

Guidance on the appropriate usage and interpretation of data from environmental (eDNA) analysis

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GLOSSARY

Term	Definition (Advisory Focus)
12S (12S Ribosomal RNA)	A gene fragment from the small subunit of the mitochondrial ribosome. Because it evolves relatively slowly but contains highly informative variable regions, it serves as an excellent diagnostic marker for vertebrates.
16S (16S Ribosomal RNA)	A component of the small subunit of prokaryotic ribosomes. The 16S rRNA gene contains highly conserved regions interspersed with species-specific variable regions, making it the standard barcode for identifying bacteria and archaea.
COI (Cytochrome c Oxidase Subunit I)	A mitochondrial gene widely used as the standard DNA barcode for identifying animals (metazoans), including invertebrates, fish, and mammals.
ddPCR (Droplet Digital PCR)	A form of PCR used to estimate how much target DNA/RNA is in a sample (not just whether it is present). It can provide more precise, repeatable counts and is often less affected by substances in environmental samples that can interfere with standard qPCR.
Decision-Support Levels	A standardised framework (Trace, Moderate, Significant) is used to categorise molecular signals and determine proportionate public health responses.
eDNA (Environmental DNA)	Genetic material (DNA) shed by organisms into the environment, water, air or soil. Can be collected from environmental samples as extracellular DNA or as cellular DNA (e.g. fragments of tissues, cells).
eRNA (Environmental RNA)	Genetic material shed by organisms that is suggested to degrade much faster than DNA. As such, its detection serves as a stronger proxy for metabolically active or viable biological threats.
False Positive/False Negative	Incorrect detection outcomes: false positives detect a hazard that is absent, while false negatives represent a failure to detect a hazard that is present.
Inhibitors (e.g., Humic Acids)	Substances common in soil and water that interfere with the PCR process, often leading to false negatives (missed detections).
ITS (Internal Transcribed Spacer)	A piece of non-functional RNA situated between structural ribosomal RNAs. The ITS region (specifically ITS1 and ITS2) has a high degree of variation even between closely related species, making it the primary barcode for fungi and communities of plants.
Legacy/Relic DNA	DNA that persists in the environment after an organism has died or moved from the location of sampling; a key factor when assessing if a detection represents a current/active health risk.
Metabarcoding	DNA sequencing-based method aimed at identifying multiple species from an environmental sample by targeting a gene/amplicon shared across the species. Also referred to as amplicon-based sequencing.

Term	Definition (Advisory Focus)
Nucleic Acids	Biopolymers (DNA and RNA) that carry genetic information. In environmental surveillance, detecting the nucleic acids present allows identification of pathogens or vectors without the need for physical capture or culture.
Passive Surveillance	A broad-scope monitoring strategy used for early warning and baseline monitoring of unexpected species.
PCR (Polymerase Chain Reaction)	A lab test used to detect a specific organism (or gene) in a sample by looking for its genetic material (DNA or RNA) and copying it until it can be measured. A positive result means the target genetic material was detected (it does not, by itself, confirm the organism is alive or infectious).
Reference Library	Regional or global databases used to match DNA sequences present in a sample to known taxa via sequence identity. Robustness of the library used is required to be able to identify down to species level present.
Sequencing Depth	The extent of data generated per sample. Insufficient depth may result in "missing" rare pathogens, increasing the risk of false negatives.
Spike-ins/Internal Standards	Synthetic DNA added during testing to verify that the assay is working correctly and to account for any environmental interference (inhibition).
Targeted Surveillance	The use of specific assays (qPCR or ddPCR) to confirm the presence and quantity of a high-priority pathogen or vector.
Taxonomic Resolution	The level of taxa identification achieved (e.g., genera, , species, strain).
TICI	Taxon Independent Community Index. A scoring system that uses environmental DNA (eDNA) and machine learning to measure the biological health of rivers and streams. By scanning genetic codes from microorganisms, plants, and animals in a single water sample, it provides a highly sensitive, early-warning health check for freshwater ecosystems.
Viable	The state of an organism being alive and capable of replicating or causing infection. eDNA alone cannot confirm viability, as it detects genetic material from both living and dead cells.

EXECUTIVE SUMMARY

This report provides technical guidance for Te Whatu Ora and the Ministry of Health on the appropriate use and interpretation of environmental DNA (eDNA) and environmental RNA (eRNA) results for public health and biosecurity surveillance in Aotearoa New Zealand. While eDNA/eRNA methods are widely used for biodiversity monitoring, their application to health protection differs because decisions (e.g., public alerts, vector control, follow-up sampling) must be timely, defensible, and proportionate to risk.

Key message: eDNA/eRNA is best treated as a high-sensitivity *sentinel* signal rather than a stand-alone diagnostic. Public health use requires clear definition of the surveillance objective (broad early warning vs targeted confirmation), careful sampling design, and an interpretation workflow that accounts for (1) sample matrix effects and inhibitors, (2) transport and persistence (including legacy/relic DNA), (3) taxonomic resolution limits from metabarcoding and incomplete reference libraries, and (4) the fact that molecular detection does not confirm organism viability or infectiousness. These constraints increase the risk of false positives (unnecessary escalation) and false negatives (missed hazards) if results are interpreted without a technical and epidemiological context.

Implications for decision-making: for routine programmes, eDNA/eRNA should be integrated with conventional surveillance (physical vector surveillance, culture/clinical testing where relevant, and water quality/MST investigations) using a tiered response approach. Initial detections – particularly low-level or unexpected metabarcoding signals – should trigger verification rather than immediate health action, through replication, expanded spatial/temporal resampling, and confirmatory targeted assays (qPCR/ddPCR) where a specific hazard is suspected. For higher-consequence scenarios (e.g., invasive mosquito vectors or priority pathogens), agencies should pre-establish decision thresholds, quality assurance expectations (controls, spike-ins, minimum sequencing depth), and reporting standards that translate molecular results into actionable categories (e.g., trace/moderate/significant) linked to proportionate response options. Building capability for expert interpretation (or ensuring access to it) is essential for consistent, ministry-grade risk assessment and communication.

1. SCOPE

Environmental DNA (eDNA) analysis is an emerging tool for detecting species of concern, including organisms that may pose risks to human health. In New Zealand, its non-invasive sampling and sensitivity for low-abundance targets make eDNA a promising option for rapid biosecurity and public health surveillance (e.g., invasive mosquito vectors). This review provides guidance on interpreting eDNA results for environmental and public health risk assessment and management, with a focus on how detections should be linked to proportionate, risk-based responses (e.g., targeted physical surveillance, health alerts, or management action).

Objectives:

1. Provide an overview of the fundamentals of eDNA analysis, specifically focusing on the suitability of this tool for monitoring species of particular concern for human health, as identified in conjunction with Te Whatu Ora (e.g., *Anopheles* mosquitoes).
2. Discuss the challenges associated with using eDNA methods for pathogen/vector surveillance, including required taxonomic resolution (species/subspecies), high background non-target DNA, and other limitations relevant to public health surveillance.
3. Provide an overview of the commercial eDNA testing landscape in New Zealand, focusing on providers offering eDNA services (e.g., Wilderlab and SeQuench), including the methods used and assay design/sequencing depth, the likelihood of false positive/negative results for health-related targets, and how results are validated (e.g., against bioassay or physical surveillance data versus reliance on reference libraries).

2. METHODOLOGY

Grey and published literature^{1,2} was reviewed to identify information pertinent to the usage of eDNA analysis in surveillance for species of concern for human health, particularly those identified as priority species by Te Whatu Ora.

The review considered the suitability and emerging use of environmental DNA (eDNA) analysis across various environmental matrices relevant to human health surveillance, including water, air, soils, and sediments. Emphasis was placed on identifying New Zealand-based studies where eDNA analysis was employed. A dedicated portion of the review focused on international and local best practices for establishing an evidence-based link between eDNA data (e.g., presence/absence, relative quantification) and the subsequent determination of environmental or public health risk.

A review of commercial entities offering eDNA services in New Zealand was also conducted. This section includes a comparative analysis of technical approaches (e.g., targeted qPCR/ddPCR vs. metabarcoding), the adequacy of reference libraries for health targets, and the level of taxonomic resolution reliably achievable for pathogenic species (e.g., species vs. subspecies).

¹ Search engines: Web of Science, Google Scholar, Science Direct and PubMed.

² Search terms: Methodology (e.g., “environmental DNA”, “environmental RNA”, “air/water/soil surveillance”, “metabarcoding”, “primer performance”, “sequencing depth”, “PCR inhibition”); Targets (e.g., “pathogen”, “vector”, “mosquito”, *Anopheles*, “invasive species”, “biosecurity”); and Context (e.g., “New Zealand”, “Aotearoa”, “taxonomic resolution”, “reference libraries”).

3. FUNDAMENTALS OF ENVIRONMENTAL DNA

Environmental DNA (eDNA) surveillance is a tool that enables collection and characterisation of genetic material directly from the environment (water, soil, air) without the need for collection of the target organism itself (Barnes et al 2016).

Collection of eDNA is emerging as a transformative tool for biodiversity monitoring (Beng et al 2020; Bohmann et al 2014; Dickie et al 2018a; Goldberg et al 2015; Hermans et al 2018; Li et al 2018; Ruppert et al 2019; Saccò et al 2022a; Senapati et al 2018; Stat et al 2017; Thomsen et al 2015). More recently, it has been used to assist in biosecurity and public health monitoring (Alfaro-Perez et al 2025; Bowers et al 2021; Cook et al 2025; Darling et al 2020; Farrell et al 2021; Rees et al 2014; Rishan et al 2023). In New Zealand, its non-invasive sampling and sensitivity for low-abundance targets make eDNA a promising option for rapid biosecurity and public health surveillance (Scriver et al 2024). There is, however, further research needed to standardise and validate these methods before eDNA surveillance can be considered a robust tool for public health and regulatory frameworks.

The aim of eDNA/eRNA characterisation is to detect extracellular DNA/RNA or cell debris that organisms leave behind in environmental samples, such as soil, water, air or sediment (Bohmann et al., 2014; Saccò et al., 2022b). For certain combinations of sampling regime and target organism (s), the entire organism may be collected (e.g. bacteria in soil samples, viruses in air samples). After sample collection, methods that target specific genetic material using primers³ and polymerase chain reaction (PCR) to amplify the DNA/RNA are used to generate thousands to millions of copies of the target genetic region. Metabarcoding⁴ enables the characterisation of eDNA from multiple species within a single environmental sample (Takahashi et al., 2023). Subsequent analysis assigns taxonomies to the sequence reads so that individual host organisms can be identified, species prevalence determined, and overall biodiversity assessed (Figure 1).

When using eDNA as an approach for surveillance and monitoring there are a number of steps (as visualised in Figure 1) that need to be undertaken.

The first step is sampling of the environment. Most methods used for collection of samples use whole volume sampling (e.g. water and soils/sediments), filtration methods to concentrate eDNA (e.g. water filtration, air filtration), or precipitation techniques (Bowers et al 2021; Dickie et al 2018a; Rees et al 2014; Saccò et al 2022b; Scriver et al 2024; Zaiko et al 2022). Any of the methods employed needs to be able to capture the DNA (or RNA for viruses) and preserve and store the DNA/RNA until analysis can be undertaken in a laboratory environment. This is a critical step to prevent DNA/RNA degradation and ensure sample integrity for the downstream analysis (Bowers et al 2021; Scriver et al 2024).

³ Primers are short sequences of DNA that are found in specific organisms (barcoding), groups of organisms or a range of organisms (metabarcoding). The primers bind to specific regions in the target organisms DNA and are then copied (amplified) many times so the organism can be detected. We can think of primers as barcodes that you see on food packaging to identify a product.

⁴ Metabarcoding is the use of a primer sequence (or multiple primers) that enables multiple taxa (types of organisms) to be identified in one sample via high-throughput sequencing of amplicons.

Once collected, samples are then processed using molecular detection and quantification methods. The most widely used methods are quantitative PCR⁵ (qPCR), droplet digital PCR (ddPCR), and, more recently, high-throughput sequencing (metabarcoding) which targets conservative gene regions (e.g. 16S, COI, ITS) (Cook et al 2025; Farrell et al 2021; Wu et al 2026). Choice of method will be informed by the aims of surveillance, for

Metabarcoding is a broad-scope screening approach used to provide early warning signals (Fediajevaite et al., 2021; Schneider et al., 2016; Takahashi et al., 2023). By using universal markers to detect many different taxa simultaneously, it offers an overview of the biological community present in a sample. This is most effective for detecting unknown taxa or monitoring shifts in ecosystem health that might precede a disease outbreak.

Targeted Surveillance (qPCR / ddPCR) is an approach focusing on the detection and quantification of a single, specific hazard. This is utilised when a high-priority pathogen or vector (such as *Anopheles* mosquitoes) has already been identified as a risk. Targeted assays, particularly droplet digital PCR (ddPCR), are generally more sensitive and resilient to environmental interference than broad screening methods (Nicholson et al., 2020).

example, species-specific detection (e.g., a known target pathogen or vector) versus biodiversity or community-wide surveillance, including pathogens or vector/invasive species (Goldberg et al 2015; Hermans et al 2018; Rees et al 2014). If using qPCR or ddPCR methods and standards of known concentration, quantitative estimates can be obtained (e.g. gene copy numbers), which in some cases may be correlated with organism abundance. In contrast, metabarcoding enables simultaneous detection of multiple taxa but is generally considered semi-quantitative at best, as read counts are influenced by amplification and sequencing biases and do not reliably reflect organism abundance.(Cook et al 2025; Wu et al 2026).

Once PCR amplified and/or sequenced, samples undergo data analysis, quality control and interpretation, all key parts of the respective methodology. Laboratory protocols rely on the use of controls (internal controls, negative controls), and, for metabarcoding, the use of validated reference databases, to reduce the risk of contamination, false detections, and assurances that data is reliable and up to date (Darling et al 2020; Wu et al 2026).

Established bioinformatic pipelines process the sequenced data, assign taxonomy (identification), and, if possible (depending on the sequencing method), enable quantification. This is a key area that requires careful curation to avoid false detections or missed detections (Lear et al 2018; Wu et al 2026).

In the public health context, each step of the above methodologies requires careful consideration.

⁵ PCR – Polymerase Chain Reaction. Well established method that targets DNA and amplifies it to detectable level Buckingham L. 2019. *Molecular Diagnostics: Fundamentals, Methods and Clinical Applications*. F.A. Davis,;

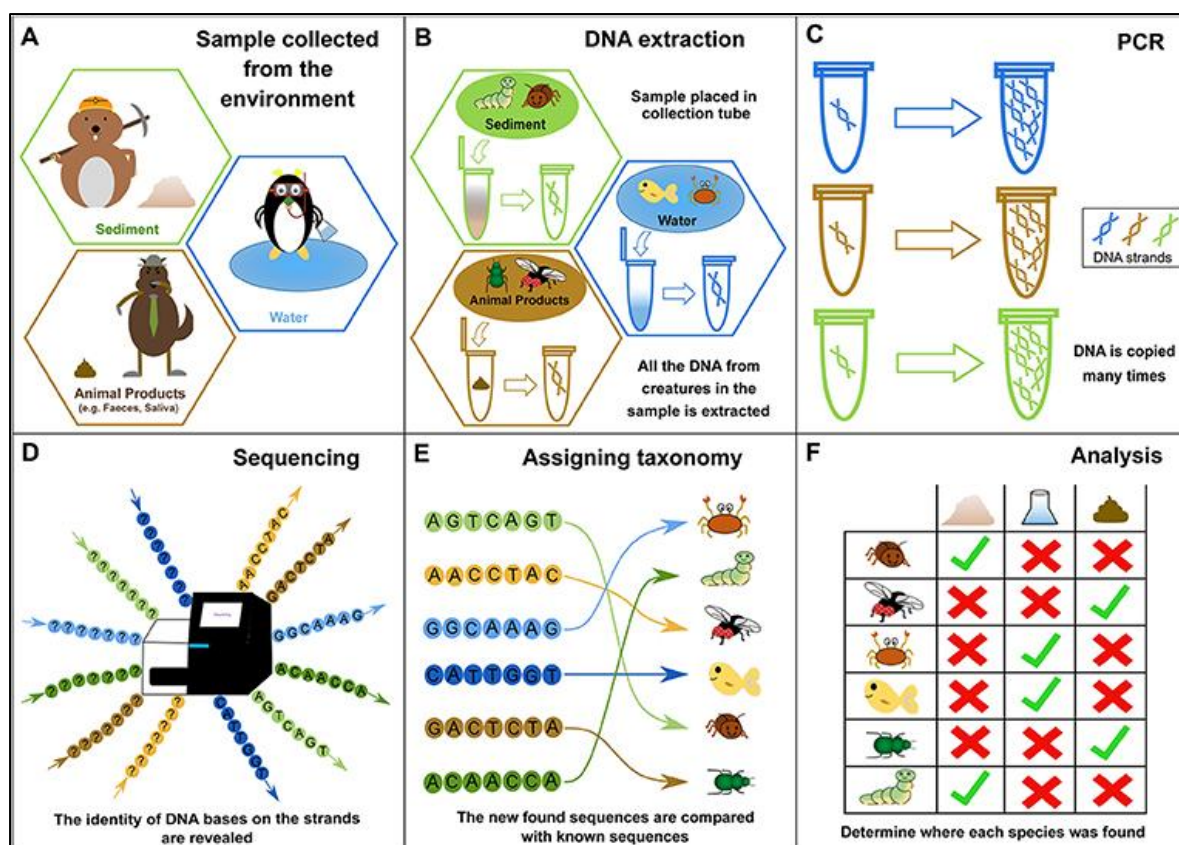


Figure 1. How can environmental DNA be used to identify species? A) Samples are collected from the environment (e.g., water, soil, animal products). B) eDNA is extracted from the samples. C) Target DNA sequences are multiplied using PCR. D) The sequences are read on a sequencing machine, showing the order of bases on the DNA strands. E) Sequences are then matched to known sequences in worldwide databases. F) A list is produced of species found in that environment (Figure taken from Schallenberg et al., 2020)

4. TECHNICAL CHALLENGES OF ENVIRONMENTAL DNA IN PUBLIC HEALTH SURVEILLANCE

While eDNA operates as an early-warning tool for monitoring ecosystems, its application in public health, where management actions such as vector control or boil-water notices are costly, requires a rigorous understanding of the limitations (Farrell et al 2021; Zaiko et al 2022). To address the objective of evaluating eDNA-based pathogen and vector surveillance, the primary methodological challenges are discussed below.

1.1 Sampling, preservation, environmental influences

Several factors can significantly affect the persistence and detection of target DNA/RNA in environmental samples. These include degradation of DNA or RNA (both between deposition and collection, and between collection and analysis), and the risk of contamination during sampling. Certain environmental factors such as temperature, pH, microbial activity, UV (sunlight) can significantly affect the persistence of environmental DNA/RNA prior- and post-collection. Degradation and contamination of eDNA samples can produce highly variable target detection and potential for false negatives (Darling et al 2020; Scriver et al 2024).

As such, careful consideration needs to be made when using eDNA/eRNA for public health surveillance, from choice of sampling location, timing (e.g., tidal cycles, diurnal cycles), and sampling method (e.g. filtration vs whole sample) to sample preservation methods. Each is critical to ensuring maximum sensitivity and reliability, and minimisation of loss or contamination (Dickie et al 2018a; Rees et al 2014; Scriver et al 2024).

1.2 Methodological and analytical challenges

There is a lack of standardised methods for DNA/RNA extraction, purification, and amplification, which results in inconsistencies across studies available (Dickie et al 2018b; Wu et al 2026). This lack of consistency also makes cross site or cross laboratory comparisons difficult (Bowers et al 2021; De Brauwer et al 2023; Rees et al 2014; Wu et al 2026).

The sensitivity and specificity of detection are strongly influenced by the methods used to detect the target (e.g. choice of primer/probe), PCR inhibition (e.g., presence of humic, fluvic acids), and background DNA/RNA presence. These factors can risk both false positive (e.g., contamination, degraded DNA/historic DNA) or negatives (e.g., inefficient extraction, low abundance) occurring (Farrell et al 2021; Schneider et al 2016; Zhu et al 2024). It is essential to include quality control measures (e.g., internal control or spike-in standards) although this is presently not established as routine across current surveillance for eDNA/eRNA. This means current tools are vulnerable to undetected errors which are pertinent to health surveillance (De Brauwer et al 2023; Zhu et al 2024).

1.3 Data quality, reference databases (libraries), and interpretation

One of the main issues when assigning a taxon to eDNA sequencing reads from metabarcoding is the lack of robust databases or libraries. Many databases are limited or incomplete, especially in under-studied environments (e.g., groundwater), regions, or

for rare or emerging species (Dickie et al 2018b; Rees et al 2014; Saccò et al 2024; Weaver 2024; Wu et al 2026). In other words, a species can be present in an environmental sample but not identified because it is not present in the database used for species identification.

In addition, interpreting eDNA/eRNA quantities remains challenging (e.g., relating DNA/RNA concentration to organism abundance or infection risk).

This is due to unknown dynamics for environmental degradation or decay rates across sampling domains (e.g. water, soils/sediments, air), environmental transport of DNA/RNA (i.e., where it originated from, and when), and the shedding rates of DNA/RNA for a specific organism (Duan et al 2025; Korbel et al 2024; Saccò et al 2022a; Wambui et al 2026).

The use of eDNA/eRNA for health-based action also depends on whether the method can achieve the taxonomic resolution required for specific risk assessments. For screening, genus-level detection (e.g., *Anopheles* mosquitoes or *Vibrio* bacteria) may be informative. However, management decisions often require species- or subspecies-level identification (e.g., *Vibrio cholerae*). Insufficient resolution increases the risk of undetected threats and false positives (misassigned taxa), the latter of which can trigger unnecessary public health escalations.

1.4 Validation, standardisation and regulation

Although many studies now highlight the need for rigorous validation and standardisation, few have moved towards this approach within the biosecurity or biodiversity surveillance space (Bowers et al 2021; Darling et al 2020; De Brauwer et al 2023; Zaiko et al 2022).

International guidelines are beginning to emerge, but further harmonisation of methodologies, protocols, quality assurance, and reporting is required before adoption into biodiversity, regulatory and public health surveillance programmes can be achieved (De Brauwer et al 2023; Lopez et al 2025).

1.5 False positives, negatives and reliable data

Environmental sampling is fraught with risks of contamination and inherent difficulties in obtaining reliable data for public health surveillance. False positives can result from sample contamination, persistence of DNA from dead organisms or sloughing/shedding of tissue/cells, as well as methodological issues such as cross-reactivity in the molecular methodologies used (Farrell et al 2021; Rishan et al 2023; Zaiko et al 2022; Zhu et al 2024).

The persistence of relic (legacy) DNA can lead to false-positive detections (Barnes & Turner, 2016; Foucher et al., 2020; Takahashi et al., 2023). In these instances, naked nucleic acids or particle-bound DNA may remain in the environment long after an organism has died or moved, generating molecular signals that no longer represent an active biological threat. Because standard eDNA analyses often cannot distinguish between this historical extracellular material and the intracellular DNA of active, transmissible cells, environmental RNA (eRNA) is increasingly suggested as a closer proxy for metabolically active biological threats due to its rapid degradation (Pochon et al., 2017). Further research into the decay rates of eDNA and eRNA in different

substrates and under different environmental conditions would help to resolve such questions.

On the other hand, false negatives can arise from environmental conditions that degrade eDNA/RNA, along with inefficient extraction methods, inhibition from the sample matrix, sub-optimal sampling strategies, and masking of the target signal by background DNA/RNA (Bunholi et al 2023; De Brauwer et al 2023; Wambui et al 2026; Weaver 2024; Zhu et al 2024).

False negatives frequently arise from environmental matrices, such as sediment-heavy water, which contain substances like humic acids that suppress PCR reactions (Zhu et al 2024). To mitigate this risk, especially for low abundance targets such as pathogens, the use of internal spike-in standards is recommended to verify assay performance. Beyond laboratory constraints, the spatial context of a detection must be carefully evaluated. For example, as a passive tracer, eDNA can travel significant distances through aquifers or Heating, Ventilation, and Air Conditioning (HVAC) systems (Ruppert et al 2019). Determining whether a hazard originated locally, was transported from upstream, or represents a historical population protected from degradation (e.g. by adsorption to clay particles) is critical for an accurate risk assessment.

Finally, biological differences, such as the structural variations between Gram-positive and Gram-negative bacteria, or enveloped and non-enveloped viruses, directly affect extraction efficiency. Some cell walls (e.g. fungal cells and bacterial endospores) resist lysing agents more effectively than others, which can lead to an inaccurate representation of the microbial community and the potential underrepresentation of certain pathogens in the final data. It is also important to note that eDNA cannot currently provide precise organism counts. An approximation can be made from certain organisms with known molecular level information and sequencing efficiency (copy number), but it cannot provide a definitive number of organisms present.

5. NEW ZEALAND COMMERCIAL ENVIRONMENTAL DNA LANDSCAPE AND PROVIDER REVIEW

This section provides a comparative review of the New Zealand eDNA commercial landscape as of 2026, focusing on the technical trade-offs between broad-spectrum ecological monitoring and targeted public health surveillance.

1.6 Commercial Testing Landscape

The New Zealand market has developed into a two-tier system: broad-spectrum ecological monitoring and high-fidelity genomic screening. While the infrastructure for biodiversity and biosecurity (e.g., lake health and marine pests) is well-established, a shift toward human-health targets requires a transition from probabilistic indices toward definitive taxonomic identification and required technical expert interpretation of the data.

1.6.1 PHF Science (formerly ESR)

PHF Science are the Public Research Organisation (PRO) for public health in New Zealand. As such, their capability spans from methodological techniques for eDNA analysis, through to specialist analysis and interpretation of data with respect to environmental health and public health risks. Methodologies used are broad, from PCR-based assays, metagenomic sequencing to whole genome sequencing (WGS). They also hold capabilities for advanced microbial source tracking (MST) and research capabilities related to environmental health and public health monitoring, surveillance and risk management.

Advances in the area of MST being developed in PHF Science offers the capability to identify sources of faecal contamination in complex sample matrices and where multiple sources are present.

Their target areas with respect to this report are population level viral loads (e.g., SARS-CoV-2, influenza), antimicrobial resistance genes, and contamination tracking through advancement of conventional MST. Samples matrices include various types of water (including drinking water, groundwater and wastewater), soil and air sampling.

As a primary research institute, their approaches can include complex interpretation and modelling leading to slower turnaround time. Their capabilities are more geared to government level interventions and surveillance rather than rapid diagnostics. That said, the capability for rapid turnaround is possible, such as was the case for the COVID-19 pandemic.

PHF Science hold the most comprehensive knowledge and capability for public health surveillance, and rapid turn-around of results can be achieved when the surveillance signals warrant.

1.6.2 Sequench

Sequench combine specialised field-filtration techniques with advanced genetic sequencing (Illumina NGS⁶, qPCR, and ddPCR). They provide bespoke automated sampling systems, which use high-efficiency electronic pumps to filter large volumes of water directly in the field, “increasing the chance of capturing rare/low abundant organisms.

Their target area is in aquatic biodiversity profiling, environmental bio-surveillance, and extraction of low-abundance target organisms from complex or large-volume water bodies.

A limitation in their approach is that they function largely as a technology and hardware provider; the client is often required to provide internal bioinformatic capabilities or hire external consultants or experts to interpret raw data outputs.

As such, they do not run routine health diagnostics, but their sampling approaches (e.g. high-volume filtering) may be beneficial for capturing low concentration pathogens that are missed in standard “grab” sample approaches.

1.6.3 BSI Manaaki Whenua Landcare EcoGene®

Manaaki Whenua, part of the Bioeconomy Science Institute (BSI), operates the specialised EcoGene® diagnostic service, which utilises targeted qPCR and forensic-grade DNA analysis, including the processing of highly degraded or historical (“ancient”) environmental samples.

Their focus is on terrestrial biosecurity, such as detecting invasive wildlife vectors (e.g., rats, possums, exotic insects), and on soil/wetland microbial biodiversity ecology.

EcoGene’s methodology is historically optimised for terrestrial and wildlife ecosystems rather than detecting active human viral or bacterial pathogens.

They are the leading choice for monitoring environmental reservoirs of disease or tracking the geographic spread of animal vectors capable of transmitting zoonotic infections (e.g., Leptospirosis, Arboviruses). There is the possibility that this could be expanded to public health relevant organisms.

As with PHF Science, as a PRO they provide more government level monitoring with longer turnaround times for commercial applications.

1.6.4 Wilderlab

Wilderlab utilise high-throughput eDNA metabarcoding, amplifying specific marker genes (such as 12S or 16S rRNA) to cross-reference environmental samples against comprehensive in-house taxonomic databases. They use a proprietary syringe-filter kit that preserves eDNA from water samples at room temperature.

⁶ Illumina NGS: Illumina Next Generation Sequencing, a metabarcoding approach.

Their primary expertise and target areas are with multi-species biodiversity snapshots, stream health assessments, and regional multi-taxa mapping across freshwater and marine environments.

Their approach using metabarcoding is predominantly semi-quantitative; it confirms if a species' DNA is present but cannot accurately calculate exact pathogen loads or tell if the genetic material came from a live or dead organism. Their standard commercial assays focus on macroorganisms, not human pathogens.

Wilderlab are highly effective for mapping the risk factors of a disease (such as locating breeding grounds of invasive pest mosquitoes) rather than diagnosing an active human outbreak. Their primary focus is on environmental biodiversity assessments and tools they have developed such as the TICI⁷ index (Wilkinson et al 2024). The tool developed is useful for assessment in changes in general biodiversity and changes occurring over time. It can identify invasive species presence in a freshwater community and is suggested as being a useful health assessment tool but currently the evidence is limited to anecdotal evidence and is still primarily an ecological assessment tool.

1.6.5 Hill Laboratories

Hill Laboratories employs standardised, IANZ-accredited quantitative PCR (qPCR) and RT-qPCR assays. This targeted approach uses specific primers to look for predefined genetic sequences.

Their target areas are withing routine commercial water quality compliance, agricultural runoff assessment, and specific target pathogen tracking (e.g., *Legionella*, *Campylobacter*).

Limitations to their methodologies include the fact that using targeted qPCR will only find the exact organism specified in the test panel; if an outbreak is caused by a novel variant or an unexpected pathogen, this method will not detect it. Heavy sediment or chemical "mud" in raw environmental samples can also inhibit the PCR reaction.

Hill Laboratories approach is ideal for routine, legally defensible compliance testing of drinking water supply networks and commercial water infrastructure to check for known bacterial threats. It is less suitable for use in public health surveillance unless a specific target is required.

1.6.6 Cawthron Institute

Currently Cawthron Institute do not offer an eDNA service, but they do offer specialised Microbial Source Tracking (MST) using qPCR. They analyse the specific gut-microbiota DNA (such as *Bacteroidales* markers) present in a water sample to match it to a host species.

Their primary areas of interest are in coastal and freshwater health, aquaculture biosecurity, marine pest detection, and faecal contamination source attribution.

⁷ TICI Taxon Independent Community Index.

MST uses faecal indicators rather than human pathogenic viruses. Additionally, because tests are tailored to specific environmental inquiries, the custom laboratory setups are relatively expensive and less suited for continuous, automated national monitoring.

For public health applications, Cawthron offers microbial source tracking investigations that may help indicate possible causes of contamination in beaches or waterways, such as human sewage, birds, or livestock. However, MST is mainly focused on faecal contamination and may be less informative where contamination levels are low or where sources are mixed. Identifying multiple sources generally requires more advanced genomic approaches and specialist interpretation.

Table 1 Current landscape of commercial providers of eDNA monitoring in New Zealand. Assessment for suitability considered the robustness of the methods used, application to target or unknown or emerging species and the ability to interpret the data with a public health context.

Provider	Primary Method(s)	Public Health Advantages	Surveillance Limitations	Suitability for Health Surveillance
PHF Science (formerly ESR)	Genomic Source Tracking, MST, Pathogen Surrogates & WBE	Traceability: Uses DNA-labelled surrogates to physically track how and where pathogens travel through groundwater and aquifers. National Intel: Can correlate local contamination events with national genomic databases to identify the exact strain origin.	Research Focus: Novel methods for monitoring and surveillance across all environmental domains. Specific expertise in groundwater (source water), WBE, and vector surveillance. Surrogate tracking as well as source tracking method capability. Access: Services are often geared toward governmental, regional councils and government investigations rather than private users.	High: High technical expertise from sampling, methodological and analytical considerations to interpretation of data for public health risk. Source tracking is best for complex investigations requiring transport modelling (e.g., "Did the virus travel from this specific farm to the well?").
Sequenach	Illumina NGS & qPCR/ddPCR	Advanced Sampling: Exclusive NZ reseller of Smith-Root automated samplers, crucial for detecting low-abundance pathogens in large water volumes.	Equipment Focus: Primary value is hardware and sequencing support; users often need their own bioinformatic capabilities to interpret public health risk.	Medium: Critical for enabling surveillance through superior sampling tech. Specialised for biosecurity targets.
Manaaki Whenua (Landcare)	EcoGene® Diagnostics & qPCR	Biosecurity Focus: Deep expertise in identifying invasive vectors (e.g., mosquitoes, rats) and reservoirs (e.g., soil microbes).	Terrestrial Bias: Historically focused more on land/pest management than human aquatic pathogens.	Medium: Best for monitoring environmental reservoirs of disease.

Provider	Primary Method(s)	Public Health Advantages	Surveillance Limitations	Suitability for Health Surveillance
Wilderlab	eDNA Metabarcoding	Vector Tracking: Mapping the spread of disease vectors (mosquitoes, rats) across large regions.	Not Pathogen-Specific: Targets larger organisms (hosts), not viruses themselves. Results are semi-quantitative.	Low-Medium: Best for monitoring biodiversity and possible specific eukaryotic targets (vectors/hosts).
Hill Laboratories	Targeted qPCR / RT-qPCR	Accredited Accuracy: IANZ-accredited methods that hold up for regulatory compliance.	Narrow Scope: Only finds exactly what you test for. Misses novel pathogens.	Medium-High: Ideal for routine compliance checks for specific targets.
Cawthron Institute	Microbial Source Tracking (MST)	Pollution Sourcing: A commercial option for distinguishing Human vs. Animal faecal markers to determine pollution sources.	Cost & Scale: Bespoke nature makes it expensive for continuous monitoring.	Medium-High: Detect sources with respect to distinguishing human or animal sources. Currently not a focus of the organisation as interpretation skills are not available for health risk assessments.

The New Zealand landscape currently offers powerful tools that are not inherently interchangeable. For public health advisory, the transition from broad ecological screens to high-fidelity, validated genomics is a critical requirement for accurate risk assessment. Selecting the appropriate platform is essential for mitigating the risk of false-positive-driven public health escalations.

For broad-scale surveillance, including identification of emerging or novel public health threats it would be more cost effective to employ a service that can be adaptive and have technical and public health expertise.

For specific target pathogens or vectors employing a commercial service is possible but caution needs to be applied in interpreting the data and understanding the limitations of the methods employed.

6. EMERGING SOLUTIONS AND CONSIDERATIONS

Accurate interpretation of eDNA/eRNA data for public health requires a comprehensive understanding of the ecology of the genetic material being detected (Takahashi et al., 2023). While eDNA/eRNA is a powerful, high-sensitivity sentinel, it is most effective when utilised as a proactive, defensible, and complementary tool alongside traditional physical surveillance and ecological surveys.

1.7 Sampling techniques

There are numerous studies improving the collection and detection DNA/RNA in the environment, for both total community and targeted surveillance applications. Some of the recent advances include the use of CRISPR/Cas-⁸based technologies, lateral flow systems, and miniaturised labs (Figure 2). Some methods aim to reduce chain of custody issues by developing in-field sampling and analysis techniques (D'Agnese et al 2023; Durán-Vinet et al 2025; Kim et al 2026; Rieder et al 2025). Certain newer techniques offer promise for sensitive targeted species identification, therefore require knowledge of expected target(s) and so are not viable for use as a wider community-based approach.

Even without these technologies, advances have been made in sampling methods themselves, such as high-volume sampling devices such as air filters and passive samplers. Other advances such as lab-on-a-chip or microfluidic devices are also being developed as sensitive concentration systems.

1.8 Interpreting the Molecular Signal

Interpretation of an eDNA "detection" should be carefully linked to the specific environmental or public health risk management context. A simple presence/absence result does not automatically equate to an immediate public health threat; rather, it requires context. For example, knowledge of the minimum infectious dose of the target pathogen, the potential for legacy DNA signals from dead or inactive organisms, and knowledge of the temporal, spatial and/or quantitative trends (is the detection unusual in the context of other sampling?). Quantifying the amount of target DNA, primarily through ddPCR, provides a more rigorous basis for health-based decisions by determining the actual concentration of genetic material in a sample, offering a more precise understanding of the molecular load. Advances in the ability to link detection with abundance are being researched in the biodiversity space and could be adapted to public health surveillance requirements (Bylemans et al 2018; Dickie et al 2018b; Li et al 2018; Wu et al 2026).

1.9 Interpreting Detections

Several environmental and technical factors should be considered in the risk assessment to avoid over-interpretation of molecular signals (De Brauwer et al 2023;

⁸ CRISPR/Cas: CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats, Cas = CRISPR associated proteins. This method employs high precision gene editing technology to specifically bind to a target eDNA sequence using specific Cas proteins. Once bound, they cleave or cut the nearby reporter molecules introduced to the sample and are detected through a visual signal in the detector. Similar to a pregnancy test or the COVID-19 RAT test.

Veilleux et al 2021; Wambui et al 2026). Factors such as UV-mediated degradation, pH levels, and temperature can break down DNA within days, meaning a positive signal usually indicates a relatively recent presence unless the DNA has been preserved in an "environmental sink" like anaerobic sediment. Furthermore, as a passive tracer, eDNA can travel significant distances through currents, aquifers, or through the air in outdoor or indoor environments; therefore, detections must consider whether the hazard originated locally or was transported from an upstream or distant source. Finally, because molecular signals alone cannot currently distinguish between a live, infectious pathogen and legacy DNA, the use of eRNA is being suggested as a closer proxy for active biological threats when viability is a primary concern.

Smart Environmental Nucleic-acid Tracking using Inference from Neural-networks for Early-warning Localization

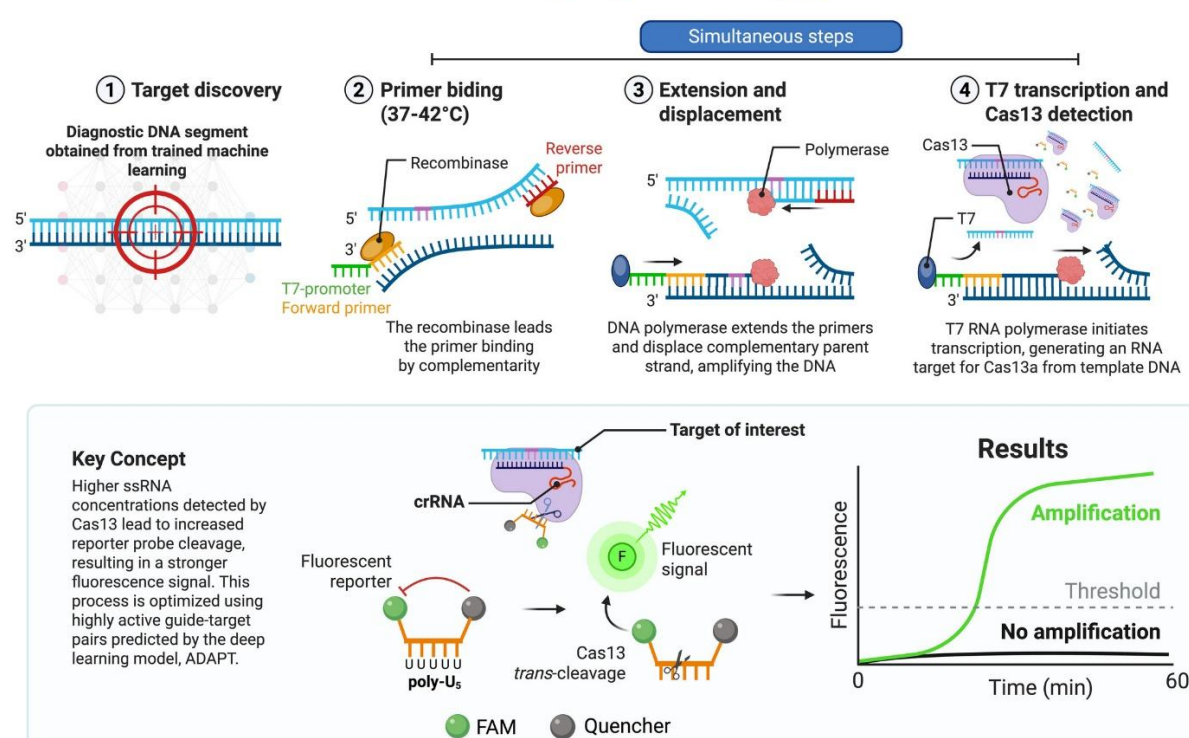


Figure 2 An example of a CRISPR/Cas approach for invasive species detection (Durán-Vinet et al 2025).

7. GAPS IN THE RESEARCH AND FUTURE NEEDS

1.10 Gaps in the research

Currently, although advances are being made at a rapid rate, there is still a lack of universally accepted protocols and methods for sampling the different environments, preservation methods, extraction techniques, and downstream analyses. These factors all limit the capability of using eDNA or eRNA as a public health surveillance tool (De Brauwier et al 2023; Zaiko et al 2022).

For many taxa, sequence information in databases and repositories is missing or limited, impacting the ability to accurately identify certain pathogens, vectors or invasive species (Sengupta et al 2022).

For these reasons, the interpretation of eDNA/RNA results for quantitative assessment or infection risk remains poorly resolved, especially in complex or dynamic environments (e.g., groundwater, air) (Amarasiri et al 2021; Wambui et al 2026).

1.11 Future needs

As eDNA is increasingly utilised for targeted surveillance alongside broader biodiversity monitoring, the emphasis for health-related applications remains focused on fit-for-purpose study design and defensible interpretation. To support robust public health decision-making, minimum sequencing depth requirements need to be standardised to improve the recovery of low-abundance targets. Furthermore, the inclusion of internal spike-in standards or synthetic DNA, which is added before molecular characterisation, is essential for verifying assay sensitivity and identifying potential PCR inhibition.

Strategic expansion of future monitoring programs needs to move beyond water matrices to include air, soils, and sediments, thereby capturing a more comprehensive profile of environmental pathogens and vectors. This shift would support a transition in reporting from simple presence/absence results toward a risk-based assessment that factors in molecular load and environmental context to inform proportionate responses.

To maintain high levels of data integrity, eDNA results should be supported by replication and appropriate controls. Detections by eDNA/eRNA need to be validated initially through dual-method protocols, such as targeted qPCR/ddPCR or physical surveillance, to ensure the molecular signal represents a current, viable health risk. After an initial eDNA/eRNA detection of public health concern, expanded sampling (spatially and/or temporal) would help to identify the scale of concern. Finally, to mitigate the risk of false positives originating from legacy DNA, prioritising the use of eRNA where possible would provide a more reliable proxy for current or recent biological activity and infectious potential. Improvements need to be made in the assurance of continued secure long term data storage as well as the need to upload to and improve databases or libraries to improve the detection of target species, especially when detecting emerging or novel pathogens or vectors.

8. CONCLUSIONS

While eDNA and eRNA offer powerful new advances and capabilities for public health surveillance, there are significant technical challenges remaining that need to be addressed before being included in any future strategies. The use of harmonised guidelines and standardised protocols, robust quality control measures, improved references or libraries, are essential to make inclusion of these approaches suitable for inclusion in routine public health surveillance.

There is an increasing landscape of commercial biodiversity monitoring providers in New Zealand, which offers exciting opportunities for including eDNA/RNA into routine surveillance. There are still, however, key factors to be considered. The biggest is the lack of specialist public health-focussed knowledge held within the commercial providers, which brings risks in terms of accurate interpretation of results and appropriate recommendations to health authorities. The use of a provider with both the technical expertise to undertake the analysis of the samples but also the downstream interpretation of the data specifically for public health implications is vital. With these considerations in place, eDNA/eRNA analysis is set to become an increasingly important tool for public health surveillance in the coming years.

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