General Enquiries on the form should be made to:
Defra, Procurements and Commercial Function (Evidence Procurement Team)
E-mail: research.competitions@defra.gsi.gov.uk

# Evidence Project Final Report

- **Note**
  In line with the Freedom of Information Act 2000, Defra aims to place the results of its completed research projects in the public domain wherever possible.
  The Evidence Project Final Report is designed to capture the information on the results and outputs of Defra-funded research in a format that is easily publishable through the Defra website
  An Evidence Project Final Report must be completed for all projects.
- This form is in Word format and the boxes may be expanded, as appropriate.
- **ACCESS TO INFORMATION**
  The information collected on this form will be stored electronically and may be sent to any part of Defra, or to individual researchers or organisations outside Defra for the purposes of reviewing the project. Defra may also disclose the information to any outside organisation acting as an agent authorised by Defra to process final research reports on its behalf. Defra intends to publish this form on its website, unless there are strong reasons not to, which fully comply with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.
  Defra may be required to release information, including personal data and commercial information, on request under the Environmental Information Regulations or the Freedom of Information Act 2000. However, Defra will not permit any unwarranted breach of confidentiality or act in contravention of its obligations under the Data Protection Act 1998. Defra or its appointed agents may use the name, address or other details on your form to contact you in connection with occasional customer research aimed at improving the processes through which Defra works with its contractors.

## Project identification

1. Defra Project code: OZ0621

2. Project title:
   Science Driven, Industry Informed Feasibility Study for the Development of a Rapid On-Farm test for Campylobacter

3. Contractor organisation(s):
   Animal Health and Veterinary Laboratories Agency (AHVLA)
   Woodham Lane
   Addlestone
   Surrey, KT15 3NB

4. Total Defra project costs (agreed fixed price): £ 52,737

5. Project: start date ..... 01/04/2011

   end date ..... 30/11/2011

6. It is Defra's intention to publish this form.

Please confirm your agreement to do so.....**YES** ☒ **NO** ☐

(a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the Evidence Project Final Report can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

(b) If you have answered NO, please explain why the Final report should not be released into public domain

## Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.


**INTRODUCTION**

- The work carried out in Project OZ0621 was a review and consultation that aimed to establish how a rapid-test for *Campylobacter* could be used to benefit *Campylobacter* controls in the UK broiler industry
- The project aimed to provide a feasibility study that reviewed the rapid testing approaches that are available, in development or in concept stage for a simple test that could be applied in the industrial setting of broiler production to detect *Campylobacter*.
- There are many possible applications for a rapid test for *Campylobacter* and potential methods that could be used. This project has explored options and attempted to identify approaches that are viable and have greatest support from parties interested in reducing *Campylobacter* in UK broilers products.
- A consultation exercise with the poultry industry, regulatory bodies, the Animal Health and Veterinary Laboratories Agency (AHVLA) cross-disciplinary expert group, researchers and other stakeholders was undertaken to define the scenarios in which a rapid test would be used and establish the parameters that a rapid test should achieve.
- A review of testing options with most potential for application was undertaken to assess efficacy and cost-effectiveness, by considering data available in literature, mathematical modelling, production costs and expected benefits.
- A second consultation exercise with stakeholders was undertaken to gather opinion on the best testing options and possible future developments for rapid testing and was used to inform recommendations for rapid on-farm tests for *Campylobacter*.

***KEY FINDINGS***

***Objective 1:*** **Consultation and discussion with the poultry industry and other stakeholders to define the scenarios for a rapid test, the parameters a test would need to cover and potential options**

- The preliminary review identified 4 methods that appear to have potential for a rapid test on-farm and also highlighted several possible testing scenarios where a rapid test could be applied to benefit *Campylobacter* controls within the poultry industry. The testing approaches identified are polymerase chain reaction (PCR), isothermal, lateral flow immuno-assay (LFD), and biosensor.
- The first consultation process gathered responses from 22 participants from a range of stakeholder groups including the poultry production industry, retail sector, government bodies (Defra, FSA), the AHVLA cross disciplinary expert group for *Campylobacter*, other research institutions and diagnostic device companies. The consultation showed the following:
  - There was widespread support for the use of a rapid test to support the control

programme for *Campylobacter* in broilers.

- A test should be available within a year.
- The maximum acceptable cost for a test is in the region of £15 per flock.
- The purpose of the rapid test is to provide information on the *Campylobacter* status of the flock to the company and the 2nd aim is to feedback to the farmer for motivational purposes.
- The test should be able to detect flocks highly contaminated with *C. jejuni* and/or *C. coli* and testing and result collection should be farm based, with minimal preparation steps.
- The use of a lateral flow device had the strongest support from all respondents.
- There was no general agreement reached on the frequency or time of test application.

***Objective 2:*** **Objective 2. Focussed review and assessment of best options, production considerations, modelling of cost benefits**

- This focussed review suggests the method with most potential to meet the demand for a rapid on-farm test is the lateral flow immuno-assay (LFD). The LFD test protocol is relatively simple and was considered achievable in the farm environment by a focus group of farm personnel. The LFD is also the cheapest option currently available with a test cost of £17.50.
- Generation of good data on the sensitivity of a LFD to detect *Campylobacter* in faecal samples is essential before further development of a testing programme. Generation of these data will clarify exactly how LFD testing can be used effectively to complement *Campylobacter* control programmes and to make savings over conventional testing methods. Therefore laboratory validation of LFD tests currently available followed by field trials should be a first step before widespread application by industry.
- The LFD appears the best option for the industry in terms of cost. A major part of the current test cost for the LFD method is the £10 cost for a commercially available LFD unit (Designed for medical and food testing markets). The development of a LFD specifically for application in the poultry farm environment could be a cheaper option in the long term. However this approach would require collaboration with a LFD manufacturer and a further investment of time and money (*ca*. £25000).
- The cost-effectiveness analyses highlighted that rapid-test methodologies may be more efficient than conventional culture methods. A LFD test may be *ca*. 70% cheaper to implement than a culture test.
- On-farm testing could enhance employee education and motivation to control *Campylobacter*. Additionally methods have the potential for quantification or simultaneous detection of other pathogens.
- If it is foreseen that *Campylobacter* testing on-farm will be a continual requirement, then it would be strategic to invest in method development and accept some equipment set-up costs on each farm.

***Objective 3:*** **Final consultation with industry and stakeholders and final report**

- A final consultation process gathered responses from 18 participants from a range of stakeholder groups including the poultry production industry, retail sector, government bodies (Defra, FSA) the AHVLA cross-disciplinary research group, other research institutions and diagnostic device companies.
- This project has shown clear support of the use of rapid testing for *Campylobacter* in the poultry industry. The project has established a demand for a farm-based test that is simple to perform and should be able to identify flocks that are highly colonised with *Campylobacter*. The test should be rapid, providing results within 1-2 hours, it should cost £5-£15 per test, and be ready for use on farms within the next year.
- The project has identified and discussed several different applications for a rapid test in an attempt to define the primary purpose for a rapid on-farm test for *Campylobacter,* a range of options were available. The project has also investigated potential rapid testing methodologies in terms of performance and costs and has found the information available to be incomplete with regard to use on-farm.
- Due the range of potential test applications and the current uncertainty over performance and costs it has not been possible to attach a specific purpose for the test beyond the basic provision of feedback to the farmer/company on *Campylobacter* status of the flock at the point of removal for slaughter.
- However the cost-effectiveness analyses highlight the potential for economic benefits from using rapid on-farm testing compared with conventional culture methods. These cost savings coupled to a range of potential test applications and other additional benefits such as farmer motivation should justify the further pursuit of rapid on-farm tests.
- There was clear demand for a very simple on-farm testing protocol and this dictates that sample collection should be straightforward, collection and testing of faecal samples is the most obvious approach. The LFD appears the most obvious method to meet the criteria for a simple test that is available now, however good data on LFD performance with the faecal sample is lacking.
- This projects primary recommendation is the generation of more robust data on the use of a LFD on poultry faecal samples. This project also recommends continued development of the alternative methodologies discussed in this project, particularly the isothermal and biosensor systems, as they may offer a viable alternative, if the LFD fails to deliver. These alternative rapid methods may have other applications that strengthen the case for further development, such as inclusion of a *Salmonella* test
- The generation of more data for potential testing methodologies could up-date the cost-effectiveness models allowing more certain assessment of the economic benefits. The enhanced test performance data should also allow more specific definition of the primary objective for the test.
- Once test purpose is finalised, an in-field validation of the complete testing protocol from should be undertaken, to confirm that the selected test is fit for purpose.

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Exchange).

***OBJECTIVES***

**Objective 1. Consultation and discussion with the poultry industry and other stakeholders to define the scenarios for a rapid test, the parameters a test would need to cover and potential options**

Objective 1.1 Preliminary review (Annex 1)
Objective 1.2 1st consultation "A rapid on-farm test for *Campylobacter*: Potential applications and methods"
Objective 1.3 Outcome of the 1st consultation

**Objective 2. Focussed review and assessment of best options, production considerations, modelling of cost benefits**

Sub objective 2.1 Focussed review of best options
Sub objective 2.2 Cost-effectiveness analyses
Sub objective 2.3 Assessment of best options
Sub objective 2.4 Further considerations for the use of a LFD
Sub objective 2.5 Opinion and insight on the implementation of a rapid test on-farm
Sub objective 2.6 Conclusions from the focussed review and assessment of best options, production considerations and modelling of cost benefits and options for future development

**Objective 3. Final consultation with industry and stakeholders and final report**

Objective 3.1 2nd consultation "A rapid on-farm test for *Campylobacter*: Focussed review of best options"
Objective 3.2 Final report project conclusions

***INTRODUCTION***

Campylobacter is the most common cause of food poisoning in the United Kingdom (UK) and at the European Union (EU) level is the most commonly reported gastrointestinal bacterial infection in people. Campylobacter accounted for a third of the cost of food-borne illness in England and Wales, estimated at £583 million in 2008. In a recently published EU baseline survey carried out in 2008, UK reported a prevalence of 75.3% for contaminated broiler batches (caecal samples) and 86.3% for contaminated carcasses (neck skin samples). At retail level, a survey (May 2007 to September 2008) reported that *Campylobacter* was present in 65% of the fresh chicken samples tested in the UK. It is well established that poultry is the most significant potentially controllable source of *Campylobacter* for humans.

Defra's first structural reform priority (Business Plan 2011-15) is to support and develop British farming and encourage sustainable food production by helping to ensure a secure, environmentally sustainable and healthy supply of food. With the Food Standards Agency (FSA) and Biotechnology and Biological Sciences Research Council (BBSRC) it has published a joint research strategy aimed at coordinating research efforts on *Campylobacter* with the overarching aim of reduction of the incidence of *Campylobacter* infection in humans.

As part of the joint research strategy FSA is implementing a *Campylobacter* Risk Management Programme, which encompasses a range of projects focused at different points across the food chain with the aim of reducing the level of *Campylobacter* contamination. A new target has been set to reduce levels of *Campylobacter* on chicken carcasses by April 2015, with an ultimate aim of reducing the number of human infections. In the food supply chain (producers, processors and retailers) work needs to be done to control *Campylobacter* and efforts are being made to identify and implement measures to control this food-borne pathogen in the broiler industry.

At the farm level, *Campylobacter* control interventions including enhanced biosecurity, fly screening, water and feed treatment are being investigated and will need to be evaluated for their efficacy. Conventional methods for efficacy assessment would involve the collection of samples on farm, and subsequent culture testing at a separate testing laboratory. Laboratory based culture methods can be expensive and the results, which can take several days to generate may never be fed back to farm personnel. To accurately assess the impact of an intervention, studies will often require multiple testing, making the overall cost of a well designed study prohibitively expensive. As interventions are an essential part of the strategy to reduce *Campylobacter*, then it is important that cheaper testing options are explored whether these options are at the farm, abattoir or the laboratory. It is also important to consider the benefits of producing rapid test results (regardless of the test location) for *Campylobacter* status in a flock or batch of birds, as they could allow reactive decisions to be made on the management/processing of a flock. Such decisions may involve the implementation of *Campylobacter* controls.

On-farm *Campylobacter* control measures will demand diligent application by farm personnel, who therefore need to be involved and motivated to participate in the *Campylobacter* control intervention. A simple rapid on-farm test that can immediately determine the *Campylobacter* status of a flock could be a tool to involve and motivate farmers to buy into *Campylobacter* interventions thereby promoting best practices.

In addition to the variety of potential applications of a rapid test, there is also a wide range of technologies that can detect *Campylobacter* presence using proteins or at the nucleic acid level. Careful assessment of the characteristics of these rapid testing technologies will be required to ensure suitability for any intended rapid test application.

This project aimed to provide a feasibility study that reviews the rapid testing approaches that are available, in development or in concept stage for a simple test that could be applied in the industrial setting to detect *Campylobacter*.

A consultation exercise with the poultry industry, regulatory bodies, the AHVLA cross-disciplinary expert group, researchers and other stakeholders was undertaken to define the scenarios in which a rapid test would be used and establish the parameters that a rapid test should achieve. A review of "state of the art" detection methods has screened for testing options with most potential for application. These options were further assessed for efficacy and cost-effectiveness, by considering data available in literature, mathematical modelling, production costs and expected benefits. The assessment of best testing options was shared with stakeholders in a second consultation. The assessment findings and stakeholder opinion feed back were used to inform recommendations for the development of rapid on-farm tests for *Campylobacter*.

**Objective 1. Consultation and discussion with the poultry industry and other stakeholders to define the scenarios for a rapid test, the parameters a test would need to cover and potential options**

This objective aimed to explore the potential applications by which a rapid test for *Campylobacter* could support and benefit *Campylobacter* control strategies in the poultry industry. A preliminary review of potential test methods was compiled by the AHVLA project team. A 1st consultation document detailing available options for test methods and test applications was circulated to stakeholders from AHVLA, government, industry and research. Feedback and opinion from the consultation document was gathered via an on-line questionnaire. The outcomes of the preliminary review and the 1st consultation process were then documented and used to inform the focussed review of best options for testing in objective 2.

***Objective 1.1 Preliminary review of rapid test methods*** **(Annex 1)**

Introduction

The objective of the preliminary review was to screen all available literature on rapid testing for *Campylobacter* to assess the "state of the art" rapid detection methods that are potentially suitable for testing in the on-farm situation. The review provided back-ground information for the 1st consultation with stakeholders on the potential methods and applications of a rapid-test (Objective 1.2).

Screening the current state of the art rapid detection methods for *Campylobacter*

Information was gathered by direct contact with commercial companies and researchers with an interest in this field. Candidates for the research call on a feasibility study for rapid on-farm tests for *Campylobacter* were invited to contribute information to assist. Internet searches of peer-reviewed scientific journals and other sources were made using search terms (*Campylobacter* +/or Bacteria +/or detection +/or rapid +/or test +/or method), via PubMed, Web of Science, Google and Bing. Abstracts of references from the year 2000 onwards were screened for any information on; 1) methods presently available to detect *Campylobacter* rapidly, 2) potential methods (with development) for *Campylobacter* detection, 3) methods for detection of other bacteria transferable to *Campylobacter,* 4) novel technology with promise that is not yet proven. Two post-doctoral scientists with experience in *Campylobacter* detection and rapid testing methods assessed references with regard to the principle of the test, the sample and resource requirements, test results and anticipated performance. For each method viewed as having potential for on farm testing a summary of advantages and disadvantages was compiled.

Potential methods and advantages and disadvantages

- *PCR (polymerase chain reaction) test*: A molecular technique that can detect *Campylobacter* by amplification of DNA from the target cell: *Advantages:* An established method with good specificity and an intermediate detection threshold that allows reasonable sensitivity for most sample types. Some methods are well validated and simplified and portable test platforms are available commercially, results within 2-3 hours. *Disadvantages:* Equipment and reagent kits may be expensive, demanding DNA extraction process required for faecal type samples, some technical expertise needed.
- *Isothermal test:* A molecular technique that can detect *Campylobacter* by amplification of nucleic acids from the target organism. *Advantages:* Molecular test that should allow good specificity and reasonable sensitivity with results within 2-4 hours. Compatible with a simple DNA extraction process, simple and portable equipment may be available: *Disadvantages:* Less data on performance and validation is available relative to PCR, some technical expertise required, final costs unclear.
- *Lateral flow device (LFD) test:* Technology is based on an immuno-assay, antibodies against target are used to detect *Campylobacter*, a simple visual read out is obtained (similar to a pregnancy test). *Advantages*: A 'pen-side' test with no laboratory equipment needed, simple to perform, no special skills needed, rapid result within 30 minutes, has been used in some poultry samples. *Disadvantages*: Detection threshold may be high, resulting in poor sensitivity for some sample types. The availability of suitable antibodies may be a potential problem.
- *Biosensor test:* Technology is based on an immuno-assay, antibodies against *Campylobacter* are used to detect target and result readout is via an electronic device. *Advantages:* Potentially a 'pen-side' test, should be simple to perform, no special skills needed, result within 30 minutes. *Disadvantages*: Not commercially available, cost of sensors. The biosensor is dependant on suitable antibodies being available.

Unsuitable methods for rapid testing on-farm

Some rapid testing methods were identified in the literature searches, but were deemed unsuitable for application in the farm situation and therefore were not fully assessed. Enzyme linked immuno-sorbent assay (ELISA) methodology was a relatively rapid method with instant potential for application if rapid testing was located in a general laboratory. If rapid testing was located in a specialised laboratory more advanced options are available including micro-array, phage mediated detection, microscopy, Matrix assisted laser desorption ionisation-time of flight (MALDI-TOF) and volatile organic compound detection (VOC).

Conclusions and the way forward

This preliminary review identified four general methodologies with definite potential for the rapid detection of *Campylobacter* on the farm (PCR, isothermal, LFD, biosensor). This review also identified some other methods that have the ability to detect *Campylobacter* rapidly but are not suitable for on-farm application and these were not recommended as a potential on-farm rapid test at this stage. Suitability of each testing method identified will become clearer when the application and the required specification of a rapid test is defined in the 1st consultation (Objective 1.2)

***Objective 1.2 First consultation "A rapid on-farm test for Campylobacter: Potential applications and methods"***

Overview

The objective of this first consultation process was to stimulate discussion on the applications for a rapid on-farm test for *Campylobacter*. Rapid tests have the potential to benefit *Campylobacter* control strategies through a variety of applications and this consultation highlighted these scenarios and then encouraged stakeholders to feedback their opinions on test application. This consultation also asked stakeholders to consider the qualities required for the rapid test, in terms of cost, performance and ease of use. This feedback was used to provide some definition of purpose and specification for the rapid test, and therefore allow potential test methodologies to be assessed against these criteria in the subsequent focussed review (Objective 2).

Consultation process

On the 17th June, 2011, a consultation document (Annex 2) on potential applications and methods for a rapid on-farm test was circulated amongst the stakeholder group which included the Animal Health and Veterinary Laboratories Agency (AHVLA) cross-disciplinary working group on *Campylobacter*, researchers Agri-Food and Biosciences Institute (AFBI) and other researchers who were interested in the original feasibility study call (September 2010). Stakeholders also included the Government-Industry Joint Working Group (JWG) for *Campylobacter*, contained representative from the British Poultry Council, major UK poultry producers, major UK poultry retailers, FSA and Defra. An oral presentation and discussion (Annex 3) of the consultation document was made to the JWG on 20th of June, 2011. Further feedback was invited from all stakeholders via an on-line questionnaire that remained open until the 11th of July, 2011 (Annex 4).

Consultation document

The consultation document contained two sections and a questionnaire embedded within the document.

- *Section 1: Potential applications of a rapid on-farm test:* This section considered scenarios where rapid tests could be used to motivate the farmer/company, to inform flock management decisions or poultry processing decisions, research studies or application at other points of the food chain. This section also investigated what type of result is required? Qualitative or quantitative? Detect all *Campylobacters* or single species or types? What sensitivity is needed? How rapid? How often will the test be used and when?
- *Section 2: Potential methods for a rapid on-farm test:* This section investigated the sample types that could be used for rapid *Campylobacter* detection on farm and asked consultants to assess what is achievable in the on-farm environment in terms of sample collection, preparation and testing. Based on preliminary review findings, the positive and negative aspects of each potential method for rapid on-farm testing were presented. Alternative options to on-farm testing were also discussed, consultants were asked to provide opinion on the need for testing on-farm and their preferred methodology.
- *On-line questionnaire:* Consultants were asked to provide feedback to questions raised in the consultation document via an on-line questionnaire. This was designed using the SurveyMonkey website (www.surveymonkey.co.uk) and contained 16 questions were consultants could rank rapid testing options/applications and provide open comment. This feedback was then documented and analysed to inform the focussed review in objective 2.

***Objective 1.3 Outcome of the 1st consultation***

Following the consultation, feedback was received from 22 respondents; from research (10), government (2), industry (4), retail (5) and other (1). Responses were analysed to identify areas of agreement between consultation participants and are documented (Annex 5 and 6), therefore providing signals for the intended application of a rapid test. **This completed milestone 01/01 and project output 1.** The majority views of the consultants were:

- There was widespread support for the use of a rapid test to support the control programme for *Campylobacter* in broilers.
- A test should be available within a year.
- The maximum acceptable cost for a test is in the region of £15 per flock.
- The purpose of the rapid test is to provide information on the *Campylobacter* status of the flock to the poultry company and the secondary aim is to provide feedback to the farmer for motivational purposes.
- The test should be able to detect flocks highly contaminated with *C. jejuni* and/or *C. coli* and testing and result collection should be farm based, with minimal preparation steps.
- The use of a LFD had the strongest support from all respondents.

Analysis of responses when divided by respondent group did not change for many of the majority views. For some questions regarding the frequency and time of testing, there were divergent responses and hence no clear agreement. However industry and retail were more in favour of a single test at the end of the production cycle.

**Objective 2. Focussed review and assessment of best options, production considerations, modelling of cost benefits**

Based on the initial consultation findings the best method options available were reviewed in greater depth to determine if they could meet stakeholder requirements. Additionally, these method options and the conventional culture method were included in a cost-effectiveness analysis to estimate the economic value of each test option. This allowed for an assessment of each options potential to meet the stakeholder criteria from the initial consultation. In agreement with the majority of this stakeholder opinion, this assessment identified lateral flow devices (LFDs) as the most promising method currently available. Therefore further information on the application of a LFD, its production and potential for implementation in the field was sought. Finally, some conclusions and suggestions are made for further development of rapid tests. This objective is documented in the project documents “review of best options” (Annex 7) and “Report of a rapid on-farm test for *Campylobacter* focus group” (Annex 8) and **this completed the project milestone 02/01 and meets project output 2**.

***Sub objective 2.1 Focussed review of best options* (Annex 7)**

Introduction

The objective of this review was to examine in detail literature on the four potential methods identified for rapid on-farm testing. The review provided information on the performance and cost of potential methods, providing input data for the cost-effective analysis (Objective 2.2) and also informing the 2nd consultation with stakeholders on the potential methods and applications of a rapid-test (Objective 3.1).

*Literature review*

Internet searches of peer-reviewed scientific journals and other information were made. The following search terms were submitted *Campylobacter* and/or bacteria and/or detection and/or rapid and/or test and/or method. Searches were made in PubMed, Web of Science, Google and Bing databases and search engines.

The searches considered results back as far as the year 2000. Considering the outcome of the initial consultation it was felt that only references regarding LFD tests, isothermal tests, PCR tests and biosensor tests were suitable for further review at this stage.
Further information was requested from authors of publications concerning LFDs. Information was also sought from manufacturers of commercially available testing kits, with a view to performance, costs and production. Further information from research groups with an interest in rapid-testing on farm was invited and rapid-test information was gathered from scientific meetings including the 16th International Workshop on Campylobacter Helicobacter and Related Organisms (2011), and the AHVLA International Conference (2011). Two post-doctoral scientists with experience in *Campylobacter* detection extracted information from the reference material to compile an overview of each testing method and generate informed estimates of test performance and cost.

Overview of LFD testing

LFDs work on immuno-chromatographic principles, in which gold-labelled anti-*Campylobacter* antibodies are used to capture antigen from a drop of sample applied directly to the test unit. The device is similar to home pregnancy tests and the results of the test is interpreted by eye, looking for the presence of a control and positive test line. The tests can provide rapid, qualitative results and require no laboratory equipment. They can be considered 'pen-side' or 'dip-stick' tests. There are three tests commercially available; the ImmunocardStat™ is aimed at patient diagnosis, whilst two other products are aimed at food microbiology Singlepath™ and NH Immunochromato™. Both the NH Immunochromato™ and ImmunocardStat™ assays have been used successfully for detection of *Campylobacter* directly from stool samples. The results of the LFD tests are currently read and interpreted by the human eye.

- *Test complexity:* Simple tests, with minimal sample preparation, no special skills or equipment required.
- *Result read-out:* Simply read by eye of tester for presence of indicative lines. Using an electronic reader to scan the LFD test lines may be developed to improve sensitivity. Only a qualitative result is possible. All three tests above can detect (but not differentiate) *C. jejuni, C. coli* and *C. lari*. LFDs are dependent on the specificities of the antibodies used, so in theory they could differentiate between serotypes or subspecies. Use of a combination of antibodies (or possibly a single antibody with broad specificity) could enable the identification of multiple species, not limited to *Campylobacter*.
- *Rapidity:* These assays are very quick, typically 15- 20 minutes reaction time.
- *Costs:* Current LFDs including reagents will cost approximately £10 per test. An estimated 15 minutes is required for sample collection and preparation and a further 15 minutes to run the test. Basic training required.
- *Reported sensitivities/ specificities:* These tests should deal with any type of *Campylobacter*-containing sample with minimal sample processing. However, the limit of detection is quite high at *ca.* $10^5$-$10^7$ colony forming units per gram of sample (cfu/g). The FSA target definition of highly contaminate carcases is $10^3$ cfu/g of neck-skin, this test would not be suitable to screen for these carcases.
- Non-commercial LFDs have been used directly on poultry caecal content with reasonable sensitivities of 85-89% and in studies on human faeces sensitivity has ranged from 72.7% to 98.5%. There is only one small study published on LFD testing in poultry faeces, the results were disappointing with only 3 of 10 positive samples detected. This is a potential problem for the application of a LFD on farm samples and needs further investigation. Specificity data for the LFD has been reported as between 96-100% for human faecal samples.

Overview of isothermal testing

Isothermal testing is a molecular technique that can detect *Campylobacter* by amplification of nucleic acids. The most common isothermal methodology is the loop mediated isothermal amplification assay (LAMP) but other methods are under development including the recombinase polymerase amplification assay (RPA). Isothermal testing uses simple heating block technology to create the test reaction conditions and the reaction enzymes are quite resistant to inhibition from faecal samples. Therefore equipment and sample preparation can be less complex and costly relative to PCR testing.
A commercial LAMP kit (Eiken™) for *Campylobacter* has been used for human faecal samples and preliminary assessment of this kit at AHVLA showed promising results for the detection of *Campylobacter* in broiler caecal samples. There are no complete integrated solutions for isothermal methods. Commercial test platforms which are relatively simple and user friendly are available (illumigene™, Optigene™, Twist DX™), and some platforms are highly portable (Lumora-Bart™). Results can be read by eye with a fluorescent light or by simple turbidimeter. It may be possible to use a LFD for the detection of the amplified product using an integrated unit such as the AMPlite™ concept.

- *Test complexity:* Commercial reagent kits offer the greatest simplicity, but still require medium level technical expertise. Without an integrated sample preparation and testing method, the liquid handling and technical expertise needed is unlikely to be practical in a farm situation.
- *Result read-out:* A single qualitative output is possible that can be read by eye, or a simple electronic device. Development for quantitative results is possible.
- *Rapidity:* Results could be available within 1- 3 hours of sample collection.

- *Costs:* Equipment costs of the isothermal platform are *ca*. £1000-£2000. Reagents cost/test is *ca*. £10. For cost-effectiveness analysis an estimated 15 minutes is required for sample collection, 15 minutes for sample preparation plus a further 60 minutes to run the test. Intermediate technical training is required for tests currently available. Simplified readout systems by eye or LFD may reduce costs. If developed an integrated unit with a LFD (AMPlite™) would cost *ca*. £250 for the test platform and *ca*. £25 for individual test reagents.
- *Reported sensitivities/specificities:* Direct detection from chicken caecal contents reported 86% sensitivity and specificity whilst 80% sensitivity and 96.5% specificity is reported for human faeces. Given the relative simplicity of sample preparation and testing this method and the reported detection limits in human faeces of between $10^3$-$10^4$ cfu/g, this method may have some promise, including the detection of highly contaminated carcases.

Overview of PCR testing

PCR is an established molecular technique that detects nucleic acids from *Campylobacter* following amplification via a PCR reaction. The amplified nucleic acid is visualised by gel-based electrophoresis or more complex fluorescence-based chemistries known as real-time PCR. The test can be designed to detect *Campylobacter* to the genus level, species level, or even to sub-species level. Using real-time PCR results can be obtained that are specific and relatively sensitive within 2-3 hours. There is potential for multiple read-out results for both species and in some assays quantification of target is possible. The platform required to facilitate the PCR reaction and the read-out system is complex and expensive, and therefore would represent a significant investment for farm or laboratory.

Several PCR tests have been described that detect *Campylobacter* directly from complex sample types, like faecal content but intensive sample processing and preparation is required prior to the PCR test that is typically unsuitable for field application. The BAX™ system[23] (DuPont Qualicon) is a laboratory based method that has been validated in Demark for detection of *Campylobacter* in cloacal swabs and Enigma Diagnostics have a Mini-Lab™ (ML) system that is validated for human faecal samples. However there are integrated PCR testing solutions (for both sample processing and testing) available at a considerable price (>£10000), which when coupled with technical expertise could be used in a farm setting such as the R.A.P.I.D™ system and Razor™ systems (Idaho Technology) or the *Campy* FL™ system (Enigma Diagnostics). A possible alternative to faecal sampling for PCR that avoids the complex sample processing is aerosol sampling. However standardised collection of this sample type may be difficult and it did not receive much support in the initial consultation.

- *Test complexity:* Commercially available integrated solutions offer the greatest simplicity, although these methods would still need a medium level of technical expertise to ensure a reliable result. Cheaper non-integrated solutions would only be possible in the laboratory environment.
- *Result read-out:* A real-time PCR platform will provide results via computer or electronic device, can give multiple results per test. Multiple species identification and quantification is possible.
- *Rapidity:* Commercial brochures suggest results in less than 1 hour from sample collection however a conservative estimate would be that results are available within 2 hours.
- *Costs:* Equipment costs of the PCR platform and readout system may be around £10000. Reagent costs per test would be *ca.* £10. For cost-effectiveness analysis an estimate of 15 minutes is required for sample collection, 30 minutes for sample preparation and 75 minutes to run the test. Intermediate technical training is required.
- *Reported sensitivities/ specificities:* Sensitivity and specificity of PCR based systems are good (*ca.* 90%) in highly contaminated broiler faecal samples (*ca.* $10^7$cfu/g). In faecal samples detection limits ranging from $10^2$-$10^6$, making screening for highly contaminated carcases a possibility. The detection of DNA from non-viable *Campylobacters* that could result in a false positive result is possible for PCR and thus could affect specificity. However methods are in development to minimise this risk and additionally this problem is not exclusive to PCR and theoretically could affect all rapid methods considered in this report.

Overview of biosensor testing

Biosensors are analytical devices that combine a biological element (e.g. antibodies, nucleic acids, enzymes) with a physicochemical element (e.g. piezoelectric, electrochemical, photometric) to detect specific analytes. A signal generated when the analyte and the biological element bind is measured and quantified by a sensor. Biosensors are the focus of much research and development as they have considerable potential for rapid and specific detection with low cost and high sensitivity, that can be used on-site (factory floor, pen-side, farm floor). Most biosensors with food-safety applications are still in the development phase with none currently commercially available. It is envisaged a final biosensor would be relatively simple to use, but may cost *ca*. £1000-£2000 which is a considerable investment for a farm. Georgia Technology Research Institute USA (GTRI) has developed a biosensor to detect up to 12 different pathogens, including *Campylobacter* and *Salmonella*. This biosensor is reported to detect 5 x $10^2$ cfu/ml of target organism in under an hour. The production of specific antibody to detect *Campylobacter* is a constraint, this can be problematic and expensive and alternatives to antibody such as DNA aptamers are now being developed.

- *Test complexity:* Although the technology is complex, testing protocols should be simple. Minimal sample preparation and testing protocols are envisaged. A handheld device with similar properties to the adenosine tri-phosphate (ATP) meters used for hygiene monitoring would be the goal for biosensor

developers.

- *Result read-out:* Qualitative and quantitative results are possible. Appropriate biological component (antibodies) should enable identification of multiple species, subspecies or types.
- *Rapidity:* Very quick, typically minutes with little sample preparation.
- *Costs:* Unclear at present. The GTRI sensor is estimated to cost between £700 and £3500, with minimal reagent cost per test. Estimates for a Stratophase (UK) biosensor (in development) were between £2000 and £10000 for the sensor and £1 reagent cost per test. For the cost-effectiveness analysis sample collection and preparation was estimated at 15 minutes plus 15 minutes for the test. Basic technical training is required.
- *Reported sensitivities/ specificities:* The GTRI biosensor has a limit of detection *ca*.$10^3$-$10^4$ cfu/g of food sample but other data is not readily available. Potentially such a method may be suitable to for screen for highly contaminated carcases as defined by the FSA ($10^3$ cfu/g). In theory sensitivities and specificities that are similar to ELISA based methods may be possible *ca.* 96-99%.

***Sub objective 2.2 Cost-effectiveness analyses*** **(Annex 7)**

Introduction

Any new rapid test should ideally be more cost-effective than current laboratory tests. This study aimed to develop a mathematical model that could compare how cost-effective different tests are for the detection of *Campylobacter* in broiler flocks in various testing situations. The model considered test costs (equipment and staff) and test effectiveness (sensitivity and specificity) to create a relative cost-effectiveness value for each test. The model determined cost-effectiveness in the most probable testing scenarios identified from the 1st consultation.

Model scenarios and timescales

- *Scenario A:* The model assessed how accurately each test can determine flock prevalence and the costs involved (One test per flock).
- *Scenario B:* The model assessed how accurately the test can determine an average 50% reduction in flock prevalence prior to slaughter and the costs involved (One test per flock).
- *Scenario C:* The model assessed how accurately each test can determine the day on which a flock has become positive, when testing once per week during the life of the flock and the costs involved (Up to six tests per flock).

For each test, *r*, we determined estimates for the cost, $C_H(r)$, and effectiveness, $E_H(r)$, under a number of different scenarios, *H*, to derive a cost-effectiveness estimate, $B_H(r)$.

$$B_H(r) = E_H(r) * C_H(r). \quad (1)$$

Two timescales where considered for each scenario, thus six cost-effectiveness comparisons were made in total:

- *Short-term view*: The model considered testing 100 flocks on a single farm. For a farm of 6 units, this would roughly cover 2 years of testing.
- *Long-term view*: The model considered testing 1000 flocks on a single farm. (20 years of testing).

Model Inputs (Table 1) derived from the focussed literature review

- *Effectiveness:* The sensitivity and specificity of each method for detection of *Campylobacter* in farm samples were estimated. It is important to note the following regarding the model inputs: When a range of equally plausible values were available for the estimates, a uniform distribution was used. As limited data were available for LFD and isothermal assays, we included data from trials of human faecal content. This should contain similar levels of target organism to poultry samples, although the matrices may differ. The data on LFD testing for faecal samples in poultry was excluded due to poor sample size and the sensitivity observed. Generation of more data on this test and sample type should be a future priority. More data for biosensor detection in faecal samples is also necessary as none were available. The biosensor inputs for this model are hypothetical and are based on an assumption that a biosensor may have similar performance to ELISA tests.
- *Costs:* Cost estimates were made for a single test considering the protocol from sample collection to the generation of a test result. It is important to note the following regarding the model inputs. The same sampling procedure was considered for each test, this is based on the collection of 10 freshly laid faecal droppings and estimated to take 15 minutes. Sample processing and testing time and single test reagent costs are assessed individually for each test. The model also accounted for one-off set-up costs (equipment) associated with each test. The laboratory culture estimate includes transport costs and a fixed test cost of £45 per test.

Table 1. Cost-effectiveness model inputs

| Test | Sensitivity % | Specificity % | LOD (cfu/g) | Total single cost | Additional cost/farm |
|---|---|---|---|---|---|
| Culture | 82-100 | 99-100 | $10^2$ | £57 | n/a |
| LFD | 72.7-98.5 | 96-99.2 | $10^5$-$10^7$ | £17.50 | £0 |
| ISO | 81.3-86.4 | 85.7-96.6 | $10^4$ | £32.50 | £1500 |
| PCR | 88-93.6 | 100 | $10^2$-$10^6$ | £40 | £10000 |
| Biosensor | 96 | 96 | $10^4$ | £8.50 | £5000 |

LOD= Limit of detection in *Campylobacter* colony forming units/gram of sample

Model assumptions

- The model assumes that all input variables are comparable.
- The model does not consider that tests may have different limits of detection. The estimates for sensitivity and specificity are when tests are applied at the removal for slaughter. The model assumes this sensitivity and specificity is unchanged when tests are applied earlier in the production or post-intervention.
- Biosensor data is purely hypothetical.
- Equipment costs are a one-off cost and will last for the lifetime of the model.

Predicting the perfect test

The data on sensitivity and the specificity of the rapid tests used in this model are currently limited therefore findings carry a degree of uncertainty. However as we are more confident of the estimates for the established culture based tests we can ask the question: *Given that we know the accepted and anticipated costs for a rapid test, how effective must it be in order to be more cost-effective than the lab based test?*

*Accepted and anticipated costs:* We have considered an individual test cost of £12.50 (an acceptable cost based on 1st consultation findings) plus three different one-off set-up costs of £0, £500 and £5,000. A one-off set-up cost of between £0 and £500 may be the most realistic option.

The cost-effectiveness of culture for prevalence determination (Scenario A) with a short-term view was the target that our 'perfect test' must improve upon. As cost was known for the 'perfect test', we can establish what effectiveness must be to improve on the efficiency of the culture based test. This effectiveness value can then be used to establish targets for sensitivity and specificity that 'perfect test' should have, if it will be more cost-effective than culture.

Model outputs and discussion

- The LFD is the most cost-effective for all 100 flock scenarios, and the biosensor is the most cost-effective for all 1,000 flock scenarios (Figure 1). Laboratory culture is only competitively cost-effective when used to determine prevalence or intervention efficacy in the short-term (Scenarios A and B). The biosensor set up costs (£5000) mean that it is slightly less cost-effective than LFD in the short-term. However, the biosensor has the lowest cost per test (£1 per test, when the next lowest is £10) and by the time 1,000 flocks have been tested it is the most cost-effective option.
- PCR and isothermal tests are reasonably cost-effective for some scenarios but not others. The isothermal test coupled with a LFD was considered in a preliminary analysis (Data not shown) but initial results suggested this approach will not offer any saving over the current isothermal tests when 100 or more tests are used.

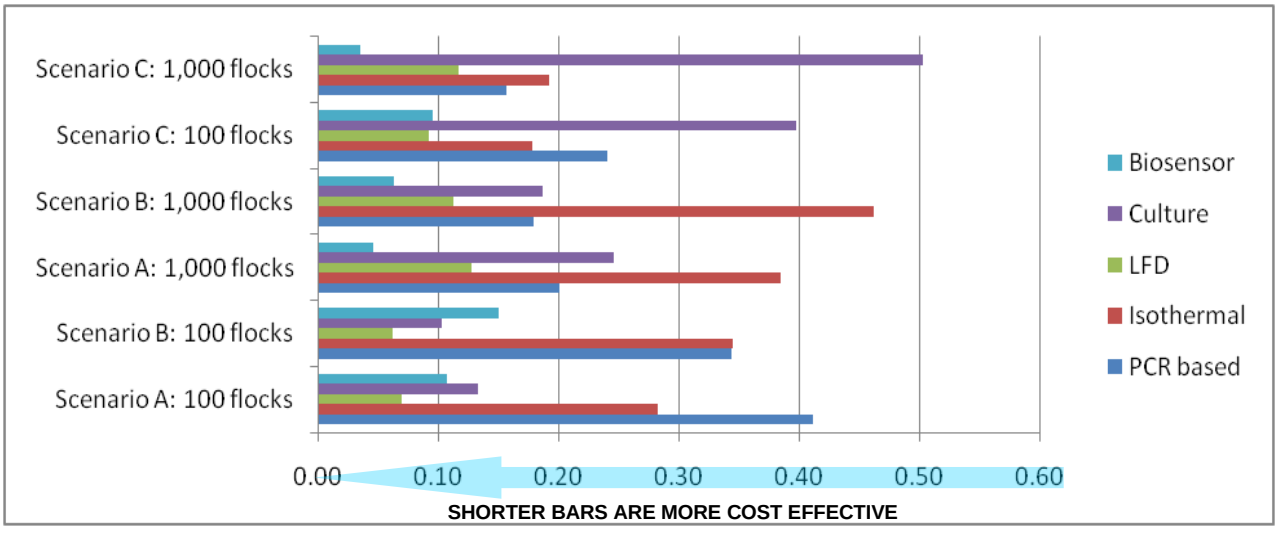

Figure 1. Cost-effectiveness analysis for different testing situations. Results have been normalised to allow comparisons between tests for each situation, do not compare between situations.) For example, in Scenario A, the PCR test is relatively more cost-effective for 1,000 flocks *compared to the other tests* than it is for 100 flocks. The figure does not show that PCR testing is more cost-effective for 1,000 flocks relative to testing 100 flocks.

- There is a high level of uncertainty regarding the cost-effectiveness estimates, due to the accuracy of the cost and effectiveness input parameter estimates. All the sensitivities and specificities of the tests have a reasonable degree of uncertainty associated with them, as different reports quote different values. If our estimates for any these values are inaccurate, this could significantly impact the results. It is particularly important to note that the estimates associated with the biosensor are hypothetical, with no *Campylobacter* specific data available.
- The estimated sensitivities for the tests in this model are for testing samples expected to have high levels of *Campylobacter* ($10^5$-$10^9$ cfu/g). However the limit of detection (LOD) for each test is not the same (Table 1). Thus the sensitivity estimate inputs for our model are not necessarily valid at other concentrations. Thus for Scenario B "intervention efficacy" or Scenario C "longitudinal investigation" it is possible that tests with lower LODs (i.e. culture) may be more cost-effective, however it has not been possible to include this effect in the current model.
- This project has not determined relative importance of false positives in this analysis and for the model it is assumed they have the same importance as false negatives. This model could be adjusted to reflect the importance of these discrepant results if data on this area becomes available.
- Variation in the results is a very important consideration if the test is used to assess the efficacy of an intervention. Analysis of the model results (not shown) suggested that the biosensors determination of flock prevalence in the short-term could be 4% lower or 6% higher than the actual flock prevalence. Thus, while results from 100 tests will show a reduction in flock prevalence when the intervention has a large effect (>10%), it could fail to detect a moderate effect (i.e. 5-10%). Similar effects were seen for the other rapid tests, the model suggested that isothermal tests may only reliably detect a reduction in flock prevalence when the intervention had a large effect (>20% prevalence). When using tests over the longer term (1000 tests), the effect of variation is reduced and reliability is increased.
- The cost-effectiveness model also determined performance characteristics of a 'perfect test' (A more cost–effective test than culture, with a £12.50 cost per individual test). It was determined that a perfect test with a one-off set-up cost of £500 would need to have sensitivity and specificity values of *ca*. 90%.

<u>Conclusions of cost-effectiveness analyses</u>

- In the short-term (100 flocks over two years) the LFD appears most cost-effective in all testing scenarios. However as the number of tests increases biosensor type tests become more cost effective. The biosensor is only a hypothetical test but it does demonstrate what could be achieved if tests are developed for use over a longer term.
- A "perfect test" that is more cost-effective than culture to determine the flock prevalence and has a one-off set-up cost of £500 and individual test costs of £12.50, only needs 90% sensitivity and specificity, this should be an achievable target for test developers.
- The model highlights the potential for savings using rapid tests rather than culture particularly over the longer term. The number of tests to be done is an important consideration; as more tests are done, the impact of the initial one-off equipment costs decrease and the cost per test (staff and equipment) becomes more important.
- Tests with good sensitivity and specificity but low costs per test (reagents and staff time) will, in the long term, become the most cost-effective, regardless of the one-off set-up cost. This cost per test should be in the range of the consultation target of £5 to £15 pounds. The hypothetical "perfect test" or biosensor tests are examples of this.
- The LFD is available now and should be more cost-effective than culture in most testing situations, thereby offering savings however it will cost £17.50 per individual test (above the consultation target), unless the test unit can be obtained for less than the current market price of *ca*. £10.
- The cost-effectiveness model is newly developed and could be improved through more specific data on test performance on poultry faecal samples, and through modification to consider the limit of detection for each test.

***Sub objective 2.3 Assessment of best options*** **(Annex 7)**

This section assesses if each rapid test can meet the criteria established by the 1st consultation.

<u>Does the method have potential for application in the poultry industry environment? In on-farm testing situation, capable for use by farm personnel, will it be simple to use?</u>

- *LFD:* Yes, the simplest method on review and could be applied on many farms within a short period of time
- *Isothermal:* Potentially yes, but currently unlikely for farm personnel. However development of

integrated solutions with ready to use reagents and a cheap test platform could have potential. Technical skills still required.

- *PCR:* Yes, integrated solutions are available but are unlikely to be suitable for widespread use on farms due to equipment and reagent costs and the skills required.
- *Biosensor:* Potentially yes but the technology and protocol need much development before use on-farm.

How does the method meet the requirements for testing scenarios described? Will the method be able to detect high levels of *Campylobacter* (*C. jejuni* / *C. coli*) with sufficient accuracy? And in faecal type samples that can be easily collected on farm?

- *LFD:* Yes, but the performance data currently available is limited. Evidence to suggest that can work for human faeces and chicken caecal contents. More data is needed, specifically for chicken faecal samples.
- *Isothermal:* Yes, but performance data available is limited, more data needed on the final sample matrix.
- *PCR:* Yes, the performance data is available.
- *Biosensor:* Yes in theory, but no data for *Campylobacter* detection in poultry faecal type samples available.

*How much more development is required for a method to meet the requirements? Is a validated method available now for application? Does it appear realistic to develop and validate the test within 1 year?*

- *LFD:* Yes, development of validated sampling and testing protocol is possible, as LFDs are available.
- *Isothermal:* Maybe, but an integrated testing solution and satisfactory field validation is required.
- *PCR:* Yes, development of validated field sampling and testing protocol is possible, as tests are available.
- *Biosensor:* No, this would be very challenging as a *Campylobacter* specific assay is not yet available. This method would then need development into an integrated testing solution that could undergo field validation.

*How much would the test cost to produce and what are the likely benefits? How much will the test process cost, from sample collection through to production of the result? Will implementation of such a method offer useful benefits? Any potential for enumeration or multiple sampling?*

- *LFD:* The cost of a single test is *ca.* £17.50 (£10 in reagents) which is just above the £15 limit identified in the initial consultation. A simple method that could involve farm staff, introducing opportunities for involvement in *Campylobacter* controls. There is scope for detection of other micro-organisms of interest (i.e. *Salmonella*).
- *Isothermal:* The cost of a single test is *ca.* £17.50 (£10 in reagents) which is just above the £15 limit identified in the initial consultation. However a further cost of £1500 for equipment must be considered. Pending development the test could provide a quantitative estimate of *Campylobacter* in the flock or screen for highly contaminated carcases ($>10^3$ cfu/g).
- *PCR:* The cost of a single test is *ca.* £17.50 (£10 in reagents) which is just above the £15 limit identified in the initial consultation. However a further cost of equipment (*ca.* £10,000) must be considered. Test could provide a quantitative estimate of *Campylobacter* in the flock or screen for highly contaminated carcases ($>10^3$ cfu/g). The test could also detect other micro-organisms of interest (i.e. *Salmonella*).
- *Biosensor:* Method could potentially cost as little as £8.50 per test (£1 in reagents). However a further cost of a base unit of *ca.* £5,000 must be considered. Pending development the test could provide a quantitative estimate of *Campylobacter* in the flock or possibly screen for highly contaminated carcases. Potentially the test could also detect other micro-organisms and provide an easy method for farm staff.

*Would the test if developed offer value for money? Will the test offer any significant saving over conventional culture methods with regard to anticipated testing scenarios such as determination of flock prevalence at time of slaughter, assessment of intervention efficacy?*

- *LFD:* Analyses suggests that using a LFD would offer a considerable improvement in cost-effectiveness relative to culture for the assessment of baseline prevalence in flocks. This increased value is seen in just 100 flock tests. The LFD is also more cost-effective than culture for intervention assessments and longitudinal testing.
- *Isothermal:* In a baseline study of 100 flocks, the test is less cost-effective than culture methods. The test offers similar value to culture after 1000 flock tests and is better value for longitudinal studies.
- *PCR:* In a baseline study or an intervention study of 100 flocks, the test is less cost-effective than for culture methods. In longitudinal studies or 1000 flock studies PCR offers better value that culture.
- *Biosensor:* A successful biosensor (96% sensitivity and specificity) with £1 reagent costs would be very cost-effective. Despite a one-off set-up cost (£5000), it would be better value than culture in most testing situations.

Which test has best potential for application on-farm within 1 year?

- The evidence available to date would suggest that the most feasible option available now to provide a simple and rapid on-farm test for *Campylobacter* is the LFD.

***Sub objective 2.4 Further considerations for the use of a LFD*** **(Annex 7)**

The criteria identified via the initial consultation were clear in the demand for a rapid on-farm test that was simple enough for use by farm personnel. Therefore the whole testing protocol has to be simple from sample collection to test processing to result read-out and recording. A hypothetical protocol was created for the use of a LFD in the on-farm situation and is described in Annex 8. This simplified protocol used faecal droppings samples only as the collection of caecal contents would involve culling and opening birds which would be more difficult for farm personnel.

Although performance of the LFD with chicken caecal contents appears to be acceptable, the evidence for LFD performance with fresh faecal droppings is very limited and not encouraging. Correspondence from researchers during the project suggest the numbers of *Campylobacter* present in faecal samples collected from positive broiler flocks may be variable and could be lower than in caecal contents. These lower numbers in faeces and interfering effects of this matrix could affect LFD sensitivity and specificity. Therefore first steps in the application of a LFD would be to quantify the severity of these faecal associated effects and develop strategies to counter-act them.

This project estimates an individual cost per test for a LFD of £17.50 which was slightly higher than the criteria of £5-£15 identified from the initial consultation. This biggest component of this cost is the LFD unit that is currently available to buy at *ca.* £10 per test (designed for medical and food markets). It may be possible to reduce the cost per test if a LFD designed specifically for *Campylobacter* in poultry samples was developed

A commercial company (Foresite) was contacted to assess the costs and time-scales involved with the development and production of a LFD and an outline plan was kindly provided. This plan was estimated to cost £21500 to implement and take six months to complete. This plan would be dependant on a partner to provide verified samples for validation purposes at an extra cost of *ca*. £5000. The plan is also dependant on availability of suitable antibodies, if required the production of a panel of 20 monoclonal antibodies would add approximately £7000 and 3 months to the plan.

***Sub objective 2.5 Opinion and insight on the implementation of a rapid test on-farm*** **(Annex 8)**

Introduction

The aim of this section was to conduct exploratory research using a focus group to gather opinion and insight on implementing a rapid on-farm test for *Campylobacter* particularly from farm managers who would be responsible for implementing the test. The detail on the methodology and data gathered from this section can be found in Annex 8.

Based on findings from the 1st consultation “Potential applications and methods” (Annex 5) the subsequent focussed review “Review and assessment of best options” (Annex 7), a hypothetical protocol for a simple on-farm test for use by farm personnel was developed. The protocol was kept as simple as possible in terms of sample collection and test method. Faecal samples and a LFD test were used. A focus group of prospective testers (mainly farm managers) discussed the implementation of a rapid testing programme on farm, using a hypothetical protocol. The practicalities and frequency of test implementation were discussed. Additionally the strengths of rapid on-farm testing and uses of the results were considered as were concerns with the application of any new test.

It was necessary to recruit a poultry company to assist with the formation of the focus group of farm personnel who would be expected to use the rapid test. A candidate company was identified for the focus group and the Technical Manager was contacted regarding the creation of a focus group. The company manages a significant proportion of the broiler farms in the UK and supplies leading retail and foodservice customers within Europe. The company kindly agreed to participate and the focus group was held on the 6th September 2011.

The group comprised six company employees; a technical advisor, an area manager and four farm managers. The session was facilitated a social scientist from AHVLA. Prior to the meeting, participants were sent the hypothetical test protocol and it was checked that all participants had read and understood the protocol. Examples of the equipment required to perform the test were also available at the session. Participants were invited to express their views openly and the commitment was made that any views expressed in the group and summarised in this report would be presented anonymously.

The main aim of this exploratory research was to gather opinion and insight on implementing a rapid on-farm test for *Campylobacter* particularly from farm managers who would be responsible for implementing the test.

*All focus group participants acknowledged Campylobacter as an issue and recognised a need to improve the control of it.*

Method

The Focus Group technique was used as it has advantages over other social research methodologies, such as, observation and in depth interviews especially for topics more associated with social psychology; some processes, such as attitude formation and decision making, are inherently unobservable or the effort required for participant observation would be excessive both in time and resource or there is a need for rapid data collection.

Focus groups are defined as a research technique that collects data through group interaction on a topic determined by the researcher. In essence it is the researcher's interest that provides the focus, whereas the data themselves come from the group interaction, (Morgan 1996).

This qualitative method is also a proficient method for generating ideas and questions; the technique aims to gain insight and understanding by hearing from representatives from the target population and therefore is not representative and the purpose is not to generalise findings.

Session Structure and Content

The session was semi-structured to facilitate open discussion and elicit opinion and insight around the test. The format included exploration of five themes: Discussion under each of the five themes was initiated using the prompt questions that are in brackets below.

- *Practicalities (Does the method have potential for application in the poultry industry?)*
- *Strengths (What are the strengths for implementing the test?)*
- *Concerns (What are your concerns for implementing the test?)*
- *Frequency of use (How often would you use this test and at what stages in the broiler production cycle?)*
- *Use of results (How would the results of the test be used in the production cycle?)*

Theme Analysis and Reporting

The session was transcribed using audio recording (with permission from the participants); non-verbal communication was not included in the transcript, the discussion lasted two hours. A thematic analysis (a structured method commonly used for analysis of text), was performed.

Barriers and motivators identified

Some topics emerged repeatedly from the analyses of the themes and are listed below.

- *Time management and financial cost* were recurring barriers across many of the themes. It was noted that the cost implication would be different between company owned farms and independent farms.
- *The frequency of testing* was identified as a potential barrier to implementing the test but an optimal frequency was agreed at 3 times a crop (if flock remained negative). It was felt that more frequent testing was not cost effective due to the sensitivity of the test.
- *Ability to react* was identified as a barrier, there was a consensus that biosecurity was a way of controlling *Campylobacter*. The implementation of these biosecurity procedures was included in the company's protocols and therefore there was a majority feeling that farm managers did not have the ability to change these procedures only to ensure the implementation of them by all staff. Thus the results of the rapid test may not enable, even motivated, farm managers to change these procedures but it might be used to highlight cross contamination routes.
- *Knowledge and education* of knowing the status was identified as a benefit as it could motivate and empower both the company and the farm manager. The company would be able to implement certain interventions including, freezing, cooking or reducing/ minimising risk of contamination by de-prioritising the killings and collection of the *Campylobacter* positive flocks. The Farm Manager could use the knowledge as a management tool to promote awareness, potentially resulting in stricter adherence to biosecurity or greater compliance with protocol(s). This status knowledge of flocks was also highlighted as a benefit of implementing the test as it would allow the industry to potentially baseline the current situation.
- *Other concerns/barriers raised*: impact on the health of staff implementing the test; harmful chemicals released when testing, disposing of the test equipment and impact of sampling on the birds; additional stress to the birds when staff collected the sample. In general the test protocol was viewed as achievable but several technical/practical concerns were raised, these are listed in the focus group report (Annex 8).

Main findings

- This focus group of prospective on-farm testers (mainly farm managers) viewed rapid on-farm testing as a positive development.
- The hypothetical testing protocol that was presented to the focus group was generally believed to be achievable in the on-farm environment. Some modifications and extra resources on farm would be required. Training would be needed to address safety and biosecurity concerns.

- A re-current concern of the focus group was the extra demand on resources for implementation of the test, the time taken for a single test (or multiple tests per farm), how it will fit with current work practice. Who will pay for the test and the time involved?
- The involvement of farm personnel in the rapid testing scheme was viewed as beneficial for the knowledge and education of *Campylobacter* to those working on farm, which should benefit *Campylobacter* control programs.
- The active participation in testing and then knowing in real-time the *Campylobacter* status of flocks under your control could also help to empower and motivate personnel to implement the farm controls for *Campylobacter* effectively. In the current high prevalence situation, farmers did not feel that knowing the *Campylobacter* status of the flock was a barrier for testing, it was felt there would be no stigma associated with having positive flocks.
- The focus group also uncovered some issues that could negatively impact *Campylobacter* controls on farm. Staff may be de-motivated if they feel their ability to react effectively to the test result is limited. This may be due to restrictive company protocols. Or in the current situation of high *Campylobacter* prevalence, staff may associate (rightly or wrongly) that control measures employed are ineffective and then lose motivation to implement them properly. There is also a possibility that staff may feel interventions have not been effective if testing was not sufficiently sensitive, a false sense of security may be created on-farm and biosecurity standards may fall.
- This exercise provided rich insight into the groups attitudes and highlighted potential barriers and motivators on implementing a rapid on-farm test for *Campylobacter* however the group's opinions may not be representative of all stakeholder views as only a single company was consulted. Further focus groups with other groups of prospective testers including private farmers would be recommended.

***Sub objective 2.6 Conclusions from the focussed review and assessment of best options, production considerations and modelling of cost benefits and options for future development***

- The stakeholders' desirable criteria for a rapid on-farm *Campylobacter* test are that it is simple test available within 1 year and relatively low cost (£5-£15/test). The test must also be rapid, detecting highly colonised flocks within two hours. The test should feed-back to the poultry industry and farmers on the *Campylobacter* status of a flock after an intervention.
- This focussed review suggests the method with most potential to meet the demand for a rapid on-farm test is the LFD. The LFD test protocol is relatively simple and was considered achievable in the farm environment by a focus group of farm personnel. The LFD is also the cheapest option currently available with a test cost of £17.50. Although the other methods assessed in this review have similar test costs, they also demand an initial and potentially significant outlay for specialised equipment.
- It should be noted that the application of an LFD test may need some compromises in terms of what can be achieved by a rapid on-farm testing programme. Ultimately any test that is implemented needs to be fit for purpose. However this purpose has yet to be fully defined. This review has indicated that the LFD may fulfil the purpose of determining the *Campylobacter* status of flocks at slaughter in a high prevalence situation like that presently found in the UK. It could also be applied when used to determine efficacy of intervention or in a longitudinal study to determine the time of infection, however these testing scenarios may be more challenging as the level of *Campylobacter* in the test samples could be lower. At present it is still unknown if the limit of *Campylobacter* detection for the LFD in faecal samples (most easily collectable on farm) will be sufficient for these testing scenarios and this is a critical piece of evidence that is lacking before any firm recommendations can be made.
- Therefore generation of good data on the sensitivity of a LFD to detect *Campylobacter* on faecal samples is essential before further development of a testing programme. Generation of these data will clarify exactly how LFD testing can be used effectively to complement *Campylobacter* control programmes and to make savings over conventional testing methods. Therefore laboratory validation of LFD tests currently available followed by field trials should be a first step before widespread application by industry. In general more comparative data for all of the test options in this review would benefit the decision-making process.
- Cost will be a key consideration for the development of any on-farm test, and the LFD appears the best option for the industry in terms of cost. A major part of the current test cost for the LFD method is the £10 cost for a commercially available LFD unit. These LFDs were made available for the medical and food microbiology markets and development of a LFD specifically for application in the poultry farm environment could be a cheaper option in the long term, potentially bringing the total test cost into the £5-£15 bracket. However this approach would require further investment *ca*. £25000 and development time before application of the test.
- The cost-effectiveness analysis in this review has highlighted the potential saving that can be made by the use of rapid-test methodologies in place of conventional culture methods, particularly over the longer term. In addition, most of the test options considered here have potential extra associated benefits that have not been considered in the analysis. These benefits include involving the farm worker in the testing process, the findings of this review suggest this could enhance employee education and motivation to control *Campylobacter*. Additionally many of the testing methodologies have the potential to be developed further with a view a quantitative result or the simultaneous detection of other pathogens of interest such as Salmonella testing (*ca.* £20 per test) as part of the broiler national control programme for Salmonella. Such an

inclusion could improve the cost-effectiveness of a test. These latter benefits are particularly applicable to some alternative test methods to a LFD, and provide some justification for their continued development.

- If it is foreseen that *Campylobacter* testing on-farm will be a continual requirement, then it would be strategic to invest more in method development and accept some set-up costs on each farm. The biosensor in this review document is only a theoretical example, but demonstrates how a test with low individual costs could save money in the long-term despite a relatively high initial set up cost. The modelling of a "perfect test" supports this view. A test with a set-up cost of £500, and individual test costs of £12.50, will only need to have modest sensitivities and specificities (90%) to be more cost-effective than current culture methods. This should be a realistic and achievable target for test developers**.**

**Objective 3. Final consultation with industry and stakeholders and final report**

***Objective 3.1 Second consultation "A rapid on-farm test for Campylobacter: Focussed review of best options"***

Overview

The objective of 2nd consultation process was to update stakeholders on the findings of the 1st consultation and the subsequent focussed review on best options for a rapid on-farm test. The 2nd consultation aimed to clarify which rapid testing options were most likely to meet the desirable criteria previously defined by stakeholders. The consultation also aimed to assess if the use of a rapid test on-farm is achievable and if it could benefit *Campylobacter* controls on farm. A final aim was to provide some direction for the future development of rapid tests. In light of the information presented in the 2nd consultation document, participants were asked to provide further opinion, reaffirming on the requirement for a rapid on test, its intended purpose and application, the most viable methods and finally the direct of future developments.

Consultation process

On the 28th October, 2011, a consultation document (Annex 9) on a focussed review of best options for rapid on-farm testing was circulated amongst the stakeholder group which included the AHVLA cross-disciplinary working group on *Campylobacter*, the Government-Industry Joint Working Group (JWG) for *Campylobacter*, the British Poultry Council Growers Committee and other researchers who had previously provided information to this project. An oral presentation and discussion (Annex 10) of the consultation document was made to the British Poultry Council Growers Committee 1at of November, 2011. Further feedback was invited from all stakeholders via an on-line questionnaire that remained open until the 11th of November, 2011 (Annex 11).

Consultation document (Annex 9)

The consultation document contained 5 sections.

- *Section 1: A review of the 1st consultation (Potential methods and applications).* (Objective 1.1-2)
- *Section 2: Summary of the focussed review of best options for rapid testing.* (Objective 2.1-4)
- *Section 3: Summary of the benefits and concerns of implementing rapid tests on-farm.* (Objective 2.5)
- *Section 4: Conclusions and the way forward.* (Objective 2.6)
- *Section 5: On-line questionnaire:* Consultants were asked to provide feedback to 9 questions via an on-line questionnaire using the SurveyMonkey website (www.surveymonkey.co.uk).

***Sub objective 3.1.1 Outcomes from the 2nd consultation***

Following the 2nd consultation phase, feedback was received from 18 respondents; Government/AHVLA/Non-Departmental Government Bodies (GOV) (9), industry (4), diagnostic research (5) and others (2). Responses were analysed to identify areas of agreement between consultation participants and are documented (Annex 12, 13). **This meets project milestone 03/02.** The findings of this 2nd consultation have been shared with the researchers from AFBINI who are also investigating the use of a rapid on-farm test.

Previous documents (Focussed review of best options and 2nd consultation document on best options) have recommended that the most appropriate method that is available now to meet the criteria for a rapid on-farm test is the LFD. The project to date has not been able to finalise a definite purpose for the rapid test, as there are several options available. However the cost-effectiveness analyses have shown that the application of rapid tests on farm could be considerably more efficient than conventional culture methods for expected testing scenarios such as the determination of flock prevalence at time of removal for slaughter.

This 2nd consultation has shown that stakeholder opinion is broadly in agreement with this viewpoint. There is a clear opinion that the use of rapid on-farm tests will be of benefit, particularly as they are cheaper than conventional culture methods. There is also a clear opinion that the method best placed to provide a rapid on-farm testing option now is the LFD test.

There is also clear opinion that work should progress now to provide the validation data regarding the application of a LFD on-farm as this is currently lacking. This can be either with commercially available LFDs or with some new poultry specific LFDs in development. This is particularly important with regard to the

testing of poultry (broiler) faecal samples, as testing this sample type will allow a simple testing protocol to be followed by farm personnel. It is encouraging that some work is presently being undertaken in this area that will hopefully supply the performance data for the LFD in the farm environment.

Although the LFD is the clear first option for the present situation, there is a longer term development plan needed for rapid testing. The alternative technologies explored in this consultation process were all shown to have potential benefits over culture is particular testing scenarios. They may also offer some advantages over the LFD, when developed and ready to apply. There was a clear signal from this consultation that work should continue on these methods, given their potential for the future. Hence it is also encouraging that some isothermal methods (LAMP linked to a LFD) are being explored further. This method may offer improved test performance over the typical anti-body mediated LFD approach. However, if large numbers of tests are necessary then test developers will also need to consider carefully the costs involved. The cost-effectiveness modelling in this consultation suggested that for a large number of tests, the method with the lowest individual reagent costs will be the best approach. If this can be achieved then relatively high set-up expenses can be recovered as large numbers of tests are completed. The biosensor when used in the long-term is a hypothetical example of an efficient test and responses to the consultation indicate support to develop this technology for the longer term. However caution is required in the long term developments, to ensure the technology invested in know does not become redundant or obsolete before the benefits of cheap testing have clawed backed the initial funds invested.

The focussed review of best methods, has modelled the range of test effectiveness / test cost combinations that will be more efficient than conventional culture, even when testing in the short-term. These analyses indicate that for a test of £12.50 with a set up-cost of £500, then specificity and sensitivity need only be 90%, to produce a more efficient test than culture over a two year period. This presents an obtainable goal and justifies the further work on alternative methodologies, in case the LFD cannot deliver. An extra justification for further development is the opportunity for quantification or multi-pathogen testing. The value of these approaches have not been explored in detail in this project, however any test that can deliver added value in one of these areas, may ultimately prove the most viable.

Through the course of this project different testing scenarios and test applications have been discussed, and it has not been able to definitively establish a final purpose or objective behind a rapid testing programme. This is due to the multiple options and uses of a rapid test and also because the expected performance of the potential rapid methods identified is still largely unknown. It has became apparent that it will not be possible to lay a full purpose to a test until the performance and costs associated with application in the farm situation are properly measured through laboratory and farm validation studies.

The obvious application of the rapid on-farm test appears to be the provision of data regarding the *Campylobacter* status of the flock. In this context, the rapid test should be well validated and more efficient than conventional culture methods. When sufficient validation data is gained it should be possible to design rapid testing programmes to support, prevalence trials, intervention efficacy assessments, investigations to determine the time of colonisation, or further research questions. The ability to do these investigations at a fraction of the costs associated with culture is clearly very attractive. These types of investigations will require either testing every batch at removal for slaughter or testing longitudinally. This consultation indicated support for these testing strategies including the industry sector. This is very encouraging and indicates that if viable rapid test becomes available it facilitate many purposes. An additional benefit would be the positive impact of involving farm personnel in the testing programme, the majority opinion of this consultation group was that this would benefit *Campylobacter* controls.

However a common theme from feedback received in this consultation, were concerns regarding the impact of rapid test results. Specifically when a rapid test results could directly impact on the profitability of that individual crop (rather than just providing data for a research question). Example scenarios would be 1) if test results were used to direct flocks to a specific intervention that has a cost implication to the famer, or 2) if the *Campylobacter* status of a flock as determined by the rapid test will have an impact on the cash value of that crop. In these situations, concerns have been raised on the reliability a rapid on-farm test delivered by a farmer. Will the method be robust enough? Will a result generated by a farmer be valid, if he/she has a financial stake in the outcome of the test result? A group of opinion in this consultation process has suggested that testing schemes with these implications might be better placed in the hands of independent testers/laboratories.

***Objective 3.2 Final report and project conclusions***

This final report document has collated and summarised the review and consultation processes undertaken for this project. In this final section conclusions are made on the definition of need for a rapid test, the criterion for use, potential methods that could meet the industry/research needs for a rapid test and the commendations for further development work. **This meets project milestone 03/02 and project output 3.**

The key finding of this project has been the establishment of criteria for the use of a rapid test to support the *Campylobacter* control strategies that are being undertaken within the UK poultry industry. Through consultation with the scientific, industry and government communities which have considered the application of a rapid test for *Campylobacter* and the methodology required, this project has shown clear support of the use of rapid testing for *Campylobacter* in the poultry industry. The project has established a demand for a farm-based test that is simple to perform and should be able to identify flocks that are highly colonised with *Campylobacter*. The test should be rapid, providing results within 1-2 hours of sample collection, it should cost in the region of £5-£15 per test, and it should be ready for use on farms in the UK within the next year.

The project has identified and discussed several different applications for a rapid test in an attempt to define the primary purpose for a rapid on-farm test for *Campylobacter,* a range of options were available. The project has also investigated potential rapid testing methodologies in terms of performance and costs and has found the information available to be incomplete with regard to use in a farm environment. Due the range of potential test applications and the current uncertainty over performance and costs it has not been possible at this stage to attach a specific purpose for the test beyond the basic provision of feedback to the farmer/company on the *Campylobacter* status of the flock at the point of removal for slaughter. However the cost-effectiveness analyses undertaken in this report, has highlighted the potential for economic benefits from using rapid on-farm testing compared with conventional culture methods (The cost of a LFD test may be 70% less than a culture test). Additionally the farmer focus group suggested that on-farm controls may benefit from a on-farm rapid testing programme. These potential benefits should justify the further pursuit of rapid on-farm tests.

There was clear demand for a very simple on-farm testing protocol and this dictates that sample collection should be straightforward, collection and testing of faecal samples is the most obvious approach. The LFD appears the most obvious method to meet the criteria for a simple test that is available now. However good data on LFD performance with the faecal sample is lacking. This project's' primary recommendation is the generation of more robust data regarding the use of a LFD on poultry faecal samples. This can be done immediately with LFD products that are currently available to purchase, and can be followed by validation of poultry specific LFDs that are currently in development.

This project also recommends continued development of the alternative methodologies discussed in this project, particularly the isothermal and biosensor systems, as they will offer a viable alternative if the LFD fails to deliver the required performance. These alternative rapid methods may have other applications that strengthen the case for further development, such as inclusion of a *Salmonella* test or as a screen for highly contaminated carcases as part of the industry *Campylobacter* monitoring scheme. Additionally, the cost-effectiveness analyses in the project have provides test developers with a performance target for a test that is more efficient than current culture methods for detection *Campylobacter* in flocks at removal for slaughter. A rapid test that costs £12.50 with a one-off set-up cost of £500, only needs to be 90% sensitive and specific to improve on culture, this should be an achievable target.

The generation of more relevant and comparable data for these testing methodologies could then be used to update the cost-effectiveness model developed in this project allowing a more certain assessment of the potential economic benefits to be obtained prior to further development and application of the tests. The enhanced test performance data should also allow more specific definition of the primary objective for the test. Once this purpose is finalised, then a full in-field validation of the complete testing protocol from sample collection to result collection and action should be undertaken, to confirm that the selected test is fit for purpose.

## References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Project OZ0621 List of Annexes
Annex 1: Preliminary review of methodology available for rapid on-farm detection of *Campylobacter* in broiler chickens
Annex 2: First consultation document. A rapid on-farm test for *Campylobacter*: Potential applications and methods
Annex 3: First consultation presentation to *Campylobacter* joint Government-Industry working group (June 2011).
Annex 4: First consultation questionnaire
Annex 5: First consultation opinion documented
Annex 6: First consultation raw data of opinion gathered
Annex 7: Review and assessment of best options for a rapid test
Annex 8: Report of a rapid on-farm test for *Campylobacter* Focus Group held on the 6th September 2011
Annex 9: Second consultation document. A rapid on-farm test for *Campylobacter*: Focussed review of best options
Annex 10: Second consultation presentation to the British Poultry Council Growers Committee (November 2011).
Annex 11: Second consultation questionnaire
Annex 12: Second consultation opinion documented
Annex 13: Second consultation raw data of opinion gathered

It is anticipated that an abstract will be submitted to Campy UK 2012, with regard to the cost-effectiveness model that was developed in Objective 2.
Two presentations were made in Objective 2, and are available in the annexes.