Assay	AIV example	Nucleic acid extraction required (once assay is optimised)?	Amplification (e.g., PCR) required?
Singleplex qPCR	Fouchier et al 2000 (matrix gene detection). Hubbard et al 2024 (environmental samples)	Yes	Yes
Multiplex qPCR	Spackman et al 2003 (H5 and H7 subtype detection)	Yes	Yes
Digital droplet polymerase chain reaction (ddPCR)	Lou et al 2023 (H7N9 neuraminidase mutation detection)	Yes	Yes
Oxford Nanopore Technologies sequencing	DeVries et al 2022 (multiple subtype detection). Croville et al 2024 (environmental	Yes	Yes
loop-mediated isothermal amplification (LAMP)	Luo et al 2015 (H10 subtype detection)	No	No, depends on sample type, but is recommended to achieve optimal results and is likely required for direct RNA analysis (e.g., samples directly taken from the environment).
Padlock rolling circle amplification (PL-RCA)	Hamidi et al 2015 (H5N1 subtype detection)	Yes	No, depends on sample type, but is recommended to achieve optimal results and is likely required for direct RNA analysis (e.g., samples directly taken from the environment).
Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) / RPA-Cas13	Chen et al 2024 (H9N2 subtype detection)	Yes, however, raw/unextracted samples can be used.	No, depends on sample type, but is recommended to achieve optimal results and is likely required for direct RNA analysis (e.g., samples directly taken from the environment)
Combinatorial arrayed reactions for multiplexed evaluation of nucleic acids (CARMEN) -Cas13	Ackerman et al 2020 (multiple subtype detection)	Yes	Yes
Recombinase polymerase amplification (RPA) with CRISPR/Cas12a	Zhou et al 2024 (H5 subtype detection)	Yes	No
Recombinase-aided amplification (RAA) with lateral flow dipstick (LFD)	Zhang et al 2024 (H5/H7/H9 subtype detection). Cui et al 2023 (matrix gene detection)	Yes	No
NanoString (hybridization-based detection method)	Huang et al 2017 (matrix, H1N1, and H3N2 subtype detection)	Yes	No
Metagenomics	Waller et al 2025 (H1N9 detection)	Yes	Yes

Heating/cooling required?	Other equipment needed	Laboratory requirements
Yes	qPCR instrument, qPCR reagents (often require temperature control, i.e., cold-chain support), pipettes, gloves, and tubes.	Often performed in the laboratory; however, portable qPCR instruments are available.
Yes	qPCR machine with detection capabilities for multiple dyes (e.g., FAM, CY, TAM, HEX), cold-chain-supported reagents, pipettes, gloves, and tubes.	Often performed in the laboratory; however, portable qPCR instruments are available.
Yes	ddPCR machine	Often performed in the laboratory, but new portable methods are being produced (e.g., SPEED by Zhang et al 2024).
Yes. Multiple temperature steps are required for	Minion (or other ONT instrument), computer capable of base-calling/reference alignment. Equipment for detecting RNA concentration. Pipettes, tubes, and cold-chain-supported reagents. PCR device.	Often performed in the laboratory, but a van, truck, or car could be used as a portable lab.
Yes (~65°C for 30–60mins)	Incubator, pipettors, tubes, cold-chain-supported reagents, detection device for fluoro readings (fluometer or turbidity meter).	A lab space is not required after it is established and optimised for end-users.
Yes (30/37/45°C depending on enzyme used)	Pipettors, tubes, cold-chain-supported reagents.	A lab space is not required after it is established and optimised for end-users.
Yes (37/39°C)	Depends on the preferred final results format, but often includes fluorescence and lateral flow.	A lab space is not required after it is established and optimised for end-users.
Yes (37/39/4°C)	Depends on assay but can include PCR, droplet emulsion device, and fluorescence microscope.	This is for massively multiplexed detection intended to be performed in a lab.
Yes (37/39/42°C)	Heat source and fluorescence reader.	A lab space is not required after it is established and optimised for end-users.
Yes (37/39°C)	Heat source and LFD.	A lab space is not required after it is established and optimised for end-users.
Yes (for hybridization with probes)	NanoString equipment (e.g., NanoString nCounter Digital Analyzer).	A lab space is required.
No	Extraction kit (may also require beadbeater or homogeniser), Library prep kit, Next-generation sequencer, Viral discovery computational pipelines.	No

Expertise required?	**Sample concentration requirement (i.e., what nucleic acid concentration is required for the assay to detect it?)** (Low, Med, High)
Yes, basic laboratory training needed. The type of qPCR instrument determines the amount of training.	Low
Yes, basic laboratory training needed. The type of qPCR instrument determines the amount of training.	Low
Yes, basic laboratory training needed. User-friendly interfaces are being developed for portable systems.	Low
Yes. The data analysis is complex and basic lab training is needed.	Med
Not after optimisation.	Low
Not after optimisation.	Low
Not after optimisation.	Low
Yes, basic laboratory training and fluorescence microscopy training is needed.	Low
Not after optimisation. Only detection method (e.g., lateral flow device [LFD]) training.	Low
Not after optimisation. Only lateral flow device (LFD) training.	Low
Yes, or will require a contract with a lab that can perform NanoString analysis.	Low
Yes. The data analysis is complex and basic lab training is needed.	Med

Start-up resource requirement (High/Med/Low; ex High = the assay requires a lot of time/money/equipment to develop assuming no prior work has been completed)	**Long-term resource requirement** (High/Med/Low, i.e. the amount of resources/costs needed to maintain the assay once it is developed)
Low, as assays for specific primers are already established.	Low–Med, often requires a lab and trained staff. Scalable.
Low, as assays for specific primers are already established.	Low–Med, often requires a lab and trained staff. Scalable.
Med, fewer assays are developed for ddPCR than other PCR methods and thus assay optimisation is likely required. The ddPCR system consists of several machines (droplet generator, thermocycler, and droplet reader) that are generally more expensive than PCR and qPCR machines, take up space, and require trained staff.	Med, reagents and machines are expensive to run by trained staff. Scalable.
Med–High (High for the portable lab/van setup).	Med, requires trained staff and specialised lab equipment. Not easily scalable.
High; optimisation often requires a lot of time, trained staff, and resources.	Low; once methods are optimised little resources are needed to maintain and use it. Scalable.
High; optimisation often requires a lot of time, trained staff, and resources.	Low; once methods are optimised little resources are needed to maintain and use it. Scalable.
High; reagents can be expensive and optimisation often requires a lot of time, trained staff, and resources.	Med (fluorescence-based approach); Low (lateral flow-based approach). Scalable.
High; reagents can be expensive and optimisation often requires a lot of time, trained staff, and resources.	Med–High; a lab component (with trained technicians) is required. Scalable.
High; reagents can be expensive and optimisation often requires a lot of time, trained staff, and resources.	Med (fluorescence-based approach); Low (lateral flow-based approach), the main cost is the LFD production. Scalable.
High; reagents can be expensive and optimisation often requires a lot of time, trained staff, and resources.	Low; once LFDs are created little maintenance is needed. The main cost is the LFD production. Scalable.
High; probe design is labour intensive and expensive. However, published probes for AIV are available.	Med–High; once probes are designed little maintenance and less time is required, but samples will still need to be processed in a lab with NanoString equipment. Scalable.
High; requires access to a Next Generation Sequencer and is expensive	High; expensive to pay for Next Generation sequencing, not easily scalable without a robot for RNA extractions

How applicable is it for management end-users? (10 is easy and 0 is not possible with the hypothetical end-user set as a regional council or middle-income gov agency)	**How easy is it to be deployed in the field by end-users?** (10 is easy and 0 is not possible with the hypothetical end-user set as a standard field ranger)
8, training can be resource intensive and trained staff can be difficult to maintain.	6; In-field equipment/reagents are often expensive.
7, training can be resource intensive and trained staff can be difficult to maintain. qPCR instruments that are calibrated and capable of detecting multiple dyes are more difficult to obtain than singleplex-only qPCR instruments.	6; In-field equipment/reagents are often expensive.
6	0 currently; however, if systems like SPEED (Zhang et al, 2023) are further developed and made easily-available for purchase, then ddPCR technology has the potential to be extremely user friendly.
2–3	3
8	8
7	7
8 (optimised lateral-flow detection) or 3 (fluorescence detection)	8
3–4, may be useful to end-users who need quick information about subtype-level diversity.	0, it may be an option for end-users who prefer sending samples to a lab for processing.
8 (optimised lateral-flow detection) or 3 (fluorescence detection)	8
7/8	9
3–4, may be useful to end-users who need to quickly gain information about subtype-level diversity.	0, it may be an option for end-users who prefer sending samples to a lab for processing.
1	0, currently can't be sequenced in field

Has it been used for environmental AIV detection?	**Environmental sample application. (10 is very applicable and 0 is not)**	**Overall advantages** (ideal assay = cheap, easy, and able to detect AIV from low concentration samples)
Yes	9	It is a well-tested method proven to produce true results (e.g., popular during the covid pandemic). It can detect low concentration targets in a sample, and a cold-chain is not required. Equipment requirements for its point of need application are readily available. Robust to various sample
Yes	8	Once an assay is optimised, it is a quicker method than singleplex qPCR for obtaining multiple detection results (e.g., H7 and H5 gene presence in a sample); it is possible to obtain multiple answers from a single reaction. It is well tested and proven to produce true results. There are
Yes. In some places, such as Estonia, a H5N1-specific ddPCR test was developed	5	Unlike PCR and qPCR, ddPCR directly counts the number of positive droplets and allows for absolute quantification of the target molecules without depending on a standard curve. It offers high sensitivity (often more precise than qPCR) and is often more tolerant to sample inhibitors than other PCR methods.
Yes	2	It can provide rich information (e.g., whole genomes) and it is able to run sequence data against reference genomes, potentially providing instant subtype/strain-specific detection results (e.g., haemagglutinin [HA] subtype). It can obtain this information in the field. Robust to various sample
Not to our knowledge	5	End-user friendly after training. Easy to read results with the right conditions. LAMP products can be quantified. Implementation is easier if similar optimised LAMP assays (e.g., those with closely-related target organisms) are already established. Interchangeable with PL-RCA in most cases but the format is slightly more user friendly than PL-RCA.
Not to our knowledge	5	Can provide SNP-level detection. Interchangeable with LAMP in most cases but massive multiplexing is easier with PL-RCA than with LAMP. Is highly specific/sensitive. Good for detecting very specific sequence types. Possible to receive results in < 1 hour.
Not to our knowledge	7	Offers an ultrasensitive and specific approach to detect any DNA/RNA down to the attomolar range. Highly portable and isothermal at 39°C. Artificial intelligence pipelines are available that can facilitate the full assay design. It can provide a result (within attomolar range) in < 1 hour.
Not to our knowledge	6	Scalable, low-cost deployment of Cas13-based diagnostics. crRNA multiplexing can overcome the challenges of sequence heterogeneity of the target locus (e.g., variances between different virus targets). Can detect up to 169 different subtypes in one reaction in 4 hours. Robust to
Not to our knowledge	7	No PCR machine needed. Ultrasensitive and specific approach to detect any DNA/RNA down to the attomolar range. Potentially lower costs to run. Rapid detection (< 30 min is possible). Highly-portable/field-friendly application. Robust to various sample types.
Not to our knowledge	7	No PCR machine needed. Potentially lower costs to run. Rapid detection (> 30 min is possible). Field-friendly application. Obtaining RAA reagents may be easier than RPA reagents. Robust to various sample types.
Not to our knowledge	6	No PCR/amplification needed. Has the potential to assess up to 800 targets and analyse low-grade samples (e.g., formalin-fixed paraffin-embedded). NanoString nCounter tech note recommends samples with "5-10 ng in a 100 ng sample no larger than 5 µL". There is often
Yes	6	NGS enables unbiased detection of both known and novel viruses, making it highly sensitive even for low-abundance pathogens. It provides whole-genome characterization, allowing for phylogenetic analysis, mutation tracking, and monitoring of reassortment events in influenza viruses. Additionally, NGS can detect multiple pathogens simultaneously, making it ideal for studying co-infections and conducting virome analyses

Overall disadvantages (ideal assay = cheap, easy, and able to detect AIV from low concentration samples)
Potentially slow (dependant on qPCR device, sometimes takes a full day). Slower assays result if they are not optimised appropriately or need to be developed. As with most methods, cross contamination is possible.
Developing a multiplex qPCR assay is often complicated and needs to work with the requirements of the multiplex qPCR instrument. Published assays are easier to use, but still must be optimised (e.g., for the machine and master mix). Commercial kits are quick, reliable, and easy to use, but
The ddPCR system requires more machines and is often more expensive than qPCR and PCR systems. The workflow is more time consuming than other PCR methods. It requires more pipetting and transfer steps that can expose the sample to contamination and other errors. Droplet variability in size and shape can affect the assay robustness/reproducibility. Generally lower throughput than
It requires trained operators, specific equipment, lab infrastructure, cold-chain reagent storage, and it is relatively expensive. Its data analysis is often complex and time consuming unless specific sequencing alternatives (such as adaptive sequencing) are used.
Usually not as sensitive as qPCR. Often needs optimization dependant on target, sample, and primers. It is pH sensitive so certain sample types/extraction buffers can affect the colorimetric result. Colour metric assay detection can not be used with wide ranging sample types (not robust); it is nearly impossible to know what is being detected in the field. LAMP is extremely sensitive but primer design is the limiting factor to achieving sensitivity. It can take months of design work, target sequence selection, and assay optimisation to achieve a successful end user friendly test
Multiple steps not easily adaptable to one-pot/one reaction formats (although possible). Likely more expensive than other isothermal methods. Read-out requires extra equipment, but colorimetric reactions possible. Colorimetric assay detection has same limitations as LAMP (e.g., sample difficulties with inconsistent sample types/robustness). Attomolar range possible, but only with
Prone to RNAse contamination and requires molecular grade components to work smoothly, which increases deployment costs.
Specific equipment required. Only fluorescence readout is available. Not designed for in-field detection of samples.
Is generally more efficient for shorter amplicons (~100-200 bp) and can have lower amplification efficiency when dealing with longer sequences. Often limited reagent availability. Short primers (30-35 bp) can limit target selection. Since reaction occurs at a constant temperature there is a higher risk of cross-contamination compared to PCR. Occasionally the visual interpretation of results can be less precise than with qPCR. Protocols are not standardised and optimization is needed for
Largely the same cons/limitations as RPA method. Prone to RNAse contamination and requires highly pure components to work smoothly, which increases deployment costs.
Requires NanoString equipment, panel/probe design, and laboratory space. Not as sensitive as qPCR. Reagents and equipment are expensive.
One major drawback is that NGS cannot be easily used in the field due to the need for specialized laboratory equipment, controlled environments, and trained personnel, making real-time outbreak surveillance challenging. The cost of NGS remains significantly higher than traditional diagnostic methods like PCR or rapid antigen tests, with expenses arising from sequencing reagents, high-performance computing resources, and bioinformatics expertise required for data analysis. Additionally, the turnaround time for results is longer, as sequencing, processing, and interpretation