Capacity of the Scientific Research Organisation of Samoa to Undertake Food Safety and Quality Testing for Export Products – Part 4

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TABLE OF CONTENTS

EXECUTIVE SUMMARY.................................................................................................................................. IV
1 BACKGROUND............................................................................................................................................... 1
1.1 Aim of the training .................................................................................................................................. 1
2 ACTIVITIES................................................................................................................................................ 2
2.1 Validation support for methods to gain ISO17025 accreditation ......................................................... 2
  2.1.1 *Vibrioparahaemolyticus in food* ................................................................................................... 2
  2.1.2 *Vibrio cholerae in water* ............................................................................................................ 2
  2.1.3 *Campylobacter in foods* ............................................................................................................. 2
  2.1.4 *Aflatoxins in copra* .................................................................................................................... 3
2.2 Support implementation of quality systems .......................................................................................... 3
  2.2.1 *Calibration*.................................................................................................................................. 4
  2.2.2 *Internal Audits* ........................................................................................................................... 4
  2.2.3 *Quality systems review* ............................................................................................................. 5
  2.3 Review of hardware/instrumentation .................................................................................................. 5
2.4 Follow up calibration and chemistry training Stage 2 and 3 ............................................................. 6
2.5 Training provided and outcomes achieved ......................................................................................... 7
  2.5.1 *Microbiology*................................................................................................................................ 7
  2.5.2 *Aflatoxin training*....................................................................................................................... 7
  2.5.3 *Quality Systems Presentation* ..................................................................................................... 7
2.6 Further support towards ISO17025 accreditation ............................................................................. 7
2.7 Conclusions .......................................................................................................................................... 8
3 RECOMMENDATIONS................................................................................................................................ 9
4 LIMITATIONS ............................................................................................................................................ 10

TABLES

Table D-1 Spike sample under normal conditions ..................................................................................... 15
Table D-2 “Stressed” spike samples ............................................................................................................ 16

APPENDICES

Appendix A Calibration Audit report
Appendix B Internal Quality Audit report
Appendix C Quality Manual Review
Appendix D Method Validation *Vibrio parahaemolyticus* Presence/Absence in food (modified)
Appendix E Method Validation *Campylobacter* Presence/Absence
Appendix F Method Validation *Campylobacter* in raw chicken samples
Appendix G  Method Validation *Vibrio cholerae* Presence/Absence in water (modified)
Appendix H  Verification of Aflatoxin test in Copra
Appendix I  Microbiology Training Report
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Atomic absorption spectroscopy</td>
</tr>
<tr>
<td>APC</td>
<td>Aerobic Plate Count</td>
</tr>
<tr>
<td>APHA</td>
<td>American Public Health Association</td>
</tr>
<tr>
<td>APW</td>
<td>Alkaline Peptone Water</td>
</tr>
<tr>
<td>BG</td>
<td>Brilliant green (agar)</td>
</tr>
<tr>
<td>CCDA</td>
<td>Campylobacter charcoal differential agar</td>
</tr>
<tr>
<td>CEO</td>
<td>Chief Executive Officer</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming unit</td>
</tr>
<tr>
<td>FAPAS</td>
<td>Food Analysis Performance Assessment Scheme</td>
</tr>
<tr>
<td>FDA</td>
<td>(United States) Food and Drug Administration</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Pressure Liquid Chromatography</td>
</tr>
<tr>
<td>IANZ</td>
<td>International Accreditation New Zealand</td>
</tr>
<tr>
<td>ILCP</td>
<td>Inter-laboratory Comparison Programme</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>ISO 17025</td>
<td>International standard for testing laboratories</td>
</tr>
<tr>
<td>KTP</td>
<td>Key Technical Personnel</td>
</tr>
<tr>
<td>NMD</td>
<td>National Meat Database – New Zealand Food Safety governmental methods</td>
</tr>
<tr>
<td>NZRM</td>
<td>New Zealand Reference Material</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Saline</td>
</tr>
<tr>
<td>PHAMA</td>
<td>Pacific Horticultural and Agricultural Market Access (PHAMA) Program</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SROS</td>
<td>Scientific Research Organisation of Samoa</td>
</tr>
<tr>
<td>SROS TS</td>
<td>Technical Services within SROS, performing analytical testing services</td>
</tr>
<tr>
<td>TCBS</td>
<td>Thiosulfate-citrate-bile salts-sucrose (agar)</td>
</tr>
<tr>
<td>TNTC</td>
<td>Too numerous to count</td>
</tr>
<tr>
<td>URS</td>
<td>URS Australia Pty Ltd</td>
</tr>
<tr>
<td>XLD</td>
<td>Xylose lyseine deoxycholate (agar)</td>
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EXECUTIVE SUMMARY

- Scientific Research Organisation of Samoa (SROS) technical and scientific staff have been trained in the method for the detection of *Vibrio parahaemolyticus* in foods and in the updated method for detection of *Vibrio cholerae* in water supplies.

- SROS staff have been trained for the detection of *Campylobacter* in foods and the enumeration of *Campylobacter* in raw chicken.

- SROS staff have participated in the verification of the R-Biopharm Rhone Aflatoxin kit on copra. Due to the high toxicity of the aflatoxins standard required to fully validate this method, it was agreed to postpone the further validation to a later date.

- SROS staff have written draft validation reports for the first and second bullet points above and are awaiting results from the Inter-Laboratory Comparison Programme (ILCP) rounds to complete the reports. Some more data needs to be generated for the enumeration of *Campylobacter* from raw chicken. SROS staff have reviewed the methods and updated where necessary.

- The consultant spent considerable time following up on the calibration training and ensuring that staff are performing calibrations required to ensure continuing accreditation.

- The consultant was involved with reviewing the SROS Quality Systems and suggested improvements that need to be implemented by the end of September to send to International Accreditation New Zealand (IANZ) by 7 October.

- The consultant was appointed as an internal auditor and performed an internal audit on the micro section. This audit was observed by the SROS Quality Manager, Samani Tupufia, for training purposes. Samani Tupufia audited the chemistry section with assistance from the consultant.

- The consultant gave two Quality Seminar presentations – one to the chemistry staff and a repeat with the micro staff. The micro team attended a presentation on the new bacterial methods.

- Follow up on the chemistry method validations was hampered by equipment failure (column on the High Pressure Liquid Chromatography (HPLC) unit) and the late delivery of the heating block for the sample digestions for the metals. The block had a missing part that was only received the day before the mission ended. SROS needs to complete the chemistry validations.

- The consultant prepared the following reports for SROS management: *Calibration – Internal Review; SROS Quality Manual Review; and Internal Quality Audit – Micro Section.*

All validations of the Microbiological methods included in this report are at a stage where they can be considered for accreditation. Although adequate training was provided for the aflatoxin method, a positive control will need to be implemented before the method can be considered for accreditation.

The Histamine method is likely to be at a stage where it can be considered for accreditation with a few more test runs to be completed before the audit. The testing of heavy metals also needs some work before it can be considered for accreditation – it is possible that this will be achieved before the audit in December 2013.
The training provided on a number of topics was received well and attended by a large number of staff.

Follow up on the validation of chemistry method, while very valuable, was constrained due to the unavailability of critical instrumentation. Despite this, methods for heavy metal analysis will potentially be ready for assessment at the audit in December 2013.

The following recommendations are made:

- SROS would benefit from a short intervention (one week) by the chemical technical expert before the IANZ audit, focussed on instrument set up, calibration and some final work on the validation of the methods.

- It is recommended that Samani, as Quality Manager, attends New Zealand Quality College courses in Laboratory Quality Management and Internal Auditing, as well as visiting a larger accredited consulting laboratory.

- Implementation of Health and Safety Standards in all labs should be considered – training would be required.

- Continuing collaboration with Cawthron for ongoing assistance would be beneficial to ensure that ISO17025 accreditation is achieved and maintained.

- The purchase of a five-place balance should be considered, providing suitable accommodation and controlled access can be guaranteed.

- The purchase of suitable reference thermometer/s (digital with probes) should be considered.

- The calibration team should be allowed to spend the required time to keep the system up to date and should report regularly to the Quality Manager.

- Further external support should be considered after the IANZ audit, depending on the audit report, with a focus on clearing any Corrective Action Requests.
1 BACKGROUND

1.1 Aim of the training

The aim of Stage 4 of the training was to support the micro section of the Scientific Research Organisation of Samoa (SROS) lab to validate new tests that would have relevance for potential customers of SROS. The new food act specifies *Vibrio parahaemolyticus*; hence the decision to perform a detection method for this species. It was also considered relevant for the lab to update the existing method for *Vibrio cholerae* detection in water supplies. This means that if the Ministry of Health should be dealing with an outbreak of cholera, the SROS lab could be engaged to test water supplies.

*Campylobacter* in foods is also a high risk pathogen and once the test for this is included in the scope, the lab would be able to offer a comprehensive range of pathogen tests. A count method on raw chicken (based on the New Zealand Animal Products Specifications Notice 2012) was also added, as raw chicken is a known source of *Campylobacter* infections and Samoa has a mobile chicken processing plant.

Provision of aflatoxin testing in copra is another customer-driven requirement, and the use of a kit was trialled.

The consultant followed up on the training for calibration procedures provided to SROS staff members in Nelson, New Zealand, and checked progress on the validation of the chemistry methods (stage 2 and 3). Ensuring that the lab maintains its accreditation is considered to be vital for the lab’s viability.

The scope of the mission was very wide, including the addition of new tests to SROS’s scope, as well as activities to support the lab to maintain its accreditation with compliance to all aspects of ISO17025. To ensure that all aspects of the mission were comprehensively covered, Cawthron Institute decided that a senior microbiology technician (Ryan Hunter) would be joining the consultant (Joy Oakly) to assist with the microbiological test validations, at no cost to PHAMA or SROS. This allowed the consultant to focus more on all the Quality issues that were highlighted in the first few days of the mission. The benefit of this arrangement has been a much more in-depth review of the quality systems. It also provided Ryan the opportunity to gain experience with training lab staff in a Pacific lab.
2 ACTIVITIES

2.1 Validation support for methods to gain ISO17025 accreditation

Validation of the micro methods was supervised by Ryan Hunter, Cawthron Institute. All three micro staff from SROS were involved: Luanda Epa, Research Scientist and the current Key Technical Personnel (KTP) for the micro tests on the SROS scope; Annie Toailoa, Principal Research Officer (also KTP); and Faataga Jr Faataga, Technical Officer. All participated in the validations performed for all four methods.

An aflatoxin kit was demonstrated and a number of staff assisted with the two extractions performed.

The method validation reports are attached.

2.1.1 Vibrioparahaemolyticus in food

The SROS micro team was involved with the validation of the method for the detection of Vibrio parahaemolyticus in a number of types of seafood. The method chosen is presence/absence rather than a Most Probable Number count method, as it uses a larger sample size but requires less consumables. Samples were spiked with low numbers of the target organism. Vibrio parahaemolyticus was successfully recovered from sixteen of the twenty one samples and from five out of six samples where the organism had been “stressed” overnight in the refrigerator. The samples from which the organism was not successfully recovered were small shrimps, which appeared to contain high numbers of Aeromonas spp that may have out-grown the Vibrio parahaemolyticus. A set of Inter-Laboratory Comparison Programme (ILCP) samples delivered by Cawthron were also tested and results submitted. A draft validation report was completed by 6 September (attached). The ILCP sample results (once available), together with the results from the April 2013 ILCP round in which the lab had also successfully recovered and identified Vibrio parahaemolyticus, will need be added before the International Accreditation New Zealand (IANZ) audit.

It is expected that this test will meet requirements for accreditation.

2.1.2 Vibriocholerae in water

All of the SROS micro team were involved with the validation of the method for the detection of Vibrio cholerae in water supplies. A number of samples were spiked with the target organism and a set were also “stressed”. Vibrio cholerae were recovered from twelve out of fourteen samples. The ILCP samples were also analysed with this method and the results for recovery of Vibrio cholerae have been submitted to the provider. These results will be added to the draft validation report, which is attached.

It is expected that this test will meet requirements for accreditation.

2.1.3 Campylobacter in foods

The validation of the Campylobacter method was performed in two parts. The presence/absence method was used for a range of meat products, from which 25 gram samples were tested by the micro team. Campylobacter can be an issue in raw chicken; because Samoa may get a mobile processing plant, it was decided that an enumeration
method on raw chicken could also be included in the validation plan. The method is based on the one documented in the New Zealand Animal Products Notice 2012 – National Microbiological Database Specification.

Twelve samples were spiked with the target organism and all 12 returned a positive detection. Three samples were spiked for the enumeration procedure, with two out of three giving acceptable recoveries. The third sample was heavily contaminated with many organisms but *Campylobacter* was also recovered. Draft validation reports were written for both methods (attached). ILCP samples have also been tested.

It is expected that this test will meet requirements for accreditation.

### 2.1.4 Aflatoxins in copra

A number of staff from the Technical Services and other Divisions participated in the verification of the R-Biopharm Rhone Aflatoxin Kit. Cawthron received the kit and a Research & Development Technical Officer had trialled the kit in Nelson using some dried coconut. The only reservation was that the colour development for the sample result was not as intense as that shown on the kit. The process of following the instructions from the manufacturer is relatively simple. The filtration process worked well at Cawthron, but using the copra samples at SROS the filtration proved to be very slow. SROS did not have the correct filter paper and this will have made some difference. The use of a vacuum pump did improve the flow rate.

The other option recommended by the kit was centrifuging at 4000 rpm, but the SROS centrifuges do not have that capacity.

To overcome this problem, a smaller sample was taken and centrifuged at a lower speed – this increased the detection limit for the kit from <2ppb to <4ppb. Colour development was also not very intense, as noted at Cawthron. We are currently seeking advice from the suppliers of the kit regarding the colour intensity.

The kit chosen does not include any positive controls. A “spiked sample” can be purchased to use as a positive control; this option is being investigated. IANZ has indicated that it would expect the lab to have and use a positive control if it is to be accredited for this test. SROS will need to decide if it is necessary to have the test accredited. They will also need to consider the health and safety issues surrounding the test (aflatoxins are very toxic) and ensure that all materials are disposed of as recommended in the procedure. Testing needs to be performed in fume-hoods wherever necessary. The consultant will write a summary of the use of the kit.

### 2.2 Support implementation of quality systems

Because SROS has recently made management changes, with the establishment of a separate division for Technical Services, assistance was required to support the new Manager of the Division (Samani Tupufia) in his role. A significant amount of the consultant’s time was spent on quality-related issues that are crucial to ensure that SROS maintains its accreditation and is able to add new tests of value to its scope. Failure to comply with the requirements of ISO17025 at the scheduled IANZ audit in December would have consequences for the operation of the Division. The consultant gave two Quality Seminar presentations on the requirements of ISO17025, detailing the importance of adhering to and documenting all the quality systems.
2.2.1 Calibration

The previous calibration officer left in January 2013 after providing very limited training to another staff member, who did a few calibrations in February and March but was then transferred to another division. To fill the gap, two staff members from SROS had two weeks’ training at Cawthron in May. Unfortunately, due to staffing availability constraints, no calibrations had been performed at the time the consultant arrived. This is a major non-conformance against the ISO17025 standard. Guidance was given to Phillip Reti and Faataga Jr Faataga to ensure better compliance in the future. SROS must ensure that the calibration schedule is adhered to and needs to resolve a number of issues listed in the microbiology internal report and the calibration internal review documents in particular:

- The lab needs to invest in some equipment and reference materials to assist with the calibrations. Currently there is only one reference thermometer, which cannot be used for all applications, particularly in chemistry, as it was originally purchased for micro use. Ideally the lab needs a digital thermometer with a selection of probes, which could then be used in ovens and furnaces as well as incubators. None of the other reference thermometers could be found and some of the larger calibrated weights have been damaged by having asset numbers painted on them.

- Some of the auto-pipettes that were in use were damaged and not performing accurately. However, a large number of new ones were found and will be brought into use as required. Calibration certificates for the four-place balances that the lab currently has indicate that they are not performing very well and two should only be used to 3 decimal places. If the lab has to calibrate auto-pipettes at volumes of 100\(\mu\)L or less, then it should be using a five-place balance. If such a balance was purchased, it needs to be in a controlled environment with restricted access in order to ensure that it is handled with the appropriate care.

- All reference materials should be stored in a safe location and only used by the trained calibration staff.

- The time taken to get repairs and replacement parts for the autoclaves is of concern for continued accreditation. While the lab has been able to use the Ministry of Health and the brewery autoclaves, this is not desirable as it would be unlikely that either of these are calibrated. If the lab is successful in increasing the number of tests it can offer to customers, it must have a working, calibrated autoclave on site. Fortunately, a number of the tests currently on the scope do not need autoclaved media, but the new tests do require this and accreditation is unlikely to be given unless the situation has been resolved by the time of the audit.

- Calibration staff have started performing calibrations but will need to set up a schedule for continuing calibrations as specified. This needs to be monitored by the Quality Manager.

A report of the review of the calibration function within SROS is attached.

2.2.2 Internal Audits

Internal audits for 2013 had not been started, although they were planned for September and October. However, this timeframe needs to be revised so that they are performed several months before an IANZ ISO17025 audit, and any corrective actions completed. Ideally they should be completed by the end of August. Samani, as new Technical Manager and Quality
Manager, will be organising these audits. As he has not had any experience performing internal audits, the consultant was appointed as one of SROS’s internal auditors. Cawthron has a template for performing internal audits and this was used for the microbiology and chemistry audits.

The consultant performed the micro audit with Samani observing, and then Samani performed the chemistry audit, assisted by the consultant. The micro internal audit is attached. Samani was encouraged to perform in-depth audits so that the lab identifies their own issues and can show the external auditors that they are managing these issues effectively.

Samani would benefit from attending New Zealand Quality College courses in Laboratory Quality Management and Internal Auditing, and visiting a larger accredited consulting laboratory like Cawthron to see how the systems are implemented.

2.2.3 Quality systems review

The consultant was asked by the lab management to perform a review of their Quality Systems, in particular a review of their Quality Manual. This document needs to be updated and supplied to IANZ by 7 October.

The management structure for the division has been changed recently, which needs to be reflected in the Quality Manual. Better document control is required in a number of areas, which were specified in the internal audit report (see attached).

Some other issues were identified:

- **Purchasing** – put measures in place to get any items cleared through customs and delivered quickly so that their integrity is not compromised.
- **Corrective actions and complaints** – these systems need to be better controlled, have a unique number and be in easily accessible files.
- **Housekeeping** – the expectations for the lab housekeeping need to be documented and staff must ensure that they do adhere to any regime that is implemented.
- **Equipment management** – due to issues already noted in the calibration report, all records need to be checked to ensure that they reflect what is documented in the Quality Manual.
- **Reporting** – a number of suggestions were made regarding the templates for the reports. The number of signatures on the reports was raised in the exit meeting with the CEO. It is not necessary for the CEO to sign off reports – an IANZ endorsed report must contain the signature of the Key Technical Personnel for the test. Another signature can be a checker or the Quality Manager if the lab considers this important for their customers.

2.3 Review of hardware/instrumentation

SROS continues to experience problems with supply and service of laboratory instrumentation and equipment, such as the digestion block (see section 2.4) and calibration certificates.

A calibrated autoclave is essential for retaining accreditation. A fully functioning fume-cupboard is required for tests requiring working with acids.
A five-place balance, as well as more reference thermometers and/or probes, would improve the quality of the calibrations performed.

2.4 Follow up calibration and chemistry training Stage 2 and 3

The consultant spent a considerable amount of time with the calibration staff helping them with the implementation of the systems and encouraging them to start all outstanding calibrations (follow up stage 2). Cards were purchased to record each item requiring calibration and to use as a simple call up system to ensure future calibrations are performed on schedule. A number of records have been water damaged in the cyclone (December 2012), and these should be kept until the IANZ audit before being disposed of if appropriate. Finding the reference materials and certificates was time consuming. Certificates for calibrations performed by an external calibration agency in February were initially not available. At the request of the consultant, most of these were emailed to the lab, with hard copies delivered later. The length of time it has taken for the lab to receive these certificates is not appropriate and the calibration agency needs to improve on this.

Follow up on the chemistry training and continuing validation work on the methods was provided (follow up stage 30).

Staff had performed some more runs for histamine on High Pressure Liquid Chromatography (HPLC) before the consultant arrived and were working on the Detection limit. Runs were scheduled but unfortunately, as a result of the column being contaminated, the instrument couldn’t be used. Chemistry staff spent a week trying to clean the column using various techniques, but when histamine standards were run no peaks were observed. Arrangements were made to send over a new column with a guard from New Zealand, but it only arrived on 9 September. SROS was also making arrangements to purchase Food Analysis Performance Assessment Scheme (FAPAS) ILCP samples to add to their validation data. Some more runs can progress now before the validation report can be completed by the end of September.

It is likely that accreditation for the histamine test will be achieved once the additional tests and ILCP have been completed successfully.

As the lab wanted to proceed with the acid digestion for the heavy metal testing, PHAMA had purchased a heating block. Issues with delivery (it was sent to the PHAMA office in Apia instead of SROS) significantly delayed progress on the validation of the heavy metal tests. Another issue emerged when the block was commissioned and a very small part – Donegal – was found to be missing. This was critical for the operation of the unit and the supplier admitted that it had not been sent. This part arrived on 5 September and calibration of the block was in progress at the time the consultant left SROS.

Acid digestion requires the heating block to be used in a suitable fume hood. The fume hood in chemistry was not working and a replacement has been ordered. The fume hood in another building was going to be used to continue the validation work. As a back-up solution, SROS will be approaching the University of the South Pacific to see if they could perform the digestion step in one of their fume hoods.

Recent communications between the consultant and SROS technical staff (late September 2013) confirm that progress is being made with the validation of these tests using the digestion block. With ongoing efforts in this area between now and the audit, it is likely that the heavy
metals tests can be submitted for accreditation, provided that initial validation data has been sent to IANZ by 7 October.

2.5 Training provided and outcomes achieved

2.5.1 Microbiology

Training was completed for the new micro methods – *Vibrio parahaemolyticus* in foods, *Vibrio cholerae* in waters and *Campylobacter* in foods – for the following staff:

- Luanda Epa, Research Scientist
- Annie Toailoa, Senior Research Officer
- Faataga Jr Faataga, Technical Officer.

Luanda and Annie were signed off to Level 4 and Faataga to Level 3 in their training records by the Cawthron trainer.

2.5.2 Aflatoxin training

The following staff attended the training session or parts of the sessions:

- Phillip Reti, Professional Officer
- Annie Toailoa, Senior Research Officer
- Oiner Leutu Moa, Technical Officer
- Agape Papalii, Professional Officer
- Militini Tagoai, Professional Officer
- Nadia McFall, Professional Officer
- Siope Pele, Research Officer.

Those staff who attended at least one complete session would be able to perform the test. A draft method was supplied to staff and an easy-to-follow flowchart.

2.5.3 Quality Systems Presentation

Attendees:

- Chemistry – Samani Tupufia, Kuinimeri A Finau, Phillip Reti, Militini Tagoai, Siope Pele, Kilom Ishiguro, Luano F Iosefa, Gaufa S Fetu, and Vanda Faasoa Chan-Ting
- Microbiology – Luanda Epa, Annie Toailoa, Faataga Jr Faataga and Tilafono David Hunter

2.6 Further support towards ISO17025 accreditation

Cawthron staff are continuing to support SROS staff with reviewing the validation reports and adding comments to these. The micro methods for the tests need to be revised and these
should be sent to Cawthron for review. The consultant will review the responses requested for the micro internal audit.

2.7 Conclusions

Overall, the mission was very successful. All the microbiological methods are now validated to a level where ISO17025 accreditation can be considered. With an extra consultant present, significant support was able to be provided on general Quality Systems requirements, with a focus on calibration, internal audits and the Quality Manual.

At this stage, the method for aflatoxin testing is not considered to be ready for ISO accreditation. This issue has been discussed with the accreditation agency and they indicated that they would expect that the lab had a positive control for use. The kit has a detection limit of 2ppb or 4ppb depending on the amount of sample taken. The use of a positive control sample would raise health and safety issues that the lab staff would need to be conscious of.

The training provided on a number of topics was received well and attended by a large number of staff.

Follow up on the validation of chemistry method, while very valuable, was constrained due to the unavailability of critical instrumentation. Despite this, methods for heavy metal analysis will potentially be ready for assessment at the audit in December 2013.
3 RECOMMENDATIONS

Recommendations are as follows:

- SROS would benefit from a short intervention (one week) by the chemical technical expert before the IANZ audit, focussed on instrument set up, calibration and some final work on the validation of the methods.

- It is recommended that Samani, as Quality Manager, attends New Zealand Quality College courses in Laboratory Quality Management and Internal Auditing, as well as visiting a larger accredited consulting laboratory.

- Implementation of Health and Safety Standards in all labs should be considered. Training would be required.

- Continuing collaboration with Cawthron for ongoing assistance would be beneficial to ensure that ISO17025 accreditation is achieved and maintained.

- The purchase of a five-place balance should be considered, if suitable accommodation and controlled access can be guaranteed.

- Purchase of suitable reference thermometer/s (digital with probes) should be considered.

- The calibration team should be allowed to spend the required time to keep the system up to date and should report regularly to the Quality Manager.

- Further external support after the IANZ audit should be considered, depending on the audit report, with the focus on clearing any Corrective Action Requests.
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APPENDIX A  CALIBRATION AUDIT REPORT

CALIBRATION – Internal Audit SROS

This report contains a summary of findings regarding the calibration section within SROS. While new staff have been trained, it will take a while to complete outstanding calibrations and implement new systems.

The quality system is new to Samani, the new Quality Manager, and he needs to be kept informed of any issues regarding calibration. If funding can be obtained, it is strongly recommended that he attends a Quality College Laboratory Quality Management Course in New Zealand and spend time in an IANZ accredited lab to see how the systems work and observe some of the chemistry instrumental methods.

The calibration section needs to organise the calibration activities in both the micro and chemistry sections urgently and demonstrate that they are capable of performing the calibration activities.

Issues to be resolved to ensure that calibration requirements are met now and in the future:

- Calibration staff to report each month to Quality Manager, outlining the tests performed and whether they are up to date with their calibrations.
- Schedule to be established (simple cards have been supplied for each item needing calibration). These to be placed in yearly/biannual box, clearly labelled.
- Source some more green stickers to use for indicating calibration of the equipment.
- All autopipettes in use in the micro and chemistry labs to be calibrated by 6 September and again in December before IANZ audit.
- All certificate reference materials to be found and a new list prepared, and the certificates to be kept in a designated office and in a clearly labelled file.
- Reference materials only to be used by those trained in calibration procedures.
- Reference materials to be stored safely, preferably in Quality Manager’s office.
- All equipment needing calibration to be listed.
- Certificates from Calibration services to be viewed and equipment labelled appropriately if the calibration is only partially passed.
- Check volumetric glassware in the labs and remove un-calibrated glassware. Plan the checking/labelling of the items collected.
- Review all methods to ensure they have enough detail and reflect current IANZ/reference requirements.
- The recordings in the recently re-instated log books to be checked each month.
- Waterbaths – those in micro to have temperature check sheets assigned for each bath.

It would appear that the lab no longer has all the calibrated temperature devices it has the certificates for. If the Ebro 420 is the only calibrated thermometer available, it limits the lab’s ability to carry out six-monthly temperature checks.
It is recommended that a probe is bought for the fluke or a new fluke and probe is purchased and calibrated over the ranges required in chemistry as well as micro.

Calibrated weights – the large weights have recently been damaged by having the asset number painted on them. This re-enforces the stance that calibrated equipment must be stored appropriately and access to them must be limited. Two of the 500 mg weights are missing from the set.

A.1 Review of Calibration Standard Operating Procedures (SOPs)

This is a general comment regarding these SOPs as well as any other SOP. When a second or later issue of the SOP is issued, then remove the signature space from the top half of the front page. Amendment details must be filled in the table and whoever makes the amendment should sign in the table. Unfortunately, a number of methods have been updated with no information recorded in the table – this is not very helpful for tracking changes. It was noted that there are several versions of some methods in circulation and they may have the same headings but they are different. Only calibration staff need access to these methods and they should be using the current version. Staff are currently working on some of these SOPs.

SOP No. 1 Autopipettes

This method needs extensive revision and advice was given when training was undertaken in Nelson. It should include partial passes when an adjustable pipette may only be used at a specific volume rather than other its entire range. The calculation section is not easy to follow and advice from Cawthron is recommended.

SOP No. 2 Volumetric glassware

Amendment record and issue date don’t match.

SOP No. 5 pH meters

This SOP should include detail on recording and accepting the slope or mV – whichever is appropriate. Only commercial buffers should be used.

SOP No. 6 – Thermometers

This should include information about six-monthly calibration of digital thermometers used for fridges. Could also include checks on incubator and waterbath probes.

SOP No. 7 Balances

Need to make changes to the weights used for six-monthly repeatability and monthly single point checks based on the performance of the current balances. Why you are choosing different weights needs to be documented.

Worksheet 7.1 – Monthly single point checks – this should be used on all balances, not just four-decimal place ones. Use reference weights at 1 g, 10 g, and 20 g. The expected weight must be taken from the reference weight certificate for the weights used. The weights on the current worksheet are not what the certificate states for those weights together with their measurement of uncertainty.

Worksheet 7.3 should be removed as this is not relevant to your method.
A.2 Equipment Issues

Autoclave: This will need to be fixed and calibrated prior to IANZ audit as they may restrict the micro lab’s operation if a calibrated autoclave is not available. Some tests that require media which is autoclaved could be suspended until this is fixed and calibrated.

Heating Block: The Donegal was received in September so calibration could be performed with some more runs for AAS method and the validation report. Temperature required is 85°C for all elements except Mercury, which requires 80°C and 30°C. Calibration is needed to check what temperature setting is required to achieve the higher temperatures. The lower temperature of 30°C is not so important.

HPLC: Use of this instrument must be restricted to trained users so that expensive errors do not reoccur. The new column is in transit and further runs should be completed as soon as possible to add to the validation report. The testing of old FAPAS ILCP samples is encouraged. If two rounds are purchased, then the lab should test the one Cawthron have results for initially and keep the other sample as a backup.

Five-place balance: It is expected that an accredited lab that uses autopipettes with volumes of <100µL calibrates these on a five-place balance. Currently the lab only has four-place balances, but these do not perform well at weights over 20 g and two should probably only be used as three-place balances. It is recommended that a five-place balance is purchased. However, the lab must control the use of this balance and its location, which is also critical for acceptable performance.

A.3 Staff training

During the training with the aflatoxin kit, it was noted that some staff didn’t appear to know how to use autopipettes correctly. Recommended that Phillip and Faataga perform training for staff in Technical Services on the use of these pipettes – they have been supplied with training information during their own training.
APPENDIX B INTERNAL QUALITY AUDIT REPORT

Internal Quality Audit for Scientific Research Organisation of Samoa

Section Audited: Microbiology Section

Reason for Audit: Annual & Training exercise.

Audit performed by: J. Oakly (Consultant), observed by Samani Tupufia – Manager of Technical Services.

Date: 26 August 2013 with some observations from the week of 19 August.

Reviewed by:

Signed by:

Date:

General comment:

The section shows good compliance and understanding of the ISO17025 principles. The amount of testing (other than ILCP samples) that the lab performs for external clients is still very limited and this needs to be considered when making comments about the operation of the lab.

Organisation of Staff

There have been a number of changes since the internal audit of 2012, particularly in relation to the structure of the group. Seeseei Molimau and Tulia Molimau Alesana who were KTPs have both gone to New Zealand for further study. Luanda Epa has been appointed as the new KTP and has Faataga Faataga and Annie Toailoa to assist her. Luanda reports to Samani Tupufia – Manager of Technical Services. The Technical Services division has only recently been established and will cover the operation of the accredited laboratories.

Staff Training Records

These were reviewed and it was noted that Faataga and Annie’s should be updated. All staff will need new training forms for the new tests currently being developed. It was noted that the training forms are very comprehensive for the pathogen tests and the ones for Vibrio and Campylobacter will follow the same format.

Documentation and record control

The micro section had filled out the worksheets very well with names, times and dates. It is expected that all pro-formas will have a little more document control on them. Some are missing the page number, issue date and issue number. It is also a good idea to have the name of the person who has authorised the pro-forma on the sheet. Dates and issue numbers help ensure that only the most recent version of the pro-forma is in use.
It was noted that when a recent batch of bottled waters were tested for Aerobic Plate Count (APC) 25 ml and 5 ml amounts were filtered in duplicate. All results were recorded as TNTC – too numerous to count. The lab needs to be flexible in the amounts of water used to test for APC. This can be based on previous results for the same bottler – if these indicate that high numbers are expected, the lab should revise the volumes accordingly – 1 ml amounts should be considered as well as 5 ml amounts from a 1/10 dilution. Three different volumes could be tested instead of 2 volumes in duplicate and those filters within the correct counting range should be used. This option should be noted in your method. While this will save the lab some costs it will also give the client a more meaningful result and thereby improve your profile with the client.

Monthly Temperature Charts – these are filled out daily but recommend that the temperature ranges are spaced out more for each tolerance range. Staff can then more accurately record the reading each day. A space for the correction factor of the probes should be added to the header.

It was noted on the Report for TS327 that the counts used for calculating the Enterobacteriaceae were lower than the recommended count range. The counts obtained from the higher dilution should have been used as these were within the count range. However the reported result was similar to that which would have been reported with the correct counting range.

Environment

Our initial observations of the pathogen lab, in particular, showed a lack of regular cleaning. All benches should be cleaned before and after use and spills in incubators, microwaves, fridges and the laminar flow cabinets must be cleaned up when they occur. The pathogen benches are extremely deep which makes cleaning difficult. The floor should also be regularly mopped as noted in your housekeeping procedure. All affected equipment has since been cleaned. Extensive environmental monitoring of the air is being performed at the documented frequency and the results are generally satisfactory. If the lab is successful in obtaining more pathogen work then a swabbing programme in the pathogen lab would be necessary. This would ensure that the work areas are not contaminated with samples containing pathogens or spills from the control cultures.

Staff are reminded that any media stored in the fridge must be in sealed plastic bags, named and dated when appropriate.

Test Equipment

There are some issues with equipment at the moment – the autoclave has been out of action since March and the lab is awaiting a new element. The back-up autoclave was damaged in the cyclone and more recently the wiring has been damaged. As most of the work performed in the lab is filtering or Petrifilm this has only caused minor issues and either Ministry of Health or the Brewery autoclaves have been used. While this is not ideal there are very few options.

Calibration Status

Since the beginning of 2013 there have been a number of changes in the calibration area. Timo who had been trained has left and Oiner who was taking over from Timo has decided to remain in another division. Philip Reti and Faataga Faataga were trained at Cawthron in May
but are now only just starting to perform the calibrations. This has meant that the autopipettes were not calibrated in April/May as required. The lab is to raise a Corrective Action which will outline why this non-conformance occurred, what measures they have taken to calibrate the pipettes as soon as possible and what preventative measures will also be put in place. Implementation of a calibration call up system such as cards or a calendar are recommended.

Annual calibration certificates from the external calibration agency for work performed in February had not been received until this week (had been posted but not received). It was unclear what calibrations had been performed as communication with Oiner may not have been passed onto the appropriate staff. Correction factors on the incubators/waterbaths were done by Timo in September but not dated or signed. Correction factors have now been received from the calibration agency and written on the daily charts. SROS calibration staff will have to perform a six monthly check of the probes in the incubators in the near future.

Six monthly checks on the balance and the digital thermometers are also due. Micro lab does not need a four-place balance – a two-place top pan balance is all that is necessary for a micro lab. If some small quantities of a chemical are needed then they could use one of the chemistry balances.

**Test Procedures**

It is recommended that the amendment section of all the methods has an amendment number column added to help keep track of amendment numbers. The issue number should be the amendment number + 1.

Method MA 2.1 Total, faecal coliforms and *E.coli* – recommend that the procedure section is expanded to document the use of the Partition method. Its use is only noted in the principle of the method.

Method MA 2.2 APC by MF – see the comment in documentation regarding adding options for volumes used based on sample history so that the client gets an actual result.

**Petrifilm methods**

This comment refers to all Petrifilm methods MA 3.1, MA 3.2, MA 3.3, MA 3.4 and MA 3.5. While testing in triplicate is commendable it is not considered necessary and duplicate plating would be considered as satisfactory in most commercial labs. The acceptable count ranges should also be specified in the methods. The manufacturer also has comments on estimating the counts when large numbers of colonies are present which the lab may wish to consider adding to the method. It was also noted in all these methods, except for Staph Express MA 3.5, that the method and worksheets state the result is reported as cfu/mL. However, the documented methods all state “take 25 g of sample”, hence the final result should be reported as cfu/g. There needs to be option of reporting as cfu/mL if liquid samples are tested. The Staph Express method also states take 50 g of sample – it is much more practical to use the same preparation weight for all tests i.e. 25 g unless there is some special requirement to take 50 g sample.

Worksheet for yeast and mould method MA 3.3 is labelled MA 3.4. Worksheet for MA 3.5 Staph express also has just cfu/mL for results.

The ISO Salmonella method for foods MB 1.1 has brilliant green (BG) agar incubated at 35°C but this should be 37°C the same as for xylose lyseine deoxycholate (XLD) agar. The lab may
wish to consider using Chromagar for the Salmonella in water method as it is considered to be appropriate for *Salmonella typhi*.

MB 2.1 Listeria – need to formalise the hand amendment incubation temperature of Palcam to 35°C and ensure that this change is reflected in the flow chart. Recommend adding the carbohydrates used for confirmation to the media list in section 3. The diagram in section 6.4 also needs updating to show the current Reveal windows and reactions (image supplied for inserting). Do not change to Reveal 2 – the kit you are using is still available in New Zealand.

**Media validation methods**

The lab is encouraged to update their methods for the evaluation of agars used in detection methods to that specified in the latest edition of the IANZ Specific Criteria. This would mean that future batches of Oxford, Palcam, thiosulfate citrate bile salts sucrose (TCBS), XLD and BG agars could be validated qualitatively rather than quantitatively. Hence method MC 2.1 needs changes to the agars on page 5. Method MC 12.1 Quantitative evaluations will also need to be updated. The change to the method will save on media and time for staff.

**Quality Control of Procedures**

**ILCP performance**

The lab participates in six programmes and takes two sets of samples for each matrix. The costs associated with freight of the samples from New Zealand makes it prohibitive to take any more rounds that the lab currently is testing. Performance over the past year has been very good with only one alert for Listeria and one for Yeast and moulds in 2012. A corrective action request was completed for these alerts. It would be helpful if the Corrective Action Request system had numbers for easy tracking – this would then also be written on the ILCP sheets.

**Media validations**

Lab is reminded to complete the validations when the media are initially opened. It would be helpful if the lab did use the coloured stickers as described in the SOP MC 6.1 to indicate the validation status of the media. Lab must complete validation of m-FC agar Batch 1220626; ½ Fraser Lot 42183 and Colitag Kit 010713A. Flexibility is required when looking at confirmatory media expiry dates due to the low samples numbers that the lab currently processes. Lab will always need to include control cultures if the media used was considered to be expired for the test but within the manufacturer’s expiry date.

**Control Cultures**

The lab has a documented three Tier system with monthly tier 2 and weekly tier 3 plates. However, as the lab currently is only testing very small numbers of samples and only pathogen ILCP samples, weekly transfer of all cultures in considered unnecessary. It is recommended that the lab documents an option that only cultures used routinely (like those for the coliform and water APC methods) are transferred weekly. This will also save time and blood agar plates. As the new Campylobacter control culture has some different maintenance requirements these will need to be added to the method and also the culture will need to be added to the lists in the appendices. The use of a -80°C freezer will ensure that the cultures can be kept for 5 years. However the lab needs to ensure that it has enough Protect beads on hand when any new culture is resuscitated – a minimum of 6 per organism is recommended.
Detergent Residue Test

This was performed satisfactorily in October 2012 and will be due again in October 2013. Recommend lab updates the bottom of the pro-forma MC 3.1 “Differences of less than 15% between G & B” – this should be A & B.

Reagent Grade Water Test

This was performed satisfactorily in October 2012 and will be due again in October 2013.

Monthly APC on media water

Performed as documented and counts are <100 cfu/ml.

Measurement of Uncertainty

If the lab has data to add to the current Memorandum of Understanding, it is recommended that this is done before the IANZ audit.

Corrective Action System

The labs system is very well documented – the micro lab has had very few raised during the past year. It would be helpful if a common numbering system was introduced which would help with the tracking of the Corrective Action Requests.

General Summary

Staff demonstrated a sound knowledge of the methods and the requirements for IANZ accreditation. They are commended on their attitude and it is hoped that a suitable increase in testing will be achievable.

Corrective action requests

(To be completed by 30 October unless specified)

1. Review of training records and the inclusion of new tests.
2. Calibration – the Corrective Action Request is to document the events in 2013, what measures are going to be implemented to manage calibration in the future to ensure that the frequency of the calibrations is maintained.
3. Complete calibration of Autopipettes currently in use by 6 September.
4. Calibrate the digital thermometers for the fridges and the probes in incubators and waterbaths.
5. Methods – ensure that the reporting options for the Petrifilm methods includes cfu/g and that the incubation temperature of the BG is 37°C.
6. Media validations to be performed on m-FC agar, Colitag and ½ Fraser broth.

Recommendations and Reminders

1. When updating pro-formas to include more document control if necessary. Lab should also ensure that the amendment section is completed at each review and number these if appropriate to help keep track of issue numbers.
2. Strongly recommend that you document the filtering of different volumes of water for APC count and only perform methods in duplicate rather than triplicate. (see report for detail)
3. Review the temperature charts to have more divisions between the temperature ranges.
4. Reminder to have a regular cleaning programme in the lab, including the equipment.
   Label and date media stored in the fridge.
5. Recommend a simple call up system for the calibration team – cards for each item is an easy reliable system.
6. Recommend that the lab reviews the test method section of this report and considers the suggestions outlined.
7. It is noted that the micro lab only needs a 2 decimal place balance.
8. Recommend that the lab updates its media validation procedures as outlined to perform qualitative validations only on selective/streaking agars. Lab to use the coloured stickers for indicating validation status.
9. Recommend that the lab reviews its culture method to include the Campylobacter culture and reviews its frequency of all the Tier 3 cultures.
10. Recommend that the lab considers a common numbering system for the Corrective Action Request.
This review has been at the request of SROS as part of the PHAMA project to support the lab in its accreditation process. It will include some comments relating to the implementation of the Quality manual procedures as well comments on changes required. As this year SROS is having a routine re-assessment for IANZ accreditation a copy of the manual must be included in the material submitted by 7th October.

The lab is expected to be able to demonstrate the implementation of the policies and procedures contained in their Quality Manual.

Please ensure that the issue number will be Number 9 as the current edition as currently there is a hand amendment with Issue 8. There is inconsistency with the headers for all the appendices – ideally they should have page number, issue number and date. If they have issued by at the bottom a name should be typed or a signature used.

Section 2 Page 6

I think this page has lost its header and should be section 2 – so needs a title. Then the SROS structure needs updating.

Appendix 3.0

SROS Organisational Structure needs updating.

Section 4 Management System

Page 8 – those with staff responsibilities in 4.3.3 needs updating.

4.9.3 Hand amendments

While six months updating is commendable it’s actually quite time consuming and if documented at that frequency it should be done every six months. I would recommend that you change that to 12 months and then making changes can be tied in with audits.

Appendix 4

4.2.1, 4.2.2, 4.5.1, 4.5.2 and 4.6 as these have no dates of issue on them make sure that these are the latest versions in circulation and update them with more document control.

4.7.1 KTP list – this needs updating and it should be noted that V. parahaemolyticus is not an IANZ accredited test yet. Staff can be appointed to a KTP to sign off non-accredited tests but you probably don’t want them listed in the manual

4.7.2 – There does appear to be several versions of training records in circulation so perhaps there should be some discussion and uniformity. This one needs a header like “Staff Training Record” and the name of the person who is being trained as it has two spaces for trainers.

Appendix 5

5.1 – Point 5 on the chart on page 2 – suggest change to Technical Services

5.1.1 – needs more document control.
Appendix 6

6.1 – Purchasing - recommend that this procedure is reviewed to ensure that it reflects current practice. In light of some of the difficulties being experienced with receipt of consumables this procedure should have some more detail for ensuring speedy clearance at the border. It would appear that this issue is having some impact on the quality of materials received for microtesting. The current process is also very costly for some items which are sent frozen from New Zealand.

The labelling of some assets should be supervised by the lab staff to ensure that the integrity of the equipment is not compromised by inappropriate labelling.

6.2.1 – needs document control

6.3 – List of Suppliers – needs updating as last done in July 2010.

Section 7 – Customer service

This section is very brief – check ISO 17025 for a little more detail. Suggest that you add a comment about confidentiality. Good communication with the customer is essential and this should also be added.

Appendix 7.1 – Survey form – no document control – this has a return by date of 23rd January 2012 so is out of date. Does SROS have any current information on customer satisfaction or comments that they could use if asked by IANZ. You do not need to do another survey but may be able to use some of the information collected in Stage one of this PHAMA project.

If you are not going to routinely conduct surveys then I would suggest that you don’t include this type of form in your manual. Just delete reference to it in Section 7.

Section 8 – Control of Non-conforming work and Complaints and Section 9 Corrective/Preventive Action.

It would be helpful if the Corrective action system was more controlled, with numbers used on the Corrective Action form and Complaints. Micro, Chemistry and Calibration could all have their own file for Corrective Action forms, with a unique numbering system in each group. Complaints should all be numbered and in one file – preferably with the Technical Services Manager. If a Corrective Action form is raised for any reason, then its unique number should be recorded on the paperwork. These suggestions may make the system more user-friendly.

Appendix 8.1 – more document control needed

Appendices 9.1 & 9.2 – more uniform document control.

Section 10 Control of records

The exception to keeping records for only three years should be documented. This relates to calibration data – especially any reference equipment which may be used for an extended number of years. This applies to balance records, reference thermometers, weights and autopipettes to ensure that you have the complete history.
Appendix 10

Needs document control

Section 11 Internal audits

You need to ensure that these are scheduled no later than the end of August each year. This will mean that any Corrective action requests will be completed prior to the IANZ audit in December.

It is recommended that any appointed internal auditor has a full understanding of ISO 17025 to ensure that all aspects of this standard are covered in each lab – particularly focusing on the different Quality Control requirements in chemistry and micro.

Appendix 11

This is good but probably has too much detail and needs to be kept up to date each year.

Section 12 – Management Group Review

Lab needs to ensure that this annual meeting is scheduled in October when the lab has had a chance to address any Corrective Action Requests from internal audits.

The 2012 minutes will have to be sent to IANZ with this year’s application.

Appendix 12.1 – should have more document control.

Section 14 – Accommodation and Environment

Recommend that the lab includes its pest control programme in this section – please see the following from the IANZ Specific Criteria for Biological Laboratories. SROS also needs to emphasis housekeeping in 14.4 and ensure that it is performed regularly – housekeeping SOPs for each lab.

4.1 Monitoring of the Environment

The laboratory environment, where relevant, shall be microbiologically monitored for trends and anomalies and records shall be kept. Laboratories should devise appropriate programmes of monitoring with respect to the type of testing being carried out. As a minimum, monitoring should be of airborne contamination e.g. exposure plates. Swabbing of critical surfaces such as sampling and testing benches, utensils, balances, stomachers, etc. are also recommended, and in pathogen testing laboratories this would be considered essential. Acceptable background counts shall be assigned and there shall be a documented procedure for dealing with situations in which these limits are exceeded.

Where necessary, appropriate pest and vermin control measures are expected to be in place. The suitability of the accommodation will be judged on whether it is likely to adversely affect the samples, equipment, staff performance or final test results.

Section 16 Equipment management

SROS has correctly documented that calibration records should contain specific details – Lab needs to ensure that this system is in place and that all equipment records are carefully
stored. Some records may need to be disposed due to cyclone damage but should be kept until the IANZ audit when the assessor has had a chance to examine and agree on disposal.

Appendix 16

16.1 – The list is incomplete and should at least include waterbaths and incubators. See IANZ Specific Criteria’s for Chemistry and Biological.

Lab needs to ensure that the records for each item of equipment are as specified in the manual. Calibration team have to work on updating these records.

16.2 – list is OK but not up to date. Wouldn’t recommend putting calibration information onto these sheets as it needs to be updated all the time.

SROS – Monthly check list doesn’t need to be in Quality Manual.

Appendix 17 – Sampling

17.1 – both pages are labelled as pg 1 of 2. Header is good but issued by should have a name or signature.

Appendix 18 – Assuring the Quality of test result

18.1 – needs 2013 ILCP programme. Recommend that the lab only does two rounds of Salmonella/Listeria in 2014 until the volume of work increases. This would be offset by the addition of Campylobacter rounds if the lab achieves accreditation for the new test.

Section 19 Reporting the result and Appendix 19

The report template needs updating and so the lab may wish to consider the number of signatures on the reports. Small labs would often have two signatures – analyst and checked by – one would be a KTP for that test or tests. SROS could change to KTP and Quality Manager and drop the CEO’s signature. As Sam is a chemistry KTP as well as Quality Manager then one of the other chemistry KTPs would need to sign off the tests.

IANZ have changed their logo and labs are encouraged to follow suit and use the new logo on their reports especially if other changes are being made to the template. An electronic version can be obtained from IANZ and will need to be down sized to fit on your header.

Another suggestion would be to not distinguish between quantitative and qualitative test results – hence only have one table of results. (Cawthron have all the results together – micro and chemistry as a list of results)

19.1B & 19.1C – amended reports have different report number spaces which are required and a comments header. It is recommended that the lab changes the comments section to “Reason for amendment” – this will ensure that the reason for the issuing of the amended report is recorded as required. It was noted during the micro audit that one amended report was issued without a reason being noted. While the reason was due to the customer changing the sample description this still must be recorded.

19.2B & 19.2C – the template appears to have a sample reference number already in the box – TS058/1112. This may need to be removed.
A series of spike trials, together with proficiency testing (ILCP samples) were performed to validate the Laboratory’s ability to carry out this test method and that the method is suitable.

The Laboratory validated the Methodology.

All micro lab staff including the KTP performed spike testing.

The laboratory participated in ILCP analysis.

**Method**

The Laboratory chose to use the FDA Bacterial Analytical Manual 2004 online version and Compendium of Methods for the Microbiological Examination of Foods 2005 – Chapter 40, Vibrio. The method has been modified from enumeration to a presence or absence detection, consequently eliminating the need to produce a blended 1:10 dilution and the use of double strength Alkaline Peptone Water (APW) tubes. A presence/absence test was deemed to be a more suitable method for use for SROS customers.

**Spike Methodology**

**Control cultures**

Vibrio parahaemolyticus NZRM 820 was used in the spike trials. V. parahaemolyticus is stored on beads in -80°C freezer. A bead is plated onto Blood agar and stored in refrigerator for one month. Prior to spike testing, V. parahaemolyticus is incubated in APW for 18–24 hours, to produce a fresh overnight culture that can be diluted to appropriate spike levels.

**Media**

- Alkaline peptone water – prepared in the Laboratory
- TCBS – prepared in the Laboratory
- Vibrio Chromagar – ready-made, purchased from Fort Richard Laboratories Limited. An optional agar used mainly to facilitate differentiation and identification.
- Marine Agar – prepared in the Laboratory. An alternative to Plate Count Agar and Blood Agar for spread plate counts.
- Phosphate Buffer Saline (PBS) – prepared in the Laboratory

**Samples**

The samples were inoculated with <100 CFU/ml (as per spike testing protocol). Actual spike counts as determined by spread plate on Marine agar were all <20 CFU/ml.

Some samples were inoculated with V. parahaemolyticus and kept overnight in the refrigerator to “stress” the V. parahaemolyticus bacteria. These were considered “stressed samples”.


Different types of matrices were used to reflect expected sample types. A variety of sample types are required e.g. fish, shellfish, processed seafood, etc.

**Spike Testing**

All spike samples were analysed as per the amended V. parahaemolyticus Presence/Absence SOP (MB 3.1).

Twenty out of twenty six spike samples produced acceptable recovery results for V. parahaemolyticus and were confirmed by the following tests.

- **Chromagar Vibrio**: suspected green isolates from TCBS were streaked onto Chromagar Vibrio, opaque mauve colonies were observed.
- **Oxidase**: positive
- **Catalase**: positive
- **Gram stain**: gram negative, curved rod

**Results**

Table D-1  Spike sample under normal conditions

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample description</th>
<th>Analyst</th>
<th>Spike dilution</th>
<th>Inoculum Count (cfu/ml)</th>
<th>Recovery on TCBS</th>
<th>Recovery on Chromagar</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/08/13</td>
<td>Tuna</td>
<td>Faataga Faataga</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Tuna</td>
<td>Faataga Faataga</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Tuna</td>
<td>Faataga Faataga</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Small shrimp</td>
<td>Faataga Faataga</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Fail</td>
<td>Fail</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Small shrimp</td>
<td>Faataga Faataga</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Fail</td>
<td>Fail</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Small shrimp</td>
<td>Faataga Faataga</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Fail</td>
<td>Fail</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Shrimp</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Shrimp</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Shrimp</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22/08/13</td>
<td>Shrimp</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>12</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Tuna</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Small shrimp</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Fail</td>
<td>Fail</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Small shrimp</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Fail</td>
<td>Fail</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Shrimp</td>
<td>Luanda Epa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Shrimp</td>
<td>Luanda Epa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
</tbody>
</table>
Table D-2  “Stressed” spike samples

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample description (Stressed samples)</th>
<th>Analyst</th>
<th>Spike dilution</th>
<th>Inoculum Count (cfu/ml)</th>
<th>Recovery on TCBS</th>
<th>Recovery on Chromagar</th>
</tr>
</thead>
<tbody>
<tr>
<td>28/08/13</td>
<td>Tuna</td>
<td>Faataga</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/08/13</td>
<td>Tuna</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/08/13</td>
<td>Tuna and small shrimp</td>
<td>Annie Toailoa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/08/13</td>
<td>Shrimp</td>
<td>Luanda Epa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/08/13</td>
<td>Small shrimp</td>
<td>Luanda Epa</td>
<td>1 ml of 7.5</td>
<td>20</td>
<td>Fail</td>
<td>Fail</td>
</tr>
</tbody>
</table>

ILCP Results

Awaiting completion of analyses.

CONCLUSION

It was determined that unsatisfactory recovery of V. parahaemolyticus in the six small shrimp trials was likely due to the high numbers of Aeromonas spp originally present in the sample, which may have out competed the V. parahaemolyticus. As Aeromonas spp are marine organisms they are ideally suited to growing in the APW broth used for the test.

As can be seen from the preceding tables, satisfactory recovery was achieved in sixteen out of twenty one spike samples that were under normal conditions and four out of five of the “stressed samples”. This demonstrates that the Laboratory can perform this test method to detect and recover V. parahaemolyticus in food samples such as fish and processed shellfish.
A series of trials were undertaken, to validate the Laboratory’s ability to carry out this method and that the method is suitable to detect the presence/absence of Campylobacter.

The Laboratory validated the Methodology.

All micro staff including the KTPs under took spike testing.

The laboratory participated in ILCP testing.

**Method**

The Laboratory chose to use the Campylobacter FDA 8th edition, and modified method to exclude incubation temperature of 37 °C. This method was chosen due to the availability of equipment and Media.

**Spike Methodology**

**Control Culture:**

*Campylobacter jejuni* NZRM 2379 was used in the spike testing.

*C jejuni* is stored on beads in -80°C freezer. A bead is removed from the freezer and stored in the 35°C incubator in Bolton Broth for one month. Prior to spike testing a fresh 48 – 72 hour culture is prepared.

**Medium:**

Bolton Broth – purchased ready made from Fort Richard and stored in freezer.

Campylobacter charcoal differential agar (CCDA) – purchased ready made from Fort Richard.

**Samples:**

The samples were inoculated with <100 CFU as determined by spread plate on CCDA agar.

Different types of sample matrices were tested to reflect the type of samples expected.

**Spike Testing:**

All spike samples, were tested by culture method.

All samples were verified as Campylobacter jejuni by the below stated reactions.

- Gram stain: -ve rods
- Motility: Wiggly in zig zags
- Oxidase: +ve
- Catalase: +ve
- Hippurate: +ve
## Results

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample description</th>
<th>Analyst</th>
<th>Spike dilution</th>
<th>Inoculum Count (cfu/ml)</th>
<th>Recovery on CCDA</th>
<th>Recovery on Blood agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/8/13</td>
<td>Pork mince</td>
<td>Luanda</td>
<td>1 ml of -6.5</td>
<td>177</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>23/8/13</td>
<td>Sausage</td>
<td>Faataga</td>
<td>1 ml of -6.5</td>
<td>177</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>23/8/13</td>
<td>Beef steak</td>
<td>Annie</td>
<td>1 ml of -6.5</td>
<td>177</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>23/8/13</td>
<td>Chicken breast</td>
<td>Luanda</td>
<td>1 ml of -6.5</td>
<td>177</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Beef steak</td>
<td>Faataga</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Beef steak</td>
<td>Annie</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Sausage</td>
<td>Luanda</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Sausage</td>
<td>Faataga</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Pork mince</td>
<td>Annie</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Pork mince</td>
<td>Luanda</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Chicken breast</td>
<td>Faataga</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/8/13</td>
<td>Chicken breast</td>
<td>Annie</td>
<td>1 ml of -6.5</td>
<td>45</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
</tbody>
</table>

## CONCLUSION

Acceptable recovery was achieved from all 12 trials with differing sample matrices and spike levels, demonstrating the suitability of this method to detect the presence or absence of the Campylobacter species. While it is noted that the first four spikes had counts higher than 100 cfu/ml, these sample matrices were repeated with lower counts giving satisfactory results.

### Initial Problems with spike testing

Prior to initiating spike testing series of dilutions were done to establish the best use of the control and dilutions required. It was established that a culture that was 48–72 hours old consistently gave similar results.

It was discovered (and well documented in reference methods) that Campylobacter is sensitive to dry plates.

The laboratory now stores CCDA agar in re-sealable bags.

### Proficiency testing

Awaiting ILCP results to be attached.
A series of trials were undertaken to validate the Laboratory’s ability to carry out this test method, and that the method is suitable for the detection and counting of Campylobacter in raw chicken.

The Laboratory validated the Methodology.

All micro staff and well as the KTP under took spike testing.

The laboratory participated in proficiency testing.

Method

The Laboratory chose to use the method from the National Microbiological Database Programme (New Zealand).

Spike Methodology

Control Culture:

*Campylobacter jejuni* NZRM 2379 was used in the spike testing

*C jejuni* is stored on beads in -80°C freezer. A bead is removed from the freezer and stored in the 35°C incubator in Bolton Broth for one month. Prior to spike testing a fresh 48 – 72 hour culture is prepared.

Media:

Buffered Peptone Water – made on site

CCDA – purchased ready made from Fort Richard

Samples:

The raw chicken samples were inoculated with sufficient colonies to be within a good counting range when 2 mls of broth is spread on 6 CCDA plates.

The spiked diluent was added to the chicken carcass in the plastic bag and the chicken was massaged as per the National Meat Database (NMD) procedure.

Spike Testing:

All spike samples, were tested by culture method.

All samples were verified as *Campylobacter jejuni* by the below stated reactions.

Gram stain: -ve rods

Motility: Wiggly in zig zags

Oxidase: +ve

Catalase: +ve
Hippurate +ve

Results

<table>
<thead>
<tr>
<th>Date</th>
<th>Analyst</th>
<th>CFU spiked</th>
<th>CFU recovered</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>26/8/13</td>
<td>Luanda</td>
<td>131,000</td>
<td>98,800</td>
<td>71.6</td>
</tr>
<tr>
<td>30/8/13</td>
<td>Faataga</td>
<td>301,000</td>
<td>338,800</td>
<td>112.5</td>
</tr>
<tr>
<td>30/8/13</td>
<td>Annie</td>
<td>301,000</td>
<td>Positive for Campylobacter but too contaminated to count</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

Acceptable recovery for two out of three trials carried out were achieved. More trials to be carried out to validate the proficiency of the method.

 Problems

Plates from the third sample were too contaminated to count. It was likely that the chicken sample purchased was already contaminated prior to it being used in the lab for the trial.
A series of spike trials, together with proficiency testing (ILCP samples) were performed to validate the Laboratory’s ability to carry out this modified test method and that the method is suitable for the detection of *V. cholerae* in water samples.

The Laboratory validated the Methodology.

All micro lab staff including the KTP performed spike testing.

The laboratory participated in ILCP analysis.

**Method**

The Laboratory chose to use the APHA Standards for the Examination of Water and Wastewater 2005, method 9260B *Vibrio cholerae*, and modified it to incorporate 18–24 hours incubation of the enrichment broth before streaking onto TCBS agar.

**Spike Methodology**

**Control cultures**

*Vibrio cholerae* NZRM 1099 was used in the spike trials. *V. cholerae* is stored on beads in -80°C freezer. A bead is plated onto Blood agar and stored in refrigerator for one month. Prior to spike testing, *V. cholerae* is incubated in Alkaline Peptone Water (APW) for 18–24 hours, to produce a fresh overnight culture that can be diluted to appropriate spike levels.

**Media**

- APW – prepared in the Laboratory
- TCBS – prepared in the Laboratory
- Vibrio Chromagar – ready-made, purchased from Fort Richard Laboratories Limited. An optional agar used mainly to facilitate differentiation and identification.
- Marine Agar – prepared in the Laboratory. An alternative to Plate Count Agar and Blood Agar for spread plate counts.

**Samples**

The samples were inoculated with <100 CFU (as per spike testing protocol). Actual spike counts as determined by spread plate on Marine agar were all <60 CFU/ml.

Some samples were inoculated with Vibrio and kept overnight in the refrigerator to “stress” the *V. cholerae* bacteria. These were considered “stressed samples”.

Some samples were inoculated with *V. cholerae* and *E. coli* in greater numbers than the Vibrio. This was done to gauge if *V. cholerae* can still be recovered in the presence of other bacteria.
Different types of matrices were used to reflect expected sample types. A variety of sample types are required e.g. untreated water, treated water, river water, brackish surface waters, etc.

**Spike Testing**

All spike samples were analysed as per the amended *Vibrio cholerae* Presence/Absence SOP (MB 5.1).

Twelve out of fourteen spike samples produced positive/acceptable recovery results for *V. cholerae* and were confirmed by the following tests.

Chromagar Vibrio: suspected yellow isolated colonies from TCBS were streaked onto Chromagar Vibrio and produced aqua blue colonies.

Oxidase: positive

Catalase: positive

Gram stain: gram negative, curved rod

**Results**

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample description</th>
<th>Analyst</th>
<th>Spike dilution</th>
<th>Inoculum Count (<em>V. cholerae</em>) (cfu/ml)</th>
<th>Recovery on TCBS</th>
<th>Recovery on Chromagar</th>
</tr>
</thead>
<tbody>
<tr>
<td>21/08/13</td>
<td>Treated water</td>
<td>Luanda</td>
<td>1 ml of 7.5</td>
<td>46</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>21/08/13</td>
<td>Treated water</td>
<td>Luanda</td>
<td>1 ml of 7.5</td>
<td>46</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>21/08/13</td>
<td>Treated water</td>
<td>Faataga</td>
<td>1 ml of 7.5</td>
<td>46</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>21/08/13</td>
<td>Treated water</td>
<td>Faataga</td>
<td>1 ml of 7.5</td>
<td>46</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>21/08/13</td>
<td>Treated water mixed</td>
<td>Annie</td>
<td>1 ml of 7.5</td>
<td>46</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>21/08/13</td>
<td>Treated water mixed</td>
<td>Annie</td>
<td>1 ml of 7.5</td>
<td>46</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Untreated water</td>
<td>Annie</td>
<td>1 ml of 7.5</td>
<td>50</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Untreated water</td>
<td>Annie</td>
<td>1 ml of 7.5</td>
<td>50</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Untreated water</td>
<td>Luanda</td>
<td>1 ml of 7.5</td>
<td>50</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>27/08/13</td>
<td>Untreated water</td>
<td>Luanda</td>
<td>1 ml of 7.5</td>
<td>50</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>28/08/13</td>
<td>Untreated water-stressed</td>
<td>Faataga</td>
<td>1 ml of 7.5</td>
<td>50</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
</tbody>
</table>
ILCP Results

Awaiting completion of analyses.

CONCLUSION

As can be seen from the preceding table, satisfactory recovery was observed in nine of the ten spike samples that were under normal conditions and three out of four of the stressed spike samples.

We did not recover *V. cholerae* after 6 hours incubation of the enrichment broth however when incubated for 18–24 hours, *V. cholerae* was successfully recovered.

The spike results demonstrate the ability of the Laboratory to perform this modified test method for the detection of the presence or absence of *Vibrio cholerae* in water samples. The spike trials proved the improvement in the recovery of the test organism when incubated for 18–24 hours as compared to a 6–8 hour incubation (the modification in the method).
APPENDIX H VERIFICATION OF AFLATOXIN TEST IN COPRA

The R-Biopharm Aflacard for the detection of total aflatoxins was chosen for the test. The kit is based on monoclonal antibody technology which has the advantage of being highly specific and sensitive while the test format is rapid and simple to perform. Each of the 10 cards has two sample ports – one purple spot at the sample site is the control spot which must appear to indicate that the test is valid. The second purple spot indicates that the contamination is less than the cut off value of the card. No colour at the sample site indicates contamination at a higher level than the cut off of the card.

Trial at Cawthron 7 August

A 50 g sample of desiccated coconut was blended with 100 mL of 80% methanol. As the sample was not homogenous another 100 mL of 80% methanol was added. The sample homogenate was filtered through #4 filter paper. The filtering was slow and then 5 mL of the filtrate was passed through the clean-up column provided. The filtrate was then placed in the port and solutions applied according to the kit instructions. Two purple colour spots developed in each port thereby giving a negative result for the sample. However the intensity of the colour was less than shown on the kit and this observation has been communicated to the kit supplier. As 50 g of sample was blended in a total of 200 mL of solvent the limit of detection would be <4ppb.

Health and Safety Considerations

Blending and filtering were performed in the fume hood. The disposal of all the items was as per the kit instruction. Decontamination of the pipette tips, sample containers, etc., is by immersing in 5% sodium hypochlorite solution for 30 minutes, followed by 5% acetone for 30 minutes. Flush with water and thoroughly wash. Solvents are to be retained and treated with at least 10% of their volume with 5% sodium hypochlorite before controlled disposal.

At SROS

The kit's performance was demonstrated out on two separate days 3 and 5 September. A number of staff participated in both days and some in only one.

A number of issues arose in the first run.

1. The Lab did not have the correct filter paper – either #113 or #4. No 40 was used and this has a much smaller μm size of 8 as compared with 20–30 μm size for 113 and 4. This may have made a difference in the rate the solution was able to be filtered.
2. The dried sample felt very oily and this may also have affected the rate the solution filtered.
3. SROS’s centrifuge would only go up to 3000 rpm rather than the 400 rpm documented in the kit instructions.

Trial 3 September

Initially 50 g of sample was added to 100 mL of 80% methanol and blended with Waring Blender in the fume cupboard. The sample (A) was well mixed. Filtering was then attempted – again in the fume cupboard. This took over an hour and only enough sample was obtained for use in the rest of the test.
A duplicate 25 g of sample (B) was added to 100 ml of 80% methanol and blended. Aliquots were then centrifuged at 3000 rpm for 20 minutes instead of 4000 rpm for 10 minutes. Separation was evident but could have been better and there was a small oily layer on the surface.

The 5 mL aliquot from the first sample was put through the same clean up column twice (this is allowed in the kit instructions) and produced a slightly cloudy solution. Another 5 mL aliquot was taken from the methanol layer of the centrifuged sample and again put through the clean-up column twice and was still slightly cloudy.

Both samples were added to the separate ports and both sample and control ports had purple spots appear – again the intensity of colour was less than the pictures – for a negative result. Sample A would be <2ppb while sample B would be <4ppb.

**Trial 5 September**

A new batch of coconut was dried for the second trial and this was very dark compared with the first lot used.

Again 50 g of sample (C) was taken and blended with 100 mL of 80% methanol. A vacuum filtering apparatus was found and while the filtering still took a long time it was quicker and filtrate was clearer but darker than the filtrate used on the first day.

Sample (D) was prepared using only 25 g with 100 mL of methanol and after blending was centrifuged for 40 minutes at 3000 rpm. The longer time period did not significantly improve the quality of the sample taken to pass through the clean-up column which was also very dark.

Both samples A and B were put through the clean-up column twice and then added to separate ports as the kit manufacturer’s instructions followed.

Two pale purple spots appeared in both ports indicating negative results. Sample A of <2ppb and Sample B < 4ppb.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Prep</th>
<th>Sample comment</th>
<th>Filtration technique</th>
<th>Clean up column</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copra A</td>
<td>50 g sample: 100 mL methanol</td>
<td>Very viscous and oily</td>
<td>Filter paper # 40</td>
<td>Twice</td>
<td>Neg &lt;2ppb</td>
</tr>
<tr>
<td>Copra B</td>
<td>25 g sample: 100 mL methanol</td>
<td>Viscous and oily</td>
<td>Centrifuge 3000 rpm / 20 min</td>
<td>Twice</td>
<td>Neg &lt;4ppb</td>
</tr>
<tr>
<td>Copra C</td>
<td>50 g sample: 100 mL methanol</td>
<td>Very viscous and dark colour</td>
<td>Vacuum filter paper #40</td>
<td>Twice</td>
<td>Neg &lt;2ppb</td>
</tr>
<tr>
<td>Copra D</td>
<td>25 g sample: 100 mL methanol</td>
<td>Viscous and oily</td>
<td>Centrifuge 3000 rpm / 40 min</td>
<td>Twice</td>
<td>Neg &lt;4ppb</td>
</tr>
</tbody>
</table>

**Screening Levels**

The screening level of the test can be adjusted according to requirements. At the lowest dilution possible, the screening level is 2ppb. At the highest documented dilution, the screening level is 30 ppb.
Health and Safety

The blending and the filtering were done in the fume hood. Initially the lab did not have any sodium hypochlorite for decontamination but procured some janola and staff were instructed to follow the decontamination procedures. Cawthron staff had taken face masks which we encouraged the SROS staff to use and have left these at SROS. All samples should be treated as contaminated and handled appropriately.

ISO 17025 accreditation

Discussions have been held with the Programme Manager at IANZ who indicated that the lab must validate the kit’s performance using a spiked control sample. It is possible to purchase these from the manufacturer and the purchase of a Corn one from the Product list might be suitable. The New Zealand agent has indicated that the manufacturer could make a specific copra positive control sample if required. Consideration should be given to purchasing one at a concentration that could be diluted to a low level of 4–5 ppb and frozen in aliquots for future use. The frequency of use of the control sample would need to be established. Attention to Health and Safety issues when using a positive control will also need to be considered.
Samoa Microbiology Training Report

We arrived to a friendly welcome at SROS and headed to the microbiology lab where we were introduced to staff and given a quick tour of the facilities. We started by looking at the methods that we were concerned with, namely the *Campylobacter* and *Vibrio* methods.

It was at once discovered that the *Vibrio* method that we had been given earlier was not the most recent, and consequently not the one currently being used in the lab. The method had been changed in response to an IANZ audit request. It was decided that the changes were not necessary if the method was labelled as ‘modified’ and that spike testing was performed. Consequently spike testing was performed on the two separate *Vibrio* methods.

I needed to organise fresh broth cultures, media and samples, so that they all came together at the right time. Most of the media required were made in the first couple of days. This was because the autoclaving was performed off site at the Vailima brewery and the media were returned the following day.

A similar procedure followed with the *Campylobacter* method. It was decided it was best to have a presence/absence test for general foodstuffs (meat, chicken pieces), and another method for the testing of chicken carcasses as the islands may be obtaining a portable abattoir in the near future. The NMD chicken carcass rinse method was chosen as the best enumeration methodology for the lab.

A draft of the NMD method was written and given to Luanda to add the requisite headers, footers and title numbers as applicable for SROS methods. As I was not familiar with the NMD enumeration method, I had to do some study of my own to acquaint myself with the techniques. The method requires whole chicken to be rinsed and an aliquot of the rinsate spread onto CCDA. There were limited resources so I let the staff perform the method under close supervision from me as opposed to me demonstrating in the first instance. They had sufficient resources to perform the test and did a good job. I also made amendments to the draft *Campylobacter* presence/absence method to remove the need for duplicate samples which would simplify the test procedure and decrease the volumes of media used.

Meat and chicken samples were acquired from the local supermarket for spiking in the *Campylobacter* presence/absence test. As with the other testing this required more planning as the test can take 5 or 6 days and the broth from which you obtain the spike has to be incubated for 48 – 72 hours in advance. Some work was required on the weekend, and I had confidence in the staff to perform these basic tasks unsupervised.

The identification steps of both tests proved to be the most interesting part of the methods for the staff. Almost everyone was involved and engaging and interested in learning more about this particular aspect of the training. I went through the identification process systematically, ensuring my instructions were clear and understood, demonstrating techniques then supervising and advising on their attempts. We covered microscopy, antibiotic sensitivity testing, oxidase, catalase, temperature tolerance testing and the use of API identification kits.

There were a few items that we were waiting on from New Zealand; most important of these was a bulb for the microscope. Both microscopes in the lab were not functioning because there were no bulbs for them. I had tried altering the microscope to use a torch as a light and
also a combination of sunlight and a mirror but neither method was overly successful. The Gram stain is one of the most definite tests for the identification of *Campylobacter* and a good microscope is essential part of the test. *Campylobacter* has a distinctive shape which can be difficult to decipher or focus on. The new bulbs were eventually installed, there were some other missing parts that were tracked down, but I was still not happy with the clarity of the field of vision. I cleaned both microscopes and changed some objectives and that seemed to make the difference and the equipment is now in good working order. I am unsure how long the microscopes had been faulty and consequently how much experience they have had with microscopy. It is one area where I felt they needed more practice.

The first week of work was frustrating and busy. I had to manage my time and plan things well in advance to maximise the chance of getting results for the spike testing. Agar and cultures were organised and I had to take stock of their resources to make sure I had everything that was required. The second week went very smoothly. Almost all spike testing proved successful and I delivered training in identification techniques. The third week involved testing the *Vibrio* and *Campylobacter* ILCP samples using the modified methods that were introduced, and ensuring a draft validation report was produced before the weeks end. I altered the training units that I had with me and wrote up new training records to reflect the testing that had been undertaken by the staff. These were completed by the micro team members and signed off by myself. I gave a brief presentation about *Vibrio* and *Campylobacter* with regards to disease and identification to the lab staff and the senior management team, including the CEO.
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