Frozen Storage of _Escherichia coli_ O157 in Buffered Peptone Water and Its Detection on Bovine Carcasses

H. E. TERNENT,1,2 G. T. INNOCENT,1,2 L. M. FILSHIE,1,2 D. J. TAYLOR,3 W. B. STEELE,3 S. A. McEWEN,4 W. J. REILLY,5 G. J. GUNN,6 S. W. J. REID,1,2* AND D. J. MELLOR1,2

1Comparative Epidemiology and Informatics, Department of Statistics and Modelling Science, University of Strathclyde, 26 Richmond Street, Glasgow G1 1XH, UK; 2Comparative Epidemiology and Informatics, Department of Veterinary Clinical Studies and 3Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK; 4Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada; 5Scottish Centre for Infection and Environmental Health, Clifton House, Clifton Place, Glasgow G3 7LN, UK; and 6SAC Veterinary Science Division, Drummondhill, Stratherrick Road, Inverness IV2 4JZ, UK

ABSTRACT

The adaptation of a standard _Escherichia coli_ O157 isolation method involving immunomagnetic separation and a period of frozen storage was investigated. A series of experiments was designed to test the recovery of a bovine strain of _E. coli_ O157 from buffered peptone water after a period of frozen storage at –80°C. The effects of the addition of glycerol at 5 and 10%, freezing time, the number of freeze-thaw cycles, the method of freezing and the method of thawing, the inclusion of a resuscitation-and-incubation step, and the sensitivity of the isolation method were investigated. The most effective method of storing frozen samples for 6 months and recovering strains of _E. coli_ O157 after storage was found to involve 6 h of incubation of sample material in buffered peptone water at 37°C before frozen storage at –80°C with 10% glycerol, a rapid thaw after frozen storage, and resuscitation at 27°C for 1 h and incubation at 37°C for 1 h to allow freeze-injured and stressed bacteria to recover with a period of growth prior to immunomagnetic separation isolation. There was no significant decrease in log counts of a bovine strain _E. coli_ O157 over 6 months of frozen storage in buffered peptone water with 10% glycerol. With this method, it was possible to isolate _E. coli_ O157 from naturally infected bovine carcasses after a period of frozen storage.

The processing of cattle from the farm of origin to chilling in an abattoir has been highlighted as one of the most important potential ways for verocytotoxin-producing _Escherichia coli_ O157 (VTEC O157) strains and other enteric pathogens to contaminate food destined for human consumption (1, 3). The logistics of carrying out a comprehensive study of beef carcasses in multiple slaughterhouses can become difficult, since such studies can result in large numbers of samples that must be processed for the isolation of the target organism in a limited time. The introduction of a period of frozen storage allows samples to be processed at a convenient time and in manageable numbers. The frozen storage of samples also provides a library of samples that can be accessed at a later date for reference, repeatability testing, or further investigations.

The process of freezing has been shown to induce irreversible damage to both the outer and the cytoplasmic membranes of bacteria, including _E. coli_ (8). The injury caused during frozen storage creates an important problem in the detection of strains of VTEC O157 in stored samples. It has been shown that the survival of VTEC O157 strains during frozen storage and thawing can be highly variable, depending on the strain of bacteria, the origin of the sample, and the method of freezing and thawing of the sample (2, 15, 16). The present study describes a series of experiments designed to assess and develop existing techniques for the frozen storage and isolation of VTEC O157 (18). The purpose of these experiments was to find a sensitive and specific method to ensure the survival and detection of very small numbers of VTEC O157 organisms in samples (cattle feces and carcass swabs collected from beef animals in the abattoir) that have undergone frozen storage. Nutrient enrichment was considered an important processing step for increasing the numbers of the target organism prior to the frozen storage of samples. The replication of bacteria is appropriate when the aim is to detect the presence of strains of _E. coli_ O157 by immunomagnetic separation (IMS) and not to enumerate the bacteria. Consideration was given to the use of a cryoprotectant, the method of freezing and thawing, the resuscitation of stressed bacteria, the duration of the freezing period, and the effects of repeated freeze-thaw cycles.

MATERIALS AND METHODS

Table 1 outlines the six experiments designed to investigate the sensitivity of an isolation method following a period of frozen storage. The effects of the addition of glycerol as a cryoprotectant, the thawing method, and the introduction of a resuscitation step were investigated.

_E. coli_ O157 strain and cultivation. A bovine strain of _E. coli_ O157 (strain 6252) reported to be verocytotoxin negative was supplied by the Scottish _E. coli_ O157 Reference Laboratory in Aberdeen and was used to inoculate bovine fecal and sterile medium samples. The strain was maintained at –80°C with the use of the Microbank storage system (Pro-Lab Diagnostics, Wirral,
A stock suspension of *E. coli* O157 strain 6252 was produced for each experiment. Cultures were activated by inoculating a single bead from the Microbank onto MacConkey agar (Oxoid, Basingstoke, UK) and incubating it at 37°C for 18 h. One colony from this culture was inoculated into 20 ml of buffered peptone water (BPW) and incubated at 37°C for 3 h, after which the optical density of the sample at 650 nm was read. The concentration of the suspension was interpolated from a standard growth curve. The suspension was diluted with sterile BPW to provide the required concentration for each experiment.

**Isolation and enumeration.** Fecal and carcass swab samples were examined for *E. coli* O157 strains with IMS as described by Chapman et al. (4) but with the use of BPW without added antibiotics during enrichment (17). Non–sorbitol-fermenting colonies on sorbitol MacConkey agar with added cefixime and tellurite (CT-SMAC) were confirmed to be *E. coli* O157 colonies by the *E. coli* O157 latex agglutination test (DR 620M, Oxoid). When field samples were used, the confirmation of positive colonies was carried out by the Scottish *E. coli* O157 Reference Laboratory Edinburgh with a biochemical API system (bioMerieux, Basingstoke, UK), a Wellcolex *E. coli* O157 latex agglutination kit (Murex Biotech Ltd., Kent, UK), and slide agglutination with *E. coli* O157 antisera (Mast, Merseyside, UK), along with phage typing and polymerase chain reaction to determine the presence of VT1 and VT2 genes.

When sterile medium samples were inoculated with *E. coli* O157 strain 6252, plate counts were determined in triplicate after 0.05 ml of sample suspension had been inoculated onto CT-SMAC plates and incubated at 37°C for 18 to 24 h.

**Experiment 1: recovery of *E. coli* O157 from spiked bovine feces and media.** A fresh bovine fecal sample confirmed to be negative for *E. coli* O157 strains by culture was divided into four samples. Three of these samples were inoculated with *E. coli* O157 strain 6252 at concentrations of 10^1, 10^2, and 10^3 CFU/g of feces, and one served as a negative feces control sample. Fecal samples were inoculated with quantities of bacterial suspension amounting to 1/10 of their mass and mixed thoroughly with a sterile spatula. Sterile BPW was used to provide a negative medium control and was also inoculated with *E. coli* O157 strain 6252 at concentrations of 10^1, 10^2, and 10^3 CFU/ml in BPW. Twenty samples (5 ml each) of the respective negative controls and 50, 30, and 20 samples with concentrations of 10^1, 10^2, and 10^3 CFU/g of feces, respectively, and 10^1, 10^2, and 10^3 CFU/ml of BPW, respectively, were prepared. The numbers of samples were calculated to give similar-sized 95% confidence intervals for the predicted recovery rates of 0.0, 0.5, 0.75, and 1.0, respectively. *E. coli* O157 strain 6252 was recovered from the fecal samples with the use of preenrichment (1 g of feces in 20 ml of BPW incubated at 37°C for 6 h) followed by IMS on 1 ml of enriched medium. *E. coli* O157 strain 6252 was recovered directly from spiked BPW samples by the IMS technique for 1 ml of the medium without an incubation step for preenrichment. Following IMS, the beads were suspended in 20 μl of phosphate-buffered saline with 20% Tween and were inoculated onto CT-SMAC plates. Non–sorbitol-fermenting colonies were confirmed to be *E. coli* O157 colonies by the *E. coli* O157 latex agglutination test (DR 620M, Oxoid).

**Experiment 2: recovery of *E. coli* O157 from spiked media stored at −80°C.** Sterile BPW was inoculated with *E. coli* O157 strain 6252 at 10^1, 10^2, and 10^3 CFU/ml. Samples (5 ml each) were frozen either by being placed directly into a freezer at −80°C or by flash freezing with liquid nitrogen and stored at −80°C for 1 month. Replicate samples for each combination of the two methods of freezing with and without the addition of 10% glycerol were prepared. The numbers of replicate samples with concentrations of 10^1, 10^2, and 10^3 were 50, 30, and 20, respect-
tively. The numbers of samples were calculated to give similar-sized 95% confidence intervals for the predicted recovery rates of 0.5, 0.75, and 1.0, respectively. Twenty replicate samples of sterile BPW with and without 10% glycerol were used as controls. Control samples were placed directly into a freezer at −80°C. Samples (4 ml each) were frozen with or without 1 ml of 50% glycerol. Samples stored at −80°C were batched and coded so that the operator could not identify the origin of the sample during thawing and IMS. Batches comprised 44 samples incorporating all 14 combinations of cryoprotectant, freezing method, and concentration. Non–sorbitol-fermenting colonies on CT-SMAC plates following IMS were confirmed to be E. coli O157 colonies by the E. coli O157 latex agglutination test (DR 620M, Oxoid).

Experiment 3: comparison of the effects of thawing method on the recovery of E. coli O157. A stock suspension of E. coli O157 strain 6252 with a concentration of 10^3 CFU/ml was produced. This stock suspension was then measured into sterile 6-ml cryotubes each containing 4 ml of E. coli O157 strain 6252 suspension with 1 ml of 25% glycerol. The 60 samples provided three sets of 20 replicates. All samples were stored at −80°C for 1 week. Two sets of samples were then thawed rapidly in a 50°C water bath until the ice had melted (13). One of these sets of samples was then subjected to 1 h of resuscitation at 27°C and 1 h of incubation at 37°C. The third set was thawed slowly, at room temperature. The number of CFU per 50 μl was counted for each sample to determine any differences in recovery levels due to the thawing method.

Experiment 4: effects of freezing on E. coli O157 over time. A stock suspension of E. coli O157 strain 6252 with a concentration of 10^3 CFU/ml was produced and measured into sterile 6-ml cryotubes each containing 4 ml of E. coli O157 strain 6252 suspension with 1 ml of 25% glycerol. The 55 samples provided five replicates for each of the 11 time periods. The samples were tested before freezing at −80°C, at the point of freezing (15 min), and at set intervals after freezing (1, 2, 4, 12, 24, and 48 h, 1 week, and 1 and 6 months). Frozen samples were thawed rapidly as described previously without the resuscitation step. The number of CFU per 50 μl was counted for each sample to determine whether there was any difference in recovery levels due to frozen storage time.

Experiment 5: effect of freeze-thaw cycles on E. coli O157. A stock suspension of E. coli O157 strain 6252 with a concentration of 10^3 CFU/ml was produced. Fifteen 4-ml stock suspension samples were divided into three groups of five samples. Glycerol was used as a cryoprotectant at two concentrations: 1 ml of 25% glycerol was added to samples in one group, 1 ml of 50% glycerol was added to samples in the second group, and samples in the third group were frozen without added glycerol. The three sets of samples provided five replicates: a prefreezing sample and samples that had undergone one, two, three, and four of the freeze-thaw cycles described above. Samples were stored at −80°C, and for each freeze-thaw cycle, a set of samples was removed from storage and thawed rapidly as described above. Fifty microliters of each sample was removed for the enumeration of CFU, and the sample was returned to storage at −80°C.

Experiment 6: recovery of E. coli O157 from bovine carcase swabs before and after storage at −80°C. Carcass swabs were taken from each side (half) of the first 20 carcases through the slaughter process during each visit to the abattoir. A moist sterile polywipe with a sterile disposable glove (MW729/B Medical Wire & Equipment Co. [Bath] Ltd., UK) was used for carcase swabbing. Each carcase side was vigorously wiped over the whole brisket and neck area, which has previously been demonstrated to be the area most likely to be contaminated (1, 12), with both sides of the wipe. The wipe was then placed in 80 ml of BPW in a 120-ml plastic container (LW5495, Alpha, Hampshire, UK) and shaken. Samples were transported back to the laboratory in a cool box containing ice packs and refrigerated at 4°C overnight. Following incubation at 37°C for 6 h, 1 ml of each sample was subjected to IMS and 4 ml was stored with 1 ml of 25% glycerol at −80°C for 1 month. After 1 month of storage, samples were thawed rapidly and subjected to a resuscitation period followed by an incubation period (as described above) before being subjected to IMS. Suspected E. coli O157 colonies on CT-SMAC plates were confirmed by culturing on Chromocult agar (Merk), and pink colonies were tested with the E. coli O157 latex agglutination test (DR 620M, Oxoid). Positive isolates were then sent to the Scottish E. coli O157 Reference Laboratory Edinburgh for further characterization.

Statistical analysis. The results for all samples subjected to IMS were analyzed with a logistic form of a generalized linear model. For plate counts (CFU/50 μl), analyses were carried out with a Poisson form of a generalized linear model. Significance was set at the 5% level with the statistical package R (9).

RESULTS

Experiment 1: recovery of E. coli O157 from spiked bovine feces and media. The recovery rates for E. coli O157 strain 6252 in inoculated media and feces were far higher than anticipated. The rate of recovery of E. coli O157 strain 6252 from inoculated media down to a concentration of 10 CFU/ml was 100%. The rates of recovery of E. coli O157 strain 6252 from inoculated fecal samples were 96% (95% confidence interval, 0.87 to 0.99) for an inoculum concentration of 10 CFU/g and 100% for higher concentrations.

Experiment 2: recovery of E. coli O157 from spiked bovine carcase media stored at −80°C. The method of freezing had no significant effect on the recovery of E. coli O157 strain 6252 from samples frozen with glycerol. However, for samples with a concentration of 10 CFU/ml that were frozen without glycerol, there was a significant increase in recovery for samples that were flash frozen in liquid nitrogen compared with the recovery rate for samples directly placed into a freezer at −80°C (P < 0.05). Both the addition of glycerol as a cryoprotectant and an increase in the concentration of E. coli O157 significantly increased the recovery rate. The recovery rate for samples with a concentration of 10^3 CFU/ml stored with 5% glycerol at −80°C was 99% (Table 2).

Experiment 3: comparison of the effects of thawing method on the recovery of E. coli O157. The levels of E. coli O157 strain 6252 recovered with a rapid thaw followed by a resuscitation and incubation step (averaging 4.6 CFU/50 μl) were significantly higher (P < 0.001) than those recovered with a rapid thaw alone (averaging 3.6 log CFU/
Experiment 4: effects of freezing on *E. coli* O157 over time. There was no significant difference between *E. coli* O157 counts for the prefreezing samples and those for the samples that had been stored at −80°C for 15 min. There was a significant decrease in counts for all samples at ≥1 h of storage, and the counts for these samples were significantly lower than those for prefreezing samples and for samples that had been stored at −80°C for 15 min. After 1 h of frozen storage, there was no further significant decrease in *E. coli* O157 counts for samples stored for longer periods, although counts for samples that had been stored for 6 months were significantly higher than those for samples that had been stored for 1 h to 1 week (Fig. 1).

Experiment 5: effect of freeze-thaw cycles on *E. coli* O157. In the absence of glycerol, *E. coli* O157 strain 6252 counts decreased by an average of 2.4 log CFU/50 μl per freeze-thaw cycle. This equates to a 91% reduction in the *E. coli* O157 count per freeze-thaw cycle ([1 − e^{−2.4}] × 100%). The addition of glycerol at either 5 or 10% significantly reduced the *E. coli* O157 count decrease per freeze-thaw cycle. When samples contained 5% glycerol, the *E. coli* O157 counts decreased by 0.17 log CFU/50 μl (a decrease of 16%) per freeze-thaw cycle; for samples containing 10% glycerol, there was a small but significant increase in *E. coli* O157 counts for each freeze-thaw cycle.

Experiment 6: recovery of *E. coli* O157 from bovine carcass swabs before and after storage at −80°C. The samples collected during the first two visits to the abattoir (80 samples from 40 carcasses) were all found to test negative for *E. coli* O157 both before and after frozen storage. Seven of the 40 samples collected on the third visit and tested directly after enrichment were found to test positive for VTEC O157 of phage type 21/28, with genes for the production of VT2 being present. Following 1 month of storage at −80°C, six of the seven samples initially testing positive were found to be positive, and one sample that had not tested positive prior to freezing was found to be positive. All isolates testing positive for VTEC O157 after 1 month of storage at −80°C were identified as being of phage type 21/28, with genes for the production of VT2 being present.

**DISCUSSION**

The aim of this study was to adapt the current commonly used standard isolation method to include a period of frozen storage and to assess the recovery sensitivity of the method. The most effective method of storing sample material for 6 months and recovering *E. coli* O157 strains after frozen storage was found to involve 6 h of incubation of sample material in BPW at 37°C prior to frozen storage at −80°C with 10% glycerol. Frozen samples were thawed rapidly and resuscitated at 27°C for 1 h prior to incubation at 37°C for 1 h to allow freeze-injured and stressed bacteria to recover with a short period of growth prior to IMS isolation. The freezing process, the thawing process, the addition of glycerol, and the number of bacteria present in the original sample all affected the recovery of *E. coli* O157.

The sensitivity of the isolation method was tested with fresh fecal and medium samples that had been inoculated and was shown to be good, with 95% confidence intervals of 0.87 to 0.99 for the recovery of *E. coli* O157 inoculated at 10^3 CFU/g from fecal samples and 0.93 to 1.00 for the recovery of *E. coli* O157 inoculated at 10^4 CFU/ml from BPW. The inoculated-medium trial was repeated with samples being stored at −80°C for 1 month and again proved to have a good sensitivity level, with 95% confidence intervals of 0.92 to 1.00 for the recovery of *E. coli* O157 inoculated at 10^3 CFU/ml from BPW. It was not surprising that the method’s sensitivity level for fecal samples was slightly lower than that for spiked BPW, likely because of the competition of other normal gut bacteria within the fecal samples.

The duration of storage at −80°C did not affect the

---

**TABLE 2. Recovery of *E. coli* O157 from medium samples inoculated with three concentrations of *E. coli* O157 strain 6252 and directly placed in the freezer or frozen with liquid nitrogen before storage at −80°C for 1 month with and without glycerol**

<table>
<thead>
<tr>
<th>Medium</th>
<th><em>E. coli</em> O157 concn (CFU/ml)</th>
<th>No. of samples</th>
<th>Directly placed in freezer</th>
<th>Frozen with liquid nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPW</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10^1</td>
<td>50</td>
<td>29</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>10^2</td>
<td>30</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>10^3</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>BPW and glycerol</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10^1</td>
<td>50</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>10^2</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>10^3</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

*—, no samples tested.*

50 μl). Levels of *E. coli* O157 strain 6252 recovered from samples that had been left on a bench at room temperature to defrost (averaging 3.4 log CFU/50 μl) were significantly lower (P < 0.001) than those recovered from samples for which either the rapid-thaw method or the rapid-thaw method with a resuscitation-and-incubation step had been used.

**FIGURE 1. Effect of duration of storage at −80°C on counts (log CFU/50 μl) of *E. coli* O157 strain 6252 in BPW (bars indicate 95% confidence intervals).**
recovery of *E. coli* O157 from BPW with 5% glycerol. It appears that any injury caused by freezing took place after 15 min to 1 h of storage, because there was no significant difference in levels of *E. coli* O157 recovered from samples stored at −80°C for 1 h and levels recovered from samples stored for >1 h for up to 6 months. The observed increase in the levels of *E. coli* O157 recovered from samples stored for 6 months is likely to be artifacts. There was no significant difference between the levels of *E. coli* O157 recovered from the prefreezing samples and those recovered from the samples stored at −80°C for 15 min. It is assumed that although the sample was in a solid state after 15 min of storage at −80°C, the suspension medium was frozen but the bacterial cytoplasm was as yet unfrozen and undamaged.

Glycerol is a commonly used cryoprotectant that limits damage to the cellular membranes of bacterial cells during frozen storage. There was a significant difference between results obtained with the use of 5% glycerol and those obtained with the use of 10% glycerol, with the addition of 10% glycerol statistically improving recovery. The reason for the increase in the level of *E. coli* O157 recovered from the samples with 10% glycerol for each freeze-thaw cycle is unclear. However, the results of the freeze-thaw experiment in the present study were comparable to those for freeze-thaw experiments involving *Lactococcus lactis* (10), although biological differences between these two organisms mean that different mechanisms may be involved. The addition of a cryoprotectant provides confidence that samples can be stored and can undergo multiple freeze-thaw cycles without major losses. Samples can therefore be thawed, tested, and refrozen for further testing if necessary.

There was no significant difference between levels of *E. coli* O157 recovered from samples that were placed directly in the −80°C freezer and those recovered from samples that were rapidly frozen with liquid nitrogen. It was therefore decided that the use of liquid nitrogen was not a beneficial step for inclusion in the final protocol.

The method of thawing did have a significant effect on the recovery of *E. coli* O157. Rapid thawing of samples in a water bath at 50°C significantly increased the levels of *E. coli* O157 recovered compared with those for samples that were left to thaw at room temperature. It is assumed that the rapid thaw decreases the recrystallization and ice crystal structure changes that occur during thawing, thus reducing the damage to cells. The addition of a step involving resuscitation and a short incubation period again increased the recovery of *E. coli* O157. The introduction of a resuscitation step allows the repair of stressed or injured bacteria and has been shown to improve recovery (7, 14). A short incubation period after resuscitation was introduced into the protocol to allow the growth and replication of bacteria in order to improve the probability of isolating strains of *E. coli* O157.

In the United Kingdom, there is no legal mandate for intervention upon the identification of a carcass contaminated with VTEC O157. Routine carcass screening for this pathogen is not compulsory, and there is no mechanism for the compensation of the farmer or the abattoir involved even if such an intervention were carried out. A possible solution to the ethical dilemma associated with identifying VTEC O157–positive carcasses destined for human consumption when there is no possibility of intervention is to use frozen storage to delay the processing of samples for research for a period long enough that all meat products from any potentially contaminated carcass would have been consumed by the time the samples are examined. In the large-scale epidemiological study of which this work constitutes a part, on the advice of a national medical ethical review panel, it was decided to delay the processing of samples derived from carcasses destined for human consumption for a minimum period of 6 months after they have been collected. Thus, a suitable method of frozen storage was essential for the success of the overall study.

Carcass swab samples collected at abattoirs are likely to contain a range of bacteria, which may reduce the probability of recovering any of the *E. coli* O157 organisms present. However, the method developed here was tested in the field at an abattoir processing >30-month-old cattle. The United Kingdom introduced a ban on beef products from >30-month-old cattle for human consumption in 1996 after a possible link between bovine spongiform encephalopathy and the variant strain of Creutzfeldt-Jakob disease in humans was suggested. The abattoir processing >30-month-old cattle was chosen to test the method in the field because it provided a source of cattle being processed in the same manner as beef cattle for human consumption but without the ethical dilemma involved in the direct processing of samples. The results from the field trial confirmed the findings of the laboratory trials. The 120 carcass swab samples collected on the abattoir visits were tested both as fresh samples and after 1 month of storage at −80°C. Isolation rates achieved before and after freezing were comparable, confirming the efficacy of the storage and recovery methods developed here.

While the decision to select an isolation method to provide results comparable to those of both current and previous studies undertaken in the United Kingdom is justified for this study, the fact remains that the medium chosen may not be the most sensitive for the recovery of freeze-damaged and stressed cells. Although SMAC is a widely accepted medium for the isolation of *E. coli* O157, previous studies have demonstrated that it does not fully support the growth of stressed *E. coli* O157 strains and that tryptic soy agar is a more favorable nonselective medium for the recovery of stressed cells (5–7, 11, 14, 15). The sensitivity tests were carried out with the use of only one bovine isolate of *E. coli* O157, and it is possible that other strains may be more prone to injury during frozen storage. However, the method selected on the basis of this series of experiments was demonstrated to have good sensitivity when it was used in field trials and could be useful for any study in which the frozen storage of sample material for bacteriological purposes is necessary or desirable.

**ACKNOWLEDGMENTS**

The authors thank Dr. David Platt, Dr. Janice Spencer, Dr. Nick Parham, Professor Mark Roberts, Associate Professor Jenny Hodgson (Syd-
J. Food Prot., Vol. 67, No. 1

FROZEN STORAGE OF E. COLI O157 IN BPW

45

ne), and Dr. Alun Williams for their helpful comments; the staff at SAC Veterinary Science Division, Inverness, for their advice and support; the former Scottish E. coli O157 Reference Laboratory, Forres terhill, Aberdeen, for contributing the bovine strain; and the current Scottish E. coli O157 Reference Laboratory, Edinburgh, for the typing of E. coli O157 strains from the field trial. The support of Professors M. Murray and J. Neil, in whose departments the research was conducted, is acknowledged. L. M. Filshie was a Wellcome Trust summer vacation student and the work described was funded by the Wellcome Trust. This work was conducted on behalf of the Wellcome Trust-funded IPRAVE consortium.

REFERENCES