SCAHLS Quality Plan for Johne's disease testing

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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 on Animal Health Laboratory Standards (SCAHLS) agreed to develop a Johne's Disease Quality Plan (JDQP) to enable the application of a national operating standard for laboratories performing Johne's disease diagnostic tests and for manufacturers supplying diagnostic reagents. The primary objective of the Johne's disease Quality Plan was to provide consistent standarisation of the tests used. The plan focused on enzyme linked immunosorbent assay (ELISA) and culture methods because they are they are the major tests used in market assurance programs.

Transparent operation of tests at a consistent high standard will promote confidence of livestock industries, laboratories and regulatory authorities in the Johne's disease diagnostic process. In particular, the Quality Plan is 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. This Quality Plan replaces the 2005 version.

1.2. Terms of reference

The Quality Plan for Johne's disease testing 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).

1.3. Structure of the Quality Plan

The Quality Plan is divided into 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 affect test quality and persons or parties responsible for implementing actions.

The second section gives a summary of ANQAP proficiency testing for Johne's disease. It refers to procedures for interlaboratory comparison and requirements for approval of animal health 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 'ANZSDP for Johne's disease' by Gwozdz 2010, is available online at http://www.scahls.org.au/procedures/anzsdps

1.4. Review of the Quality Plan

To ensure continuous improvement of diagnostic laboratory operating systems, SCAHLS monitors reports of ANQAP, regularly reviews the Johne's disease ANZSDP and updates 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 affect test quality. The areas considered are the standardisation of laboratory practices, quality control of test performance, 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 NATA accreditation in the relevant field of testing (ii) use tests complying with the ANZSDP, (iii) maintain records detailing test methods, reagents and operation, including statements of any deviations from the ANZSDP, (iv) comply with ANZSDP definitions when reporting results, and (v) participate in ANQAP proficiency testing programs for Johne's disease serological tests and culture.

The specific quality criteria that have been identified are tabulated below 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** Label all specimen containers clearly and accurately. Provide accurate and detailed specimen advice. Transport serological and bacteriological specimens, on ice, to arrive at the laboratory within 48 hours of collection. **Laboratories** Confirm that field collection data is complete. Confirm specimen identification and labelling. Store all samples for bacteriological testing at 4^{o}C and process within 2 days, otherwise store at -80^{o}C. Store all serum samples at 4^{o}C and process within 7 days, otherwise store at -20^{o}C. Maintain records of all laboratory results ensuring a specimen trail can be audited.
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 the outside of containers. Decontaminate the outside of containers if necessary. **Laboratories** Swab the tops of BACTEC bottles with 70% ethanol, or equivalent, before each reading or sampling. Thoroughly clean and sterilize, using an autoclave, any reusable equipment. Impose rigorous movement controls for different stages of polymerase

	chain reaction (PCR) procedures.
Use of valid sampling strategy	**Specimen Submitters** Refer to ANZSDP.
Availability of animals for re-sampling	**Specimen Submitters** Ensure all sampled animals are individually and permanently identified and recorded. Retain sampled animals until negative results are confirmed or when required re-testing is completed.
Apply standard for investigation of infection status of ELISA reactors in the NJDMAP	**Specimen Submitters and Laboratories** Maintain records demonstrating that the sampling and operation of the 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, Elizabeth Macarthur Agricultural Institute (EMAI), NSW and National Johne's Disease Reference Laboratory, Department of Primary Industries (DPI), Victoria.
Availability of ELISA of acceptable reproducibility	**Reagent/kit manufactures** New batches of kits are pre-tested by the National Johne's Disease Reference Laboratory, DPI, Victoria (refer to NSRP). **ANQAP** Monitor standard sera to detect long term trends as part of proficiency testing (refer to ANQAP). **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 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** Include low-positive sera in the criteria for a valid assay. **Laboratories** Monitor standard sera whenever the assay is performed. Report to the kit manufacturers in the first instance (and ANQAP 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 *Mycobacterium 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 that 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 a 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.
Monitoring of culture procedures	**Laboratories** Process negative and positive control faeces and tissues and positive BACTEC control 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 and negative faeces and tissue controls Positive PCR 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 reagent control and purified water added at time of samples/template

2.6. Schedule for use of controls in 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.
*, *M. paratuberculosis*

2.7. Common diagnostic outcomes of faecal and tissue culture: Interpretations and relevant Quality Control measures.

Diagnostic outcome	Liquid media culture	PCR on liquid media culture	Solid media culture	ZN staining of liquid media culture	Result reported*
I	No growth	Not done	Not done	Not done	Negative
II	Growth	+	Mycobactin dependent growth	+	Positive
III	Growth	+	Mycobactin independent growth	+	DNA consistent with *M. paratuberculosis*
IV	Growth	+	No growth	+	DNA consistent with *M. paratuberculosis*
V	Growth	+	No growth	-	DNA consistent with *M. paratuberculosis*
VI	Growth	-	No growth	-	Overgrown culture
VII	Growth	-	No growth	+	Overgrown culture
VIII	Growth	+	Mycobactin dependent growth	-	Positive
IX	Growth	-	Mycobactin dependent growth	+	No result
X	Growth	-	Mycobactin dependent growth	-	No result

*, Interpretations in text below; ZN, Ziehl-Neelsen.

Diagnostic outcome I

Liquid media culture:	No Growth
IS*900* PCR on liquid media culture:	Negative
Solid media culture:	No Growth
ZN staining of liquid media culture:	No acid fast organisms (AFOs) present

Result

No *M. paratuberculosis* detected.

Interpretation and QC measures

This diagnostic outcome is considered as an unequivocal negative result. Although the possibility of misidentification of samples/cultures, inappropriate (overzealous) decontamination or failure to add mycobactin J to liquid media resulting in a false negative result could never be ruled out, the likelihood of such laboratory errors is low as long as the following QC measures are in place:

1.1. The identity of samples and cultures is cross checked throughout the procedure.

1.2. A positive control (faeces or tissues spiked with *M. paratuberculosis*) is processed in parallel with the diagnostic samples.

1.3. A positive control for the liquid BACTEC medium is also included.

Diagnostic outcome II

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Positive
Solid media culture:	Mycobactin dependent growth
ZN staining of liquid media culture:	AFOs present

Result

M. paratuberculosis detected.

Interpretation and QC measures

This diagnostic outcome is considered as an unequivocal positive result. The likelihood of false positive result due to misidentification of samples or cross-contamination of liquid/primary cultures is very low as long as the following QC measures are in place:

2.1. As for 1.1.

2.2. Negative culture controls (liquid medium and faeces or tissues) are included.

Diagnostic outcome III

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Positive
Solid media culture:	Mycobactin independent growth
ZN staining of liquid media culture:	AFOs present

Result

DNA consistent with *M. paratuberculosis* detected. Growth of organisms other than *M. paratuberculosis* present.

Interpretation

This diagnostic outcome is usually considered as a positive result. Possible causes are as follows:

3.1. Number of organisms other than *M. paratuberculosis* in a sample exceeds the capacity of the decontamination process or the decontamination is inadequate (most common cause).

QC measures: Fresh HPC (≤1 week old) used.

3.2. Failure to identify mycobactin dependent growth on solid media due to the use of very heavy inoculum and carry over of large amounts of mycobactin from the primary/liquid culture.

QC measures: Small volume (≤ 0.1 mL) of liquid culture subcultured on solid media.

3.3. Failure to identify mycobactin dependent growth due to the lack of experience of an operator. It is common to observe very scanty growth of *M. paratuberculosis* at the bottom of slopes without mycobactin.

QC measures: Ensure that a trained, experienced operator reads the cultures.

3.4. Misidentification (mislabelling) of solid media subcultures or ZN smears. This may occasionally occur when several samples are processed at the same time and there is an operator error resulting in a mismatch between liquid cultures, subcultures and/or smears.

QC measures: As for 1.1.

3.5. Faulty solid media. It is worth noting that it is impossible to assess the selectiveness of the media for all possible contaminants.

QC measures: Pre-tested solid media need to be used.

3.6. Cross-contamination of the PCR. This is highly unlikely because mycobacteria have been demonstrated by ZN staining in the liquid medium. Because of a large number of copies, PCR products pose much higher risk of cross-contamination than *M. paratuberculosis* or its genomic DNA.

QC measures: Appropriate negative controls used in PCR procedures.

3.7. Isolation/detection of mycobactin non-dependent mycobacterium containing IS900-like fragment in its DNA. A is a very rare event.

QC measures: The restriction enzyme analysis of the IS*900* PCR products, IS*1311* REA strain typing and/or PCR tests targeting DNA fragments other than IS*900* are used to confirm the identity of the isolate.

Diagnostic outcome IV

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Positive
Solid media culture:	No Growth
ZN staining of liquid media culture:	AFOs present

Result

DNA consistent with *M. paratuberculosis* detected.

Interpretation

This diagnostic outcome is usually considered as a positive result. Possible causes are as follows:

4.1. Solid media used for subculture do not support growth of a particular isolate (most common).

QC measures: Herrold's medium should be used for bovine strains, 7H10 medium for ovine strains, or both media need to be set up if type of expected strains is unknown. It is recommended to repeat the PCR on liquid culture and perform strain typing. In addition, in index cases subcultures should be repeated using media on the basis of the strain typing results.

4.2. Faulty solid media.

QC measures: Pre-tested solid media need to be used.

4.3. Failure to inoculate solid media.

QC measures: As for 4.1.

4.4. Mismatch between liquid media cultures, solid media subcultures and/or ZN slides.

QC measures: As for 1.1.

4.5. Cross-contamination of the PCR. This is highly unlikely in the light of the demonstration of mycobacteria in the liquid medium by ZN staining.

QC measures: As for 3.6.

Diagnostic outcome V

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Positive
Solid media culture:	No Growth
ZN staining of liquid media culture:	No AFOs present

Result

DNA consistent with *M. paratuberculosis* detected.

Interpretation

This diagnostic outcome is usually considered as a positive result. Possible causes are as follows:

5.1. Failure to observe growth on solid media could be due to factors specified already in diagnostic outcome IV.

QC measures: As for 4.1, 4.2, 4.3, 4.4 and 4.5.

Failure to observe AFOs microscopically could be due to:

5.2. Low numbers of mycobacteria in the sample tested.

QC measures: ZN staining of cultures with low growth indices (<200) should be avoided.

5.3. Inadequate staining.

QC measures: A positive ZN control should be included.

5.4. Failure to recognise mycobacteria.

QC measures: Trained, experienced operators should read ZN smears.

Diagnostic outcome VI

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Negative
Solid media culture:	No growth
ZN staining of liquid media culture:	No AFOs present

Result

Growth of organisms other than *M. paratuberculosis* (Overgrown culture)

Interpretation

6.1. Absence of growth on solid media is likely to be due to the high selectiveness of such media or death of organisms in the liquid culture by the time of subculture.

QC measures: The delay between the detection of growth in liquid media and subculture and PCR should be kept to a minimum.

6.2. Occasionally, spurious readings between 11 to 15 GI may be produced by BACTEC machines in early stages of incubation.

QC measures: All cultures that produce GI >10 at the last reading or have two or more readings between 11 and 20 need to be followed by PCR and subculture into liquid and solid media.

6.3. Faulty solid media.

QC measures: As for 3.5.

Diagnostic outcome VII

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Negative
Solid media culture:	No growth
ZN staining of liquid media culture:	AFOs detected

Result

Growth of organisms other than *M. paratuberculosis* (Overgrown culture)

Interpretation

7.1. The overgrowth is likely to be due to mycobacteria that have failed to grow on solid media.

QC measures: As for 3.5, 5.4 and 6.1.

Diagnostic outcome VIII

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Positive
Solid media culture:	Mycobactin dependent growth
ZN staining of liquid media culture:	No AFOs present

Result

Technically, no result should be released until investigations of the failure to detect mycobacteria by ZN are completed. However, some laboratories do not perform ZN routinely and report "*M. paratuberculosis* detected" on the basis of positive results obtained by highly specific PCR and demonstration of mycobactin dependence.

Interpretation and QC measures

As for 3.3 and 5.2, 5.3 and 5.4.

Diagnostic outcome IX

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Negative
Solid media culture:	Mycobactin dependent growth
ZN staining of liquid media culture:	AFOs present

Result

No result should be released until investigations of the PCR failure to produce positive reactions are completed.

Interpretation

The possible causes are as follows:

9.1. The PCR does not work. No amplification.

QC measures: Include positive PCR controls. Repeat the PCR.

9.2. There is always a remote mismatch possibility between the liquid cultures, PCR samples solid cultures and ZN smear.

QC measures: As for 1.1. Perform the PCR on growth from both solid and liquid cultures.

9.3. Inhibition of the PCR.

QC measures: Repeat DNA extraction and PCR using standardised purification methods.

9.4. Insufficient number of *M. paratuberculosis* in samples tested.

QC measures: Avoid DNA extraction from liquid cultures with GI <200. If the GI is <200, testing multiple samples by PCR should be considered, along with application of standardised purification methods during DNA extraction.

9.5. Misdiagnosis of mycobactin dependency.

QC measures: As for 3.3.

9.6. The mycobactin-dependent isolate does not contain IS*900*.

Diagnostic outcome X

Liquid media culture:	Growth
IS*900* PCR on liquid media culture:	Negative
Solid media culture:	Mycobactin dependent growth
ZN staining of liquid media culture:	No AFOs present

Result

No result should be released until investigations to the source of the mycobactin dependence are completed.

Interpretation and QC measures

As for 9.1 to 9.6 and 5.2 to 5.4.

3. Australian National Quality Assurance Program (ANQAP) proficiency testing for Johne's disease

The ANQAP aims to:

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

This section summarises ANQAP proficiency testing for Johne's disease, including procedures for interlaboratory comparison and requirements for approval of animal health 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.

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

3.1 Procedures for 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 (e.g. for faecal culture – the National Johne's Disease Reference Laboratory). Following initial testing, or retesting at the request of the laboratory or

the ANQAP National Coordinator, results are classified as falling within an expected range from the consensus mean (satisfactory), demonstrating minor variation, or as unacceptable.

For each phase of interlaboratory comparison, the coordinator publishes results and a 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 in 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 have been developed for assessment of sensitivity under the direction of SCAHLS. A national bovine panel is held in Victoria and a national ovine panel is held in NSW. These states have endemic Johne's disease in cattle and sheep, and are experienced in the processing and testing of samples from infected animals. Collection of sera and determination of the *M. 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 the Microbiology and Immunology Section, EMAI, NSW.

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

4.1 Quality control of bovine Johne's disease ELISA kits: commercial and in house

There are three approved ELISA systems described in the ANZSDP for Johne's Disease. Commercial suppliers include Prionics (PARACHEK™), IDEXX (Pourquier Paratuberculosis Screening Test) and IDVET (ID Screen® Paratuberculosis Indirect). In addition, an ELISA developed in New Zealand is approved for use. If there are concerns about the quality of any particular kit batch, the kit manufacturer should be contacted in the first instance in an attempt to resolve the issue. If the manufacturer is unable to resolve the issue, the JD Reference Laboratory may be asked to further assess the kit batch.

All new batches of Johne's disease ELISA kits (both commercial and in-house) must be subjected to pre-testing 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 primary objective of this pre-testing is to assess reproducibility of assay performance. This is demonstrated by proving that test results on well-characterised sera fall

within an acceptable range of optical densities, and that interpretation of the results is consistent.

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

A minimum of 11 well-characterised sera from the NSRP (one serum of high OD, at least 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 3-4 different days over a 2 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 in 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 those 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.

4.2. ELISA quality standards

Results of ELISA quality control testing should fall within the following tabulated 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.