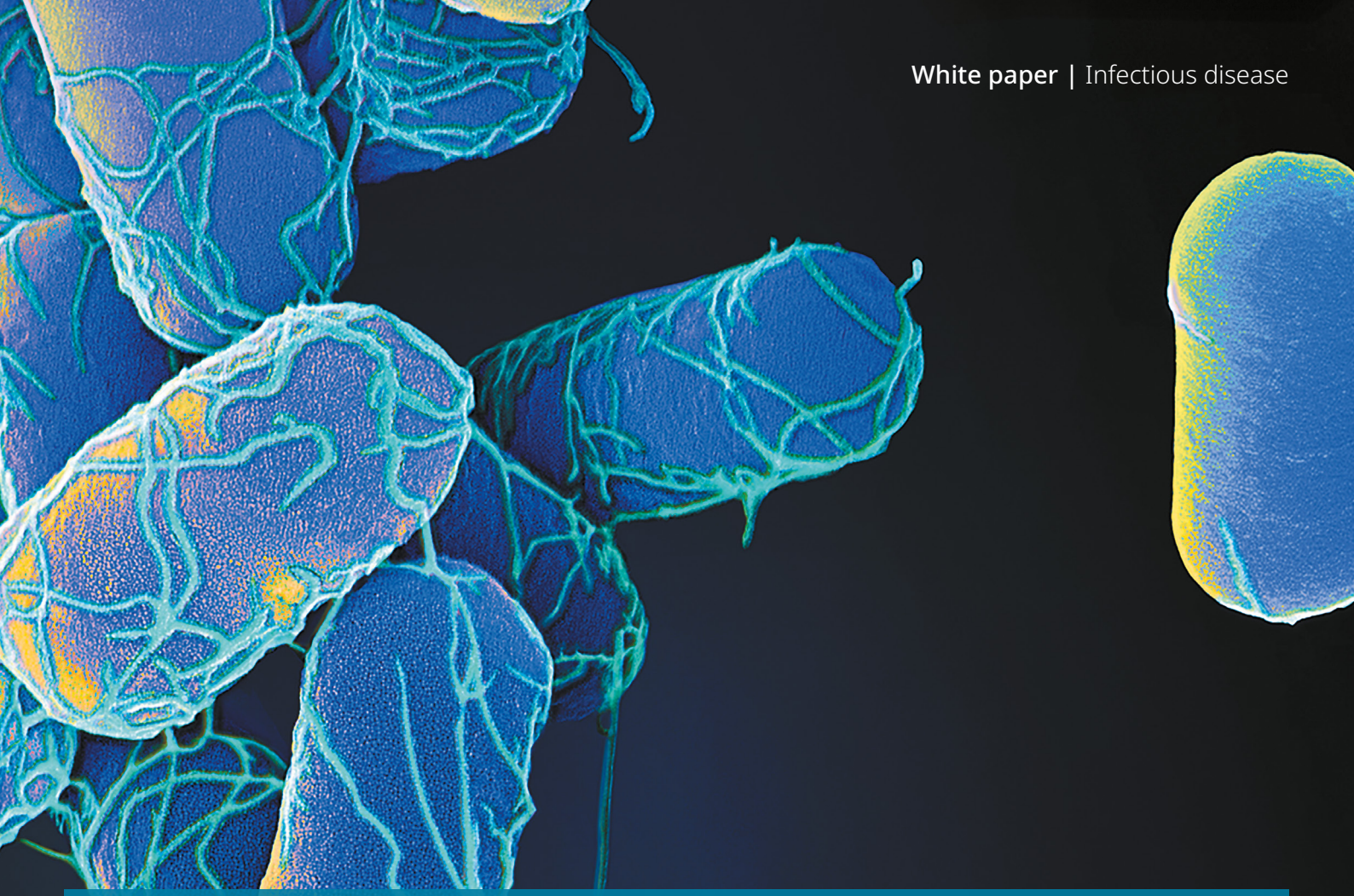
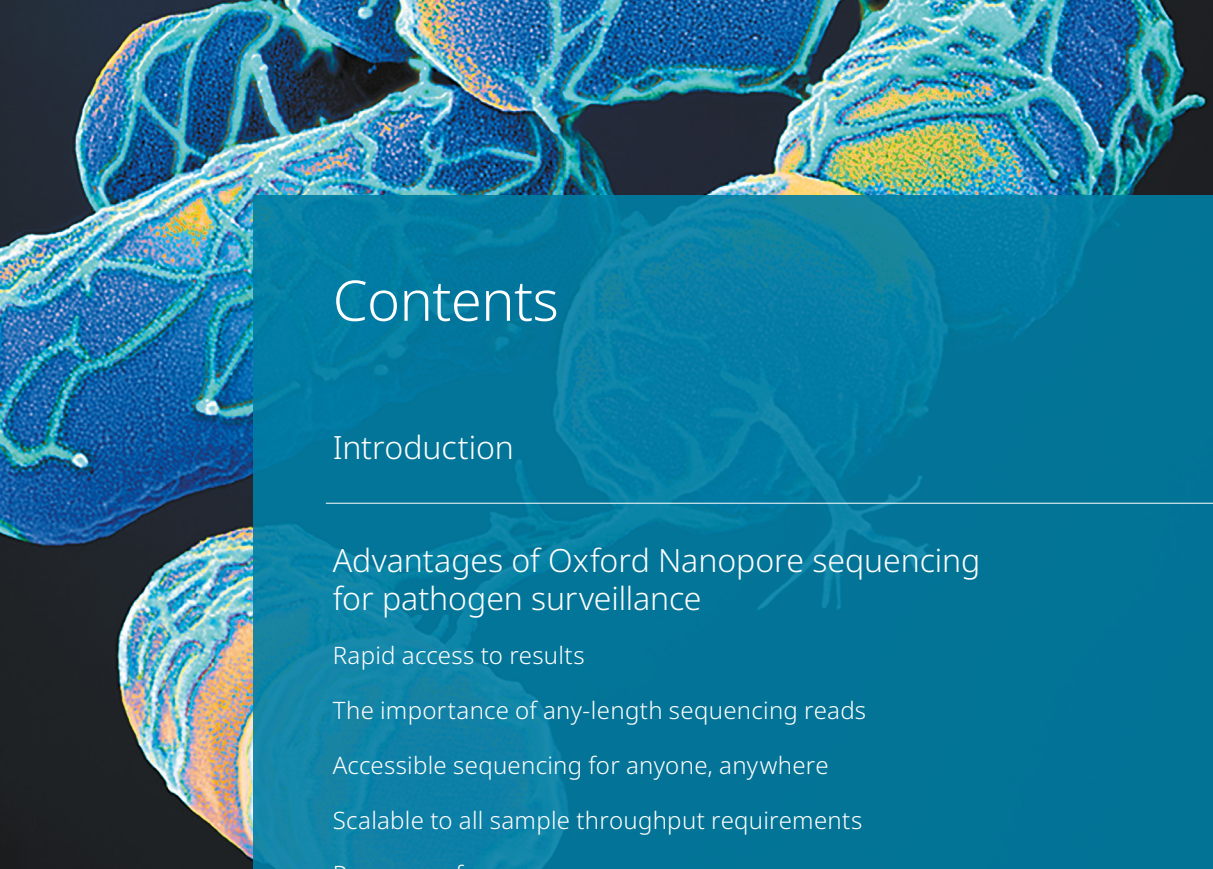


Delivering the future of genomic pathogen surveillance with Oxford Nanopore sequencing

Oxford Nanopore Technologies



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Introduction

Towards the end of 2019, news first broke of a novel, highly virulent pathogen that could cause serious human respiratory distress, and in some cases, death. That pathogen, SARS-CoV-2 — a name now familiar to all — has since been attributed to over 6.5 million deaths and will cost the global economy an estimated $28 trillion in lost output by the end of 2025[1].

Serious infectious disease outbreaks have been an ever-present threat throughout human history and, driven by factors such as increasing globalisation, population growth, urbanisation, and climate change, that threat is increasing. The last two decades alone have witnessed numerous infectious disease epidemics, including severe acute respiratory syndrome (SARS; 2003), H1N1 swine flu (2009), Ebola (2014–2018), Zika (2015), and mpox (2022–2024) — each with significant potential for global concern.

The rapid advancement of genomic sequencing technologies means that it is now possible to characterise pathogens more easily and comprehensively than ever before; however, due to high costs and infrastructure requirements, global access to such technologies has traditionally been limited to well-funded, centralised laboratories. Furthermore, to inform public health response to an infectious disease outbreak, it is critical that genomic information is generated and delivered quickly. This is a significant challenge for legacy sequencing technologies that require time-consuming sample preparation workflows and lengthy, fixed run times, only after which the data can be delivered and analysed.

This review outlines how Oxford Nanopore sequencing overcomes the challenges associated with legacy sequencing technologies to deliver comprehensive, affordable, and real-time analysis of human and animal pathogens. The faster information can be learnt about a pathogen, the faster appropriate steps to manage an outbreak can be taken. This is why effective genomic surveillance to monitor potential new disease threats is so important and why preparedness is so vital to lessen the damage infectious diseases can cause. Specific case studies reveal how researchers are deploying nanopore sequencing to better identify, characterise, and track a range of pathogens to help minimise the threat and impact of disease outbreaks.

‘Building a strong and resilient global sequencing network can maximize the public health impact of sequencing, not only for SARS-CoV-2 but also for future emerging pathogens’

World Health Organization[2]

Advantages of Oxford Nanopore sequencing or pathogen surveillance

Rapid access to results

Time to result is of critical importance during a novel infectious disease outbreak. Firstly, the causative pathogen must be identified. This is most commonly achieved using a metagenomic sequencing approach, whereby the nucleic acids from all organisms in a clinical research sample are analysed (see **Case study 1**)[3]. Metagenomic sequencing requires no prior knowledge of the infectious organism and is also useful for the identification of coinfections, where multiple different pathogens may be present in a single sample. For example, deploying real-time Oxford Nanopore metagenomic sequencing of research samples from patients with COVID-19 in intensive care units has been shown to enable the identification of secondary infections, antimicrobial resistance (AMR), and hospital-associated transmission[4].

Once the pathogen responsible for a disease has been identified, it is possible to design nanopore sequencing assays that specifically detect and amplify only the genome of the pathogen of interest (see **Case studies 1** and **3**)[5,6]. Such targeted assays often support faster time to results and lower sequencing and analysis costs. They also enable the analysis of samples with lower levels of a pathogen.

Oxford Nanopore sequencing offers several advantages over alternative sequencing platforms for pathogen genome analysis, including real-time data streaming. Unlike legacy sequencing technologies that deliver data in bulk at the end of a lengthy sequencing run, Oxford Nanopore technology provides access to data as soon as it is generated. Using nanopore sequencing, researchers have been able to identify pathogens from complex metagenomic samples in minutes, with further insights, such as AMR profiling, becoming available as soon as sufficient data has been generated (see **Case study 1**)[3,7–10]. The facility of real-time analysis to deliver significantly faster access to actionable results can be crucial in outbreak scenarios.

As discussed, turnaround times can be reduced even further when there is a known suspected pathogen. Oxford Nanopore sequencing has the unique capability of adaptive sampling, an on-device target enrichment methodology that allows researchers to target specific genomic regions without upfront sample enrichment. Adaptive sampling leverages real-time basecalling to selectively sequence or reject DNA molecules as they pass through the nanopore (**Figure 1**). Rejection of molecules that fall outside of the chosen criteria (e.g. non-homology to target sequence) is achieved through reversing the current applied to the individual nanopore, thereby making it available to sequence an alternative DNA fragment. Researchers are incorporating adaptive sampling into nanopore workflows to rapidly investigate outbreaks from foodborne pathogens without the need for time-consuming culture-based enrichment[11].

> ‘Identification of pathogens was possible after 10 minutes of sequencing and all predefined AMR-encoding genes and plasmids from monoculture experiments were detected within one hour using raw nanopore sequencing data’
>
> Taxt, A.M. *et al*.[7]

Oxford Nanopore Technologies offers a range of streamlined library preparation kits (which can be easily automated for a range of large liquid handlers) that further shorten time to result when compared with legacy sequencing technologies. For example, Rapid Sequencing Kits, which include multiplexing options for cost-effective analysis of multiple samples in a single run[12,13], allow researchers to prepare their purified sample for Oxford Nanopore sequencing in as little as 10 minutes[14]. In addition, although storage at –20°C is recommended, the kit can be safely stored at room temperature for up to two weeks or at 4°C for up to four

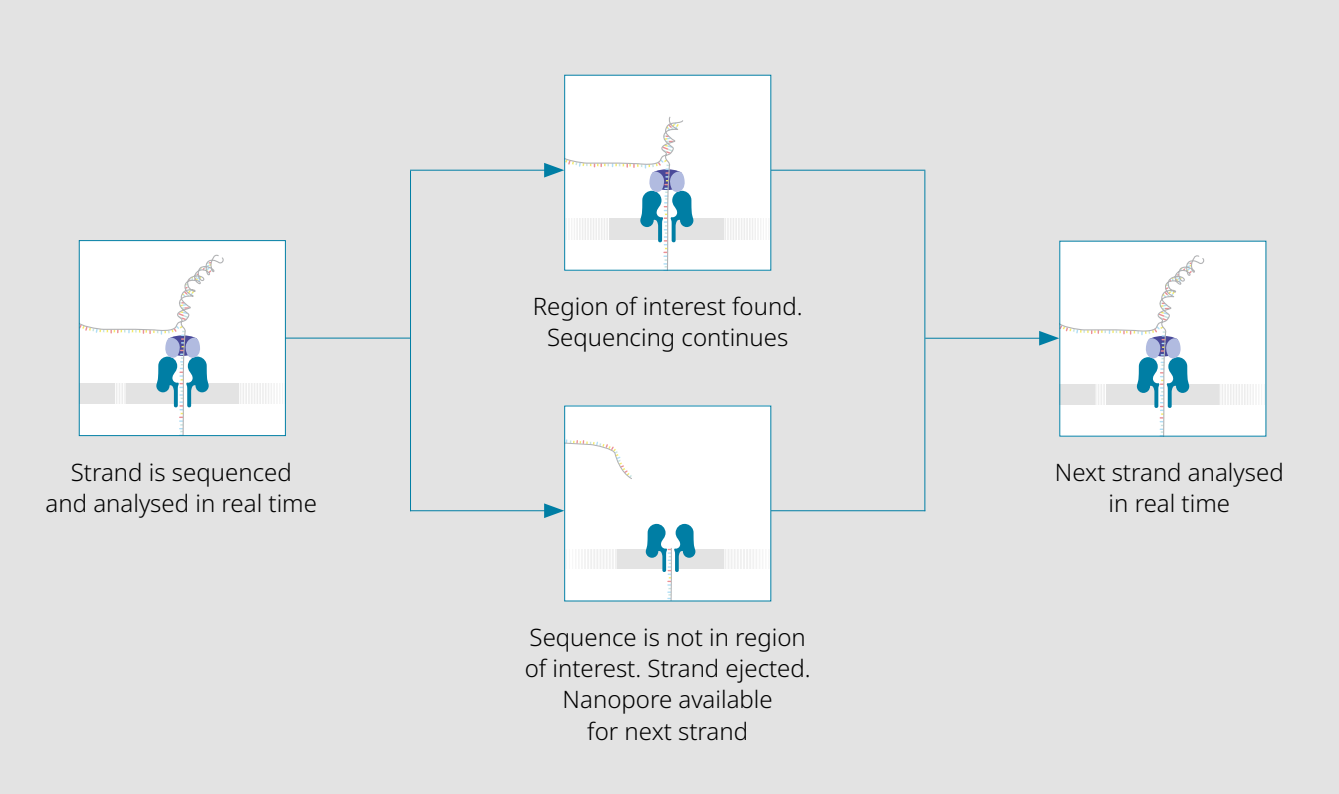


Figure 1. Adaptive sampling is a unique, on-device approach to targeted sequencing that requires no upfront library enrichment steps. Using real-time basecalling, DNA fragments can be accepted or rejected for further sequencing based on their initial sequence composition. A flexible list of regions to be enriched or rejected, provided as a BED file, is required. Adaptive sampling can be implemented in advance of — or even during — a run to increase coverage of specific targets.

weeks, enabling researchers to more easily deploy nanopore sequencing in the field or in remote, resource-limited environments.

Further efficiencies can be obtained using the EPI2ME™ data analysis platform from Oxford Nanopore. EPI2ME offers a range of best-practice workflows to enable Oxford Nanopore data analysis for all levels of expertise. The preconfigured analysis workflows are free to access from a desktop application with an intuitive, easy-to-use graphical interface or the command line and can be deployed locally or in the cloud. A growing number of workflows for pathogen surveillance are available (**Table 1**).

'The integration of adaptive sampling with metagenomics presents a valuable strategy for more efficient and targeted analysis of microbial communities in foodborne outbreaks, contributing to improved food safety and public health'

Buytaers, F.E. *et al*.[11]

Workflow	Description
wf-bacterial-genomes	Bacterial genome assembly and annotation, including AMR profiling
wf-metagenomics and wf-16s	Species identification (metagenomics and 16S)
wf-tb-amr	AMR profiling of *Mycobacterium tuberculosis*
wf-flu	Influenza typing
wf-mpx	Mpox reference alignment
wf-artic	SARS-CoV-2 variant identification

Table 1. EPI2ME workflows provide a streamlined pipeline for analysing nanopore sequencing data of potential pathogens. They offer intuitive point-and-click analysis and generate comprehensive reports for rapid insights and actionable results.

Find out more about data analysis options for nanopore sequencing: nanoporetech.com/analyse.

The importance of any-length sequencing reads

The ability of a pathogen to cause disease or evade treatment is often encoded in highly repetitive genomic regions, such as those flanking AMR genes and virulence factors. Genomic repeats also frequently serve as sites for structural variation, which contributes to rapid microbial evolution[15].

To fully characterise and functionally annotate a given pathogen, it is imperative to create a complete genome assembly; however, legacy sequencing technologies, which typically process short (<300 bp) DNA fragments, struggle to resolve repetitive regions and structural variants (SVs), resulting in highly fragmented genome assemblies[16].

In contrast, Oxford Nanopore sequencing technology processes the entire DNA (or RNA) fragment that is presented to the nanopore, regardless of its size. Complete fragments of hundreds of kilobases are routinely processed and ultra-long read lengths more than 4 Mb have been generated[17]. These any-length reads can span entire regions of repetitive DNA and SVs (**Figure 2**), making it possible to generate complete, closed, reference-quality microbial genome assemblies[18]. To take advantage of this capability, Oxford Nanopore has developed the nanopore-only microbial isolate sequencing solution (NO-MISS)[19]. This rapid, flexible, end-to-end workflow for whole-genome sequencing of microbial isolates produces reference-quality genome assemblies in one experiment and in-house, without the need to outsource or use multiple techniques for validation.

In addition, unlike legacy techniques, nanopore sequencing does not require DNA amplification, eliminating a key source of sequencing bias. In a study by Browne *et al.*, out of five sequencing technologies tested, only Oxford Nanopore sequencing exhibited no GC bias[20].

Any-length reads have been demonstrated to offer a significant advantage in microbial identification, particularly in distinguishing between closely related species or strains[21]. This facility also supports the generation of high-quality metagenome-assembled genomes (MAGs) from complex, mixed microbial samples[18].

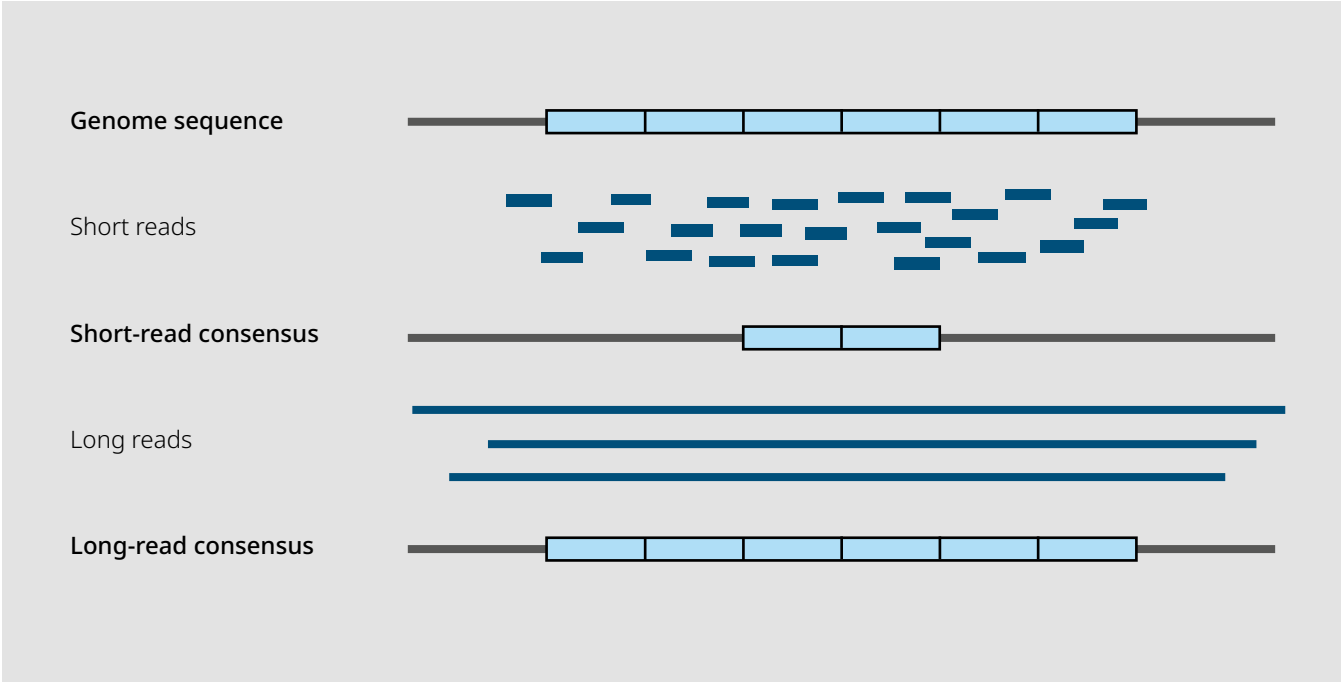


Figure 2. A schematic highlighting the advantages of long reads in *de novo* assembly of repetitive regions. Repeat and high-identity regions are often collapsed during the assembly process when using legacy short-read sequencing technology. In contrast, unrestricted read lengths are more likely to incorporate the whole repetitive region (blue boxes), allowing more accurate assembly. Figure adapted from Kellog[22].

Find out more about metagenomic research with nanopore sequencing: nanoporetech.com/metagenomics.

Accessible sequencing for anyone, anywhere

Establishing and maintaining local capacity for rapid deployment of pathogen genomic sequencing can provide a critical time advantage in containing outbreaks; however, legacy sequencing technologies are typically expensive, bulky, fragile, cumbersome to ship, and require substantial site infrastructure. This, coupled with a general lack of facilities and trained personnel, make such technologies difficult to deploy in remote locations from where many novel infectious diseases emerge. Furthermore, the requirement for precise calibration by trained engineers means that most legacy sequencing platforms are incompatible with rapid deployment.

Oxford Nanopore sequencing technology resolves these challenges, making sequencing accessible to anyone, anywhere. The MinION™ Mk1D device offers affordable, portable sequencing for every laboratory (**Figure 3**), allowing sequencing to be undertaken at sample source — including in the field, resource-limited settings, decentralised laboratories, and healthcare research settings. This saves considerable time and cost associated with shipping samples to a centralised sequencing facility.

'The portability and ease of use of the nanopore MinION sequencing system was first demonstrated by Quick and associates, who successfully developed a portable Ebola virus genome surveillance system in Guinea[23]'

Low, L. *et al*.[24]

Running off a laptop or desktop computer, USB-powered MinION devices are ideal for local implementation and resource-limited settings that ordinarily might not have access to such powerful and versatile sequencing technology.

Find out more about portable nanopore sequencing: nanoporetech.com/portable-sequencing.

Scalable to all sample throughput requirements

Routine pathogen sequencing during an outbreak is vital to identify novel variants that may increase pathogenicity or reduce the effectiveness of approved diagnostic assays or therapeutics. In addition, genomic data allows patterns of transmission to be elucidated to support outbreak management. The power of genomic epidemiology to answer these questions relies on the rapid analysis of high numbers of samples. For example, at an early stage of the COVID-19 pandemic, the European Commission urged member states to sequence 10% of SARS-CoV-2-positive test results[25].

'The key strengths of [Oxford Nanopore Technologies] sequencing platforms are that they are a low capital investment, have competitive per sample sequencing cost when multiplexing, the possibility of bias-free PCR library preparation, … fast turnaround time, use of long reads to resolve complex genomic loci, and the ability to investigate methylation status'

Dippenaar, A. *et al.*[26]

As with legacy sequencing platforms, nanopore sequencing allows multiple samples to be analysed in a single run with multiplexing. However, unlike legacy platforms, nanopore flow cells, with active pores remaining after a sequencing run, can be washed and reused. This allows throughput to be scaled to suit the number of samples available — further reducing time to result and costs.

In addition to delivering portable sequencing devices that can be deployed at sample source, Oxford Nanopore provides a range of devices that offer fully scalable, cost-effective, high-throughput sequencing to suit all requirements (**Figure 3**). The benchtop GridION™ device can run up to five MinION Flow Cells simultaneously. MinION Flow Cells typically deliver 15–35 Gb* of data and each flow cell can be operated independently or in parallel.



Figure 3. Oxford Nanopore sequencing devices (back row: from left to right): PromethION 2 Integrated with capacity for up to two PromethION Flow Cells; the high throughput PromethION 24 with capacity for up to 24 PromethION Flow Cells; and the GridION with capacity for up to five MinION Flow Cells; (front row from left to right) the portable MinION Mk1D and the PromethION 2 Solo with capacity for up to two PromethION Flow Cells.

Find out more about the full range of Oxford Nanopore sequencing devices: nanoporetech.com/sequence.

The PromethION™ family of devices offer the highest sequencing outputs, ideal for generating high-quality MAGs from complex samples, such as bodily fluids, faecal samples, and environmental samples such as wastewater, where the pathogen DNA/RNA may represent just a small fraction of the nucleic acids present. Running up to two or 24 high-output flow cells, respectively, the PromethION 2 and PromethION 24 devices provide the ultimate sequencing power and flexibility. Each PromethION Flow Cell typically delivers 100–200 Gb* of data and can be run independently or in parallel.

These compact benchtop systems offer the facility to start and stop flow cells at any time, which eliminates the delays associated with sample batching — ideal for both small- and large-scale sample analysis. These devices† also come complete with integrated, high-performance data processing modules, reducing the need for complex IT infrastructure.

*Flow cell output will vary according to library type, run conditions, and other sample-specific factors. For more information on data output, visit: nanoporetech.com/products/specifications.

†Includes GridION, PromethION 2 Integrated, and PromethION 24. The PromethION 2 Solo can be connected to a GridION device or existing computer infrastructure.

Proven performance

One of the earliest examples of the suitability of Oxford Nanopore Technology for pathogen and outbreak sequencing was evidenced in the 2015 Ebola epidemic in West Africa, where researchers from the University of Birmingham and Public Health England utilised the MinION as part of a mobile sequencing unit in Guinea[23]. Within two days of arriving on site, the team were able to perform real-time genomic surveillance, delivering sequencing data within just 15–60 minutes of sample preparation.

'We show that real-time genomic surveillance is possible in resource-limited settings and can be established rapidly to monitor outbreaks'

Quick, J. *et al*.[23]

Since then, Oxford Nanopore sequencing has delivered actionable insights for human and animal pathogen surveillance and outbreak research around the world, including viral pathogens (e.g. Zika virus[27], rabies virus[28,29], Lassa virus[30], swine influenza[31], mpox virus[32], and SARS-CoV-2[33]), bacterial pathogens (e.g. *Mycobacterium tuberculosis*[34], *Salmonella* spp.[35], and *Enterobacteria* spp.[36]), and fungal pathogens (e.g. *Candida* spp.[37,38]) (**Figure 4**).

Genomic surveillance of circulating viruses can enable the development of candidate vaccine viruses, which form the basis of a new vaccine before an outbreak occurs. Using the MinION device as part of a mobile sequencing station at a large swine exhibition, researchers in the USA identified three different swine influenza A viruses, one of which was genetically distinct to the most similar candidate vaccine virus — including differences in parts of the genome known to determine vaccine efficacy[31]. Using this information, the US Centers for Disease Control and Prevention (CDC) were able to develop a new candidate vaccine virus. Importantly, within 18 hours of setting up the mobile facility, the researchers had generated and characterised complete, full-length consensus genomes.

'Had this virus caused a severe outbreak or pandemic, our proactive surveillance efforts and vaccine derivation would have provided an approximate 8-week time advantage for vaccine manufacturing'

Rambo-Martin, B.L. and Keller, M.W. *et al*.[31]

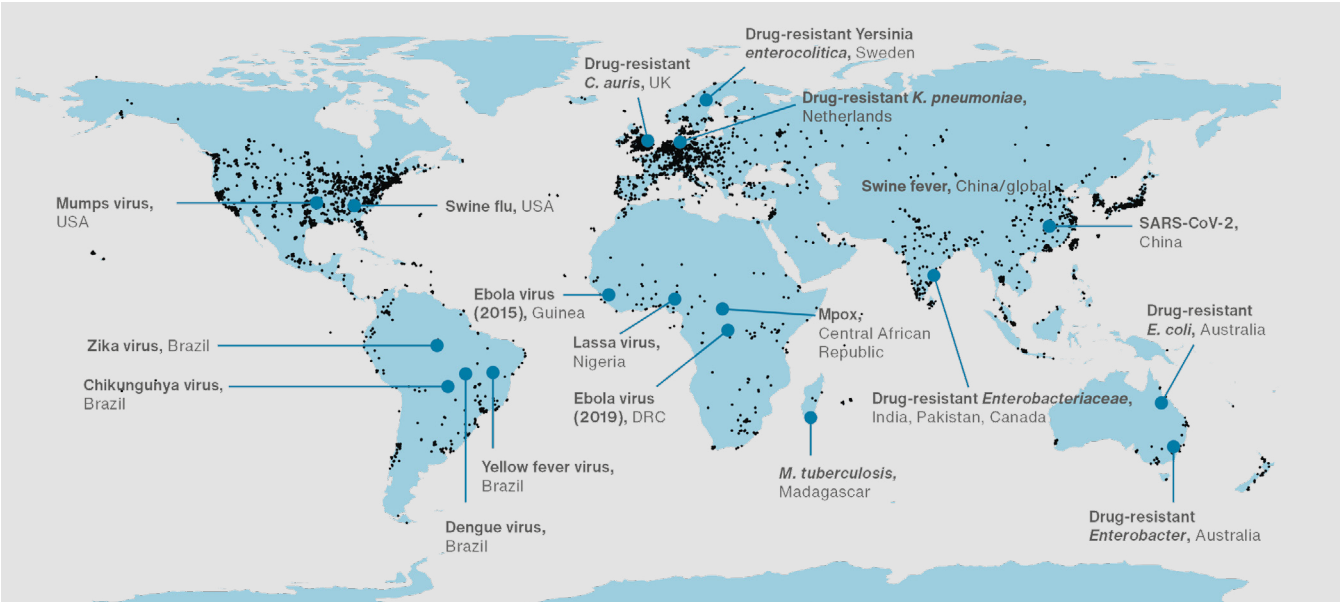


Figure 4. Selected human pathogen outbreaks analysed using Oxford Nanopore sequencing. Affordable, real-time nanopore sequencing is being used extensively around the world for rapid insights into human and animal pathogens. Dots represent Oxford Nanopore sequencing users for a range of applications, including outbreaks. Data correct as of October 2022.

Find out more about infectious disease research with nanopore sequencing: nanoporetech.com/infectious-disease.

Methylation calling as standard

Base modifications (e.g. N6-methyladenine and 5-methylcytosine) in microbial genomes have been linked to a range of functions, including gene expression, AMR, and in prokaryotes, protection against bacteriophages. Research has also suggested that modified bases within host and pathogen genomes underlie intricate biological interactions that could provide new markers for disease severity or novel therapeutic targets[39]. As a result, the detection of base modifications in the genomes of pathogens and their hosts is becoming an area of increasing interest; however, DNA/RNA amplification, which is a requirement for legacy sequencing technologies, removes these modifications, meaning they can only be studied by using additional time-consuming and costly sample processing and sequencing steps.

Oxford Nanopore sequencing does not require amplification, enabling base modifications to be preserved and directly sequenced. MinKNOW™ — the software that controls Oxford Nanopore sequencing devices — performs raw data acquisition and basecalling, meaning that both genetic and epigenetic data are generated in the same sequencing run, in real time. Base modification detection not only provides greater depth of genomic characterisation but can also be used to support metagenomic contig binning, association of mobile genetic elements with their host genomes, and the identification of misassembled metagenomic contigs.

'With longer reads as well as richer information of additional epigenetic modifications, developments in sequencing technology could bring another round of revolution in metagenome as well as virome investigations'

Cao, J. and Zhang, Y. *et al*.[40]

Find out more about direct methylation detection with nanopore sequencing: nanoporetech.com/epigenetics.

Case study 1

Oxford Nanopore sequencing improves surveillance of serious respiratory diseases and characterises the causative agents

Respiratory tract infections are a leading cause of illness and death — in particular, lower respiratory tract infections (LRTIs), which accounted for infections in over 488 million people and 2.4 million deaths in 2019[41]. Respiratory infections are caused by a variety of infectious agents — bacterial, viral, and fungal — but identifying which agent is the cause is not always feasible. In China, there are approximately 10 million new cases of LRTIs each year, with a 30% mortality rate, but the cause of 30% of total LRTI cases is unknown[3]. This is due to the limitations of successfully culturing and identifying microorganisms using traditional methods, such as PCR and serological techniques[3].

Using a metagenomic approach, Luo *et al.* tested the potential of Oxford Nanopore sequencing to identify LRTIs[3]. They performed nanopore sequencing of 70 bronchoalveolar lavage fluid research samples and compared performance with traditional culture-based methods and qPCR. Libraries were prepared using the Rapid PCR Barcoding Kit and sequenced on a MinION; real-time pathogen identification was performed during sequencing. Using Oxford Nanopore sequencing, pathogens were identified in 59 samples (**Figure 5**); qPCR identified pathogens in 56 samples, while culture-based methods found pathogens in just 18 samples. In this study, Oxford Nanopore sequencing outperformed culture-based methods and, in addition, could also identify AMR genes and viruses. Moreover, the turnaround time for the nanopore sequencing method was six hours, compared with three days using traditional methods, demonstrating the future potential for this approach to rapidly identify pathogens.

Legionnaires' disease is a serious respiratory infection caused by *Legionella* spp. bacteria and can result in severe pneumonia and death. To characterise the diverse strains of this single respiratory disease, Krøvel *et al.* used Oxford Nanopore sequencing to sequence type *Legionella pneumophila*[42]. *L. pneumophila* can be sequence typed through characterisation of seven genes, *flaA, pilE, asd, mip, mompS, proA,* and *neuA,* with at least 3,100 sequence types identified so far[42]. However, it is challenging to identify *L. pneumophila* by sequence typing using short-read technology, as the *mompS* gene is present in two copies in the genome. When the *mompS* gene copies are non-identical, they may be misassembled or non-typeable using short reads, because they cannot cover the entire *mompS* gene region. The authors sought to overcome these limitations by using Oxford Nanopore sequencing.

Of a collection of 233 *L. pneumophila* genomes that were sequence typed with short reads and Sanger sequencing, 35 were identified as being inconclusive due to discrepancies between the technologies; these were taken forward for Oxford Nanopore sequencing. Libraries were prepared with the Ligation Sequencing Kit and sequenced on a GridION device. Using long nanopore reads, they were able to resolve the *mompS* region, including in 27 isolates that contained non-identical copies of the *mompS* gene. The sequence types called with Oxford Nanopore sequencing were consistent with hybrid assemblies; the authors concluded: 'we therefore propose [nanopore] sequencing as an alternative method to perform *L. pneumophila* [sequence-based typing] to overcome the *mompS* challenge observed with short-reads'.

Avian influenza (AIV) is a highly infectious respiratory disease, which usually infects wild or domestic birds, but also has the potential to cause a serious outbreak among humans. Novel genotypes of AIV have previously caused outbreaks in humans, with a lethal variant discovered in Cambodia in 2023[43]. An outbreak of AIV in Europe in 2021–2022 was devastating to bird populations, with 40 million birds culled[43]. Given the economic impact of the disease on agriculture and the potential for a disease epidemic in humans, real-time genomic surveillance is crucial to track circulating stains of AIV. An important facet of this is the ability to rapidly identify links between outbreaks, to determine direct or indirect farm-to-farm contamination routes.

Croville *et al.* developed an amplicon-based Oxford Nanopore sequencing workflow for the rapid genetic typing of AIV strains, which they tested on 30 field samples collected from AIV outbreaks in France[5]. Libraries were sequenced in multiplex on a Flongle Flow Cell; real-time alignment was then performed using MinKNOW, which allowed the sequencing run to be stopped as soon as enough data was generated. This method enabled sequential analysis of AIV during outbreaks and the ability to detect minority variants. With a turnaround time of less than 24 hours when sequencing 30 samples, this workflow demonstrates the ability of Oxford Nanopore sequencing to provide crucial information for surveillance during an active outbreak.

'This analysis showed that all the isolates that were misassembled or untypeable when using short-read assembly methods were typeable with [Oxford Nanopore Technologies] -only assemblies'

Krøvel, A.V. *et al.*[42]

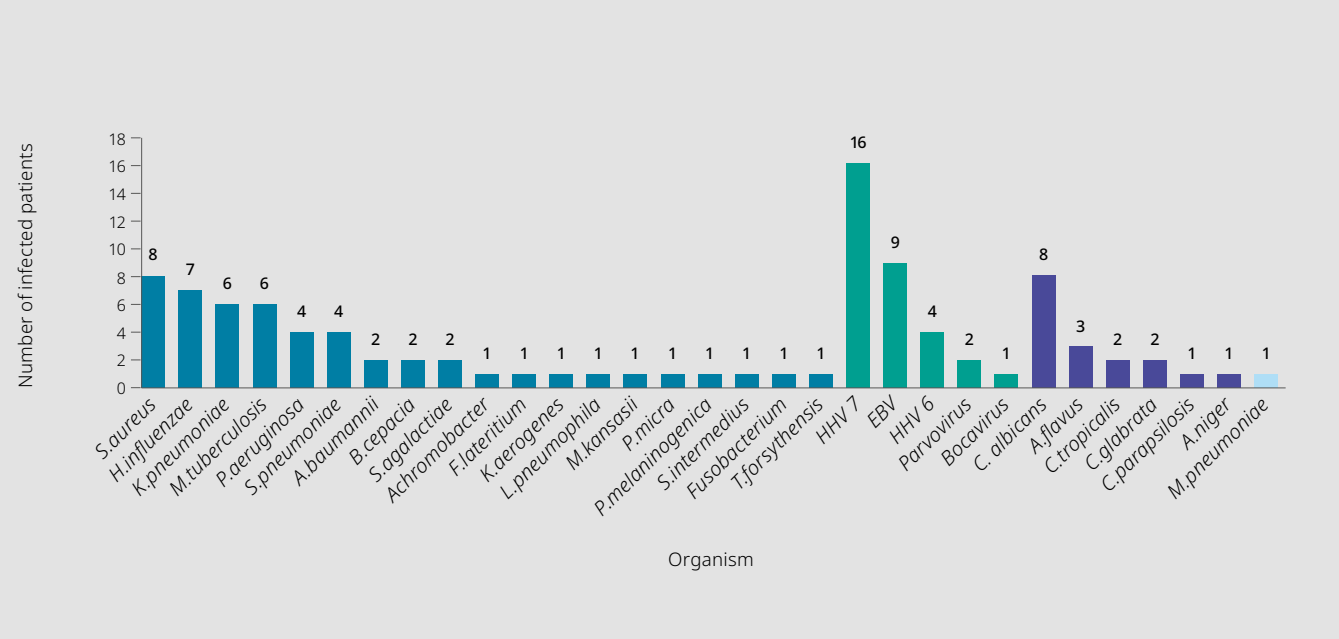


Figure 5. Detailed species classification of pathogens detected by Oxford Nanopore sequencing of bronchoalveolar lavage fluid research samples. The blue, green, purple, and light blue columns indicate bacteria, viruses, fungi, and mycoplasma, respectively. Figure from Luo *et al.*[3] and available under Creative Commons license (creativecommons.org/licenses/by/4.0).

Case study 2

Rapid epidemiological characterisation of a *Streptococcus pyogenes* outbreak using nanopore whole-genome sequencing

Streptococcus pyogenes, also known as Group A *Streptococcus* (GAS), is a gram-positive bacterium, often implicated in minor ailments such as sore throats, tonsillitis, and scarlet fever[44]. However, infection with GAS can occasionally lead to life-threatening illnesses such as streptococcal toxic shock syndrome or necrotising fasciitis[44], and in such cases is referred to as invasive GAS. GAS is susceptible to antibiotics, but treatment must be administered rapidly to avoid potentially severe complications or death; GAS infections are responsible for more than 500,000 deaths worldwide every year[44]. Ongoing surveillance of GAS is essential to limit the spread of infection. This is especially important for outbreaks among children, as they represent a high-risk group. Rapid characterisation of circulating strains is crucial for early recognition, reporting, and prompt treatments.

A higher than usual number of cases of GAS were recorded in England between 2022 and 2023 (**Figure 6**)[45], with 4,622 notifications during this period, compared with an average of 1,294 notifications over the same period in the previous five years[46]. This resulted in a higher number of cases of invasive GAS and 30 children lost their lives as a result. As it was not clear whether this outbreak was the result of a particularly invasive strain of GAS, Alcolea-Medina *et al.* characterised the epidemiology of this outbreak using Oxford Nanopore whole-genome sequencing[45]. For two weeks from the day the UK Health Security Agency announced the outbreak (06 December 2023), the team generated Oxford Nanopore sequencing data for all invasive and non-invasive GAS isolates alongside routinely collected clinical data. Isolates were prepared for nanopore sequencing with the Rapid Barcoding Kit and sequenced on a GridION device.

Of the 56 sequenced research samples, 10 were invasive isolates from blood cultures and other normally sterile sites, and 46 were non-invasive, mostly from surface wound and throat swabs. Oxford Nanopore sequencing identified the known virulence factors including *emm* genotypes, which encode the surface M protein used for strain typing, and 11 superantigen genes. As has been previously reported, the nanopore sequencing data confirmed that invasive isolates were more likely to carry the *emm1* genotype and belong to the emerging M1uk variant, but the novel finding revealed by multivariable analysis was an association between serious invasive disease and the superantigen genes *spea* and *spej* (streptococcal pyrogenic exotoxin A and J), and not the *emm1* gene or the M1uk clone.

Sequencing results were made available within three weeks at the peak of the epidemic, rather than retrospectively, highlighting the powerful impact that nanopore sequencing can have to provide rapid access to data during an ongoing outbreak when embedded in routine laboratories. The ability to rapidly produce genomic epidemiology data close to the sample source improves surveillance and informs public health measures.

In addition, Oxford Nanopore sequencing at the sample source allows the linking of pathogen genotypes with routinely collected epidemiological data and the distinctive presentation of serious disease; data on genotype-phenotype correlation can be lost when molecular analysis is performed later and away from the sample source. This provides

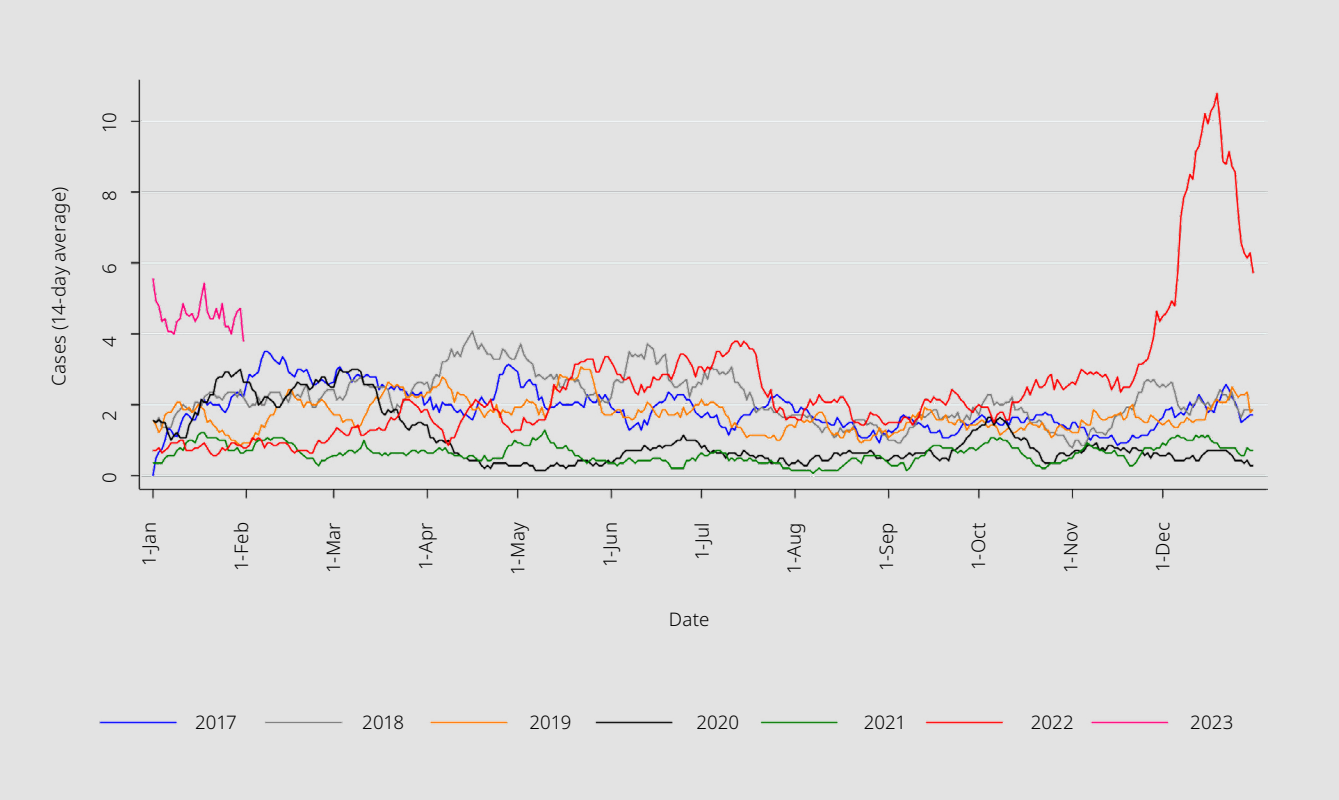


Figure 6. Number of daily cases (14-day moving average) of reported GAS between 2017 and 2023. Figure from Alcolea-Medina *et al.*[45] and available under Creative Commons license (creativecommons.org/licenses/by/4.0).

detailed information on potential molecular markers to better understand the pathogen and, potentially, the underlying mechanisms of serious disease. These insights also contribute to the future potential for the development of novel therapeutic and preventative interventions.

The authors pointed out that their findings may not be representative of the outbreak at a national level, as they only had access to isolates from their hospital network. Data from research samples collected from other hospitals would be needed to increase confidence in this novel link between specific virulence factors and invasive disease. This highlights how integrating nanopore isolate sequencing capability in routine laboratories would enable decentralised generation of genomic epidemiology data for such emerging community infections and investigation of hospital outbreaks with antimicrobial-resistant organisms. They concluded that local Oxford Nanopore pathogen sequencing provides a way forward 'allowing rapid outbreak investigation to inform clinical and public health teams'.

Case study 3

Tackling poliovirus outbreaks and eliminating cell-culture detection methods with Oxford Nanopore technology

Poliomyelitis (polio) is a viral disease transmitted via faecal-oral contamination and spreads rapidly in areas with poor sanitation. This infectious disease has varied clinical features, often starting with flu-like symptoms, leading to severe muscle spasms. However, its hallmark in children is flaccid paralysis of the limbs that can be permanent, and in extreme cases, respiratory paralysis that can cause death[47]. Polio was a global disease until the first successful large-scale poliovirus vaccine trials in 1954 in the USA, which led to widespread use of the vaccine in developed countries, significantly reducing outbreaks[48]. In 1988, the Global Polio Eradication Initiative (GPEI) was launched to further tackle this harmful disease, resulting in wild poliovirus being almost eradicated in all countries, excluding Afghanistan and Pakistan[49].

However, poliovirus is still a public health concern in countries with low vaccine coverage and, as a result, the GPEI has rolled out mass vaccination campaigns using the oral poliovirus vaccine (OPV)[50]. The live, weakened virus in the OPV spreads within a population to widen immunisation; but prolonged circulation can lead to the attenuating mutations in the weakened virus to reverse in under-vaccinated populations, resulting in outbreaks of neurovirulent circulating vaccine-derived poliovirus (cVDPV)[50,51].

The Democratic Republic of the Congo (DRC) has continued to have cases of cVDPV since their last incidence of wild poliovirus ten years ago[50]. To prevent the spread of poliovirus in the DRC, all reports of acute flaccid paralysis are investigated by collecting stool samples from the affected individual (and their close contacts) within two weeks of the onset of paralysis[50,52]. The samples are then sent to the only World Health Organization (WHO)-accredited laboratory in the DRC, which performs cell culture and intratypic differentiation (ITD) quantitative PCR to initially detect poliovirus. To confirm the presence of poliovirus and to distinguish strains, positive samples must then be sent to a laboratory in South Africa for Sanger sequencing of the VP1 capsid region[50].

This cell-culture-based process is slow and complex, increasing the time to results and leading to delays in outbreak detection and response[50,52]. For each additional week between paralysis onset and vaccination, outbreak cases are estimated to increase by 12%[52]. To effectively combat polio endemics, outbreaks must be confirmed faster to prevent delays in treatment and to limit outbreak size and duration by implementing appropriate response plans.

The WHO Polio Eradication Strategy 2022–2026 aims to remove cell-culture-based detection methods and implement country-level direct detection strategies to speed up poliovirus investigations[53]. Direct molecular detection and nanopore sequencing (DDNS) offers the possibility to detect poliovirus outbreaks locally[54]. DDNS combines fast, simple library preparation, which requires minimal laboratory equipment, with onsite sequencing and analysis using benchtop Oxford Nanopore sequencing devices to generate data in real time, 'avoiding international transport of samples and enabling quick response to outbreaks'[50]. To evaluate the DDNS method for poliovirus surveillance, Shaw *et al.* compared it with the current gold-standard culture-based method for over 2,300 stool research samples[50].

For DDNS, RNA was extracted before nested, barcoded PCR was performed, and the resulting amplicons sequenced on a GridION for 4–12 hours. The culture-based method followed the standard accredited process, including shipping poliovirus-positive samples to South Africa for Sanger sequencing.

'Direct molecular detection and nanopore sequencing (DDNS) of poliovirus in stool samples is a promising fast method'

Shaw, A.G. *et al.*[50]

DDNS provided results in an average of seven days and was able to confirm the serotype of the cVDPV outbreak. This was 23 days faster than the average turnaround time for the culture-based method, which included approximately eight days for shipping samples between countries (**Figure 7**). DDNS also offered highly accurate results, comparable to the culture-based method. However, interestingly the culture-based method produced nine false-negative results.

Furthermore, multiple viral serotypes, such as Sabin-1 and Sabin-3, from a single sample were identified with DDNS due to the improved haplotype calling, meaning viruses of importance to the eradication strategy — whether wild-type or vaccine-derived — can be detected and a poliovirus outbreak confirmed with a single workflow. With the current gold-standard method, this information can only be determined when samples are shipped to a separate laboratory for Sanger sequencing.

Shaw *et al.* confirmed that 'DDNS can be applied as a rapid, sensitive, and cost-effective tool for surveillance in the DRC' without the need for time-consuming cell culture and is feasible to implement in national laboratories due to its simple workflow, minimal infrastructure requirements, and low cost per sample for high-throughput experiments.

The authors also suggested that the DDNS method could be repurposed to detect other pathogens locally during public health emergencies because the same facilities and sequencing equipment can be used. By training more laboratories to perform nanopore sequencing, there will be increased 'opportunities to foster the development of these skills and facilitate their contribution to disease surveillance and pathogen genomics'.

Finally, Shaw *et al.* concluded that the research team are optimising the DDNS method to also analyse environmental samples from wastewater, with the aim of generating a similar degree of detection sensitivity for continuous surveillance of poliovirus. The researchers are working to meet the requirements for the WHO Global Polio Laboratory Network recommendation, with hopes for DDNS to be used to inform poliovirus outbreak responses in the future.

'The skills and facilities required for DDNS can be rapidly redeployed to other pathogens during public health emergencies'

Shaw, A.G. *et al.*[50]



Figure 7. Median time required for each step in the two methods for 27 cVDPV-positive stool samples. Figure from Shaw *et al.*[50] and available under Creative Commons license (creativecommons.org/licenses/by/4.0).

Summary

The COVID-19 pandemic brought the devastation caused by infectious diseases into sharp focus, and improving global access to genomic analysis tools is now a key objective in the WHO's ten-year pathogen surveillance strategy[55]. Pathogens know no borders, so to ensure effective genomic surveillance and timely outbreak response, it is imperative to build regional sequencing capability that interconnects with national and global infrastructure.

Oxford Nanopore technology meets these crucial needs, delivering fast, scalable, and cost-effective pathogen sequencing that can be implemented in any environment — from high-throughput public health and research laboratories through to rapidly deployable mobile laboratories at outbreak source.

Oxford Nanopore is proud to support the work of the global pathogen surveillance community to develop rapid and affordable genomic analysis solutions to mitigate the incidence and impact of future outbreaks.

'The accelerated integration of genome sequencing into the practices of the global health community is a must if we want to be better prepared for the future threats'

World Health Organization[2]

About Oxford Nanopore Technologies

The goal of Oxford Nanopore Technologies is to enable the analysis of anything, by anyone, anywhere. The company offers the only sequencing technology to combine scalability — from portable to ultra-high-throughput formats — with real-time data delivery and the ability to elucidate accurate, rich biological data through the analysis of short to ultra-long fragments of native DNA or RNA (**Figure 8**).

The capability to generate reads of any length, including long and ultra-long reads more than 4 Mb[17], enables the most comprehensive analysis of pathogen genomes available using a single technology. These benefits can be applied across all time-critical applications, such as pathogen surveillance, genomic epidemiology, and outbreak investigations — allowing researchers complete flexibility in the development of rapid, cost-effective genomic analysis workflows.

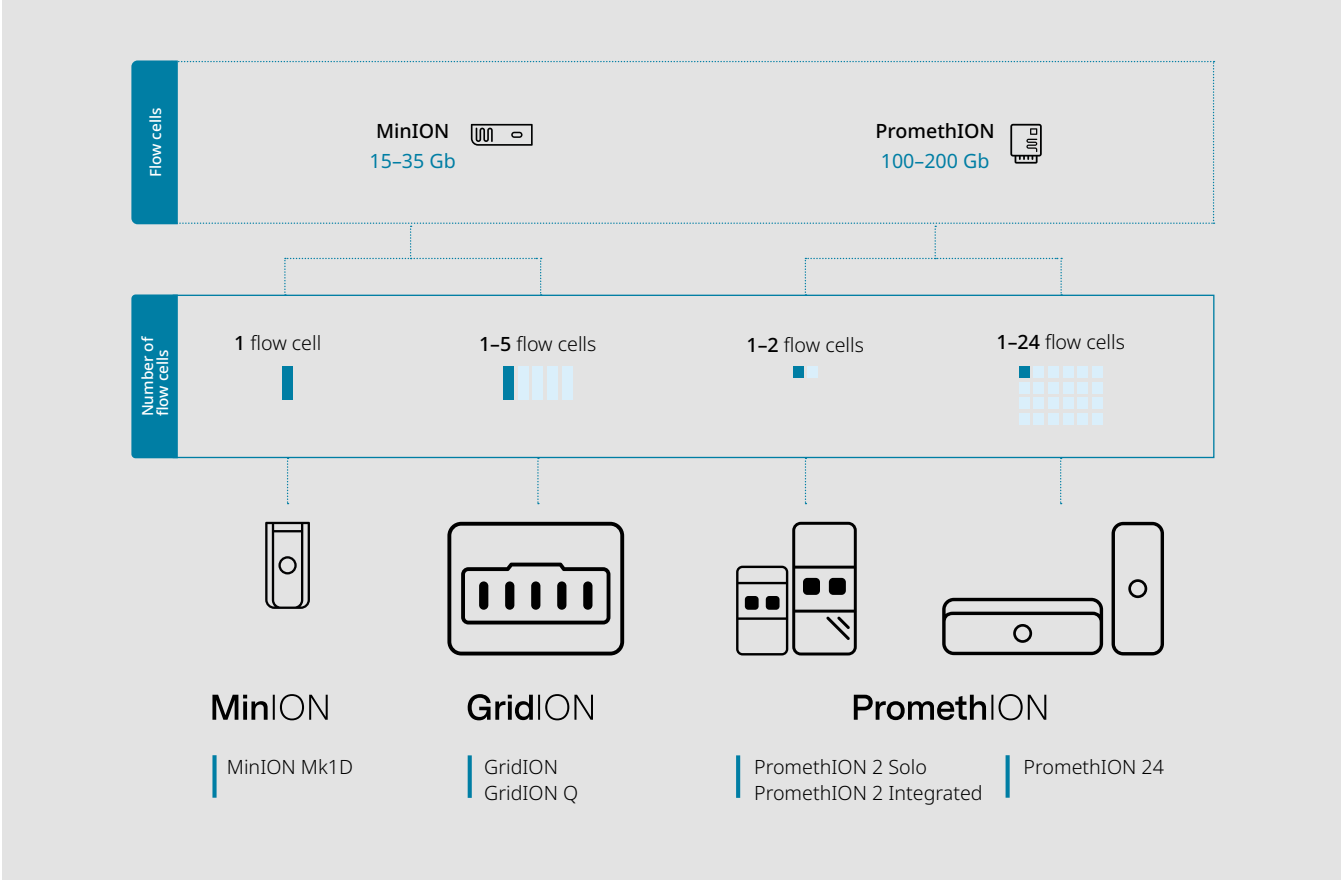


Figure 8. A range of Oxford Nanopore sequencing devices are available, providing high-output and scalable throughput to suit all requirements. The continual development of nanopore technology delivers increased output for even more cost-effective analyses. Information correct at time of publication, for the latest information, visit nanoporetech.com.

For the latest information about nanopore sequencing for pathogen surveillance, visit: nanoporetech.com/infectious-disease.

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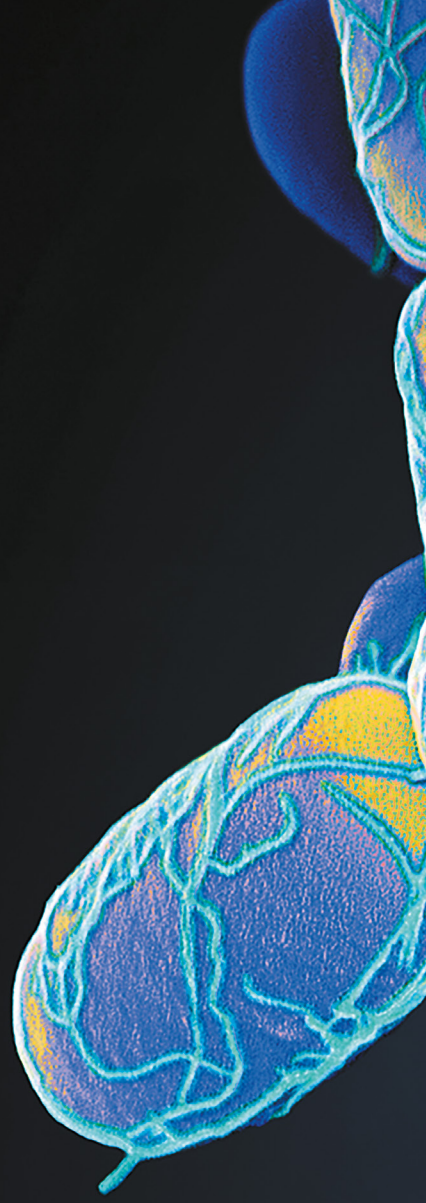
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