Approval of qPCR - A TaqMan real-time PCR (Than et al. 2013) for use in *P. agathidicida* surveillance and diagnostics

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Reviewed by committee (date 04/02/2026)

Revised following feedback (date 13/02/2026)

Endorsed by committee on (date 09/03/2026)

Approved by Tiakina Kauri (date 09/03/2026)

TIAKINA KAURI
KAURI PROTECTION

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Executive summary

Tiakina Kauri approves the use of the TaqMan real-time PCR (qPCR) assay, originally developed by Than et al. (2013), for use in surveillance of *Phytophthora agathidicida* (PA) as a diagnostic test, following the recommendation of the committee. This approval addresses a critical gap in diagnostic capability following the discontinuation of LAMP testing from the approved provider in 2024 and aims to strengthen New Zealand's surveillance network through evidence-based molecular methods. Based on available data, the Than et al. qPCR assay offers high analytical sensitivity and specificity, detecting as little as two femtograms of PA DNA from pure cultures. Its extensive use in research and confirmatory diagnostics supports its reliability and has confirmed strong analytical and diagnostic performance. Approval to perform the qPCR testing for Tiakina Kauri is contingent on laboratories meeting containment requirements, following MPI-approved SOPs for soil sampling and baiting, and passing an MPI audit to ensure proficiency and cross-contamination control. Guidelines for reporting, interpretation, and handling unexpected positives are provided to ensure consistency and validity of results.

Context and test approval process

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

Molecular testing for *Phytophthora agathidicida* (PA) has been integral to surveillance following LAMP approval in October 2022 and Ampersand's audit for test delivery in March 2023. Molecular testing expands the number of laboratories able to provide PA detection services, as proficiency in molecular methodologies is more common than expertise in species-level morphological identification of *Phytophthora* species. Furthermore, a multi-laboratory approach strengthens the testing network, promotes competition, and ensures PA detection services remain accessible during peak demand. Tiakina Kauri seeks to approve diverse laboratories and methods to enhance resilience and maintain reliable, innovative surveillance.

Following the discontinuation of LAMP testing from the approved provider in 2024, no commercial laboratories have been approved to offer LAMP testing for PA detection as part of the national surveillance programme. Several laboratories, however, possess the expertise and containment required for performing real-time PCR testing and have requested to approved for this process. Tiakina Kauri therefore proposes approving qPCR (TaqMan real-time PCR) as a diagnostic method for PA surveillance. Through this test approval process, Tiakina Kauri sought guidance on best practices for using Than et al. methods and whether the assay should be integrated as a primary surveillance method or only as a solution where the morphological test is not feasible.

This document covers the following:

- Conflict of interest for test reviewers
  - Test approval process
  - Test method
  - Development and use in scientific research
  - Approved primers and probe
  - Assay sensitivity and specificity
  - Assay limitations

- Test usage and quality control measures
  - Test usage in surveillance
  - Standard operating procedure requirements
  - Containment and permissions
  - Laboratory audits to approve test providers
  - 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 qPCR – TaqMan real-time PCR assay for *Phytophthora agathidicida* surveillance disclosed any potential conflicts of interest. A conflict of interest is 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 declared and documented. Where a potential conflict existed, appropriate measures, such as recusal from decision-making, was implemented to maintain transparency and integrity in the approval process.

Test approval process

The review process involved:

- Drafting of document by compiling evidence and processes for test approval (Tiakina Kauri - 13 Jan 2026)
- Formation of committee of experts with conflict of interests declared (13 Jan 2026)
- Draft document reviewed by committee and feedback provided (4 Feb 2026)
- Feedback integrated into document (Tiakina Kauri) (13 Feb 2026)
- Finalized document reviewed by committee (21 Feb 2026)
- Committee agreed on endorsement of the (09 March 2026)
- Test approval confirmed on by Tiakina Kauri (09 March 2026)

The evidence in subsequent sections has been compiled by Tiakina Kauri and reviewed and endorsed* by:

Group member (alphabetical by surname)	Organisation
Dr. Karyn Froud	Biosecurity Research Limited
Dr. Shannon Hunter	Auckland Council
Dr. Rebecca McDougal	Bioeconomy Science Institute - Scion Group
Dr. Maria Rovisco Correia Goncalves Monteiro	Department of Conservation
Dr. Stephanie Tomscha	Tiakina Kauri, MPI
Dr. 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 use in research

- The qPCR assay was developed in 2013 (Than et al., 2013) and validated across multiple laboratories, which have demonstrated the assay's high analytical sensitivity and specificity (Than et al., 2013; McDougal et al., 2014).
- Extensive use in research and diagnostics supports its suitability for PA detection (see Appendix 1, Table 1).
- Comparative studies are limited, but evidence shows that the qPCR assay is generally more analytically sensitive than traditional morphological tests, which rely on culturing the pathogen.
- While not previously used as a primary surveillance tool for Tiakina Kauri, it has consistently served as a confirmatory method for morphological and LAMP results.

Assay primers and probe

The primer and probe sequences were originally published by Than et al. (2013) but then identified to be published incorrectly and republished as the reverse-complement by McDougal et al. (2014).

Name	Direction	Sequence (5' - 3')	Target region
ITS_PTA_F2	Forward	AACCAATAGTTGGGGGCGA	ITS region
ITS_PTA_R3	Forward	CTCGCCATGATAGAGCTCGTC	ITS region
ITS_PTA_probe2	Probe	AGCCAAAGCCAGCAGCCG	ITS region

Assay sensitivity and specificity

- **Analytical sensitivity:** Defined as the minimum detectable concentration, the TaqMan real-time PCR assay can detect as little as two femtograms of Phytophthora agathidicida DNA from pure culture, demonstrating high sensitivity.
- **Diagnostic sensitivity:** Defined as the ability of a test to correctly identify positive cases. Where a 'test' is the end-to-end process of sample collection to test results. For the SOPs being put forward for approval, the TaqMan real-time PCR assay currently requires a soil sampling and baiting step which is the same requirement as the morphological test. Because the sample collection step likely has the greatest impact on diagnostic sensitivity, the diagnostic sensitivity is expected to be similar to the morphological test. The diagnostic sensitivity of the morphological test was examined as part of the Waitākere study (Froud, 2022). Diagnostic sensitivity will be updated as new evidence becomes available.
- **High analytical specificity:** No amplification was observed for any of the 36 non-target isolates tested in assay's original publication, confirming strong specificity and reducing the risk of false positives from other Phytophthora species. Note that late cross-reaction with other Phytophthora species (e.g. P. cinnamomi) have been observed (MPI's Plant Health and Environment Laboratory, in-house data), which means decisions based on Ct values alone can result in false positives. These false positives can be ruled out based on determining the PCR product size and setting Ct value cut-offs based on validation data obtained in each testing laboratory.
- Because this method is analytically highly sensitive, even small amounts of DNA contamination can create false positive results if protocols to avoid and detect cross contamination are not followed. These risks can be mitigated with good practice and expertise.

Assay limitations

- Specialized instruments and skilled personnel are needed to perform and interpret qPCR assays accurately, adding to operational complexity.
- Late cross-reaction with a *Phytophthora cinnamomi* and potentially other *Phytophthora* species has been observed, necessitating clear Ct thresholds and interpretation guidelines to avoid misidentification.
- These primers also have the potential to amplify additional clade 5 *Phytophthora* species. However, the cross-reactions reported to date in clade 5 have occurred with species that have not been found in New Zealand.
- Soil-derived inhibitors can reduce assay sensitivity, making sample preparation critical for reliable results. As such, the approved protocol includes a baiting step to minimize the impact of soil-derived inhibitors, along with the use of an internal control to ensure any inhibition is detected. The approved protocols for both qPCR and the morphological test require a soil baiting step, meaning they share limitations associated with earlier sampling stages, such as uneven pathogen distribution and organism viability.
- Potential PCR inhibitors in the plant baits could also reduce assay sensitivity. To minimise this risk, assay validation in the laboratories performing the assay should include experiments to determine whether the presence of bait plant DNA is inhibiting assay sensitivity, as well as an internal positive control. If inhibition is detected, troubleshooting is required to minimise the plant matrix effect.

Test usage and quality control measures

Test usage in surveillance

Than et. al. qPCR assay is recommended as one of the possible initial tests in PA surveillance with some caveats:

- Molecular tests may require independent validation. Because molecular tests, such as the Than et. al. qPCR, are highly analytically sensitive, they carry a risk of false positives from contamination and cross-reactivity. For this reason, any positive result in a new location should be confirmed using a morphological test with the sampling strategy as recommended in validation protocols (24-point sample rather than standard 8-point sample), before being accepted as definitive. See the "*Validation Requirements for Official PA Site Status*" document. See below for more details.
- The qPCR assay must include the following quality controls: positive control, non-template control and internal control. Please see Table 3 in Appendix 1 for details on what needs to be reported.

Additional uses of the qPCR assay in PA surveillance:

- When a soil bioassay and morphological assessment is provided by an approved laboratory, this qPCR can confirm presence of PA in cultures where multiple *Phytophthora* species are present and morphological identification is uncertain.
- Suitable for testing unviable baits that still require analysis for surveillance purposes.

Standard Operating Procedure (SOP) requirements

- Sample collection should follow the soil and root sampling SOP.
- The soil bioassay must follow the soil baiting SOP to reduce the impact of soil inhibitors and ensure cleaner DNA for testing.

Containment and permissions

Laboratories must meet all containment and s52/53 permission requirements before offering this test. PA is an unwanted organism under the Biosecurity Act 1993. The Biosecurity Act 1993 section 52 and section 53 are relevant to laboratories setting up to undertake PA testing. If your lab is testing soils for PA, you will need permission to handle the organism.

Lab audits to approve test providers

MPI requires that test results meet the case definition to confirm a PA site (Appendix 1, Table 2). This means the test must be an approved test carried out in an approved laboratory. In addition to having the necessary permissions and containment measures, the laboratory's testing process must be audited by an MPI diagnostic testing expert.

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

Before reporting results to the client, laboratories must confirm that the positive result passes quality control checks.

Typical steps may include:

- Check and confirm all controls passed
- Check for correct amplification curve shape and rule out drift or late cycle noise
- Verify results meet predefined criteria (Ct threshold and replicate consistency)
- If any of the above criteria are not met, the result should be classified as inconclusive and troubleshooting should be carried out. This could include:
    - Retest the same DNA
    - Re-extract DNA from the same baits (if available)
      Retest frozen baits from the same sample to determine if cross-contamination occurred after baiting

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, use only verified clean reagents and repeat extraction from that sample and rerun the qPCR assay.

If samples from multiple locations are processed at the same time in a lab, DNA sequencing the amplified PCR product and comparing the result with sequences from isolates that were known to be processed at the same time may also be informative for identifying if cross-contamination resulted in a false positive.

Validation requirements for official PA site status

Additional independent testing may be required 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. For positive test results that pass the quality control checks, the client 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.

As part of validation, the client may choose to collect new samples from the same location for morphological testing. 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. We have three documents in development.

1. Scientific process and justification
2. Plain language FAQ
3. Plain language “How to guide”

Result interpretation and reporting guidelines

Laboratories should provide clients with information to help track and interpret PA results. An overview of what should be reported to clients can be found in Appendix 1, 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 supplier should use Kete Aronui tools to automate this reporting, which should become available in 2026.

Appendix 1.

Table 1 shows a summary of studies that have used the assay and the main findings of the literature.

Study/Report	How qPCR was used	What was found
Than et al. 2013 (assay development)	Developed and validated the original TaqMan qPCR for P. agathidicida. Tested specificity, sensitivity, detection limits.	Highly sensitive and specific assay. Detected PA at low DNA concentrations. No cross-reactivity with other common *Phytophthora* spp in New Zealand.
McDougal et al. 2014 (Scion comparison study)	Compared qPCR vs soil baiting for detecting PA in field soils.	qPCR detected PA in more samples than the standard culture-based baiting method. Showed higher sensitivity.
Singh et al. 2017	Used qPCR performance results to assess diagnostic pathways alongside culture and baiting.	qPCR generally outperformed the standard culture-based baiting method regarding the assays sensitivity. Recommended multi-method approach.
Byers et al. 2021 (soil microbial community study)	Used qPCR to confirm PA absence.	qPCR validated infection status and supported subsequent work on how different soil microbial isolates inhibit PA growth.
Hunter et al. 2024 (metabarcoding validation)	Used qPCR to validate community control samples and test mock communities.	qPCR confirmed presence/absence detected by metabarcoding.
Winkworth et al. 2020 (LAMP development)	Used qPCR to benchmark LAMP performance for sensitivity and specificity.	LAMP sensitivity was similar to the reported qPCR sensitivity in Than et al. 2013. The assays were not specifically re-tested in comparison in same lab. The qPCR was also NOT tested with baited leaf samples.
Thurston 2021 MSc Thesis	Used qPCR to compare DNA extraction protocols, test inhibitors, and validate improved isolation methods.	qPCR performance varied with DNA extraction method.
Palmer et al. 2025 (oospore extraction + PCR paper)	Compared the sensitivity of the Than et al PCR to their new assay.	Primers developed in the Palmer oDNA paper showed higher sensitivity than the Than et al primers.
MPI PHEL (in-house test validation results)	Used qPCR for PA diagnostics.	The assay was more sensitive than the LAMP assay and amplified all tested PA isolates. During assay validation, late amplification was noted as a result of cross-reaction with other Phytophthora species. Further analysis showed that the length of the amplified product was larger than expected, confirming non-specific binding.

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): Collection date, 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 information (name, address, contact person, email/phone)
	Unique sample ID
	Sample type (e.g., bait, soil)
	Date received by lab

Report topic	Example of reporting details
Sample information continued	Sample condition on receipt (if relevant)
	Storage conditions prior to analysis
Test method details	Target organism (e.g., *Phytophthora agathidicida, P. cinnamomi*)
	Assay type (qPCR)
Results per sample	Result: Positive / Undetected / Inconclusive
	Ct/Cq value (each replicate or mean ± SD)
Accreditation and compliance	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
	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

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