Optimising extraction and RT-qPCR-based detection of hepatitis E virus (HEV):
Executive Summary

A critical review of published literature was performed, which compiled information on methods developed to detect HEV in foods, focussing on pork products. The most important information which was derived, was that there was one foremost candidate RT-PCR assay, used in many studies, which mediated detection of a broad range of HEV genotypes. Other information from the review included details of a variety of extraction methods for a range of matrices, and use of various SPCs.

Further to this, a review was undertaken to identify the most likely pork products consumed in the UK to select appropriate food matrices to test. In reviewing the data from the 3 datasets available, no conclusions could be arrived at with regard to specific product types consumed across the UK e.g., premium vs. economy pork sausages, salami, chorizo, hams etc. The general conclusion showed that pork sausages are one of the highest consumed products across the UK and consequently this matrix was included in the next phase of the project along with those identified in the literature to be the most likely source of HEV contamination.

The main aim of this project was to provide the FSA (and pork meat and related industries) with a means of evaluating the presence of hepatitis E virus in food products – something which has been difficult to do with any level of confidence thus far due to the absence of a standardised and validated method. The development / optimization of an extraction method from these complex matrices which are applicable for PCR detection is of major importance and can be used to incorporate new control measures into the pork industry’s food safety management plans. Since HEV will be internalized in the product e.g., in the sausage meat or liver, a non-destructive means of extracting the virus was essential. Likewise, the identification of a simple, safe and effective method was also an aim.

As previously indicated there are a number of different methods described and none are consistent. To address the specification to develop a simple and universal method, we aimed to work with current in-house methods adapted at both GCU and CBRI and compare them with each other, and to the best published protocols identified by Task 1 the literature search. In addition, the incorporation of a full suite of controls were to be included in the study as this was lacking in many of the previous reports. In summary, four protocols were tested; two in-house protocols from the partners GCU and CBRI, and two published protocols Martin-Latil et al (2014) and Di Bartolo et al (2015). In addition to the four protocols tested, 3 methods of RT-PCR detection were also used. The most appropriate method was determined via the effectiveness of recovery for the controls, for the target and the for the sample process control (SPC). Lack of inhibition in the RT-PCR, and the most cost effective, safe and simple method was also taken in to account. Overall, it was identified that the most appropriate method that met all criteria was the in-house GCU protocol. Extraction efficiencies were good in all food matrices tested and no hazardous reagents were utilised in the process. It was also shown that the use of a 2 step RT-PCR protocol gave the most consistent results. Based on these data, this method was developed as a standard operating procedure and prepared for the next stage of validation.

A collaborative trial of the final method was performed, with 4 UK and 3 European participant laboratories. Ideally, a collaborative trial should include more (at least 8) participants, but there
was not a sufficient number of suitably experienced laboratories available for the trial. However, initially it was intended only to include the UK laboratories, therefore increasing the number of participants and consequently the trial data set represented added values to the project.

The participating laboratories conducted analyses of pork liver samples artificially contaminated with various levels of HEV (including uncontaminated samples). The resulting data set was statistically analysed by procedures established in several similar international collaborative trials to evaluate the robustness of molecular-based microbial detection methods. Among other parameters, the repeatability and reproducibility of the HEV DETECT method were evaluated by determining the accordance and concordance. In plain language, accordance is the probability of getting an identical result from two unknown samples analysed in the same laboratory, in other words the repeatability. The method was 82.2% repeatable for identification of HEV-contaminated samples, and 66.7% repeatable for identification of uncontaminated samples. Concordance is the probability of getting the same result from two identical samples, where one is analysed in one laboratory and the other is analysed in a different laboratory: in other words, reproducibility. The method was 69.6% reproducible for identification of HEV-contaminated samples, and 70% reproducible for identification of uncontaminated samples. Determination of the concordance odds ratios showed that the HEV DETECT method was just as reproducible between laboratories as it was repeatable within a laboratory.