

# ITM Antwerp leverages Nextflow to obtain breakthrough clinical research results

## CUSTOMER



<https://www.itg.be/itg.be/>

## LOCATION

Antwerp, Belgium

## INDUSTRY

Public Health, Biomedical and Clinical Research

## OBJECTIVES

Rapidly sequence and analyze samples to help contain the 2022 global mpox outbreak.

## CHALLENGES

- Patient with anogenital lesions testing negative for typical STIs and low epidemiological suspicion of mpox
- Lack of available PCR testing at the Institute to confirm first mpox suspects
- Lack of MPXV WGS protocol for research purposes (molecular epidemiology)
- Need for pipeline portability and reproducibility

## SOLUTION

- Custom Nextflow pipeline
- Oxford Nanopore Technologies sequencer

## RESULTS

- Rapid pipeline development
- Belgian index case confirmation through metagenomic sequencing (2nd genome of 2022 outbreak)
- Investigation of variants through targeted sequencing

## Summary

While historically confined to remote forested regions in Central and West Africa, in 2022, a global MPXV outbreak occurred, affecting approximately 93,000 people, surpassing all preceding outbreaks. When the first suspected mpox patients presented at the HIV/STI Clinic from the Institute of Tropical Medicine (ITM), a multidisciplinary team of researchers was assembled to curb and better understand the ongoing outbreak, leveraging pre-existing knowledge stemming from long-standing partnerships with researchers in endemic countries. ITM's Unit of Clinical Virology and the Outbreak Research Team contributed by applying their viral sequencing protocols and pipelines to swiftly develop an MPXV whole-genome sequencing protocol using Nextflow, helping to understand the ongoing outbreak.

## The business

The Institute of Tropical Medicine (ITM) in Antwerp (Belgium) strives for the advancement of science and health for all, through innovative research, advanced education, professional medical services and capacity sharing with partner institutions in Africa, Asia and Latin America. The institute has a rich history of involvement in outbreak investigation, research, and response.

## The challenge

The 2022 MPXV outbreak took the world by surprise. The first case in Belgium presented to ITM's HIV/STI Clinic in May of 2022, and the country went on to log about 800 infections, primarily among the MSM (men who have sex with men) community.

After confirmation of the first case, with the help of metagenomic sequencing, the institute's MPXV research team tackled questions on i) how to best

recognize and diagnose an MPXV infection in the clinic and in the lab, ii) who was most at risk, iii) the possibility of atypical clinical presentations (including asymptotically), and iv) how the virus was transmitted. PCR was the main laboratory support tool for these investigations. However, PCR does not provide a deep understanding of viral evolution. Tracking viral genome mutations that instruct on relatedness, antiviral resistance, and immune escape required tools to investigate the MPXV genome at high resolution.

## The solution

ITM's Unit of Clinical Virology, together with ITM's Outbreak Research Team, adjusted a protocol based on metagenomic sequencing originally developed to identify viral pathogens with much shorter RNA genomes. The computational pipeline was re-designed by Antonio Mauro Rezende, a researcher from FIOCRUZ Brazil, who was working at ITM during the 2022 mpox outbreak. The pipeline was used to analyze samples obtained from a portable Oxford Nanopore Technologies sequencer.

Antonio chose Nextflow to implement his pipeline because of its ease of implementation, parallel execution features, and the widespread ability of similar reference pipelines, which enabled him to speed up development.

Portability across computing environments was another consideration in selecting Nextflow, given ITM's high connectivity with partner countries and capacity-sharing mission. While the pipeline was initially run on a single GPU-accelerated system, the research team wanted the flexibility to use the same pipeline elsewhere to scale analyses by leveraging a PBS-managed cluster or cloud resources without rewriting pipeline logic.

## Results

The Nextflow pipeline proved highly effective in enabling ITM's broader research related to MPXV genomic epidemiology and variant analysis. The team rapidly identified the MPXV variant causing the 2022 outbreak based on metagenomic sequencing of the Belgian index case's clinical samples, and immediately shared the sequence with colleagues worldwide.<sup>1</sup>

According to Associate Professor Koen Vercauteren, head of the Clinical Virology Unit at ITM, **"By analyzing the virus' DNA, we discovered that we were dealing with an MPXV of clade II, which had been reported to be the less pathogenic cousin of clade I<sub>s</sub> found in Central Africa"**. After this finding, ITM went on to deliver evidence of asymptomatic infections among individuals who visited ITM's HIV/STI Clinic and yet again shared the associated MPXV genomes in the report ["Retrospective detection of asymptomatic monkeypox virus infections among male sexual health clinic attendees in Belgium"](#).

Shortly after, MPXV WGS protocols became available—based on tiled amplicon sequencing PCR enrichment—allowing MPXV genomic investigations at higher resolution, which we analyzed by exploiting the same in silico workflow. We then applied this new method to continue our genomic investigations into post-vaccination MPXV infections<sup>2</sup>, on-therapy breakthrough infections<sup>3</sup>, and prove patient-relatedness in aerosolized viral genomes<sup>4</sup>.

Our Nextflow-implemented pipeline continues to be applied in today's new developments. Pedro Dantas, a Brazilian student enrolled in ITM's Master of Tropical Medicine program shares, **"There is a pressing need for a WGS protocol to facilitate similar investigations into MPXV of Clade I, infamous for its higher pathogenicity, and currently causing MPXV outbreaks at an unprecedented scale in the Democratic Republic of Congo. I am eager to collaborate with our local partners at the national institute for biomedical research on the development of a sequencing workflow to help curb this next threat."**

Nextflow helped contribute to ITM's research and understanding of MPXV transmission in several respects:

### RAPID PIPELINE DEVELOPMENT

Nextflow enabled rapid pipeline development on locally available hardware while ensuring that pipelines would scale to larger environments in the future. According to the pipeline's author, Antonio Mauro Rezende:

**“Nextflow was the ideal choice for pipeline development. The language is simple, has excellent parallel execution features, and has a rich library of existing modules that enable immediate productivity with a minimal learning curve.”**

### PIPELINE PORTABILITY

ITM also aimed to ensure their techniques could be leveraged for future outbreaks. Nextflow has the advantage that pipelines can run anywhere, from single computers to clusters to large-scale cloud infrastructure, with minimal adaptation. Portability is essential to support ITM's capacity-sharing mission with researchers worldwide, especially in areas more affected by tropical infectious diseases.

### ENABLING FUTURE RESEARCH

While the pipeline remains proprietary to ITM and its connected research institutes in tropical countries, researchers involved in its development aim to make it public in the near future so that other research institutions and clinics can leverage it to help understand future outbreaks.

### LEARN MORE

Visit [www.seqera.io](http://www.seqera.io) or tune into [‘Transmission’](#), ITM's thrilling podcast series on global health battles. Season one gives you a behind-the-scenes look at ITM's response to the first mpox cases.

1. [Virologocal.org: Belgian case of Monkeypox virus linked to outbreak in Portugal](https://virologocal.org/Belgian-case-of-Monkeypox-virus-linked-to-outbreak-in-Portugal)
2. [Severe mpox \(formerly monkeypox\) disease in five patients after recent vaccination with MVA-BN vaccine, Belgium, July to October 2022](#)
3. [Tecovirimat Resistance in an Immunocompromised Patient With Mpox and Prolonged Viral Shedding](#)
4. [Detection of mpox virus in ambient air in a sexual health clinic](#)