilution) |
| **Lipase** | | Semi Quantitative |

## Advice Based on Experience

**Advice Based on Experience**

Premier Analytical Services was the first UK laboratory to offer a UKAS accredited quantitative GMO testing service in the UK utilising analysis based on real-time PCR. More than 20 years of experience in molecular diagnostic techniques has meant that our scientists have been at the forefront of the detection and quantification of GMOs, providing expert advice to UK and European governments. This advice is readily available to all our customers in terms of recommendations regarding sampling, sampling frequency and testing regimes.

**Accuracy and Precision**

**An extensive DNA extraction process is followed by advanced purification, in order to remove inhibitors of the PCR reaction**

**Certified Reference Materials are used in each analysis to enable accurate quantification**

**Appropriate positive and negative controls are used with each analysis**

**Multiple primers and probes are employed. These have been designed for specific and sensitive detection of a wide range of GMOs**

**Analysis is carried out in triplicate; we do however offer the ability to run a greater number of replicates if required.**

**Sensitivity**

The limit of detection for both maize and soya in processed and raw materials can be calculated for each sample analysed. These are typically 0.01% for processed foods and 0.001% for raw materials.

**Interpretation of Results**

Premier Analytical Services's GMO Detection and Quantification Service can provide a full explanation and interpretation of all results thus enabling you to take the appropriate actions.

**Quality Assurance**

Exemplary performance in collaborative proficiency testing schemes further endorses the accuracy of our work, which is performed to the ISO 17025 standard as monitored by UKAS.

**Three-Phased Approach**

The possible combinations of GMOs that could be present in food materials are becoming increasingly complex as more GMOs receive EU approval. Premier Analytical Services has developed a three-phased approach to GMO detection and quantification in order to minimise costs. Details are tabulated below.