SCAHLS Quality Plan for Johne's disease testing

1. Introduction

1.1 Background

Laboratory testing is pivotal to national Johne's disease programs developed by the Australian livestock industries. In 1996 the Subcommittee for Animal Health Laboratory Standards (SCAHLS) agreed to develop a Quality Plan to enable the application of a national operating standard for laboratories performing Johne's disease diagnostic tests and for manufacturers supplying diagnostic reagents.

Transparent operation of tests at a consistent high standard will promote confidence of livestock industries, laboratories and regulatory authorities in Johne's disease diagnostic process. In particular, a Quality Plan needs to be available to Australian laboratories seeking approval to perform serological tests, faecal and tissue culture and histopathology for National Johne's disease Market Assurance Programs (NJDMAP).

The Quality Plan for Johne's disease is based on standard diagnostic procedures, proficiency testing of laboratories, and guidelines for assessing the performance of serological tests. Application of the Johne's disease Quality Plan and standard laboratory quality procedures provides a comprehensive quality diagnostic operating system. The Quality Plan should be reviewed regularly to enable continuous improvement of this operating system.

1.2 Terms of reference

To prepare a Quality Plan for Johne's disease testing which includes:

- The Australian and New Zealand Standard Diagnostic Procedures (ANZSDP) for Johne's disease;
- The Australian National Quality Assurance Program (ANQAP);
- Guidelines for using National Serum Reference Panels (NSRP).

The Quality Plan will be a visible, transparent document for distribution to industry.

1.3 Structure of the Quality Plan

The Quality Plan is divided in four sections.

The first section describes the Johne's disease operating system. It outlines actions required to maintain and promote a quality operating system by identifying issues that ultimately impact on test quality and persons or parties responsible for implementing actions to emphasise areas of responsibility for end-users. It will be subject to regular revisions as scientific information is updated and new issues are recognised.

The second section gives a summary of ANQAP proficiency testing for Johne's disease. It refers to procedures of interlaboratory comparison and requirements for approval of veterinary laboratories.

The third section provides guidelines for using National Serum Reference Panels for Johne's disease to enable laboratories, ELISA kit manufacturers and ANQAP to assess reproducibility of serological assays.

The final component of the Quality Plan, the "Australian and New Zealand Standard Diagnostic Procedures" for Johne's disease" by Cousins et al 2002 is available online at www.scahls.com.

1.4 Review of the Quality Plan

To ensure continuous improvement of diagnostic laboratory operating systems, SCAHLS will monitor reports of ANQAP, regularly review the Johne's disease ANZSDP and update the Quality Plan.

2. Johne's disease operating system

The Johne's disease operating system outlines actions required to maintain and promote a quality operating system by identifying issues that ultimately impact on test quality. Broadly, the areas considered are the standardisation of interlaboratory practices, quality control of test performance within laboratories, the quality assurance of diagnostic reagents, and selection of appropriate tests.

Manufacturers of reagents/kits, ANQAP, specimen submitters and laboratories are responsible for implementing actions required to maintain the quality operating system.

The laboratories are required to (i) operate under and maintain NATA accreditation (ii) use standard tests complying with the ANZSDP, (iii) maintain records detailing test methods, reagents and operation, including statement of any deviations from the ANZSDP or PFC Workshop Manual, (iv) comply with ANZSDP definitions when reporting results (refer to ANZSDP) and (v) participate in ANQAP testing phases for Johne's disease serological tests and culture.

The specific quality criteria that have been identified are tabulated against corresponding actions for quality assurance and the responsibilities of parties actively involved in the quality operating system.

2.1 Quality criteria for sampling and specimen collection and processing	Action for Quality Assurance
Maintenance of specimen integrity	**Specimen Submitters** All specimen containers clearly and accurately labelled. Provide accurate and detailed specimen advice. Transport serological and bacteriological specimens to laboratory on ice to arrive at laboratory within 48 hrs of collection. **Laboratories** Confirm field collection data complete. Confirm specimen identification and labelling. All samples for bacteriological testing to be stored at $4^{o}C$ and processed within two days otherwise store at $-80^{o}C$. All serum samples to be stored at $4^{o}C$ and processed within seven days otherwise store at $-20^{o}C$. Maintain records of all laboratory results ensuring auditable specimen trail can be followed.
Prevention of cross Contamination of specimens	**Specimen Submitters** Use new sterile disposable gloves for each pool of 50 animals. Avoid multi-use packs of gloves, which may become soiled. Avoid contamination of outside of containers. Decontaminate outside of containers if necessary. **Laboratories** Swab tops of BACTEC bottles with 70% ethanol or equivalent before each reading or sampling. Any reusable equipment is thoroughly cleaned and sterilised using an autoclave. Impose rigorous movement control for different stages of PCR procedures.
Use of valid sampling strategy	**Specimen Submitters** Select 350 sheep based on 'Sheep selection criteria' Appendix 2 SheepMAP Rules and Guidelines. One pellet or equivalent quantity of faeces per sheep (max 50 pellets per sterile container).
Availability of animals for re-sampling	**Specimen Submitters** All animals have individual, permanent identification recorded with mob. Animals retained until negative results confirmed or when required re-testing completed.
Apply standard for investigation of infection status of ELISA reactors in the NJDMAP	**Specimen Submitters and laboratories** Maintain records demonstrating sampling and operation of test complies with the ANZSDP and NJDMAP Manual of Procedures. Record any inadequacies in the process.

2.2 Quality Criteria for Serological Testing	Action for Quality Assurance
Availability of ELISA with specificity's greater than 99%	**Reagent/kit manufactures** Pre-test new batches of kits using an acceptable panel of sera. Contributions to the panel are arranged by contacting the Immunology Section, EMAI, NSW and National Johne's Disease Reference Laboratory, PIRVic, Victoria.
Availability of ELISA of acceptable reproducibility	**Reagent/kit manufactures** Pre-test new batches of kits using an acceptable panel of sera. New batches of kits are pre-tested by the National Johne's Disease Reference Laboratory, PIRVic, Victoria (refer to NSRP). **ANQAP** Monitor standard sera to detect long term trends as part of proficiency testing (refer to ANQAP). Increase the number of low positive sera assessed in ANQAP phases. **Laboratories** Record attributes which may affect the final test result, including: date of test, technician, identification of kit batch and any modifications, any deviations from recommended test protocol, stopping time, laboratory temperature, plate layout and record of ELISA optical densities. Store diagnostic sera for a minimum of four weeks at a maximum of 4˚C.
Recognition of unacceptable variability in valid ELISA assays	**Reagent/kit manufactures** Review of requirements for valid ELISA assays. Include low positive sera in the criteria for a valid assay. **Laboratories** Monitor standard sera whenever assay is performed. Report to the kit manufacturers in the first instance (and SCAHLS if necessary) when there are concerns about assay performance (refer to NSRP).

2.3 Quality Criteria for bacteriological testing	Action for Quality Assurance
Maximisation of *M. paratuberculosis* recovery	**Reagent/kit manufactures** Produce media according to specifications (refer to ANZSDP). **Laboratories** Use culture methods as recommended by the ANZSDP. Record source and identification of any media used in the diagnostic laboratory. When using new batches of media, either: confirm new batches of mycobactin-containing media support growth of *M. paratuberculosis* whereas media without mycobactin media does not, and both are free from contaminants., or use new batch in parallel with a proven batch, including at least three positive samples, to ensure consistent results, or use media that is retrospectively subjected to quality control checks. Ensure modified 7H10 media with mycobactin supports the growth of sheep (S) strains of *M. paratuberculosis*. Determine the shelf life of HEYM and 7H10 media and ensure media is not used outside the specified shelf life.
Reduction of growth of organisms other than *M. paratuberculosis*	**Reagent/kit manufactures** Produce media according to specifications (refer to ANZSDP). **Laboratories** Ensure 72 hours incubation with VAN or BHI/VAN. Always use Difco BHI.
Monitoring of culture procedures	**Laboratories** Process negative and positive control faeces with each batch of cultures.

2.4 Quality Criteria for histopathological testing	Action for Quality Assurance
Consistent histopathological assessment	**Laboratories** Comply with recommendations of the ANZSDP.

2.5 Quality Criteria for PCR testing	Action for Quality Assurance
Monitoring of PCR procedures	**Laboratories** Use the following controls with each PCR batch when using alcohol extraction and Wizard DNA purification: Positive faeces control Positive PCR control Negative faeces control (BACTEC GI negative) Negative PCR cocktail control Negative PCR reagent control and purified water added at time of samples/template Use the following controls with each PCR batch on colonies from solid media and from 12B vials without added egg yolk: Positive PCR control Negative PCR cocktail control Negative PCR reagent control and purified water added at time of samples/template

NOTE: Any new test that is developed for Johne's disease must comply with SCAHLS Policy on Guidelines for the Development and Approval of New Diagnostic Tests. The New Test approval process includes providing a written submission to SCAHLS including the method of validation, the test Sensitivity and Specificity, evidence of peer review, quality control issues and technology transfer (see SCAHLS website www.scahls.com for further information). SCAHLS may request that new ELISA kits (or other methodology) be subject to additional evaluation by the JD Reference Laboratory.

2.6 Schedule for use of controls in BACTEC faecal culture/PCR

	JD positive faeces control	JD negative faeces control	M.ptb DNA control	MQW water control	PCR reagent control
BACTEC culture	Start	Start			
Alcohol extraction	Start	Start			
Primary PCR	⁄	⁄	Start	Start	Start
Primary electrophoresis	⁄	⁄	Finish	Finish	Finish
Secondary PCR	⁄	⁄	Start	Start	Start
Secondary electrophoresis	Finish	Finish	Finish	Finish	Finish

⁄ Indicates that the original control is processed with the samples through this procedure

3. ANQAP proficiency testing for Johne's disease

The Australian National Quality Assurance Program (ANQAP) aims to:

- ensure a high standard of diagnostic test performance in Australian veterinary laboratories,
- provide standard anti-sera for quality control testing and calibration.

This section summarises ANQAP proficiency testing for Johne's disease, including procedures of interlaboratory comparison and requirements for approval of veterinary laboratories. Detailed information is available in ANQAP reports released following each phase of interlaboratory testing and in the ANQAP Procedures Manual. These documents are available from the ANQAP National Coordinator (Jan Beattie 03 92174360).

Johne's disease tests that are assessed by ANQAP include Johne's disease agar gel immunodiffusion (AGID), complement fixation (CFT), enzyme linked immunosorbent assays (ELISA), pooled (sheep) faecal culture and individual (bovine) faecal culture.

3.1 Procedures of interlaboratory comparison

Laboratories enrolling in ANQAP nominate specific tests for assessment. They are sent 'unknown' samples and diagnostic test reference samples for testing according to a timetable released by ANQAP. These samples are tested and reported using the standard operating procedure in the laboratory and the results are forwarded to the ANQAP National Coordinator or to the specific QAP coordinator (eg for faecal culture – the JD Reference Laboratory). Following initial testing, or retesting at the request of the laboratory or the ANQAP National Coordinator, results are classified as acceptable when they fall within an expected range from the consensus mean, otherwise as either demonstrating minor variation or as unacceptable.

For each phase of interlaboratory comparison, the coordinator publishes results and summary of methods of analysis. Participating laboratories are identified in coded form to ensure confidentiality.

3.2 Requirements for approved laboratories

Laboratories with ANQAP test results classified as acceptable or demonstrating minor variation, at the first test or on retest, are listed on an ANQAP endorsed list of laboratories compiled for each particular test. The lists are presented quarterly to SCAHLS, the Chief Veterinary Officers of each state and territory and to Chief Quarantine Officers.

Laboratories with ANQAP test results classified as unacceptable on retest are not included on the endorsed list and the ANQAP National Coordinator notifies the appropriate laboratory director, SCAHLS members and the CVO for that state. To be reinstated, laboratories must demonstrate improved competence by producing acceptable results when further evaluated by ANQAP.

Definitions of acceptable result ranges for ANQAP tests and a flow chart of the endorsement procedure are detailed in AN001 Endorsement of Laboratories for Export Testing through Interlaboratory Proficiency Testing of the ANQAP Procedures Manual.

4. National Serum Reference Panels for Johne's disease

National serum panels enable laboratories, ELISA kit manufacturers and ANQAP to undertake objective and comparative assessment of the reproducibility of serological assays. In the longer term, expansion of the panels may provide for independent and standard assessment of serological test accuracy.

Two serum panels are being developed for assessment of sensitivity under the direction of the Sub-Committee on Animal Health Laboratory Standards (SCAHLS). A national bovine panel is held in Victoria and national ovine panel is held in NSW. These states have endemic Johne's disease in cattle and sheep respectively, and are experienced in the processing and testing of samples from infected animals. Collection of sera and determination of the *Mycobacterium paratuberculosis* infection status of animals from all states of Australia is on going. Contributions to the panel are arranged by contacting the Johne's Disease Reference Laboratory at Attwood, Victoria or Microbiology and Immunology Section, EMAI, NSW.

This section on the NSRP for Johne's disease specifies reproducibility criteria for bovine ELISA assays and provides a protocol for testing Johne's disease ELISA kits.

4.1 Quality control of ELISA kits: commercial and in house

There are five approved ELISA systems described in the Australian and New Zealand Standard Diagnostic Procedures. Commercial suppliers include Pfizer (PARACHEK™), Institut Pourquier (Bovine Paratuberculosis Test) and IDEXX (Mycobacterium paratuberculosis antibody test kit). In addition the Elizabeth Macarthur Agriculture Institute (EMAI) JD ELISA and ELISA developed in New Zealand are approved for use. Interpretation of the various ELISA's are summarised in the ANZSDP. If there are concerns in the marketplace about the quality of any particular kit batch, the kit manufacturer should be contacted in the first instance to resolve the issue. If the manufacturer fails to satisfy the client's concerns, that client may elect to ask the JD Reference Laboratory to further assess the kit batch.

All new batches of Johne's disease ELISA kits (both commercial and in-house developed assays) must be subjected to pre-testing by the manufacturer prior to release for diagnostic purposes. A batch is considered new when there is a change in the source, production or processing of any biological component of the kit. The Subcommittee for Animal Health Laboratory Standards recommends that new batches of ELISA kits for Johne's disease must be subjected to independent pre-testing at the JD Reference Laboratory prior to release for diagnostic purposes. The primary objective of this pre-testing is to assess reproducibility of assay performance. This is demonstrated by proving that test results of well-characterised sera fall within an acceptable range of optical densities and interpretation of the results are consistent.

The quality control of ELISA kits involves assessing the within-plate variation of optical density values (OD), between-plate OD variation, between-batch OD variation, evaluation of specificity and sensitivity and percentage (rate) of diagnostic classification agreement with the previous batch. The evaluations are performed using sera that were characterised on approved ELISA kits.

A minimum of 11 well-characterised sera from the NSRP (one serum of high OD, at leat seven sera of low OD and three negative OD sera) are used to determine the within- and between-plate variations. The sera are run on assays performed on three to four different days over a two-week period. For the within-plate evaluation, six to eight replicates of each serum are assayed on one plate followed by calculation of an average coefficient of variation (CV) of OD values. For the between-plate evaluation, two replicates of each serum are assayed on three to four plates followed by calculation of an average CV of mean OD values.

The specificity and sensitivity, and between-batch variation and percentage of diagnostic classification agreement with the previous batch are evaluated by testing 135 to 150 sera from cattle from Western Australia (specificity panel) and 35 to 40 sera from infected animals (sensitivity panel). The specificity and sensitivity sera are assayed in single and duplicate wells, respectively. Average CV of specificity sera OD values, average CV of mean OD of sensitivity sera and overall average CV of OD of all sera tested in common with the previous batch are calculated to assess the between-batch reproducibility of a kit. After diagnostic classification of the OD values the test results are compared with that achieved with the previous batch to determine their consistency as measured by percentage of diagnostic agreement. Additional sera may be tested to complete evaluation of some batches that produce inconclusive results.

All laboratory records associated with testing are maintained by the reference laboratory for audit purposes. If difficulties with a particular batch of any kit cannot be resolved, the client is advised to consult with the JD Reference Laboratory and bring the issue to the notice of SCAHLS for resolution.

4.2 ELISA quality criteria

The results of quality control testing of ELISA kits should fall within the following range.

ELISA performance criteria	No. sera tested	Range of acceptable results			References
		Expected result	Minor variation from expected	Major variation from expected result	
Average CV of OD values within plate	≥11 sera	≤**7.0%**	>7 to **8.5%**	>8.5 to **10.0%**	Collins et al (1993) J Vet Diagn Invest 5:52-55. Gwozdz unpublished data.
Average CV of mean OD values between plates	≥11 sera	≤**9.0%**	>9 to **12.0%**	>12 to **15.0%**	Collins et al (1993) J Vet Diagn Invest 5:52-55. Gwozdz unpublished data.
Overal average CV of OD values between batches	35-40 sensitivity and 135-150 specificity sera	≤**13.0%**	>13 to **16.0%**	>16 to **20.0%**	Gwozdz unpublished data.
Overal % diagnostic clasification agreement with previous batch	35-40 sensitivity and 135-150 specificity sera	**100%**	**99%**	**98%**	Collins et al (1993) J Vet Diagn Invest 5:52-55. Gwozdz unpublished data.
No. specificity sera testing positive	135 to 150 specificity sera	**0**	**1** (0.6-0.7%)	**2**[a] (1.3-1.5%)	Gwozdz unpublished data.

[a], Batches that produce positive results in 2 of 135 to 150 specificity sera are considered acceptable provided that they produce no positive reaction in any of additionally tested 80 specificity sera.

NOTE: Manufacturers of batches producing results that show MAJOR VARIATION from the expected result are advised to review procedures to identify reasons for such variations.