

## Mpox viral DNA detected in scab, oropharyngeal and whole blood samples in a child after one month of infection and medical care in the Republic of Guinea

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### Abstract

This paper describes the first detection of mpox virus in the Republic of Guinea, also describing diagnostic results from diverse clinical specimens in a patient late in the course of infection. RT-PCR results showed that after one month of infection, Mpox virus DNA was detectable in skin crust samples, nasopharyngeal swabs, and whole blood.

The observation of detectable mpox virus across specimen types can improve procedures for diagnosis of mpox during the recovery phase of an infection, as well as for ensuring that sufficient infection prevention and biosafety measures are in place during specimen collection.

**Keywords:** Mpox Virus; Human; Molecular Diagnosis; Specimen Selection; Real Time-PCR; Guinea

### 1. Introduction

Human mpox disease, previously known as monkeypox, is a zoonosis caused by the monkeypox virus (MPXV). It is a double-stranded DNA virus belonging to the Poxviridae family and the genus *Orthopoxvirus*. Human infection was first identified in Central Africa (Democratic Republic of Congo) in 1970 [1-5]. Rodents (rats, mice, squirrels, etc.) are the natural reservoirs of MPXV and it is from these animals that it is transmitted to monkeys and humans.

Two genetically distinct clades of MPXV are currently circulating in human populations. The first (clade I) is that of the Congo Basin and the second (clade II) of West Africa. Both are currently divided into two subclades, Ia/Ib and IIa/IIb. The 2022-2023 multi-country epidemic, which resulted in over 60,000 reported cases in over 100 countries, was caused by clade IIb, which remains less virulent compared to clade I viruses, with a mortality rate of 10% [6]. In 2024, a resurgence of new cases of mpox was reported, initially from the Democratic Republic of Congo (DRC). Some of the DRC's neighboring countries (Burundi, Kenya, Rwanda and Uganda) that had never reported cases in the past have also

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been affected. Sequence analysis resulted in the identification of clade Ib, which is distinguished from clade Ia by evidence of adaptation to human transmission and higher virulence, especially in children [7]. Given these developments, on August 14, 2024, the World Health Organization declared the current epidemic a public health emergency of international concern, or PHEIC.

Following the WHO's PHEIC announcement for mpox, the Republic of Guinea, through its Ministry of Health and Public Hygiene, set up a surveillance network through its reference laboratories. Since then, this network has been collecting specimens from suspected cases according to the WHO's mpox case definition and transferring these specimens to reference laboratories.

In this paper, we describe the first confirmed case of human mpox in Guinea, focusing on the results of testing different specimen types taken from the patient for assessment of infection status after one month of medical care post initial diagnosis.

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## 2. Methods

A 7-year-old girl was admitted to the health center in Koyamah sub-prefecture in Macenta prefecture with pimples on her body and on the sole of her foot, in August 2024. After initial confirmation of mpox on August 30, 2024 by RT-PCR with a very high viral load (Ct=13), she was hospitalized and treated at the epidemiological treatment center in Macenta. One month after her medical treatment, the patient had almost no clinical signs, except for a few scabs on the back of her right hand. Per national policies to allow the patient to leave the treatment facility, follow-up molecular testing was performed at this time to ascertain viral persistence.

### 2.1. Sample collection

After 24 days of medical treatment, three (3) types of specimens (scab, oropharyngeal and whole blood) were taken from the patient according to national specimen collection protocols and in keeping with biosafety practices. The specimens were sent at a controlled temperature (4°C) to the INSP-Nongo High Containment Reference Laboratory in Conakry.

Laboratory staff checked the specimens and associated transfer documentation before bringing them into the nucleic acid extraction room. The extraction of genetic material was carried out after making an aliquot for repository purposes.

### 2.2. Viral DNA extraction and detection

In order to obtain purified viral DNA, an automatic extraction was carried out with the MagMax kit on the ALLsheng Auto Pure 96 automatic extractor machine according to protocols validated in the laboratory. Two hundred microliters (200ul) of samples were used for each type of specimen and then eluted in 100ul of nuclease-free water. At the end of the plate extraction, a final volume of 100 uL of nucleic acid elution was collected. The Monkeypox Real time PCR kit Shanghai Biotech (ZD-0076-02) was used for the detection of viral DNA by real-time PCR on the Biorad CFX96 machine (Hercules, USA) using the following cycling conditions: 37°C for 2 minutes; 95°C for 90 seconds; 95°C for 5 second and 60°C for 30 seconds, for 40 cycles.

For the validation of nucleic acid extraction, the human ribonuclease P (RNase P) gene was amplified. The curves were analyzed using Biorad CFX Maestro 1.1 sequence detection software (4.1.2433.1219). In addition, a positive and negative control for MPXV was used. After 56 min of amplification, the curves of MPXV DNA analysis, positive control and extraction were analyzed by examining their respective Ct values.

### 2.3. Ethical statement

The specimens were collected and tested in the context of the Guinean national surveillance program and routine clinical care, which do not require prior ethical approval in Guinea. The patient was asked to provide verbal assent for specimen collection.

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## 3. Results

Validation and interpretation of the RT-PCR results showed that MPXV DNA was detected in all specimen types collected (scab, nasopharyngeal, and whole blood) with high CTs, indicating relatively low viral load (Table 1).

**Table 1** Ct values for amplification of Mpox and RNase P genes across different tested specimen types

Type of specimen	Results Mpox	Ct Mpox Gene	Ct RNase P
Scab	Positive	33.12	undetectable
oropharyngeal swab	Positive	37.03	29.35
Whole blood	Positive	39.69	33.35

#### 4. Discussion

The experience gained during the large-scale Ebola epidemic of 2014, which resulted in many deaths, including among health workers, has enabled the Republic of Guinea to build a robust surveillance and epidemic control system. This pyramidal system covers the entire country and is based on the local, prefectural, regional and central structures of the country, coordinated by the National Agency for Health Security, under the Ministry of Health and Public Hygiene, and supported through a strengthened laboratory system that is capable of rapid detection of epidemic prone diseases. These protocols also apply to epidemic situations declared as PHEICs by the WHO even when the disease have not yet been identified in or introduced into Guinea [8-10].

The case described in this paper was the first confirmed human mpox case reported in the Republic of Guinea. After genomic sequencing and phylogenetic analysis, the virus was identified as belonging to clade IIa with a public access reference available on the GISAID database under the number EPI\_ISL\_20053691. The source of transmission to the patient is not known; the patient lived in a family of seven individuals, and no contact could be established with another patient or a suspected case, and the patient had not travelled. However, the prefecture of Macenta, where the case was detected, is a tropical rainforest area where primates and rodents live, both of which are potential reservoirs of MPXV [11], warranting further studies to investigate potential zoonotic spillover.

As described earlier, follow-up testing was performed on the patient after a month of treatment, to confirm recovery and allow for the patient to leave the treatment center. Testing by RT- PCR showed a positivity of all three types of samples, which proves the persistence of MPXV virus in diverse parts of the patient's body, albeit with a low viral load. The scab specimen had a lower Ct value compared to the oropharyngeal (Ct=37) and whole blood (Ct=39) specimens. This means that the virus was present at higher levels in the scab while the human RNaseP gene was undetectable, a sign of altered tissue that did not include living human cells.

It has already been reported that the optimal clinical samples for laboratory analysis are those taken directly from the rash – skin, fluid or scabs – collected by vigorous smear and stored in a dry and sterile place [12]. Our study confirms that in the absence of skin lesions, tests can be performed on oropharyngeal swabs and whole blood during the viremic phase, and even up to several weeks after initial confirmation.

In conclusion, the choice of specimen is always an important concern with respect to making a reliable diagnostic, and can potentially vary over the course of an infection. These results show that one can choose from at least three types of specimens (scab, oropharyngeal swab and whole blood) for the detection of MPXV in patients in the recovery phase. During this phase, viral loads vary across specimen type, with highest viral loads likely in scabs than in nasopharyngeal swabs or whole blood. Thus, even after the near disappearance of symptoms and clinical signs of mpox, the scab remains the preferred specimen for follow-up testing, but if unavailable, whole blood or nasopharyngeal swabs can be used.

We anticipate this information will contribute to improving the diagnosis of human mpox, as well as for assessing persistence of viremia even in the recovery phase, by providing additional data on the impact of specimen type on detection. It also provides important considerations for biosafety and infection prevention and control when managing patients in the recovery phase of an mpox infection.

#### Compliance with ethical standards

##### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

### *Authors' Contributions*

- **Design:** Mamadou Bhoie KEITA, Housseinatou BAH, Mahamoud Sama CHERIF, Sory CONDE and Mamadou Aliou SAMPOU
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