FINAL REPORT

POTENTIAL FOR RAPID ONSITE TESTING AT BORDER INSPECTION POSTS

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1 Executive Summary

The requirement for the monitoring/screening of chemical residues or contaminants in food imported into the EU is the subject of much EU legislation. For example, pesticides in imported foods are covered by Regulation 396/2005/EC and veterinary residues by the 'Vet Checks Directive' (Council Directive 97/78/EC). Other measures exist for contaminants such as mycotoxins (Commission Regulation 165/2010), and 3-monochloropropane-1,2-diol, polycyclic aromatic hydrocarbons, metals and nitrates (Commission Regulation 1881/2006). Furthermore, over the past 10 or so years, there has been a series of specific measures to control imported foods for a number of chemicals such as nitrofurans, chloramphenicol and melamine. The presence of elevated concentrations of these chemicals/contaminants in imported food may mean that these products are not safe to eat.

The 'traditional' approach to monitor chemical contaminants in imported food is for the Designated Port of Entry (DPE) or the Border Inspection Post (BIP) to take a sample, and then dispatch this sample to an external/accredited laboratory for assessment. Imported food products that are subject to routine surveillance at the UK borders are usually released from the inspection facility (DPE, BIP) once they have been sampled. This process is the same for products that are frozen, chilled or ambient. Products which are subject to safeguard measures or enhanced border controls, such as those identified in Regulation 669/2009 are sampled and detained at the inspection facility (DPE/BIP etc).

The time taken from sampling, to shipping the sample to the remote testing facility for analysis and reporting can range from within 24 hours to 30 days although in our experience is typically between 5 and 28 days. Produce can be kept at the facility whist testing is underway, but this system is only normally used for high risk scenarios and/or when EU legislation requires positive release of consignments (as in the case of nitrofurans in shrimp, chicken etc. in 2003/04).

The selection of commodities for analysis at DPEs and BIPs is either performed as part of statutory testing or as part of routine surveillance. This project has focussed on the implementation of the application of rapid on-site screening at Border Inspection Posts for routine surveillance purposes.

The disadvantages of the current approach with regard to routine surveillance are that:

1. When analysis results are not returned rapidly and a positive or 'non-compliant' result is obtained, there is a high likelihood that either (i) the food has

been distributed to many retail outlets, or (ii) the food has been consumed (due to the long time between sampling and analysis).

2. Only a limited number of tests on imported can be conducted due to cost/resourcing issues.

3. Although a proactive system for control of imported food is available, emerging risks may not be captured due to the low frequency of testing.

An alternative, more beneficial, approach could be for rapid screening/diagnostic method(s) to be made available for use at the BIPs. Any imported food consignment giving a 'screen positive' result by the rapid method would be held pending a confirmatory test at an experienced laboratory such as a Public Analyst. All negative (compliant) consignments could be released into the food supply with a higher degree of confidence that consumer safety limits have been met. This approach should also lessen the need for expensive product recalls by the importer or distributor of the imported food.

This project is designed to conduct a small-scale study to review the current system(s) of residue/contaminant control of imported food, with a particular focus on the use of rapid diagnostics. The project concentrated on the import of Products of Animal Origin (POAO) and therefore interviews were conducted specifically with BIP staff, although the conclusions generated should be applicable to the testing of commodities at all points of entry. The project was composed of three phases:

Objective 1. A desk-study to review rapid diagnostics methods - in relation to the testing requirements stipulated in EU Legislation.

Objective 2. Interviews with BIP staff to identify the issues and practicalities associated with conducting rapid tests at a port.

Objective 3 A mini-demonstration phase whereby a currently available rapid diagnostic method is installed at a BIP. This rapid screening method will be operated at the BIP for a short period with assistance from staff at Fera. The feedback obtained will be used to decide whether the routine/continued use of rapid screening tests at BIPs is a viable option.

Key findings

The key findings of this project were that rapid screening at BIPs is a feasible option for routine surveillance purposes although certain necessary factors need to be addressed prior to implementation. The rapid screening tests employed need to be fit for purpose, i.e. provide detection limits at the required levels, and be simplistic to use. The questionnaire and mini-demonstration exercise completed highlighted the issues that need to be addressed. The contractor recommendations for further work highlight the necessary steps that would need to be performed to implement this type of 'up-steam' screening on a range of food/feed commodities and products of animal origin.

2 Non-technical (laypersons) Executive Summary

The import of food and feed into the EU is controlled by the Port Health Authorities through the use of border inspection posts (BIPs, for food and feed products of animal origin) and Designated Points of Entry (DPE, for food and feed products of non-animal origin). To test whether food is compliant with EU regulations, analytical testing is performed on consignments by taking samples and sending these to external accredited official control laboratories. Unless specifically forbidden by EU regulations, consignments are usually released before testing results are returned to the BIP. This project sought to determine the feasibility of providing screening analyses directly at the BIPs using commercially available kits.

Firstly, a literature review was conducted to determine the breath of rapid testing methods available in the scientific literature and commercially. These are tabulated and provided in the report.

Secondly, BIP staff were interviewed to determine the current implementation of on-site testing and what issues could prevent this from happening. These are detailed in the report and responses from the authors are provided to address these concerns where possible.

Finally, a mini-demonstration of two different rapid screening technologies were taken to a single BIP and over a period of two days demonstrated on a limited number of samples. The test kits performed as expected and were successful in determining both compliant and non-compliant samples (non-compliant samples were taken to the demonstration from the authors' laboratory to provide suitable positive control material). The demonstration was well received and the staff interviewed saw the benefit of such testing. A number of issues were also raised, some of which had not been identified previously during the questionnaire stage. These are also addressed by the authors.

It is the opinion of the authors of this report that the implementation of rapid screening tests at BIPs and other up-stream testing locations is feasible once the issues raised during this project have been addressed.

3 Glossary

Adulterant a chemical substance which should not be contained within other substances (e.g. food) for legal or other reasons.

Analyte The chemical substance that is being tested for.

Animal-based food Food consisting of or containing constituents from animal products (includes e.g. milk, honey, eggs as well as meat, fish; processed foods containing animal product ingredients)

Antibody A protein produced by animals/humans that is used by the immune system to identify and neutralize foreign objects (such as bacteria, viruses) or substances (such as toxic chemicals). The antibody recognizes a unique part of the foreign object/substance, called an antigen.

Application-specific (method) A test method devised for a particular, specific use.

Aquaculture The farming of aquatic organisms such as fish, crustaceans, molluscs.

Article 24 Refers to Article 24 of the Council Directive 97/78/EC. A control procedure used following a serious or repeated infringement of the veterinary import rules. Following such an infringement the next 10 similar consignments, determined by the TRACES system, are subjected to mandatory testing. During this period of testing all similar consignments are held until satisfactory testing results are obtained.

Assay An investigative (analytical) procedure for qualitatively assessing or quantitatively measuring the presence or amount of a target entity (the analyte)

Authenticity (testing) Confirmation, by analysis, of a product's origin and composition being as claimed by the supplier.

Beta-lactam (antibiotics) A broad class of structurally-related (chemically-similar) antibiotics

Biochemical (method) An analytical method that utilises a biochemical interaction between the test device and the analyte.

Biochip A micro-array of biochemical-type sensors used to simultaneously perform a range of related tests (multiple analytes) in a single sample.

Chloramphenicol An antibiotic, prohibited by the EU for use in food –producing animals.

Class (of analyte) A grouping of chemically similar analytes (e.g. beta-lactams)

Clean-up (of sample) The analytical procedure to isolate the analyte from other potentially interfering constituents of the product being tested.

Contaminant (food) A substance (normally absent from food) which, in sufficient concentration, can adversely affect the consumer.

Contaminant/matrix combination The specific application area of a test method (e.g. beta-lactams/milk)

Enzyme-linked immunosorbent assay (ELISA) A particular format of test method based on the immunological response between the analyte and an antibody (see above) that binds to it.

Enzyme-based analyte hydrolysis A test method based on an enzyme that reacts specifically with the analyte to break it down.

Extraction (of sample) Typically, the homogenisation of a sample with solvent or water to extract the analyte (often prior to additional sample clean-up)

Gas chromatography A laboratory-based instrumental analysis method that separates chemical constituents in the gas phase prior to their detection/identification (e.g. by Mass Spectrometry).

False negative (or false compliant) A false negative result is obtained when a testing method incorrectly determines that an analyte is not present at above a declared concentration / action level.

False positive (or false non-compliant) A false positive result is obtained when a testing method incorrectly determines that an analyte is present at above a declared concentration / action level.

Immunoassay A test method based on the immunological response between the analyte and an antibody (see above) that binds to it.

Immunostrip A dipstick-type immunoassay

Ion Mobility Spectrometer (IMS) A specific type of mass spectrometer that is particularly suited to use in portable/on-site applications.

Lateral flow device (LFD) A disposable or one-shot dipstick-type format, incorporating for example a receptor or antibody for the analyte of interest. Typically, the presence of analyte is indicated by the absence of one of two coloured lines in the developed test.

Liquid chromatography A similar technique to gas chromatography, but using a separation of chemical in the liquid phase (see above).

Mass Spectrometry A very specific and sensitive (traditionally laboratory-based) instrumental analysis method that identifies chemical constituents from their molecular structure.

Maximum levels (ML). The European Union (EU) has set maximum levels for certain contaminants with a view to reducing their presence in foodstuffs to the lowest levels reasonably achievable by means of good manufacturing or agricultural practices – see Commission Regulation (EC) No 1881/2006 of 19 December 2006.

Maximum Residue Level (MRL) A Maximum Residue **Level (**MRLs, including import tolerances) provides a mechanism to verify that produce has only been treated with pesticides according to authorised agricultural practices, both for produce treated within the EU and for imported produce – see Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005

Maximum Residue Limit (MRL). The Maximum Residue **Limit** (MRL) is the maximum concentration of residue accepted by the European Union (EU) in a food product obtained from an animal that has received a veterinary medicine or that has been exposed to a biocidal product for use in animal husbandry- see Council Directive 96/23/EC of 29 April 1996.

Minimum Required Performance Limit (MRPL). The MRPL is the minimum content of an analyte which at least has to be detected and confirmed. This limit is specified for the performance of an analytical technique where the analyte in question is prohibited or not authorised to be present in a commodity – see Commission decision 2005/34/EC of 11 January 2005.

Monoclonal (antibody) Antibodies that are made from identical immune cells and that have affinity for the same specific antigen.

Multiplex (capability) The ability of a test method to simultaneously detect multiple analytes and/or classes of analytes.

Mycotoxins the toxic chemical products produced by fungi that readily colonize crops

Nanoparticle-based methods Typically, dipstick-type test methods employing very small particles (e.g. gold) coated with for example the analyte receptor or antibody.

Negative control A sample that has been confirmed to not contain a specific analyte. A negative control is used to confirm that a testing method does not give false positive results. **Polyclonal (antibody)** Obtained from the serum of animals exposed to the analyte (antigen) of interest, as opposed to from a single cell-line.

Positive control A sample that has been confirmed to contain a specific analyte. A positive is used to confirm that a testing method does not give false negative results.

Pre-screening The rapid throughput testing of samples, to identify 'suspect' samples for subjecting to more rigorous analysis (typically a qualitative, 'presence/absence' type test).

POAO Product of animal origin referring to materials that are of animal origin including but not limited to, apiculture products, bone products, cooked meats for human and non-human consumption, egg products, fresh meat, gelatin, hunting trophies, manure.

Raman spectrometer A specific type of analytical instrument that is amenable to certain portable/on-site applications (including non-destructive testing and hand held devices).

Rapid Alert System for Food and Feed notifications (RASFFs) The European Union (EU) system for rapidly communicating food safety issues between EU Member States.

Receptor-based method A similar principle to immunological-based methods, but for example using a protein as opposed to specifically an antibody.

Residue The remaining chemical in a crop or product of animal origin after an approved/unapproved treatment with a pesticide or a veterinary medicine.

Scanner/reader An electronic device (possibly hand-held) for determining the line colour density of dipsticks in order to obtain numerical, semi-quantitative results.

Semi-automated (method) An instrument-based method where at least some of the sample extraction, clean-up and/or results interpretation is performed by the machine itself.

Total aflatoxins The total concentration of all analytes belonging to the class 'aflatoxin' within a sample.

TRACES trade control and expert system. TRACES is an EU based e-government system for the certification, notification, 'help to the decision' and controls of the importations, exportations and intra community movements. It is used for the application and use of Common Veterinary Entry Documents for both animals and animal products.

4 Aims and Objectives of the Investigation

To review the state-of-art and future prospects of rapid on-site testing for food contaminants/residues via:

Objective 1. A review of the ‘state-of-art’ and future prospects of rapid ‘on-site’ testing for food contaminants/residues.

Objective 2. A review of the practical, and any other relevant issues, associated with conducting rapid tests at a Border Inspection Post (BIP).

Objective 3. A mini-demonstration study at a BIP, using a representative rapid analytical technique.

5 Experimental Procedure

5.1 Review of the 'state-of-art' and future prospects of rapid 'on-site' testing for food contaminants/residues.

5.1.1 Literature Search of Primary Journals

A literature search was conducted as follows:

Scope of search: Rapid onsite test method for veterinary drugs, pesticides, metals

Databases used:

BIOSIS Previews (1985 to 2012 week 25)

CAB Abstracts (19723 to 2012 week 20)

Food Science and Technology Abstracts (1969 to 2012 May week 3)

OVID MEDLINE® (without Revisions, 1996 to May week 3 2012)

Concepts included:

1. The contaminants:

veterinary and (medicine* or drug* or pharm* or antibiotic* or steroid*)

pesticid* and (residu* or contamin*)

(metal or metals) and contamin*

adulterant*

2. It's rapid / on-site

(rapid* or onsite or on-site or quick)

Real adj time "On the spot"

Mobile adj device*

3. Methods, of which lots...

Analyt* or Analys*

(assay adj kit*)

(test* adj kit*)

biochip* or chip*

(immunochem* or immunodiag* or ELISA

immunoassay*

immuno* adj assay*

lateral adj flow*

polyclonal adj antibod*)

(monoclonal adj antibod*)

immunostrip*

4. food adj5 (safety or quality)
5. Test* or Enforc* or Inspect* or monitor* or Alert* or Screen* or Assay* OR AUDIT*
6. 4 and 5
7. 1 AND 2 AND
8. 1 AND 2 AND 6
9. 7 OR 8.

5.1.2 Internet search for commercially-available methods

An internet search was also conducted, using Google advanced search facility and similar combinations of search terms to the above.

5.2 Review of the practical and other relevant issues associated with conducting rapid tests at a Border Inspection Post.

5.2.1 Questionnaire completed by BIP personnel

A questionnaire was devised to determine the practical and relevant issues with regard to using on-site testing at BIPs. The most pertinent responses to this questionnaire are provided in Section 6.3. The fully completed questionnaires are collated and provided in Annex II **(Note: these have been made anonymous)**. In total five questionnaires were completed, four of which completed on site by a member of Fera staff at the BIP and the fifth questionnaire was completed over the phone as no suitable time could be arranged for an on-site visitation.

5.3 Mini-demonstration study at a BIP, using a representative rapid analytical technique.

The mini demonstration exercise was conducted over two days at one Border Inspection Post. Two systems were demonstrated. Firstly, the Evidence Investigator supplied by Randox Food Diagnostics and secondly an immunostrip based assay supplied from the EU FP7 project Conffidence (www.conffidence.eu)[1].

The evidence investigator is a biochip based system designed for research, clinical, forensic and veterinary application. Biochips capable of analysing an array of analytes were used which provided detection of analytes using the principle of chemiluminescence. Two different biochips were used, one containing array suitable for analysing anthelmintics (avermectins, benzimidazoles, amino–benzimidazoles, levamisole, thiabendazole, moxidectin and triclabendazole) and a second array suitable for analysing antimicrobial agents (sulphachlorpyridazine, sulphadiazine, sulphadimethoxine, sulphadoxine, sulphamerazine, sulphamethazine, sulphamethizole, sulphamethoxazole, sulphamethoxypyridazine, sulphamonomethoxine, sulphapyridine, sulphaquinoxaline, sulphathiazole, sulphisoxazole and trimethoprim). The anthelmintics array was used to analyse three samples of corned beef and one sample of cooked beef. The antimicrobial agent array was used to screen 6 samples of honey.

The dip-stick based assay (bee4sensor) is a competition based immunochromatographic assay that is able to detect the presence of antimicrobial agents (sulfonamides, tylosin, (fluoro)quinolones and chloramphenicol antibiotics). The presence of one of the described antimicrobial agents is determined through the absence of a test line visible by eye on the test strip. After demonstration of the

[1] http://www.unisensor.be/en/catalog/antibiotics-28/bee4sensor-45.php

described methodologies the BIP provided feedback on their utilisation. Suitable positive and negative control material was provided by Fera to ensure confidence of the analytical methods employed. The immunostrip based assay was used to screen 6 samples of honey.

6 Results and Discussion

6.1 Literature Search of Primary Journals

6.1.1. Results

The initial search yielded a total of around 1400 references, once duplicate entries were removed. From an initial assessment of each publication based on the abstract, references identified not relevant were sifted out. In particular, the sift was based on the following criteria for removal:

Review papers

Non-food applications only (e.g. water analysis)

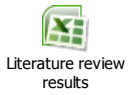
Chromatographic techniques (unless specifically reported as for use on-site/in the field etc.)

Microbiological contaminant detection (this project concentrated on the detection of chemical analytes not microbiological ones)

Detection/identification of bulk constituents (e.g. adulteration by another species/authenticity)

This resulted in a total of 138 relevant references (Annex 1). Because of the limited scope of this study and the need to concentrate on state-of-the art and future methods, the list was further limited to publications from 2011 onwards. This gave a final list of 47 papers for appraisal (Section 9 and Table 1).

Table 1. Literature review results 2011.



6.1.2 Discussion

The most frequent techniques cited were immunological-based methods, followed by receptor-based and nanoparticle-based methods (19, 9 and 5 papers, respectively)

Within these 33 publications, 13 applications are either lateral flow devices (LFDs or 'Dipsticks') or colorimetric type tests that don't require instrumentation (other than possibly a scanner/reader if semi-quantitative results rather than qualitative results are required).

One disadvantage with LFD/Dipstick-type devices is that they generally lack a multiplex capability (i.e. they are most often highly specific tests for one analyte or one class of analyte). They would therefore be best applied as simple, low-cost and rapid targeted test methods for specific 'problem' contaminants: for example beta-lactams in milk, banned antibiotics such as chloramphenicol, 'total aflatoxins' or specific pesticides or metals of particular concern. (A commercially-available LFD kit capable of detecting both beta-lactams and tetracyclines is listed in Table 2.)

Of the pre-2011 publications included in Annex 1, the vast majority relate to methods similar to those covered in Tables 1 and 2. Exceptions are: a biosensor which incorporates calls (Annex 1, reference 30); and a biosensor which incorporates bioluminescent bacteria (Annex 1, reference 110).

6.2 Internet search for commercially-available methods

Limitations with internet searching precluded an exhaustive search for commercially-available products. Where relevant products were found, the webpage was bookmarked for further investigation, including an evaluation of other relevant products from the product manufacturer via company web-sites. A total number of 31 products were identified (Table 2).

Table 2. Internet review results.

Products that are commercially available fall into two broad categories: disposable kit-based methods and methods requiring analysers/instrumentation. With the exception of some of the instrument-based methods, products are application-specific. Exceptions include products such as Raman spectrometers or Ion Mobility spectrometers (IMS) that have been developed for military or industrial chemistry applications but could potentially be employed in detecting certain food contaminants. Hand-held versions of such instruments are unlikely to currently offer the sensitivity required for many food contaminant applications. For example, although Raman instruments have some potential as non-destructive remote-

sensors, sensitivity often needs to be enhanced by modification of the sample surface (Surface Enhanced Raman Spectroscopy). Therefore in practise, sample preparation is likely to be required for most applications.

LFD/Dipstick-type devices again dominate and most of these devices are for specific single analytes or single classes of analyte. It is interesting to note that two 'rapid Enzyme-Linked Immunosorbent Assay (ELISA)' products are included in Table 2, as a previous disadvantage of ELISA has been the relatively long analysis time, including the need for multiple wash-steps. Several instrument-based biochemical-type methods are included in Table 2, at least some of which offer semi-automated analysis whilst retaining the advantage of relatively low cost and simplicity of use compared to laboratory-based instrumental methods such as liquid or gas chromatography with mass spectrometry.

6.3 Review of the practical and other relevant issues associated with conducting rapid tests at a Border Inspection Post.

The following section contains the questionnaire questions and a summary of all the relevant responses given.

1) What screening technologies, if any, does your BIP currently use?

None of the BIPs questioned were currently using screening technologies. Some BIPs had tested some specific screening technologies (aflatoxins and bacterial contamination) in the past but their use was discontinued. No specific time was given for when their use was discontinued although it is the authors opinion that this was several years ago. The use of bacterial contamination rapid screening was discontinued as the results it provided were inconclusive. The use of afloxatin screening was discontinued as it was trialled by a member of staff and not taken forward.

2) If screening technologies are currently used at your BIP how was their use decided? If screening technologies are not employed have they been previously considered?

In the majority of cases screening technologies had not been investigated thoroughly although most people questioned were interested in their application. The tests are currently non-official, do not have legal standing and advice from analytical laboratories has been that these testing technologies are unreliable. It was unclear to the BIP as to whether their use would be beneficial.*

**Note that no specific tests were discussed during this response, these were the comments of the BIP staff to all screening testing including statutory testing and surveillance screening.*

3) What accredited control systems are currently used at your BIP (e.g. ISO17025)?

Accreditation varied amongst BIPs with some organisations having ISO 9001 accreditation, others having individuals accredited and some that had neither.

4) What facilities, if any, currently exist for on-site testing (e.g. clean rooms, non-hazardous waste disposal, solvent disposal)?

The following facilities were available at the BIPs; inspection / clean rooms; fridge and freezer storage; sterilisers; non-hazardous waste disposal. No BIPS had facilities for handling solvents (specific air handling units and solvent waste disposal). Some BIPs had available space that could be converted into a laboratory area. The costs of conversion and upkeep were not considered.

5) What sampling protocols are currently employed at your BIP?

The question of sampling protocols provided answers from two different aspects of the analysis of imports. Firstly, the question of which commodities and analytes were to be chosen (e.g. analysis of corned beef to determine levels of anthelmintics antibiotics). Secondly, relating to what number and the location of samples within a shipping container should be taken.

To determine which commodities and analytes should be sampled: a variety of techniques were used including annual targets with tracking using spreadsheets, use of the RASFFs / TRACES, visual inspection of consignments when performing physical checks, internal knowledge, external knowledge shared between BIPs, emerging risk information.

The number of samples taken, unless specified otherwise by EU regulations, was three representative samples; one for the testing laboratory and two samples kept in storage at the BIP. The two samples were retained in case the testing laboratory required a separate sample for analysis and the remaining sample was kept in case the importer required external secondary analysis. To take these samples, full turnout of a consignment was rarely practically possible.

A lack of official guidance on what sampling to perform, and how to take this sample from a consignment was quoted by one of the BIPs and others sought guidance from testing laboratories.

6) What are acceptable turnaround times for the production of a screening result from a submitted sample?

The majority of BIPs specified that the maximum turnaround time for sampling would be two hours to fit in with current practises.

7) What numbers of samples (analyte/matrix combinations) are / would be tested per annum using on-site testing?

The majority of BIPs were unable to provide specific figures for the number of samples and matrix combinations that would be tested per annum. This was because specific information about such tests (e.g. time taken to test and to determine a result and the cost of tests) was not available. Several matrices and analytes were identified including: histamine analysis in fish and fishery products; mycotoxin testing in cereals; bacterial testing in meat and fish; anthelmintics testing in corned beef; antibiotic residues in honey; illegal dyes in spices. One BIP estimated that approximately 30 samples per month would be screened using on-site testing.

8) What format should results from an on-site test take (Pass/Fail, analyte concentration, analyte concentration with uncertainty)?

The majority of BIPs specified that a pass / fail result would be acceptable providing this complied with legislation although most would also want the absolute concentration if available.*

**Pass fail results would be applicable to the relevant EU legislation for specific analytes.*

9) What are the cost implications associated with on-site testing (e.g. kits, staff costs, facility maintenance costs)?

No specific figures could be supplied for determining the costs of on-site testing but the following aspects were raised as having cost implications: cost to purchase the test; cost of any instrumentation / machinery required to implement the tests; costs to provide dedicated rooms for analysis; staff training; hazardous waste disposal; costs to accredit tests and staff; costs of confirmatory analysis; personnel protection equipment.

10) How would you expect funding to be provided for on-site testing?

The costs to start-up such facilities were generally seen as requiring external funding (e.g. from the FSA) although once implemented testing costs and upkeep would be passed on to the importers through fees.*

**This answer is specific to POAO*

11) What staff resourcing issues would be faced at a BIP that performed on-site testing?

The majority of BIPs thought that such testing would become 'part of the day-job' assuming that the tests could be implemented around current practises. Further staffing may be required if the number of analytical tests and their complexity were significant.

12) What barriers, if any, exist to prevent the routine implementation of screening technologies?

The following statements were provided as current barriers that prevented the routine implementation of screening technologies.

- Costs.
- Lack of legal recognition of the screening tests.
- Test reliability.
- Extra sampling required, currently three samples are taken (one for analytical laboratory, one for importer and one stored at the BIP), whereas this would require four samples to be taken (fourth sample required for screening).
- Obtaining and maintaining accreditation.
- If testing was not universally performed in the UK (and to some extent the EU) the increased testing may result in 'BIP shopping' where importers favour certain BIPs over another based on costs and experiences had at the BIP.
- Applicability of the test to multiple matrices (where trade variations may make specific tests obsolete).

6.4 Mini-demonstration study at a BIP, using a representative rapid analytical technique.

Four samples of corned beef were provided by the BIP, of which one had an unacceptable level (47 ± 16 µg/kg) of ivermectin, as determined by an external testing laboratory. Two samples of corned beef that had been previously analysed by LC-MS/MS at Fera were supplied; one that was confirmed as being free of anthelmintics and one that was shown to contain ivermectin at 73 µg/kg. These samples were used as negative and positive controls.

The positive sample and the positive control were screened positive using the screening technology at the BIP, containing an avermectin residue (this class of compounds includes ivermectin) whereas all other samples were screened negative. Therefore no false positive or false negative results were obtained using this methodology.

Six samples of honey were provided by the BIP, all samples had been previously analysed by an external testing laboratory and were shown to be negative for the antimicrobial agents listed in Section 5.3.

A positive control sample was prepared to confirm that the screening technology was operating correctly. This sample was screened positive for the antimicrobial agents listed. All known negative samples were screened negative. Therefore no false positive or negative results were obtained using this methodology.

The use of dipsticks is a qualitative test and requires visual inspection by the operator. All test strips on the honey samples analysed were visible for all honey samples analysed (excluding the positive control sample) therefore all samples were screened as negative.

The feedback obtained while demonstrating the test determined that the extended sample preparation required for the anthelminitc assay (solvent extraction and subsequent solvent removal step prior to analysis) would mean that in its current form this assay would not be suitable for deployment at the BIPs. The antimicrobial array that required minimal sample preparation (dilution of honey into buffer prior to analysis) was deemed more suitable for deployment at the BIPs but the time taken to obtain a reading (approximately two hours) was deemed too long and therefore it is unlikely that this assay would be routinely used at the BIP in its current format. The ability to generate specific residue levels was seen as a positive for this method. The dipstick assay for honey required minimal sample preparation (dilution) and the total time taken to obtain a reading from receiving a sample (approximately 30 minutes) was deemed suitable for deployment at the BIP but the detection limits of this assay (sulfonamides 50 µg/kg; tylosin/macrolide 25 µg/kg; (fluoro)quinolones 25 µg/kg; chloramphenicol 100 µg/kg) in the ‘field’ format (without the requirement of solvent extraction) were not relevant to the MRPL limit associated with these compounds in honey.

Following the demonstration feedback was sought from the BIP and is given below. Where clarification has been added by the authors, these are presented in parentheses and in italic text.

“All attending were very interested in the possibility of having this kind of technique available, and the actual operational aspects of processing and testing the samples was found very appealing to many, although there were comments that within a laboratory environment the understanding of "rapid" might be different than at a BIP, and some concerns were raised about the amount of time that carrying out the tests would take. An operational limitation we have is that the number and the frequency of consignments is unpredictable, making difficult to organise a sensible way to maximise the tests without penalising with delays other consignments. Other limitations were voiced in the case of the tests for mycotoxins (*during the demonstration, the availability of rapid test kits for mycotoxins was discussed*). We would not be able to homogenise the aggregate sample unless we invest in suitable kit. Taking a small sample of nuts would not be representative and is easily distorted by the discrete nature of contamination

Everyone valued the increased assurance that the test would provide for the products tested, and the positive impact that this on the spot tests it would have in the recall or withdrawal of consignments that at present are being released pending results from the lab and that are found unsatisfactory at a later stage.

Also comments were put forward about the resources that would be required, apart from time as mentioned above, also space, investment in equipment, staff and training. These would have to be recovered resulting in an increase of the fees assuming that neither the FSA/DEFRA or the EU Commission would be funding this initiative. There would also be an extra liability for false positives due to human errors at our end.

If this initiative was going to be implemented at the discretion of the BIPs and not as an EU wide project, those BIPs with the rapid tests would increase the possibility to detect more non compliant consignments, this will make those BIPs less attractive for the trade and could result in loss of trade.

The organisation of official controls is incorporating the principles of risk management, and this approach to testing all consignments does not seem to fit well with a risk based approach to the implementation of the border veterinary checks.

Many raised the question about having other substances and products, and as Mariclare (*Randox Installation Scientist, present during demonstration*) explained Randox can develop biochips to include different matrix and substances without much problem.

There were some concerns raised about the confidence in the accuracy of the tests and the interpretation of the results, but these I suppose could be supported by Randox with reports and various data plus training of the staff that involved in the tests would ensure a correct interpretation of the results.”

The issues raised by the BIP have been addressed in the contractor recommendations.

7 Contractor recommendations for further work

Biochemical-type methods based on LFD/Dipstick-type devices appear to have considerable potential for use on-site at the ports for pre-screening samples for specific contaminants of concern. The European Union Rapid Alert System for Food and Feed notifications (RASFFs) continue to be dominated by for example: mycotoxins in various grains, nuts and fruits; nitrofurans in aquaculture; malachite green in aquaculture; chloramphenicol in animal-based foods from the Far East; histamine in fish etc. Such devices are highly applicable to the rapid, targeted screening analysis of these types of contaminant in food.

Automating or at least partially automating this type of analysis would yield additional advantages, but of course at increased cost in terms of investing in and maintaining the instrument. For this to be economically-viable, the instrument would need to be useable over a range of relevant contaminant/matrix combinations. Multiplex instruments that can meet this requirement are now becoming commercially available.

Several of the concerns raised by the BIPs could be addressed through the selection of inexpensive robust screening methodologies. Non-validated methods would require validation data to be generated which would be used to deem the screening methodologies suitable for use by EU law. Assuming the majority of samples do not contain illegal contaminants (as is currently reflected), cost savings would be realised through the lowered requirement for external surveillance screening tests. The cost of on-site screening tests is lower than those required for external screening tests. These savings would be passed to the importer through reduced import fees therefore increasing business through the port. Reduced fees for the importer and rapid confirmation of consignments as being negative for the tested analyte would likely result in increased import through the BIP and be advantageous rather than detrimental.

The demonstration exercise was successful in providing a mini-demonstration of two different types of on-site screening technologies. It was well received by the BIP who stated that all attending were very interested in the possibility of having these techniques available. Several concerns were raised from the demonstration and those that are not addressed in the section relating to the questionnaire responses are here:

It was apparent that techniques that require clean up using solvent extraction were not suitable at a BIP without the implementation of solvent handling and disposal facilities. The time taken to complete the demonstrated analyses and the possibility of human error was a concern. The demonstrated tests do not represent the totality of diagnostic solutions that are available. It is recommended that future work concentrate on determining rapid screening tests that are simple in their operation. The concerns about human error could be alleviated through robust training and the implementation of proficiency testing rounds. The use of a proficiency testing scheme would generate confidence in the testing performed.

It was noted that the organisation of official controls was incorporating the principles of risk management which is not be aligned with the concept of rapid screening of all

consignments. Risk management would determine those consignments most likely to contain non-compliant material and these consignments could be rapidly screened using the technologies described. It is also prudent when the cost of screening is small to perform random screening to assist in the detection of unknown risks.

An issue about the confidence in the accuracy of the results and their interpretation was raised. Validation of screening tests to the requirements set out in Commission Decision 2002/657/EC, where required, would provide the necessary confidence. In the case of result interpretation this could be demonstrated by suitable training. It should be clarified that screening tests are proposed only to be used to confirm samples as satisfactory. A sample that was revealed to be unsatisfactory would require confirmation from an accredited testing laboratory. The implementation of screening tests would require that the conditions of Article 12, Regulation 882/2004 were still met unless there was a change in legislation. The Commission is currently reviewing Regulation 882/2004. If a screening test were fully validated to current legislative standards and proven to give equivalent results to accepted methods then it could be put forward for assessment to international standardisation committees. Any proposed changes to this legislation would require harmonisation across the EU.

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Annexes/Appendices

Annex 1: Reference list prior to selecting 2011 onwards

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Note: Reference 120 also removed from final selection (water analysis: non-food application).

Note: Reference 16 also removed from final selection (GC-MS lab-based method)

Annex 2: Amalgamated and anonymous questionnaires from all Port Health Authorities questioned.

1) What screening technologies, if any, does your BIP currently use?

All Border inspection posts interviewed declared that none were currently in use. One BIP declared that several years ago some work was completed using test kits for aflatoxin in tomatoes. One BIP declared that they had used a rapid screening test to detect microbiological contamination although its use had been discontinued.

2) If screening technologies are currently used at your BIP how was their use decided? If screening technologies are not employed have they been previously considered?

Not previously considered as no benefit was observable.

Previously investigated the use of ATP test for micro-organisms although did not find a benefit from using these tests. Lack of information on what is available has hampered their use. The tests are non-official and therefore it is considered easier to take the samples and send to official control laboratories.

Not currently aware of what technologies are available and therefore their use at the BIP.

They have been previously considered although advice from the Public Analyst and Food Examiner was that they were not sufficiently accurate (in terms of false positives & false negatives) to allow enforcement action as they lacked legal standing.

Because of the potential money-saving benefits there remains a high of level of interest at the BIP, if tests were to be robust, of proven technology and if there was legislative acceptance.

The BIP was not currently aware of the current testing kits available and stated they would appreciate a final copy of the first stage of this report.

3) What accredited control systems are currently used at your BIP (e.g. ISO17025)?

No external accredited control system.

No accreditation exists for the BIP although individuals have some ISO accreditation. Samples received at the port are usually sent as pre-packed samples and these are removed without opening. Three samples are taken, one for the importer, one sent for analysis and a sample is retained at the BIP. The testing laboratory is ISO accredited.

ISO9001

ISO 9001

ISO9001, plans to implement further ISO accreditation over the next twelve months.

4) What facilities, if any, currently exist for on-site testing (e.g. clean rooms, non-hazardous waste disposal, solvent disposal)?

Clean room and examination rooms. An office at the testing facility was also available for conversion. No solvent waste disposal was available and no air extraction facilities. The stated office had an available window for venting to external atmosphere.

Inspection rooms, hygienic facilities, fridge and freezer storage, sterilisers, non-hazardous waste disposal. No solvent disposal facilities exist.

Clean rooms (6 inspection rooms in total, these are not 'laboratory clean'), Non-hazardous waste disposal (samples for disposal are stored in a quarantine detention room prior to being incinerated). No facility for solvent disposal.

No laboratory facilities, clean room for inspection and taking samples but currently no room available in for dedicated laboratory.

Clean rooms, non-hazardous waste disposal. No facilities exist for solvent disposal.

5) What sampling protocols are currently employed at your BIP?

Sampling protocols are in-house in consultation with the official control laboratories and are dependent on commodity and analytical test type (e.g. microbiological and residue testing sampling strategies are different).

Random sampling of approximately 20% of a chosen consignment, visual inspection often used whether to take a sample or not. Excel spreadsheet is used to determine sample numbers to be taken over annual periods.

Mandatory samples specified by the TRACEs system / Article 24. Surveillance testing is performed using a combination of information, an internal database, past experience, emerging risks, applying knowledge of current and past issues to analogous products, knowledge of issues typical to different processing techniques, food fraud advisory information. Dependant on the volume of import of a commodity, a sample from each country is tested an approximately bi-monthly basis. Unless specified by regulations containers are not fully turned out to retrieve samples due to time and budget constraints.

There is no official written guidance available to determine what sampling regime should be conducted.

A master spreadsheet was used to pre-determine what non-statutory samples would be taken over the year. This is informed using RASFF, the TRACES EU website, internal knowledge and external knowledge gained from discussions with other BIPs.

No official sampling protocol was used for taking sampling within a container. Practically the containers were opened and unless specified otherwise the most accessible packages opened.

For most contaminant types/scenarios, an aggregate sample is produced from sampling at various places through the container (e.g. 10 boxes are taken representatively) and this aggregate sample is then divided into three. Minimum amounts taken per container are 1 Kg/1 L. The number of samples taken depends on consignment weight/total number of packages). Protocols follow legislation such as, for example:

- 470/2009 (pharmacologically-active substances)

-333/2007 (metals)

For aflatoxins, a more rigorous 'full turnout' protocol is employed, to allow every 'nth' sample to be taken.

Small pre-packed assortments of items, such as 25g spice selections for example, are more problematic as the challenge is to take a representative sample without rendering too many of the items unfit for sale.

6) What are acceptable turnaround times for the production of a screening result from a submitted sample?

Rapid results would be more likely to be used. Due to time taken to inspect a consignment and the rotation of BIP staff the maximum test time should take no longer than 2 hours.

As regards potential on-site screening (informal samples): results would be required immediately (within 10 minutes or so). The reason for this is that the container would be moved away from the BIP immediately after an informal sample was taken. If this sample tested 'screen positive', the container would have to be brought back to the BIP.

Currently: Sampling is always formal and six working days* is generally the requirement for contaminants*. (Drivers for rapid turnaround: commercial operation, therefore need to minimise delays/cost to importers; danger that non-detained sample later tests positive for a hazardous substance.)

**Seven at airports including bank holidays.*

Currently the BIP specifies that samples that are analysed by physical inspection are returned within 24 hours (although often are returned the same day) so tests that do

not delay this process would be acceptable (maximum 24 hours). The acceptable time for this BIP was significantly longer than those of the other BIPs questioned.

Ideally dipstick tests with immediate results. Practically, due to the time taken and the number of samples required the upper limit for times was two hours.

Dependant on the test and the commodity. Rapid tests (1 – 2 hours) would fit with the time goods are usually held. Non-official tests would not be used to hold up consignments.

7) What numbers of samples (analyte/matrix combinations) are / would be tested per annum using on-site testing?

This would be based on the speed and cost of the testing.

Colours (including Sudan dyes), Dioxins/PCBs, GMOs, Heavy metals/3-MCPD/Benzo(a)pyrene/Melamine, Aflatoxins (Fruit/Nuts/Cereals, Milk, Spices), Ochratoxin A, Fusarium toxins, Patulin, Histamine, Ergot alkaloid, Microbiological, Nitrates, PAHs, Pesticide residues, Radiation, Veterinary residues, PAAs, Aluminium.

Typically, 30 samples/month including microbiology (which is currently expanding).

This would mainly depend on the cost of the test (including staff time to complete the analysis)

It was not possible to determine what sample numbers would be tested as this would be dependent on the cost, ease, reliability and sped of a test. The typical analyte and matrix combinations that would be tested include

Fishmeal – Salmonella, Enterobacteria, *E. coli* and mammalian protein.

Kitchenware (including ceramics) – PAHs, BPA

Fish products – histamine, PCBs

Cereals and nuts – mycotoxins

Milk – melamine

Corned beef – anthelmintics

Honey – antibiotics

Whey protein – protein content, presence of illegal metabolites (testosterone and analogues)

Depends on the test, for example, currently a test that was able to test for avermectins would probably be used weekly to monitor beef and beef products.

8) What format should results from an on-site test take (Pass/Fail, analyte concentration, analyte concentration with uncertainty)?

Generally: 'Less than'/'Greater than' (concentration of relevance to legislation).

Specific applications (e.g. mycotoxins/peanuts): product could fail in terms of fitness for human consumption, but pass for use as bird feed. In this situation, info on the amount and type of mycotoxin present would be required (i.e. semi-quantitative + chemical i.d.).

Pass fail is OK as long as it conformed to legislation levels.

Ideally the total concentration of the analyte in the sample.

Pass / Fail with analyte concentration would be most sought after but a pass / fail would be acceptable for screening purposes. It was noted that importers are usually aware of the issues and therefore would probably ask for analyte concentration levels.

Analyte concentration would be preferred although pass/fail would be acceptable.

9) What are the cost implications associated with on-site testing (e.g. kits, staff costs, facility maintenance costs)?

Testing costs should not exceed £20 - £30k per annum otherwise due to financial considerations they would not be implemented.

These are currently unknown

Kits, staff training, facilities, disposal (solvent waste and hazardous material), machinery, accreditation, analytical experts (chemical and biochemical), postage of screen positive samples for confirmatory analysis, PPE.

No idea as do not know what is required.

Staff training, accreditation, storage costs/lab space, staff resource (it would currently be stretching the existing staff resource to do this, but could work if there is an over-all cost-saving that would benefit importers).

10) How would you expect funding to be provided for on-site testing?

Set-up costs by FSA

If samples tested fall under statutory sampling, then costs are recoverable (most importantly: must be cheaper than what is done currently).

It is dependent on the amount of money that is required to implement. If the BIP operator was not legally required to perform on-site testing and saw no advantage to it, it would not be something they would implement.

Funding should therefore be provided wither by local or central authorities.

Funding would be provided through the Port as an extract levy on imported goods.

To initiate start-up external funding (e.g.) from the FSA would be required.

11) What staff resourcing issues would be faced at a BIP that performed on-site testing?

Staff training, accreditation, staff resource.

None foreseen, as the inspectors take the sample this would become part of their routine.

Would become part of the day job if test was simple. If testing became a significant part of the routine further staff would be required.

Depends on the level of screening performed but it was envisioned that testing would become part of the day job but if significant amounts of screening were performed more staff would be required

None, it would become part of the day job.

12) What barriers, if any, exist to prevent the routine implementation of screening technologies?

Increased workload, costs of testing and costs if screening technology had a high false positive rate. This is because this would result in more samples having to be sent unnecessarily to external labs for analysis.

Currently no barriers providing the test was efficient and met the requirements specified above.

Reliability of test; Legality of the test, is a negative sample accepted as negative, can a positive sample be legally defended as positive; Need for testing, samples are already sent to laboratories for analysis, why add an extra stage; Cost of extra samples, in effect 4 samples would be required, a sample for screening above the three samples that were described above; Obtaining and maintaining accreditation; If not universally adopted by the BIPs it may encourage “BIP shopping” within the UK.

Legislation; Accuracy; Robustness; Variation in trade (matrix-types) from one month to the next (certain products come and go); Emergency testing scenarios, e.g. Salmonella in prawns from India (how to cope with high numbers of samples without disrupting routine, legitimate trade); Potential for test kit manufacturers to go out of business and consequential unavailability of test; Potential associated downsizing of the supporting reference labs/Public Analyst labs. The law requires certificates for examination/analysis to be issued by qualified public analysts and food examiners; New contaminants: Potential associated loss of access to response capability.

The screening test would need to be recognised for enforcement purposes otherwise no need to use.