United Kingdom Accreditation Service

Audit of IAH-E

on

24 October 2001

The United Kingdom Accreditation Service is the sole accreditation body recognised by government to assess, against internationally recognised standards, organisations that provide certification, testing, inspection and calibration services.
Executive Summary

An audit of the sample handling procedures at IAH-E was carried out on 24 October 2001 at the request of the Department of the Environment, Food and Rural Affairs (DEFRA), by a team of two UKAS auditors. The scope of the audit was limited to the traceability of cow and sheep brain samples used in several experiments relating to transmissible spongiform encephalopathy (TSE) agents. In particular, the team focused on the audit trail of samples that had been sent to LGC, Teddington, the audit trail of brains collected in 1990/92 by Veterinary Investigation Centres and the audit trail for archived material held by IAH-E. In addition the audit team evaluated the IAH-E procedures against the specific requirements for sampling handling of international standard, ISO 17025 and identified opportunities for improvement.

The audit established that there was no formal documented quality system covering this work at IAH-E and that record keeping was inadequate to give confidence in the chain of custody of samples used in the various rendering, genotyping and strain typing experiments audited. It was not possible to establish clear traceability between the samples that had been used in the individual experiments carried out by IAH-E or IAH-C with those analysed at LGC or with those that had been collected in 1990/92. The sample handling procedures covered by this audit at IAH-E did not meet the requirements of ISO 17025.
UKAS Audit at IAH-E on 24 October 2001

1. Attendees

UKAS Auditors
Two UKAS Assessment Managers

Staff of IAH-E
- Inactivation Group Leader
- Inactivation Group Member
- Inactivation Group Member
- Peripheral Pathogenesis Group Leader
- Neurodegeneration Group Leader
- Head of Animal Facility
- Sheep Genetics Group Leader
- Sheep Genetics Group Member

An independent auditor appointed by IAH was also present during examination of the test samples.

2. Purpose of UKAS’ Audit

The audit was commissioned by DEFRA to perform an assessment of the chain of custody arrangements and sample handling system in place at IAH-E. The audit was directed to focus primarily on the experimental work carried out by IAH to investigate the possibilities of the BSE agent being carried in sheep.

3. Key Audit Objectives

3.1 Assessment of the audit trail of the samples sent to LGC in which only bovine material was identified and to establish if the same samples were used in the experiment in which a BSE-like agent was detected in mice.

3.2 Assessment of the audit trail within IAH-E of brains collected during the 1990/1992 period from Veterinary Investigation (VI) Centres and submitted to IAH-E for evaluation of rendering process variables on transmissible spongiform encephalopathy (TSE) agents.

3.3 Report on the audit trail for archived material in general and held by IAH-E.

3.4 Evaluate compatibility of sample handling procedures with accreditation requirements (ISO 17025, Section 5.8) and identify opportunities for improvements. [Note ISO 17025 is ISO/IEC 17025 1999 General requirements for the competence of testing and calibration laboratories.]
4. Audit Programme

- Introductions (09.30)
- UKAS’ role and purpose of the audit (UKAS)
- Background to the experiment (IAH-E)
- Examination of records
- Discussions with staff
- Examination of sample storage facilities
- Examination of sample identification and labelling
- Close (18.30)

5. Scope of the Audit

UKAS conducted the audit on 24 October 2001 by discussions with the various staff members identified above and by examination of records, storage facilities and samples at IAH-E. Technical aspects of the experimental work were not assessed. The animal facility was not visited. The more recent recording system maintained electronically was not audited.

It should be noted that some IAH-E staff members who had been involved in experimental work no longer worked at IAH-E and could not therefore be involved in the audit.

6. Background to the Audit

IAH-E gave an overview on the history of “The Experiment”, comprising trials to assess the effect of rendering conditions on the survival of the TSE agents BSE and scrapie.

The audit team was told that in 1990 IAH-E was commissioned by MAFF and others to conduct experiments as set out in a ROAME statement into the effect of varying the conditions of the rendering process on the survival of the BSE agent in cows and scrapie in sheep. Results from these trials were published separately in the Veterinary Record. The project leader for this work had now retired from IAH-E.

In 1996 IAH-E was commissioned by MAFF as set out in another ROAME statement to investigate the possibilities of the presence of the BSE agent in sheep brain material. To achieve this, IAH-E performed a strain typing experiment starting in 1997 to look for any evidence of BSE in the sheep brain sample and two meat and bone meal samples which had been retained from the rendering experiments. IAH-E also carried out a genotyping test as a species check on the material used to inoculate mice for the strain typing experiment. The initial parts of the experimental set up were under the responsibility of the same member of staff who conducted the rendering experiments described above. Other staff carried on the work following his retirement.

Inconclusive results from this strain typing experiment led to a further experiment, using a secondary passage technique into mice, which commenced in 2000. This experiment has not yet concluded. The sheep brain inoculum material was sent recently for analysis by LGC, with the intention of confirming that cross contamination with bovine material had not occurred. DEFRA informed IAH-E on 17/10/01 that the LGC results indicated the identity of test material to be bovine and not ovine.

IAH-E had explained that various VI Centres in England had collected brains from cattle and sheep showing clinical symptoms of TSE. The brains had been macerated in the presence of IAH-E staff at a pilot scale rendering plant in Doncaster. The resultant brain pool had then been used to spike various batches of intestines and bone material; each batch was subjected to a different rendering process. Samples of the brain pool and the rendered materials were transferred to IAH-E to assay the level of TSE infectivity.

7.1 Detailed Observations – Records of Brain Pool Preparation

- IAH-E holds a set of nine A4 ring folders labelled MAFF, MAFF 2 through to MAFF 9. There are also three other A4 ring binders and a box file labelled Rendering 1, Rendering 2, Rendering 3 and Solvent Extraction 1.

- The documents included numerous communications with MAFF and CVL, minutes of meetings, experimental plans etc. The files contained papers commencing in 1990 and were filed chronologically. Records pertaining to the rendering experiments were not separated or readily identifiable. Detail of the file contents was not audited.

- Published papers “Inactivation of the bovine spongiform encephalopathy agent by rendering procedures”, The Veterinary Record, December 9, 1995 and “Effect of rendering procedures on the scrapie agent”, The Veterinary Record, December 20/227 1997 describe the collection and preparation of brain pool spiking material. For both rendering experiments these papers describe that approximately 60g (a pool of 6x10g sub-samples) were retained as the laboratory sample and stored at below –20°C on each occasion. Original records were not available to confirm these details.

- IAH-E suggested that additional records might still be held by the project leader, at the VI Centres which supplied brain material, or at the contract pilot scale rendering plant, Prosper de Mulder Ltd, Doncaster. It was not possible to verify whether or not these records exist.

Summary Findings – Records of Brain Pool Preparation

Original records of the following were not readily identifiable at IAH-E to adequately detail the experimental procedures:

- Documentation of the collection and origin of the brains
- Descriptions of any identity checks on the brain material used to prepare the pooled brain macerates
- Documentation on the creation of the laboratory brain pool samples
- Records to define the identification and labelling of the laboratory samples of brain pool material and rendered products
- Records to detail transport and storage details of the laboratory samples of brain pool material and rendered products
- Record of the receipt of these laboratory samples at IAH-E
- Records of subsequent storage location(s) or the conditions of storage of these laboratory samples at IAH-E
- Details of subsequent handling, treatment, use or aliquoting of the samples
7.2. Detailed Observations – Experimental Filing System

- IAH-E has a centralised system of filing cabinets in the main corridor. Staff explained that these held collated records of experiments conducted in the animal house (this facility was referred to by IAH-E as “behind the barrier”). No documentation was provided that described the coding system or the indexing of the files.

- In the filing cabinets were logically ordered buff folders each labelled with an alphanumeric code. IAH-E explained that the code was assigned by the head of this facility following IAH-E’s established convention and was structured uniquely to identify the specific mouse experiment and any related tests within a project.

- Labelled buff folders in the coded series 372R and 272R for the mouse bioassay experiments arising from the BSE and scrapie rendering trials respectively were examined to assess the record system. Specific files identified by the auditors as most critical to the audit trail of brain pool samples were examined in more detail as reported below.

- Individual folders in the 272R series were coded A through to U. IAH-E explained to the auditors that these indicated A for the control brain pool and B to U for products resulting from the various rendering treatments. Code letters used were not explained in the folder records. A similar subset of files labelled alphabetically starting at A was seen in the 372R series.


- The papers in these experimental folders were loose-leaf sheets, attached with a paperclip to the back cover of the folder and some sheets included the experiment number. Records were made using both ink and pencil; correction fluid had been used. Entries were not all individually signed and did not all identify the individual involved.

- For the experiments in folders in the series labelled 372R and 272R the sample under test was described respectively as “BSE bovine” and “Scrapie sheep”; its storage location was recorded as “DMT” or “DMT freezer”.

- The auditors were informed that written procedures were in place to cover various aspects of work “behind the barrier”. The auditors did not enter the animal house to examine these written procedures, as they were not considered to be directly relevant to the chain of custody records.
Summary Findings – Experimental Filing System

- Experiments set up "behind the barrier" were recorded within a more structured system than other records seen
- Records of experiments set up “behind the barrier” were stored under a unique coding system but this was not defined or indexed
- IAH-E used the day number as an alternative to more conventional date forms for some of the “behind the barrier” records
- Papers were not held securely in the experimental files
- Papers were not paginated or indexed in any way to define the contents of an entire file
- Records in the experimental files were not all indelible
- Records were not all traceable by date
- Records were not all traceable to individual staff involved
- Corrections were not made in such a way as to enable traceability

7.3 Detailed Observations – Folder 272R-1A

- IAH-E indicated that the folder labelled 272R-1A held details of the mouse assay experiment on the brain pool material in the scrapie experiment. The auditors were told that these mouse-assay infectivity experiments were conducted at IAH-C under the direction of IAH-E staff.

- The auditors regarded folder 272R-1A as critical to the audit.

- There were no records to explicitly state the location of the test work. The records were not distinguished from any others included in the IAH-E filing system. Individual records on the protocol sheet were not dated or signed.

- Details on this protocol sheet recorded the sample identity as “Sample A”. There was no description of the material actually submitted, whether a brain pool macerate or a 1 in 10 homogenate. There were no records of sample quantity or container type and no information on the sample labelling. There were no records of checks on the condition of the sample or of its suitability for test.

- Inoculation was recorded on this protocol sheet as being performed on day 112.93 using dilutions $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$. There were no records of any sample preparation steps to detail how, when, by whom or in what solution the dilution series was prepared.

- There were no records to describe what happened to the sample after test. The auditors were told by IAH-E that there was a well-established record system covering all material retained in freezers “behind the barrier”. Staff confirmed that they could find nothing in their freezer log records to document the retention of samples from this experiment. From this they had concluded that the samples would have been returned to the department initiating the work, as was usual practice in this situation. The audit team accepted these statements and did not enter the animal house to examine these records, as they could see no additional benefit in checking further for themselves for the absence of records. However, later in the audit, the audit team was shown records of a sample which was labelled for use in this experiment (see Appendix 1, Item E)
• After the protocol sheet were various pages detailing the mouse raw data records, including tabulation of the regular observation, scoring and culling. Some of this raw data was dated and initialed but not all. Some records had been made using pencil and other records were in ink. Correction fluid had been used on some sheets. The last raw data record noted was a mouse scored on day 186.95.

• Post mortem and pathology details were also tabulated in the folder for 272R-1A. A unique number was allocated to each mouse when culled and this was recorded alongside the other mouse data. IAH-E explained that the mouse number was a unique sequential number assigned at the time of death and did not directly indicate the experiment in question. IAH-E also told the auditors that the mouse number was used to trace through to any further histopathology, biochemistry or other laboratory work.

• Summary sheets, typed or hand-written, were also included in folder 272R-1A showing the overall outcome of the experiment. These were not dated or signed.

• IAH-E staff explained that a computer record system had been established over the last few years and that these electronic records were backed up in accordance with a documented procedure and held in a fire-proof-safe. This aspect was not audited, as it was not directly relevant to the chain of custody being traced.

• Experimental details were presented in the published paper “Effect of rendering procedures on the scrapie agent”, The Veterinary Record, December 20/227 1997. It was not possible to confirm this information from the available original records seen at IAH-E.

Summary Findings - Folder 272R-1A

• Papers were not held securely in this experimental file
• Papers were not paginated or indexed in any way to define the contents of the entire file
• Papers did not all show the number 272R-1A
• Records were not sufficiently detailed to unambiguously define the identity of the sample tested
• Sample preparation records were not sufficiently detailed to enable the experiment to be repeated under conditions close to the original
• Records were not all indelible
• Records were not all traceable by date or to location of laboratory
• Records were not all traceable to individual staff
• Corrections were not made in such a way as to enable traceability
• There were no records of sample preparation
• Records did not adequately detail the storage location of the sample before and after test
• There were no records of the sample storage conditions before and after test

7.4 Detailed Observations – General laboratory records of mouse assay infectivity experiments

• IAH-E provided a note on A4 paper dated 6/9/91. It was not clear to whom the note was addressed. IAH-E told the auditors that the addressee was based at IAH-C. The record comprised brief instructions on sample treatment, but it did not describe exactly what samples were provided. The date coincided with the start of the mouse assay in the cow brain rendering experiment.
• There were no instructions for the experimental set up of the mouse assay on the sheep rendering trial samples set up some two years later. There were no records to define the samples used or to detail any sample preparation. IAH-E told the auditors that this experiment was also done at IAH-C.

• There was no record to show whether any samples were returned from IAH-C to IAH-E at any stage from either of the experiments.

Summary Findings - General laboratory records of mouse assay infectivity experiments

• The laboratory records available were insufficient to document the experimental design and set-up of mouse assay infectivity experiments on BSE rendering trial samples.

• The laboratory records available were insufficient to document the experimental design and set-up of mouse assay infectivity experiments on scrapie rendering trial samples.

• There were no laboratory records to detail the samples used to inoculate the mouse assay infectivity experiments set up in 1991 and 1993 on materials from the BSE and scrapie rendering trials.

7.5 Detailed Observations - Experimental folder 272R-1V

• IAH-E informed the auditors that folder 272R-1V recorded an experiment run at IAH-E in 1993. This had been performed at the same time that the mouse bioassay checks on sheep brain pool and rendered materials (272R-1A to 272R-1U) had been set up at IAH-C. IAH-E described this as a limited strain-typing exercise using different strains of mice to differentiate the type of TSE agent present.

• The auditors regarded folder 272R-1V as critical to the audit trail.

• Details on the protocol sheet described the inoculum used for this experiment as “10⁻¹ scrapie sheep 272R pool” provided from “DMT freezer”. The experiment was inoculated on day 176.93. The individual records on the protocol sheet were not dated or signed.

• There was no description on the protocol sheet of the material actually submitted “behind the barrier”. IAH-E staff told the auditors it could have been a brain pool macerate or the 1 in 10 homogenate ready for inoculation. Later in the audit, the auditors were shown records for one sample from the “behind the barrier” archives which may relate to this experiment (see Appendix 1, Item E).

• There were no records on the protocol sheet of sample quantity or container type and no records that the sample had been checked in any way for condition or suitability for test.

• Sample preparation was indicated as “reground”, “unspun”. There were no other records of any sample preparation steps to detail how, when, by whom or in what solution the 10⁻¹ dilution was prepared.

• There were no records on the protocol sheet to describe what happened to the sample after test.
Summary Findings - Experimental folder 272R-1V

- Papers were not held securely in this experimental file
- Papers in this file were not paginated or indexed and did not all show code 272R-1V
- Records were not adequately detailed to unambiguously define the sample tested
- Sample preparation records were not adequately detailed to enable the experiment to be repeated under conditions close to the original
- Records were not traceable by date or to location of laboratory
- Records were not traceable to individual staff
- There were no detailed records of sample preparation
- There were no records to show storage conditions of the sample before and after test
- Records did not adequately detail the storage location of the sample before and after test

8. Audit of Records of Strain Typing Experiments to check for the presence of BSE in sheep brain material (Started 1997)

IAH-E had explained that MAFF had commissioned work set out in a ROAME to look specifically for the presence of the BSE agent in sheep brain material and to investigate the possibility of differential survival of the TSE agents in some rendered materials. IAH-E told the auditors that the sheep brain pool and rendered variables F and H were selected for these strain typing experiments, from the samples retained after the 1993 scrapie rendering trials.

8.1 Detailed Observations - Laboratory Records of Strain Typing Experiment

- One staff member explained that within the last few days she had recorded her recollections of the strain typing experiments as an aide memoir. She referred to two A4 sheets during discussions with the audit team. No project number or experiment code was indicated on the record.
- Other records seen were papers of various experiments with which this individual had been involved whilst at IAH-E. Papers were held in a series of plastic wallets in an A4 ring binder.
- Detailed records were not readily available to describe the experimental design or the rationale for sample selection.
- An undated and unsigned A4 page with hand written notes relating to instructions from the project leader was presented. IAH-E explained that these rough notes were made as an aide memoir when locating samples from the scrapie rendering experiments (described above) and which were to be used for additional experimental work.
- These records were not detailed enough to be self-explanatory but show notes:
  - “3 McCartney bottles rendered brain pool sample A” and “? No indication of agent”
  - “F 23/9/92 G?” and “~140g”
  - “H 29/9/92 G?” and “~200g”

Although the records showed a query about the agent, there were no records of further investigation or clarification with the project leader.
• Typed notes of a sample preparation exercise were also seen. This A4 sheet was undated and not signed and did not record the identity of staff involved.

• The preparation of brain pool material and of two meat and bone meal samples was described in these notes. The samples used were not unambiguously described but were referred to only as “MBM F” and “MBM H”. IAH-E informed the auditors that the preparation steps had been based on details taken from the published paper “Inactivation of the bovine spongiform encephalopathy agent by rendering procedures”, The Veterinary Record, December 9, 1995.

• The A4 sheet also recorded that this sample preparation had taken place on 4/11/97 and that samples had been kept frozen until the experiment started. The last paragraph on this sheet recorded that the animal facility had had to ensure that the sample was homogenous when injected. IAH-E explained that staff “behind the barrier” had given them this detail when the inoculation actually took place.

**Summary Findings - Laboratory Records of Strain Typing Experiment**

• There were inadequate records describing the samples selected for test, their labelling or appearance and condition.

• There were no records of storage location or storage conditions for the samples before and after selection as the intended test materials.

• Records of laboratory sample preparation were not adequate to enable the preparation to be repeated and were not traceable by time or to staff involved.

• There were no records describing the specific sample(s) sent for assay “behind the barrier”.

**8.2 Detailed Observations – Laboratory records of a Genotyping Screen**

• The audit team was told by IAH-E that the brain pool sample intended for the strain typing experiment had been submitted to the sheep genetics group at IAH-E. The sheep genetics group had been asked to carry out a screening test to check on the species identity of the sample material, using one of their routine procedures.

• There were no written records of the work requested or of specific sample identity. There were no records to describe what happened to the samples after test.

• The sheep genetics group presented records of a genotyping experiment run on 17/3/97 which included 13 samples and some controls.

• Sample 13 was coded as “KF”. Notes across the top of this tabulated record sheet described sample 13 as a brain sample submitted by a member of IAH-E staff.

• The tabulated result for sample 13 is recorded as QR. IAH-E explained that this represents a PrP allele which was known to exist in sheep but which had never been found in cows.
Alongside the tabulated results were notes “gel number 404” and “gel number 405”. There were also notes which IAH-E explained referred to the PCR equipment used.

Individual components of the record sheet were recorded in different writing using different instruments (pencil and biro) and did not show any specific traceability to analyst or date of recording.

Attached to this record sheet were two photographs showing a number of parallel linear bar patterns. One photograph was labelled with a fine marker pen “Run 404” and the other “Run 405”. The photographs had also been annotated with small spots and the initials “KF” in the margin to identify gel line 13. These annotations had been made with a fine marker pen and were not dated or signed.

A translucent plastic sheet, described by IAH-E as an X-ray film, was also shown to the auditors. This had a number of traces showing linear separation of dark spots. Two of these lines were labelled 405A and 405B respectively.

Summary Findings - Laboratory records of Genotyping Screen

Original records were not available that identified or fully described the sample tested.

Records of laboratory work were not adequate to enable the work to be repeated under conditions close to the original and were not traceable by time or to staff involved.

8.3 Detailed Observations - Experimental folder labelled 246A-1A

This folder was presented by IAH-E as containing the records of the strain typing experiment which was commenced in 1997 to look for the BSE agent in sheep brains. This was deemed by the auditors to be an important record in the chain of custody records.

The protocol sheet recorded 3 inoculations indicated as “group A”, “group B” and “group C” with dates 296.97 for A and 316.97 for both B and C.

Sample strain was recorded as “Scrapie sheep” and agent as “Scrapie brain pool”. Against Identity was recorded “Pool of sheep brains for meat and bone meal study. ✺ Already Macerated – very fluid – quite dark for brain!”. Additional hand-written notes recorded “Universals for these two groups labelled: - Supernatant MBM GPF & GPH 4/11/97”. There were no specific records linked to sample identification for group A, B or C. The date 4/11/97 is the same as the date recorded in the laboratory notes for sample preparation described under 8.1 above.

Sample location is noted as “DMT Freezer?”

There were no records of any consideration given or check made that the samples had been suitable to test, particularly with respect to the item which had been noted to be unusual in appearance. There were no records of further investigation or clarification with laboratory staff.

There were no records in this folder of the details of sample preparation.
None of the records on this protocol sheet were dated or signed.

IAH-E explained that this experiment ended in late 1999 and that results had not been clear-cut. In order to gain further information on the identity of the TSE agent IAH-E explained that a further experiment had been started, and was recorded in experimental folder 246B-2A. This further experiment entailed setting up a mouse to mouse passage with brain material from experiment 246A-1A which had been held in the archive “behind the barrier”. IAH-E told the auditors that experiment 246B-2A had not yet been concluded. Records of experiment 246B-2A were not audited, as they did not relate directly to the historic chain of custody records.

Summary Findings - Experimental folder labelled 246A-1A

- Papers were not held securely in this experimental file 246A-1A
- Papers were not paginated or indexed and were not all labelled 246A-1A
- Records were not sufficiently detailed to unambiguously define samples tested
- There were no records to indicate any follow up of a sample of unusual appearance
- Records were not adequately detailed to enable the experiment to be repeated under conditions close to the original
- Records were not traceable by date or to location of laboratory
- Records were not traceable to individual staff involved
- There were no records of sample preparation in this folder and no reference to laboratory notes
- There were no records to show storage of the sample before or after test

8.4 Detailed Observations - Records pertaining to samples sent to LGC for DNA analysis

- The auditors were told that samples of the brain pool material used for experiment 246A-1A had been prepared to be sent for analysis by LGC, with the intention for LGC to test for cow material in the sheep brain samples. IAH-E told the auditors that LGC had requested 1ml aliquots of 50% homogenates in water of the test samples. Control materials comprising pure sheep brain and cow brain spiked into sheep brain were also prepared for analysis at LGC.

- No records were available to document the discussions relating to the experimental procedure and the samples to be used.

- The auditors were told that laboratory sample preparation records for these samples were held on the computer; these were not audited due to time constraints and earlier findings.

- A copy of the work request details sent to LGC was seen. This was not dated or signed. Typed records described the samples supplied as triplicates of SBP sample 1, SBP sample 2, SBP sample 3, pure sheep brain and a ½ log dilution series of cow brain in sheep brain from $10^{-0.5}$ to $10^{-6}$.

- The auditors were told that this note was sent to LGC packaged with the samples which were contained in Eppendorf tubes individually labelled with the contents. No records of dispatch were made available by IAH-E.
• The auditors were told by IAH-E that triplicate replicates of each sample and each control were retained frozen at IAH-E together with some residual material. See Appendix 1 for details of these samples examined at the audit.

• At the audit IAH-E made available a copy of a report from LGC addressed to DEFRA. The report was headed “preliminary report” and was not signed. The report indicated the date of issue as 19 October 2001 and related to samples described as received on 13 September 2001.

• The report listed the samples as supplied by IAH-E against assigned laboratory numbers LR/237/01 through to LR252/01, in the order: 3x SBP sample 1, 3x SBP sample 2, 3x SBP sample 3, 3x pure sheep brain, and then the triplicates of the ½ log dilution series of cow brain in sheep brain from $10^{-0.5}$ to $10^{-6}$.

• Results were presented in a second table against the LGC laboratory numbers. The first three lines reported individual numbers LR/237/01, LR/238/01 and LR/239/01 each as “bovine”. The next three sequential numbers (LR/240/01, LR/241/01, LR/242/01) were grouped on one line alongside the result “ovine” and then the final box in the table showed LR/243/01 - LR/252/01 alongside the result “ovine, bovine down to $10^{-5}$ dilution”.

Summary Findings - Records pertaining to samples sent to LGC for DNA analysis

• The records available of sample identity were inadequate and the records of samples dispatched to LGC were insufficient to give traceability.

• Computerised records were not audited. Records of sample preparation were therefore not seen

9. Audit of Samples and Storage Facilities

The audit team asked to see the samples and storage facilities relating to this audit trail. This part of the audit was carried out in conjunction with IAH’s own independent auditor.

9.1 Detailed Observations - Sample storage locations

Three items of equipment were inspected as IAH-E had informed the audit team that these were the only freezers in which the samples had been stored. The three freezers were in different locations at IAH-E, linked by a main corridor.

9.1.1 Freezer ET 0333

• A large chest freezer was indicated by IAH-E as the one in which the samples had been held from 1990. This item of equipment was now located in the main corridor but IAH-E informed the audit team that it had been moved when the laboratory in which it had previously been located was modified and upgraded to Containment level 3.

• The freezer was marked with the IAH-E inventory number ET 0333 but otherwise there were no records of its location/ relocation, use or maintenance.

• There were no records of temperature monitoring of freezer ET 0333.
• At the time of the audit the freezer contained a range of packages in tubes, racks, boxes or plastic bags variously labelled. There was no systematic labelling or coding system.

• There were no records of the freezer contents which detailed the samples used in the experiments audited.

9.1.2 Zanussi Fridge/freezer

• A fridge/freezer in the Containment level 3 laboratory was shown to the auditors. This domestic style equipment (Zanussi) had a small three star freezer compartment which on opening also contained a variety of sealed/wrapped items with various forms of labelling. No systematic sample coding was noted. The equipment was labelled 58 and did not have an ET inventory number.

• The auditors were told that the freezer compartment had been used to store the three McCartney bottles believed to be the sheep brain pool material from the rendering experiment. IAH-E told the auditors that the bottles had been moved from the chest freezer in 1996/1997 when they were identified for use in the strain typing experiment 246A-1A.

• There were no records of the location, use or maintenance of this equipment and no records of temperature monitoring.

• There were no records of the contents of the freezer which detailed the samples used in the experiments audited.

9.1.3 Ultrafreezer ET 0821

• Finally the auditors were shown a large upright two-compartment ultra-freezer marked with inventory number ET 0821 and locked with a padlock. The freezer was situated in the workshop area opening directly off the main corridor of the building. The digital display showed a reading of –68°C.

• The auditors were informed by IAH-E that all samples related to the experiment had been moved on Friday 19 October 2001 and Monday 22 October 2001 to this location when the IAH's Director had instructed secure storage.

• There were no records of the samples being moved and no records of the current contents of this freezer.

• The freezer contained a number of items, mostly bags but also one cardboard box. Each container had been sealed with masking tape that was initialled by two members of IAH-E staff and dated either 19/10/01 or 22/10/01.

• The UKAS auditors recommended that if IAH-E deemed these samples to be of continued importance a detailed log should be established with immediate effect to record the storage and any subsequent handling, actions etc. The UKAS auditors also suggested that IAH-E should consider whether photographic records should be made of the samples and sample labelling in its current form, before any further manipulations/changes occurred.
Summary Findings - Sample storage equipment

- No records were provided to detail location, use or maintenance of equipment
- There were no records for temperature monitoring of sample storage freezers
- There were no records of the storage location of experimental samples
- Stored samples were not labelled with any systematic coding

9.2 Detailed Observations – Samples and sample labelling

- A member of IAH-E staff in the presence of the audit team unlocked freezer ET 0821.

- Several items were removed by IAH-E staff one by one from freezer ET 0821 and carried down the corridor to the Containment level 3 laboratory for the audit team to examine the contents. Each of these outer packages was sealed as described at 9.1.3. The audit team was looking for the samples directly related to the audit. Examination of the various samples was performed fairly quickly to ensure that the samples did not begin to thaw. After examination, the bags were returned to freezer ET 0821.

- The laboratory labelled for category 3 work opened off the main corridor and had a pair of keys in the external lock. The laboratory was not locked when the audit team arrived at the door; the room was left unlocked when the audit team left.

- IAH-E staff wore green gowns and thin latex gloves to handle the samples within the containment 3 room. The auditors remained in the corridor and IAH-E staff displayed the items to them through the open door. None of the samples were touched by any members of the audit team. None of the sample containers were opened.

- Details of the items seen and their labelling are given in Appendix 1.

Audit Summary - Samples and sample labelling

- Sample labelling was not traceable with date or time or by individual staff involved.

- Sample labelling was not permanent and was not always affixed directly to the sample container.

- Sample labelling was brief and did not provide unambiguous identification of the contents.

- Sample labelling did not include the experimental numbers allocated by IAH-E.

- Additional details/ corrections could not be differentiated from original labelling.

- Labelled samples seen could not be directly related with any of the experimental work audited via records held by IAH-E.
10. Overall Audit Summary

The auditors gained most of the information on the sequence of experiments from information supplied verbally by IAH-E staff and from the two papers published in The Veterinary Record.

Records were not available to demonstrate a chain of custody for the samples used in these experiments.

10.1 Summary for Key Objectives 3.1 and 3.2

Objective 3.1 was assessment of the audit trail of the samples sent to LGC in which only bovine material was identified and to establish if the same samples were used in the experiment in which a BSE-like agent was detected in mice.

Objective 3.2 was assessment of the audit trail within IAH-E of brains collected during the 1990/1992 period from Veterinary Investigation Centres and submitted to IAH-E for evaluation of rendering process variables on transmissible spongiform encephalopathy (TSE) agents.

The information on both these chains of custody was gathered contemporaneously during the audit. Based on the evidence seen:

The auditors found that record keeping was inadequate to give confidence in the chain of custody of samples used in the various rendering, genotyping and strain typing experiments.

There were no co-ordinated records to link any of the individual experiments described as having been carried out at IAH-E or IAH-C with each other, or with the DNA analysis done by LGC.

There were no records to demonstrate exactly which samples had been used for the various inter-related rendering, genotyping and strain typing experiments.

Sample labelling did not provide adequate detail to indicate for which experiments the samples had been used.

Summary for Key Objective 3.3

Objective 3.3 was to report on the audit trail for archived material in general and held by IAH-E. IAH-E staff were anxious to inform the auditors that these particular experiments audited were very much "specials" and had not been carried out within the same system as the majority of their work.

The auditors had been told that work in the animal house was carried out more systematically than that in the laboratory areas. However there was inadequate time to carry out in depth auditing of "routine" work in the animal house. An example of the freezer log records presented to the auditors was indicative of a more comprehensive audit trail system for archive materials than existed for the laboratory storage facilities. The experimental files which had been audited had provided some traceability of test records and archive material.
The records seen for the experimental work “behind the barrier” were still not adequately detailed to give a full audit trail and to enable the work to be repeated under conditions close to the original if required.

Sample labelling on general material stored in the laboratory freezers seen was insufficient and was not systematic. There were no co-ordinated records of storage location and storage conditions of the samples held in these laboratory freezers.

**Summary for Key Objective 3.4**

Objective 3.4 was to evaluate compatibility of sample handling procedures with accreditation requirements (ISO 17025, Section 5.8) and to identify opportunities for improvements.

There was no formalised quality manual or documented quality system for IAH-E. Based on the elements audited, the quality system in place at IAH-E was very rudimentary and a long way from meeting UKAS accreditation requirements.

ISO 17025 defines the criteria of competence to be met by testing laboratories in a general way and permits laboratories to apply systems which are relevant to their own disciplines and to client needs. However, the auditors found that IAH-E did not meet the requirements in the key areas specifically audited i.e. technical records, equipment / equipment records and sample handling.

From the chain of custody audit there was also evidence that laboratory control of contract review, document control and subcontracting would not be compatible with ISO 17025.

Although it was not specifically audited, there was little evidence of quality control or quality assurance systems in the experimental work audited. Whilst it is appreciated that experiments in the animal house had to be designed so that animals are not used unnecessarily, there were no records in the experimental files to show that any checks were made on samples prior to inoculation into mice. There was no evidence of replicate checks on the genotyping test at a crucial stage in the experimental set-up. There was no documentation to show that the samples tested at LGC had been repeated or that the samples retained at IAH-E had been tested.

ISO 17025 also covers a number of other elements including staff and training, calibration, method documentation and validity, reports, internal audits and management review, purchasing, complaints and anomalies. The scope of this audit did not touch on these aspects, so the audit team cannot comment on full compliance with ISO 17025.

To comply with ISO 17025, IAH-E would have to develop and implement a quality system to assure the quality of experimental work/ test results.

Details of specific deficiencies related to the key audit areas of technical records, equipment / equipment records and sample handling are covered in the opportunities for improvement section below. See section 10.2.

**10.2 Opportunities for Improvement**

A number of opportunities for improvement by IAH-E were identified arising from the limited audit that was carried out:
• Establish and document a co-ordinated recording system which enables all relevant background paperwork and all laboratory records associated with experimental work to be identified and retrieved.

• Clearly document any file-coding system which has been/is adopted. Set up a detailed index to relate the code allocation to experimental/project information. Consider extending this code system to all other related paperwork.

• Ensure records in experimental files contain full details of the whole experiment and/or a cross-reference to such information.

• Establish and define criteria for control of records in all laboratory areas and in the animal house to provide a full audit trail, to contain sufficient information to enable the test to be repeated under conditions close to the original and to facilitate identification of the sources of any error.

• Establish a co-ordinated recording system in all laboratory areas and in the animal house to ensure records are clearly and permanently recorded at the time they are made and signed by staff making them. Establish a recording system to enable observations, data and calculations to be identifiable to the specific task. Make corrections in an appropriate manner to avoid loss of the original record and show when, why and by whom the alteration was made.

• Establish and define secure storage systems for both paper and computer records. Establish and define archive/retention time for both paper and computer records.

• Establish and maintain a system for recording sample receipt and sample handling which ensures appropriate and unique identification of materials and any sub-samples at all times and permits correlation of samples with work records. System should include a description of the sample and notes on its condition.

• Establish sample-labelling procedures to ensure that all samples remain uniquely identified throughout their life and cannot be confused.

• Establish and maintain a system to record the storage location and storage conditions of test items and taking precautions to prevent deterioration of test items.

• Establish a system to control and record the discard/disposal of test items.

• Establish a system to record information on the location, use, maintenance and calibration of equipment.

• Establish systems to monitor and record the temperature of relevant equipment using appropriately calibrated equipment.

• Establish systems to check and record that laboratory test materials/samples are suitable for test purposes before and/or at point of use. If materials appear unsuitable then records of follow up checks should be made.
Appendix 1

Detailed Observations – Samples and sample labelling

Item A

Shallow rectangular cardboard box labelled with marker pen on cream tape: “SH 21/3/01 246 sheep brain pool samples Checked April ’97 by W [staff member’s name] 99% certain of ovine origin”

This box contained:

a) Glass Universal bottle with a metal cap. Black marker pen labelling read “H” on lid and “Supematant MBM Gp H 4/11/97”

b) Zip-lock bag labelled with Black marker pen “Autolysed? Rendering sheep brain pool. 100mg aliquots for DNA analysis 28/11/00”. This contained three Eppendorf tubes which were not individually examined.

c) Another Zip-lock bag with another three Eppendorf tubes which were not individually examined.

d) Zip-lock bag inside which was a loose piece of masking tape labelled in black marker pen “This one used for 246A-1A”. Inside the bag was a glass McCartney bottle with a white plastic cap. The number “1” was written on the cap. The bottle had a red/orange label over wrapped with sellotape. Smudged writing on the label was just legible: “Rendering sample A (Brain Pool)”. The bottle contained about 2 cm depth of a brown material.

e) Zip-lock bag inside which was a glass McCartney bottle with a white plastic cap which was marked “2”. The bottle had a red/orange label over wrapped with sellotape. Writing on the label read: “Rendering sample A (Brain Pool)”. This bottle was nearly full to the shoulder with a brownish material. The bag was marked with black marker pen “sampled by KF 19/2/01”. The year 00 had been overwritten to 01.

f) Zip-lock bag marked with black marker pen “untouched? As of 19/2/01”. The year 00 had been overwritten to 01. The bag contained a glass McCartney bottle with a white plastic cap marked “3”. The bottle had a red/orange label over wrapped with sellotape. Writing on the label read: “Rendering sample A (Brain Pool)”. This bottle was about 3/4 full with a brownish material.

- IAH-E told the auditors that the 3 McCartney bottles (d, e and f above) were the ones that had been located in the chest freezer for use in the strain typing experiment 246A-1A and for genotyping. IAH-E stated that the three bottles were then stored in the small freezer compartment in the containment 3 laboratory, until moved very recently to −70°C storage. They had been selected, as they were believed to be the scrapie brain pool material from rendering experiment 272A-1A. No records were seen at the audit to confirm these actions.
• IAH-E also told the auditors that the McCartney bottle labelled 1(d above), was used to set up experiment 246A-1A as indicated by the loose label. IAH-E explained that the same bottle had also been used for the genotype screen. No records were seen at the audit to confirm this chain, but IAH-staff repeatedly emphasised that this particular McCartney bottle had been selected, as it had contained less material and so had been assumed to be the sample used previously for the infectivity trials (272R-1A).

• The three McCartney bottles were very similar in appearance and labelling and differed principally only in the volume of sample each contained and in the cap numbering 1, 2 and 3.

• IAH-E also told the auditors that aliquots from all 3 McCartney bottles (d, e and f above) were used to prepare the samples sent to LGC and that it was at this time that the 3 McCartney bottles were labelled 1, 2 and 3. No records were seen at the audit to confirm this chain.

• IAH-E told the auditors that the Glass Universal (a above) was the laboratory prepared sample for strain typing experiment 246A-1A. Wording on this label matches part of the sample description recorded on the protocol sheet for experiment 246A-1A.

• IAH-E told the auditors that the Eppendorf tubes, (b and c above) related to other work.

Item B

Sturdy yellow plastic bag approximately 30-40 by 50-60 cm. Inside this were several clear plastic bags containing a variety of plastic pots and other containers.

One of these inner bags was examined. Inside this thin clear plastic bag was a loose piece of paper, half an A4 sheet, on which was written in smudged black marker pen “Spare MBM samples received 8/9/95. Individually coded”. Contained in this bag were a number of miscellaneous pots of about 100ml capacity labelled PM, TM, OM, MM etc.

Two other of the items in this bag were individually examined. They were double wrapped in labelled zip-lock bags:

a) The inner zip-lock bag was labelled in black marker pen “H 24.9.92 G”. The outer bag was not marked.

b) The inner zip-lock bag was labelled in black marker pen “F 23.9.92 G”. The outer bag was not marked.

• IAH-E informed the auditors that the Zip-lock bags (a and b) were the meat and bone meal samples used for the strain typing experiment 246R-1A.
Item C

Clear large polythene bag labelled outside in black marker pen “LGC SBP control series 7/9/01”. This bag contained a rack of Eppendorf tubes.

- Contents were not examined as the auditors agreed that these control samples did not relate directly to the chain of custody for the main experimental samples.

Item D

Clear large polythene bag containing several items. The following were examined:

a) Glass Universal bottle with a metal cap labelled “F” in black marker pen. On the side of the bottle was also written directly onto the glass in black marker pen: “Supernatant MBM Gp F 4/11/97 F”.

b) Plastic pot of about 100-150 ml capacity. This was labelled indistinctly with black marker pen: “A Sh……..”. After the first two legible letters was a black smudge 2-3 times the length of the first two letters. The pot contained a material which appeared to be a granular/fibrous solid, which was unlike the contents of the other samples examined.

c) Smaller clear plastic bag labelled “SBP samples for LGC 10/9/01”. This bag loosely wrapped a rack holding nine Eppendorf tubes with spaces for many more tubes. Three tubes were labelled “SBP 1” in black marker pen on the sides of the tubes. Three tubes were labelled “SBP 2” in black marker pen on the sides of the tubes. Three tubes were labelled “SBP 3” in black marker pen on the sides of the tubes.

- IAH-E told the auditors that the Universal (a above) was the laboratory prepared sample for strain typing experiment 246A-1A. The label wording matches part of the records on the protocol sheet for experiment 246A-1A.

- IAH-E also told the auditors that the 9 Eppendorf tubes (c above) were the retained replicates of the test samples sent to LGC for DNA testing.

Item E

IAH-E explained that there was one sample in the “behind the barrier” freezer system which was relevant. A photocopy of a bottle was shown to the audit team. The label read “272R 1A 10⁻¹ homogenate Prep 70.93”. The sample was not seen as it was in the animal house.

- IAH-E told the auditors that this was the sample used as the inoculum for experiment 272R-1V.