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Article

Beyond the Host: Evaluating Methods for Environmental Biosurveillance of Viruses Using Avian Influenza Virus as a Case Study

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Abstract: Environmental DNA and RNA-based virus biosurveillance holds promise as an early warning system for detecting pathogen incursions and tracking their evolution across fields. Unlike traditional pathogen monitoring, which typically relies on direct sampling from humans and animal hosts, environmental biosurveillance offers a broader, faster, more accessible, and cost-effective approach. While recent advancements in viral detection methods have expanded their utility, the relative effectiveness and suitability of these methods for point-of-need detection remain unclear. To this end, we evaluate and compare 12 contemporary viral detection methods to assess their potential for identifying viruses in environmental samples, with a specific focus on highly pathogenic avian influenza (HPAI) virus. We examine the scalability, practicality, and future potential of each method, particularly those that have yet to be applied to environmental samples, while considering the specific requirements for each approach. Our goal is to provide guidance to end-users on selecting the most appropriate methods based on current available approaches and data. In addition, we outline key requirements for an “optimal” in-field detection method and identify the steps necessary to enhance environmental virus biosurveillance in the future.

Keywords: biosecurity; eDNA; eRNA; biomonitoring; HPAI; CRISPR; detection; environmental surveillance

1. Introduction

Viral disease outbreaks can have devastating consequences for human, animal, and plant health (Jones, 2021; Msemburi et al., 2023; Oldstone, 2020). One prominent example is the H5N1 subtype of highly pathogenic avian influenza (HPAI) virus that is currently spreading worldwide, killing millions of birds and spilling over into a broad range of non-avian hosts, including livestock and humans (Bi, Yang, Wang, Ran, & Gao, 2024; Neumann & Kawaoka, 2024; Plaza, Gamarra-Toledo, Euguí, & Lambertucci, 2024). Given the significant health risks associated with such viral outbreaks, there is an increasing need for rapid detection and continuous biosurveillance, ideally using environmental samples. Such efforts would provide a much-needed early warning system for new viral incursions while improving our understanding of pathogen transmission and evolution.

Environmental sampling of DNA and RNA (eDNA/eRNA) has emerged as a powerful tool for viral monitoring (Bass, Christison, Stentiford, Cook, & Hartikainen, 2023; Farrell et al., 2021), offering a cost-effective, scalable, and less-invasive alternative to traditional host sampling for pathogen detection (Alfano et al., 2021; Farrell et al., 2021; Huver, Koprivnikar, Johnson, & Whyard, 2015; Kawato, Mekata, Inada, & Ito, 2021). Compared to direct sampling from hosts, environmental surveillance is safer and less labour-intensive (Hood et al., 2021).

The practice of monitoring viruses in environmental samples dates back to the 1940s when cell culture techniques were used to detect human enteric viruses in water sources (Bivins et al., 2022; Metcalf, Melnick, & Estes, 1995). While slow, cell culture was the gold standard because alternative approaches such as radioimmunoassay, immunofluorescence, and complement fixation lacked the sensitivity needed for detecting low viral counts from environmental samples (Fong & Lipp, 2005; Griffin, Lipp, McLaughlin, & Rose, 2001). Other molecular detection methods including loop-mediated isothermal amplification (LAMP) and enzyme-linked immunosorbent assays (ELISA) proved useful for rapid and sensitive detection of viruses from environmental samples (Kfir & Genthe, 1993; Wong, Othman, Lau, Radu, & Chee, 2018; Zhu et al., 2021), however, it was reverse transcriptase (Temin & Mizutami, 1970) and polymerase chain reaction (PCR) (Mullis & Faloona, 1987) that revolutionised viral detection, enabling rapid, specific and highly sensitive identification of viruses, including strain-specific information, even from low-titre samples (Fu et al., 2023).

Viral environmental detection techniques have developed rapidly in the last five years, largely due to the COVID-19 pandemic. During the COVID-19 pandemic, significant efforts were directed toward optimising environmental sampling, particularly human wastewater surveillance, to detect viral variants (Kumblathan, Liu, Uppal, Hrudehy, & Li, 2021). These methodologies are now being applied to monitor a wide range of viruses, including mpox, dengue, and HPAI, with new assays and technologies continuously emerging (de Jonge et al., 2022; Louis, 2024; Wolfe et al., 2023). In response to the ongoing global spread of HPAI and its potential effect on wildlife, human health, and primary industries (Adlhoch, Baldinelli, Fusaro, & Terregino, 2022; Plaza et al., 2024), several new molecular detection methods have been deployed. These include CRISPR-based diagnostic platforms (CRISPR-Dx) (J. S. Chen et al., 2018; S.-S. Chen et al., 2024; Gootenberg et al., 2018), digital droplet PCR (ddPCR) (Wolfe et al., 2024), and Oxford Nanopore Technology (ONT)-based sequencing approaches (Croville et al., 2024). Nevertheless, to be effective in real-world settings, viral detection methods must be user-friendly, highly specific, cost-efficient for large-scale use, and capable of detecting viruses from a wide range of diverse, low-concentration environmental samples so that they can guide biosecurity investigations and public health decisions towards tactical deployments (e.g. containment or eradication) (Campbell & Hewitt, 2025). While the development of new environmental nucleic acid detection methods has provided end-users with numerous options, a clear understanding of their advantages and limitations is currently lacking. This knowledge gap presents challenges both in optimising existing methods and in selecting the most suitable technology for new monitoring programs.

To address this knowledge gap, we examine 12 molecular methods to explore their resource requirements and in-field potential for viral detection. We particularly focus on a method's utility in the current global HPAI outbreak. Herein, we discuss the advantages and disadvantages of these methods, offering end-users a practical guide to selecting the most appropriate approach for viral detection based on several of their requirements. We also discuss key barriers to achieving a cost-effective, low-resource, and field-deployable method for detecting viruses in low-concentration environmental samples, along with potential solutions to overcoming these challenges.

2. An Overview of Current Detection Methods for Monitoring Viruses in the Environment

The proliferation of nucleic acid testing (NAT) methods can make it difficult for end-users and researchers to select an approach that best suits their environmental virus monitoring needs. Methods vary in sensitivity, up-front development costs, per-method expenses, and the equipment and

expertise required for implementation. To aid in the selection process, we compiled a list of possible methods, some of which are commonly used for detecting viruses in environmental samples, and have summarised their methodological requirements, advantages and limitations (Figure 1; also see Supplementary Table S1 for more detail). While our primary focus is on detection and biosurveillance methods for avian influenza virus (AIV), these methods can, in principle, be applied to any virus (RNA and DNA) or nucleic acid target. We intend this summary to serve as a practical guide for end-users evaluating different detection methods.

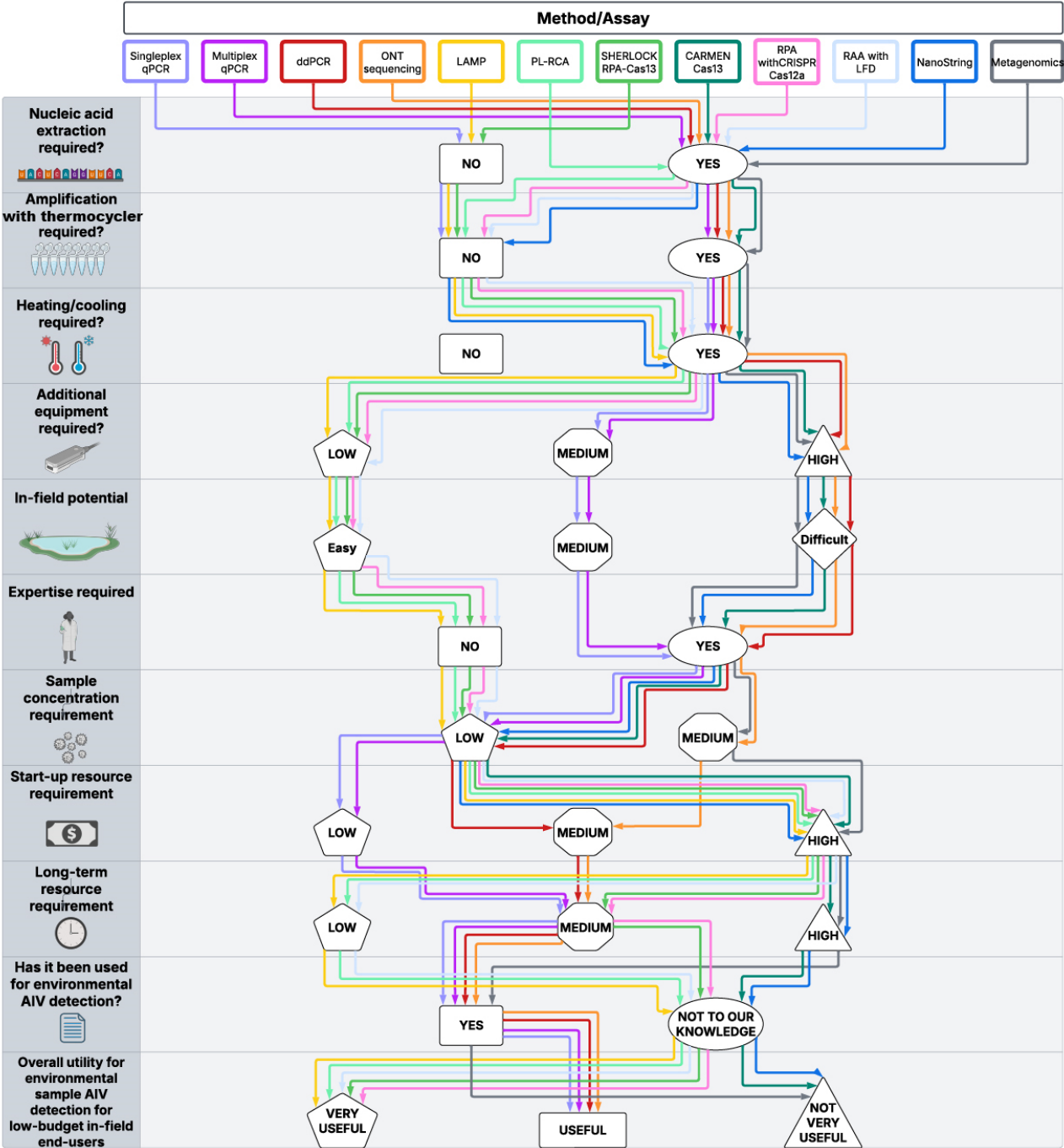


Figure 1. Workflow summarising the 12 methods evaluated. Coloured arrows correspond to 1 of the 12 environmental viral detection methods. The 11 criteria we use to assess the methods are listed in the column on the left and the corresponding responses are listed in the middle. These middle responses range from more advantageous to less advantages going from left to right (e.g., the response of “No” for “Nucleic acid extraction required?” is more advantageous than the “Yes” response). See Supplementary Table S1 for further detail. Figure created with Lucidchart and Biorender.

We identified 12 distinct viral NAT approaches for AIV detection, including CRISPR-Dx platforms (DETECTR [Chen et al., 2018]; SHERLOCK [Gootenberg et al., 2018]; and CARMEN [Ackerman et al., 2020]), loop-mediated isothermal amplification (LAMP; Kaneko, Iida, Aoki, Ohno, & Suzutani, 2005), NanoString (nCounter; Geiss et al., 2008), ONT (Ji, Aw, Van Bonn, & Rose, 2020), padlock rolling circle amplification (PL-RCA; Jain, Dandy, Geiss, & Henry, 2021), quantitative PCR (qPCR; singleplex [Ding et al., 2024] and multiplex [Vogels et al., 2021]), digital droplet PCR (ddPCR; Ding et al., 2024), recombinase-aided amplification (RAA; Nie et al., 2022), and recombinase polymerase amplification (RPA; Parameswari et al., 2025). Overall, all methods currently rely on a heat source, custom-designed primers, and probes uniquely designed for each method, and, in most cases, nucleic acid extraction. However, there are key notable differences in methodological requirements that present distinct advantages and disadvantages among these approaches. Below, we outline the commonalities and differences in detail.

2.1. Nucleic Acid Extraction

Most methods and samples require nucleic acid extraction (the process of isolating or purifying nucleic acids from a biological sample), with the potential exceptions of some LAMP, CRISPR, and PCR methods, where extraction may not be strictly necessary for target detection (e.g., Barnes et al., 2020; Lalli et al., 2021; Schellenberg, Ormond, & Keynan, 2021; Scriver et al., 2024; Wang et al., 2024). Nucleic acid extraction is needed for RNA targets to prevent degradation, and can help mitigate unwanted complications associated with non-target (e.g. DNA or cellular material) and environmental (e.g. decaying matter and tannins) components. Often this is done through methods including magnetic bead washes and DNase digestions.

2.2. Amplification with Thermocycler

Some methods discussed require resources for nucleic acid amplification. Singleplex and multiplex qPCR and ddPCR, as well as metagenomics approaches, rely on thermocyclers (PCR instruments capable of precisely controlling temperature changes at specific intervals) and sometimes other equipment (e.g. equipment to read fluorescence) for nucleic acid amplification. Some CRISPR-Dx platforms, such as SHERLOCK and DETECTR can simply be run on battery-operated portable heaters (Misra et al., 2022; Zahra, Shahid, Shamim, Khan, & Arshad, 2023), and only require thermocyclers when the reactions are fluorescence-based (Broughton et al., 2020; Myhrvold et al., 2018). Other methods, such as LAMP and RPA, do not necessarily rely on a thermocycler, but still require temperature control (Notomi et al., 2000; Piepenburg, Williams, Stemple, & Armes, 2006).

2.3. Heating and Cooling

All methods require heating steps for strand separation, probe annealing, and enzymatic activity. The complexity of heating and cooling requirements varies, however, ranging from simple heat plates (LAMP methods), to thermocyclers (PCR-based methods), and method-specific heating instruments (e.g., ONT Minion and NanoString Counter Digital Analyzer).

2.4. Additional Equipment Required

At their full optimisation, the equipment requirement needs of the different methods vary dramatically, with some such as NanoString, metagenomics, Carmen Cas13, ONT, and ddPCR often requiring a large amount of equipment compared to others such as CRISPR-Dx platforms and LAMP.

2.5. In-Field Potential

The need for laboratory space depends largely on the method stage. Most methods require a laboratory during early-stage optimisation, but some can be adapted and performed in-field once optimised. Singleplex and multiplex qPCR, ddPCR, SHERLOCK, DETECTR, and ONT sequencing can be adapted for field use with portable equipment (e.g., Boykin et al., 2019). In contrast, methods

such as CARMEN, metagenomic sequencing, and NanoString require large, non-portable equipment, making field deployment generally impractical.

2.6. Expertise

Most methods require significant expertise during the development phase, but minimal training once optimised. For example, LAMP requires well-trained staff for primer design and optimisation, but is relatively straightforward to perform afterwards. A benefit of using well-established methods (e.g. qPCR) is that there are often tools available to help with assay design, compared to novel approaches that might require more hands-on time. NanoString, ONT, and metagenomic sequencing methods, however, require specialised expertise at all stages due to their complexity and equipment requirements.

2.7. Sample Concentration

We define sample concentration as low (~1–10 copies/ μ L of target sequence), medium (~100 copies/ μ L), and high (>1000 copies/ μ L). Most methods can analyse samples across the above mentioned range, with the exception of metagenomic sequencing and ONT (in some cases) which typically recommends higher input concentrations.

2.8. Start-Up and Long-Term Resources

Start-up and long-term resources (that is, the initial resources needed to develop the method and the resources needed to maintain and run the method after the initial start-up) ranges widely and is influenced by expertise, laboratory space, and the desired targets to detect. Methods with established protocols focused on common targets will require some optimisation but have minimal start-up requirements compared to methods that target less-common/custom targets (e.g. specific viral variants). For example, there are multiple well-optimised and highly cited qPCR methods (even commercial kits) for detecting the matrix protein of AIV and thus require little trained technician time to optimise for individual needs. In comparison, methods to detect new emerging variants such as the H5N1 clade 2.3.4.4b often demand more time from trained technicians and laboratory resources to develop and optimise.

Long-term resource needs depend on reagents, technician time, software needs, equipment maintenance, and the variable nature of the target to detect. LAMP, for instance, requires minimal ongoing resources, whereas CARMEN, metagenomic sequencing, NanoString, and ONT require costly reagents, technical expertise, and dedicated laboratory equipment. Technical expertise includes the trained bioinformaticians that are needed to process data (e.g. from metagenomic and ONT sequencing) post sequencing, which can take between days and months depending on the required objective. Lastly, if the viral target is highly-dynamic (i.e. quickly evolving new subtypes/strains) then it will require additional effort to ensure that the method is able to continually detect it.

2.9. Proven Use on Environmental AIV Samples

We found it advantageous if the method had been previously demonstrated to detect AIV from environmental samples. To our knowledge the CRISPR-Dx platforms we analysed (excluding Carmen Cas13), LAMP, and NanoString have no published records of their use on environmental samples for AIV biosurveillance.

Overall advantages and disadvantages. We assessed the methods based on multiple criteria, including cost, speed, portability, sensitivity, and viral subtype detection capability (Figure 1; Supplementary Table S1). Expensive equipment, reagents and/or high technician expertise, and time were generally considered disadvantages. Rapid methods, such as PL-RCA, were considered advantageous, while ONT and metagenomic sequencing were often slower compared to other methods and, while they produce a lot of data, they were considered disadvantageous when time to

detection were key criteria. It was advantageous if a method had routinely been used for viral detection, such as singleplex and multiplex PCR, and if the method could be deployed at point-of-need.

Sensitivity, or the ability to detect viral genetic material from low concentration samples, is important, especially for environmental samples. Many methods promised high sensitivity, but were not at the level of singleplex PCR (often considered the gold standard for viral detection). In general, the ability to identify more AIV subtypes was advantageous; however, a high level of detection (e.g. CARMEN can detect up to 169 subtypes in one reaction) may be less advantageous to end-users whose requirement is to detect only a specific subtype in the environment (e.g. H5N1). We found some methods had difficulties assessing samples uniformly, for example LAMP sometimes demonstrated delayed onset of amplicon formation because of inhibitors including tannins, urea, calcium chloride, and humic acid (Nwe, Jangpromma, & Taemaitree, 2024), or was affected by pH variability of samples (Brown et al., 2021; Nwe et al., 2024), both often characteristics of environmental samples.

Other disadvantages were method-specific and were associated with the equipment or the nature of the method. For example, ddPCR has droplet variability and ONT/metagenomic sequencing has long-term equipment costs, often with sample quality requirements. By considering these factors, end-users can select the most appropriate technology for their specific application, balancing cost, ease of use, sensitivity, and field deployment potential.

3. Application of These Methods to Avian Influenza Virus Environmental Detection

PCR methods have routinely been the cornerstone for detecting specific AIV genes in environmental samples, including bird guano, wastewater, and sediment (e.g., Hubbard et al., 2023; Jallow et al., 2025; Kuchinski et al., 2024; Tisza et al., 2024). Additionally, ONT has demonstrated utility in identifying influenza viral subtype information directly from bird faeces (de Vries et al., 2022; Nabeshima et al., 2023). These established methodologies provide a strong foundation for AIV biosurveillance; however, emerging NAT approaches can potentially offer additional advantages that may enhance detection capabilities in environmental settings.

While these novel NAT approaches have not yet been shown to detect AIV from environmental samples, we considered their sensitivity, limitations, and start-up/long-term resource requirements based on their use across other sample types to estimate their potential applicability for environmental samples. It is clear that single and multiplex PCR methods have established protocols for environmental samples (e.g., Hubbard et al., 2023), are highly sensitive (Smith & Osborn, 2009), and do not require expensive specialised equipment.

Nevertheless, CRISPR-Dx platforms (e.g. DETECTR and SHERLOCK) are ultrasensitive, specific, scalable, portable, and can be free of extensive equipment when deployed with lateral flow strips (Baerwald et al., 2020; Wei et al., 2022) or even without the need for pre-amplification steps (Bhatt et al., 2022; Huang et al., 2022; Miao et al., 2023; Wang et al., 2024), increasing their point-of-need utility for in-field detection, all of which may outweigh the benefits of other technologies.

Although we focus on one virus type here, the NAT methods we examine can be applied to other viruses besides AIV, including DNA viruses. Viruses naturally vary in their genetic sequences, structures, and hosts; however, their general transmission pathways through environmental mediums such as faeces and water often make them detectable by NAT methods. For example, other avian viruses such as Newcastle disease virus, that are primarily spread through the faecal-oral route (Alexander, 2001), can be present in the environment and thus have the potential to be detected through the NAT methods listed here.

4. Limitations of Environmental Sampling for Viruses

Given recent human and animal health concerns regarding HPAI and other zoonotic pathogenic viruses, there is an urgent need for sensitive, rapid, and cost-effective environmental biosurveillance methods. Unfortunately, methods targeting specific viruses from environmental samples face significant challenges, including heating requirements, low virus concentrations, and nucleic acid isolation limitations. Indeed, a heat source was required for all of the eDNA/eRNA methods that we reviewed. Some methods, such as LAMP and PL-RCA, can function with easily-accessible heat sources (e.g. portable heater, or even body heat). However, PCR, metagenomics, and NanoString require precise temperature control provided by specialised laboratory equipment, making in-field applications difficult or requiring financial investment in portable equipment.

Environmental samples typically contain variable virus concentrations, enzyme inhibitors, and environmental contaminants that hinder detection success (Beng & Corlett, 2020; Bowers et al., 2021). Of the 12 methods reviewed, only five (singleplex qPCR, multiplex qPCR, ddPCR, metagenomics, and ONT sequencing) have been applied to environmental AIV detection. These methods have demonstrated high sensitivity, with singleplex RT-PCR capable of detecting AIV RNA at concentrations as low as 1×10^{-1} EID₅₀ (median egg embryo infectious dose) per mL in wetland water (Hubbard et al., 2024). However, most PCR-based methods require laboratory settings, increasing the delay between sampling and detection. Portable qPCR devices, such as Ubiquitome's Liberty 16 (<https://www.ubiquitomebio.com/>) device are becoming more available, but their efficacy for environmental samples remains largely under evaluation (Billington, Abeysekera, Scholes, Pickering, & Pang, 2021). ONT sequencing has emerged as a promising alternative, demonstrating the ability to recover whole hemagglutinin (HA) genes for AIV subtype and phylogenetic clade identification from environmental samples, including from dust and bird guano (Croville et al., 2024; de Vries et al., 2022). It has also demonstrated a higher sensitivity than conventional culture methods for human pathogen detection (Deng et al., 2022). However, ONT sequencing has several drawbacks, including high cost, an often medium-to-steep learning curve (especially the bioinformatic components), and the general need for RNA extractions prior to sequencing (see Figure 1). There are several extraction-free ONT methods; however, they have not yet been evaluated with environmental samples. The sensitivity of ONT is also often considered a disadvantage of the method. Sensitivity studies are generally lacking for ONT virus detection; however, Croville et al. (2024) concluded that such high throughput sequencing methods, including ONT, were not as sensitive as PCR methods, but that there are demonstrated advantages of combining PCR with high throughput sequencing (e.g., Quick et al., 2017). Lastly, adaptive sequencing is a potential option for increasing ONT detection sensitivity and has been demonstrated to improve the sensitivity of viral clinical detection (Lin et al., 2022); however, more research is needed, especially to determine its use with very low concentration targets, such as those often found in environmental samples.

Nucleic acid extraction was required for all reviewed methods, with the exception of LAMP, RCA, and some Cas12- (Wu et al., 2022) and Cas13-based (Myhrvold et al., 2018) methods when used with specific protocols and sample types. The sample types that were tested so far with extraction-free protocols are often clean of inhibitors and highly-concentrated, such as nasopharyngeal, oropharyngeal, urine, or rash (e.g., mpox) samples (Bhatt et al., 2022; Lalli et al., 2021; Schellenberg et al., 2021; Wang et al., 2024). Kang et al. (2023) demonstrated that RT-RPA with Cas12a and G4 cleavage could detect spiked wastewater samples; however, the samples were processed slightly prior to spiking (centrifuged at 9000 rpm for 10 min). Many extraction-free methods have not been tested for AIV detection in environmental samples, while portability and nucleic acid extraction remains a bottleneck in viral biosurveillance due to its time-consuming nature, laboratory space requirements, and high costs. Several recent advancements aim to facilitate in-field nucleic acid isolation, including S-TECH (<https://www.dnaitech.com/>), Bento Lab (Pillay, Calderón-Franco, & Abeel, 2024), and PDQeX (Stanton, Muralidhar, Rand, & Saul, 2019). Additionally, novel RT-PCR methods that bypass extraction are under development, such as Cytiva's RT-PCR anti-inhibitor complex (AIC) and homogenizer-based RNA extraction free methods (Ramírez-Córdova et al., 2023; Struijk et al., 2023). However, these techniques have not yet been validated for environmental

samples, which often contain high levels of contaminants, variable pH levels, and fragmented or degraded RNA and DNA (Sahu, Kumar, Singh, & Singh, 2023). In addition, they often have a limit on the number of samples they are able to process at once (often 1–24) making them unsuitable for high throughput monitoring programs when compared to laboratory-based methods that can process up to 16x as many samples in parallel.

Developing viral detection methods is inherently challenging due to the extreme sequence variation and rapid evolution of viruses. Primer and probe design was required for all reviewed methods, except for ONT and metagenomic sequencing. Designing optimised primers and probes requires skilled technicians and can take weeks, sometimes months, to ensure specificity and sensitivity. Primers and probes can be used to amplify a broad range of taxa or very specific taxa of interest. For example, they can be designed to sequence conserved regions of the AIV HA gene to detect general HA subtypes such as H5 and H7, or they can be designed to sequence specific lineages of these subtypes such as H5N1 2.3.4.4b. Tools are available for evaluating and validating targeted eDNA primer and probe methods (e.g., Thalinger et al., 2021) and can be similarly applied to eRNA and viruses).

5. Recommendations for Environmental Biosurveillance of Viruses

Although all of the 12 methods that we reviewed were previously proven to detect AIV, only five have been used with environmental samples to our knowledge. We found several methods to be more user-friendly, less resource-heavy, and/or cost-efficient than others based on their requirements (Figure 2). We found singleplex qPCR still appropriately fits into the “gold standard” rating as it is a well-tested method that has been proven to detect multiple viruses (including AIV) at low concentrations, has equipment and protocols readily available, and has been widely used for environmental samples. There are also methods to specifically enhance the RT-qPCR sensitivity for virus detection from environmental samples such as dust and air samples (Bessière et al., 2023). Multiplex qPCR is another popular option after the protocol is optimised; however, the development and optimisation of the method requires time and needs to work with the requirements of the instrument. Commercial multiplex qPCR kits exist, but are often expensive. Although ddPCR performs absolute quantification and is sometimes more sensitive and more tolerant of sample inhibitors than qPCR (Rački, Dreo, Gutierrez-Aguirre, Blejec, & Ravnikar, 2014), it requires more time, complex machines, greater expertise, and generally less throughput than qPCR. ddPCR may also be more than what is needed by end-users if absolute quantification is not necessary and sample concentration is within qPCR limits. Other methods such as the CRISPR-Dx platforms appear to hold potential to detect viruses from environmental samples (e.g., Kang et al., 2023), but many of them have not yet been tested on environmental samples. For these methods, we found that the development and optimization effort could be high depending on the computational resources available, but that their thermocycler-free, and potentially nucleic acid extraction-free, requirements allowed them to be highly-portable, low-cost, and field-friendly once optimised (e.g., Barnes et al., 2020) (Figure 2).

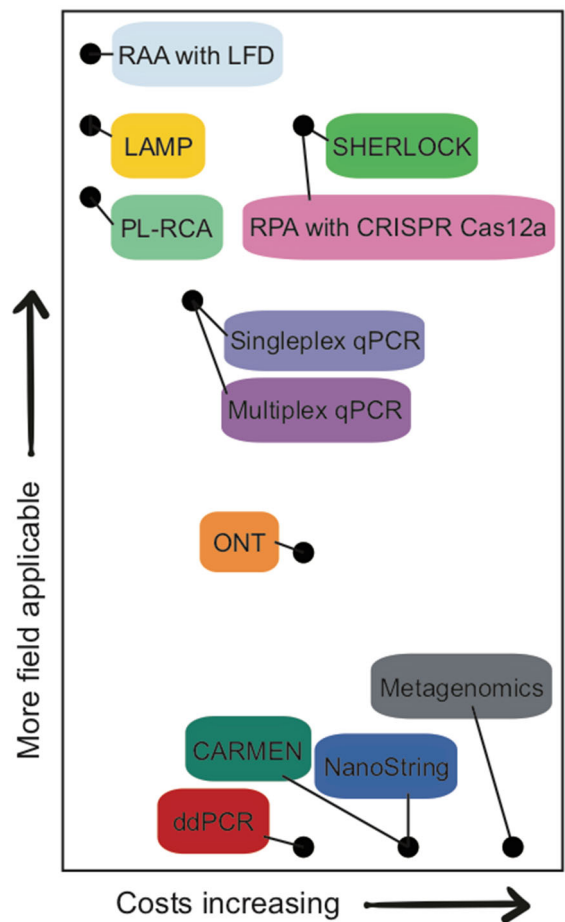


Figure 2. Cost and in-field potential of environmental viral detection methods. Twelve methods plotted by long-term resource cost and their ability to be deployed in the field using information from Supplementary Table S1. Colours correspond to the methods listed in Figure 1.

6. Conclusion

Ultimately the choice of method for environmental virus detection should align with the specific budget, expertise, and goals of the end-user and their biosurveillance program. We found that, to date, there are no methods that can be easily performed at the point-of-need by non-experts. As an example, ONT sequencing methods have been shown to detect viruses (and sometimes whole viral genomes) in the field (Croville et al., 2024; de Vries et al., 2022); however, these methods require trained technicians, specialised expensive and often inaccessible equipment (e.g. see de Vries et al 2022). We found that five methods held higher potential than others for detecting AIV in the field requiring lower budgets. Overall, methods free from nucleic acid extraction and PCR steps will ultimately allow for more rapid, sensitive, and cheap ways of detecting viruses in the field.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Allison Miller and Jemma Geoghegan: concept and design. Allison Miller: method development. Allison Miller – original draft. Allison Miller, Eddy Dowle, Benjamin Duran-Vinet, Jo-Ann Stanton, Vinko Besic, Jackson Treece, Stephanie Waller, Gert-Jan Jeunen, David Winter, Neil Gemmell, Jemma Geoghegan: manuscript preparation – review and editing. Jemma Geoghegan and Neil Gemmell: supervision and administration. David Winter and Jemma Geoghegan - Funding.

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Conflict of Interest: JS is currently COO of eXymes PLC, a nucleic acid extraction company.

Ethics statement: The research meets the ethical guidelines of the study country and the manuscript has not been submitted elsewhere.

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