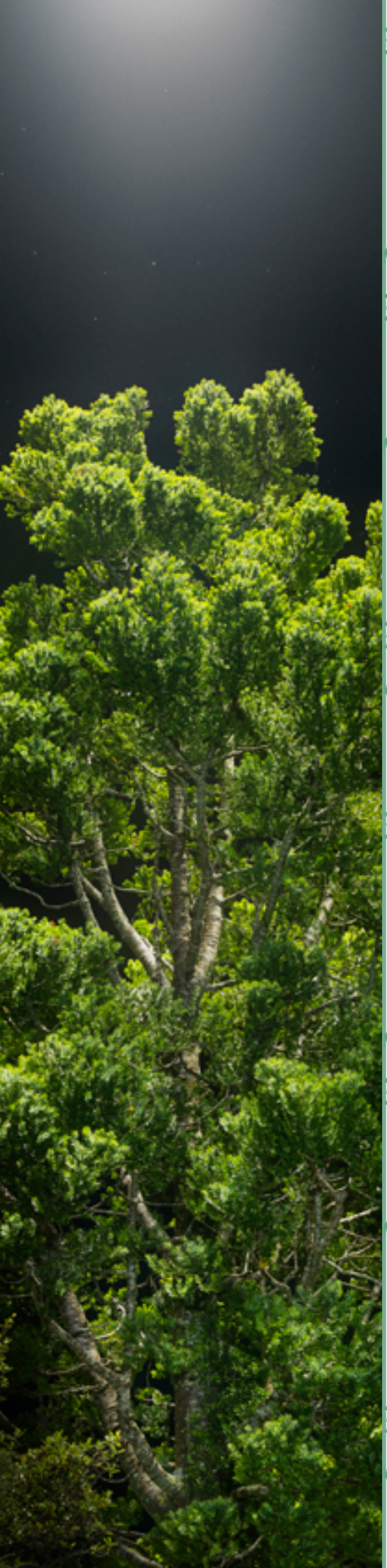

Approval of the oDNA assay for use in *P. agathidicida* surveillance and diagnostics

Drafted by Stephanie Tomscha, Science Lead - Tiakina Kauri

and Lindsay Bulman, Chair

Reviewed by committee (18 June 2026)

Revised following feedback (29 June 2026)

Endorsed by committee on (30 June 2026)

Approved by Tiakina Kauri (30 June 2026)

TIAKINA KAURI
KAURI PROTECTION

June 2026

Executive summary

Tiakina Kauri approves the use of the oDNA end-point PCR assay, developed by Palmer et al. (2025), as a diagnostic test for the surveillance of *Phytophthora agathidicida* (PA). This approval follows endorsement by the oDNA Technical Advisory Committee that the oDNA test is sensitive and specific. The evidence and decision-making processes underpinning this approval are outlined in this document.

Incorporating the oDNA assay into the diagnostic toolbox enables access to a rapid and highly sensitive method for PA detection. Increased sensitivity is achieved by using an improved oospore DNA extraction method coupled with a highly sensitive end-point PCR. The removal of the baiting step will increase efficiency by reducing the diagnostic test time. This approval also reflects Tiakina Kauri's commitment to fostering innovation in PA testing and supporting the adoption of evidence-based diagnostic approaches.

Approval to perform the oDNA assay for Tiakina Kauri surveillance diagnostics is contingent on laboratories obtaining permissions, adhering to MPI-approved standard operating procedures (SOPs), and successfully completing an MPI audit to demonstrate proficiency and effective cross-contamination control. Clear guidelines for reporting, interpretation, and the management of unexpected positives are provided to ensure consistency and validity of results.

Acknowledgements

Tiakina Kauri thanks the Gerth Lab at Victoria University of Wellington for developing the oDNA methodology and supporting its validation for application in PA surveillance. Tiakina Kauri thanks members of the oDNA Technical Advisory Committee and the Chair for their expertise and contribution to the evaluation process. We also acknowledge the laboratories involved in interlaboratory testing and the field kaimahi (staff) who collected soil samples to support validation activities.

Context and test approval process

Tiakina Kauri role in facilitating testing for *P. agathidicida*

Tiakina Kauri currently supports three approved diagnostic tests for *Phytophthora agathidicida*, all of which require baiting (e.g., Than et. al Approval). Although two of the currently approved tests are DNA based methods (Than et al. 2013, Winkworth 2020), baiting step is still used to mitigate the effects of soil inhibition.

The oDNA assay, published in 2025, introduces a novel DNA extraction approach that combines soil filtration with highly sensitive PCR, enabling direct testing of filtered soil. This approach reduces reliance on baiting and can improve both the speed and sensitivity of detection, positioning it as a valuable addition to our diagnostic toolkit.

Tiakina Kauri approves the use of assays for surveillance to ensure only high-quality evidence is used in decision making. Given the novelty of the extraction method and the use of a less-characterised genomic target, the assay presents risks distinct from existing methods. Tiakina Kauri therefore sought expert independent advice on evidence to provide us with high confidence that assay was sensitive and specific, as well as on the best-practice use of the assay in surveillance.

This document covers the following:

- Conflict of interest for test reviewers
- Test approval process
- Test method
  - Development and evidence considered in approval process
  - Approved primers and methodology
  - Assay sensitivity and specificity
  - Assay limitations
- Test usage and quality control measures
  - Use in surveillance
  - Standard operating procedure requirements
- Standards applied to all laboratories (In Appendix)
  - Permissions
  - Laboratory audits
  - Laboratory responsibilities before reporting positive results
  - Contamination assessments
  - Validation requirements for official PA site status
  - Interpretation and reporting guidelines

Conflict of interest statement for test reviewers

All individuals involved in the review and approval of the oDNA assay for Phytophthora agathidicida surveillance were asked to disclose any current or potential conflicts of interest. A conflict of interest was defined as any financial, professional, or personal relationship that could influence, or be perceived to influence, the objectivity of the review process. Any affiliations with research institutions, diagnostic service providers, or related projects were to be declared and documented. If a potential conflict existed, appropriate measures, such as recusal from decision-making, were to be implemented to maintain transparency and integrity in the approval process.

No conflicts of interest were declared during the formation of the committee and during subsequent meetings.

Test approval process

The review process involved several key processes:

- Formation of committee of experts with conflict of interests sought and declared (March 2025)
- Committee reviewed currently available evidence for the oDNA assay, assessed any gaps and recommended further validation steps (March 2025)
- Completion of additional validation, including expanded isolate testing and interlaboratory assessment (June 2025 - Jan 2026)
- Submission of compiled evidence to the committee (February 2026)
- Evidence reviewed by committee and further validation recommended (February 2026)
- Further validation evidence received on 8 June 2026
- Committee reviewed evidence and recommended test be approved (16 June 2026)
- Approval document produced and circulated to committee on (18 June 2026)
- Test approval confirmed by Tiakina Kauri (30 June 2026)

Group member (alphabetical by surname)	Organisation
Lindsay Bulman	Independent Chair
Treena Burgess	Murdoch University
Niklaus Grunwald	United States Department of Agriculture
Shannon Hunter	Auckland Council
Stephanie Tomscha	Tiakina Kauri, MPI
Merje Toome	Plant Heath and Environment Lab, MPI

*Note that endorsement does not include endorsement of the section above on *Tiakina Kauri Purpose and Role*.

Test method

Development and evidence used for approval

- The oDNA assay was published in 2025 (Palmer et al., 2025) and included validation of the assay using isolates and natural soil samples.
- The assay has several novel steps including filtration of soil to increase the concentration of oospores.
- The assay targets a high-copy LTR region of the genome, improving analytical sensitivity.
- Assay robustness was validated via interlaboratory testing, showing 93% reproducibility between participating laboratories and no false positive detections (Appendix 1, Table 1).
- Assay specificity was determined by examining existing data from the original publication and by testing a range of new isolates. All 13 tested PA isolates produced positive results, while all 23 non-target isolates tested negative (Appendix 1, Table 1).

Assay primers

Forward primer (PA-LTR-for): 5′- ACGCGCTCTGTTTCTTTAGC -3′
Reverse primer (PA-LTR-rev): 5′- GCGGGCTTCCATTCAATTCA -3′

Assay sensitivity and specificity

- **Analytical sensitivity:** The oDNA end-point PCR assay can detect as little as 1 fg of PA DNA under controlled conditions.
- **Diagnostic sensitivity:** Diagnostic sensitivity is expected to be higher than other diagnostic tests, based on preliminary evidence and will be refined as additional validation data become available, especially under field conditions.
- **Analytical specificity:** No amplification was observed for non-target isolates tested during assay development and validation, indicating strong specificity.

Assay limitations and risks

No critical limitations prevent the use of the assay. Several risks are acknowledged:

- Knowledge of *Phytophthora* diversity in New Zealand soils remains incomplete, and while false positives are unlikely, they cannot be fully ruled out. This residual uncertainty is inherent to molecular assays, but the risk for the oDNA test has been carefully mitigated through rigorous validation in the original publication as well as through enhanced specificity testing via the approval process.
- Because the method captures DNA from both viable and non-viable PA and other organisms, results need to be interpreted carefully to distinguish between current active and residual non-viable presence. Nonetheless, detection of PA DNA in soil indicates that the organism is either active, previously active, or has been introduced to that location.
- The high analytical sensitivity of the assay increases the risk of contamination-driven false positives if strict laboratory and field controls are not maintained

These risks are mitigated through validation requirements, quality control measures, and this test approval process.

Test usage and quality control measures

Test usage in surveillance

The oDNA assay may be used as an initial test in PA surveillance programmes, with the following considerations:

- Positive results in new or unexpected locations may need to be independently validated using a morphological method (See Appendix 2). This protocol aligns with standard biosecurity procedures where evidence required for management of incursions in new areas may require high levels of certainty.
- Enhanced sampling strategies (e.g. 24-point sampling), including shallower sampling where appropriate given higher oospore abundance near the surface (Palmer et al. 2026, In Review), may be required for validation to overcome the lower sensitivity of baiting and culture-based methods.

Standard Operating Procedure (SOP) requirements

- Sample collection should follow the Soil and Root sampling SOP Tiakina-Kauri-1-SOP-for-Soil-and-root-Sampling-final-June-2023.pdf
- SOP for laboratory methods will be similar to as published here. A Method for the Separation of Phytophthora Oospores from Soil for DNA-Based Detection | Springer Nature Experiments

Please see Appendix 2 for further details on Standard processes that apply to laboratories, as well as an overview of validation protocols.

Appendix 1. Evidence of assay suitability

Table 1 shows a summary of studies that have been carried out to validate the assay and demonstrate that it is fit for purpose.

Study	Primary finding
Palmer et al. 2025	PCR confirmed success of improved oospore extraction method and confirmed the performance of the new set of primers developed in this study.
Palmer et al. 2025 and additional specificity testing as part of the oDNA approval process	All 13 tested PA isolates were successfully detected, while all 23 non-target isolates tested negative.
Inter-laboratory DNA testing – oDNA approval process	Purified PA DNA from different isolates (in water) and eDNA extracted from soil was used for testing across three laboratories. Sample concentrations spanned above and below the detection limit and all labs achieved the same results (30/30 samples; 100% agreement).
Inter-laboratory soil reproducibility – oDNA approval process	The same 10 positive soil samples were tested across three laboratories using the oDNA assay. 93% reproducibility was achieved (28 of 30 positive). Of the 10 negative samples (30 tests total), 28 were conclusively negative, while two were inconclusive due to extraction control failures. No false positives were observed. These same samples were also tested using the morphological assay and 100% agreement was achieved in comparison to the most expert oDNA lab.
Effect of soil drying – oDNA approval process	Concordant results were obtained for 9 of 10 samples; one false negative was recorded following soil drying, showing potential risk of reduced sensitivity following sample drying. Thus, using fresh soils is recommended for this assay.
Additional testing to cross-validate positive results – oDNA approval process	Twenty samples collected from two natural forests, comprising both asymptomatic and mildly symptomatic trees, showed positive results with the oDNA PCR assay, although earlier sampling had yielded negative baiting results. These samples were subjected to additional testing using an orthogonal method to rule out non-specific detection. All 20 samples (100%) were positive for PA using a nested qPCR assay with the Than et al. 2013
Hadlow IC, Palmer JT, Gerth ML. 2026.	Evidence that PA was undetected outside of Kauri's natural range, provides additional evidence of specificity

Appendix 2. Standard laboratory and surveillance processes

Standards for all laboratories working with PA

Permissions

Laboratories must meet all permission requirements before offering this test for surveillance diagnostics. PA is an unwanted organism under the Biosecurity Act 1993. The Biosecurity Act 1993 section 52 and section 53 are relevant to laboratories handling PA.

Laboratory audits to approve test providers

MPI requires that test results meet the case definition to confirm a PA site. To achieve this, testing must be carried out by an approved laboratory using an approved test. In addition to having the necessary permissions, the laboratory's testing process must be audited by an MPI diagnostic testing expert to become an approved lab.

MPI audits laboratories to confirm they can perform the test to the expected standards and to check that any risks (e.g. cross contamination risk) are appropriately managed. During an audit, laboratories will need to provide SOPs and validation reports for the test method. Examples of the checks in a lab audit include, but are not limited to:

- Sample processing and reporting
- Personnel training and competency
- Interpretation of results and sign off
- Equipment
- Physical laboratory conditions
- Waste disposal (focus is on minimising cross contamination, not on containment, i.e. not overlapping with the role of verification)

Laboratory responsibilities before reporting positive results

Laboratories must confirm that all quality control criteria are met before reporting positive results, including:

- Verification that all controls performed as expected
- Repeat testing where required
- Investigation of any anomalous results

Results failing quality criteria must be reported as inconclusive.

Contamination assessment following an unvalidated positive

Laboratories may wish to rerun their tests following a contamination assessment. MPI may also ask labs to provide information to help with a contamination assessment where an unexpected positive occurs.

Things that may be reviewed include:
- Other PA positives processed or stored nearby
- Check physical set up and any associated risks

If a risk is identified, decontaminate all the areas, and repeat extraction from that sample and rerun the PCR assay using only verified clean reagents.

Validation recommendations and official PA site status

Additional independent testing may be recommended to confirm a site as positive for PA, ensuring the result meets the official case definition for a confirmed PA site before regulatory or management decisions are made (Table 2). For positive test results that pass the quality control checks, the surveillance group should have a pre-agreed validation plan that outlines specific geographical criteria (i.e., distance from nearest confirmed PA site) where validation is needed, especially when a positive result is unexpected or from a new site. Where testing has been conducted using an approved method in an approved laboratory, the decision to undertake further validation remains with the land manager and will depend on the context and the level of certainty required to support management action.

As part of validation, the surveillance manager may choose to collect new samples from the same location for morphological testing. Enhanced sampling strategies (e.g. 24-point sampling) may be required for validation. If extra soil was collected and held separately, they may also request testing of the retained soil.

Guidelines are available to help surveillance groups through the process of validating unexpected positives. Please get in touch with Tiakina Kauri for documentation on validation.

Three documents are in development.
1. Scientific process and justification
2. Plain language FAQ
3. Plain language “How to guide”

Result interpretation and reporting guidelines

Laboratories should ensure that information is available to support the ongoing tracking and interpretation of PA results. An overview of what should be reported can be found in Appendix 2, Table 3. This table is not exhaustive and may be updated based on laboratory and test innovation. Broadly, the report should identify what was tested and how the sample was handled. When possible, the laboratory should use Kete Aronui tools to automate this reporting, which should become available in late 2026.

Table 2. Recommended Tiakina Kauri P. agathidicida site case definitions with two classes of suspect sites and the recommended visual markers for mapping P. agathidicida sites (Table from Kauri surveillance test validation process -v.X).

Visual marker	Case definition
#E41A1C	A *P. agathidicida* **site - confirmed** is defined as a point location where the presence of *P. agathidicida* has been confirmed (from a tree, soil or other substrate), using an approved test at an approved laboratory. This includes historical *P. agathidicida* detections and confirmed tests following validation with the morphological test (if it was required).
#E69F00	A *P. agathidicida* **suspect site – validation recommended** is defined as a point location where the presence of *P. agathidicida* has been detected (from a tree, soil or other substrate) using an approved molecular test, that requires validation due to geographical distance from a confirmed *P. agathidicida* site, and no validation tests have yet been undertaken, or there is reason to believe that historical tests may be incorrect.
#F0E442	A *P. agathidicida* **suspect site – validation negative** is defined as a point location where the presence of *P. agathidicida* has been detected (from a tree, soil or other substrate) using an approved test, that required validation due to geographical distance from a confirmed *P. agathidicida* site, and no validation tests were positive.
#4477AA	A *P. agathidicida* **not detected** site is defined as a point location where the presence of *P. agathidicida* was not detected (from a tree, soil or other substrate), using an approved test at an approved laboratory. Note: validation is not required for not detected sites.

Table 3. Information and disclaimers that should be provided to clients when receiving test results.

Report topic	Example of reporting details
Submission information provided by client	Collection details (provided by client): Collector, Collection and dispatch dates, Location (if not blinded)
	Client name and project reference
	Any batching information
	Unique sample ID
	Storage conditions prior to sending to lab
Sample information	Laboratory name
	Unique sample ID
	Sample type (e.g., bait, soil)
	Date received by lab
	Sample condition on receipt (if relevant)
	Storage conditions prior to analysis
Test method details	Target organism (e.g., Phytophthora agathidicida, P. cinnamomi)
	Assay type (e.g., oDNA end-point PCR)
	Soil drying (y/n)
	Sample retention details for soil and DNA
Results per sample	Result: Positive / Undetected / Inconclusive
Accreditation and compliance	Laboratory information (name, address, contact person, email/phone)
	Relevant accreditation status
	Name and credentials of operator/technician
	Date of report and version control
	Signature and credentials of authorizer (must differ from operator and must be a molecular biology expert for PCR tests)
Generic guidance and information clients should receive for a batch of test results	A plain-language interpretation on what a positive means and what a negative does not mean. Reporting language must align with Tiakina Kauri programme language and interpretation standards
	Guidance on repeat sampling or validation and linkage to Tiakina Kauri documentation on validation plans
	If used for surveillance, information on how this test should be used for regulatory or management decisions. These must align with Tiakina Kauri programme language and interpretation
	Minimum retention period for DNA and samples
	Data ownership and confidentiality statement
Disclaimers	Statement on detection limits
	Environmental caveats (patchiness in soil, soil inhibition, etc.)
	Disclaimers on risks of false negatives at low pathogen load and on risk of trace contamination at high sensitivity

References

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