Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Laboratory diagnostics for monkeypox: An overview of sensitivities from various published tests

Monkeypox has been declared a "public health emergency of international concern (PHEIC)" by World Health Organization on July 23, 2022. This sudden and unexpected global outbreak of monkeypox virus (MPXV) raises significant concern [1], particularly in view of the on-going COVID-19 pandemic. From January 1 through June 22, 2022, a total of fifty countries with a total of 3413 laboratory confirmed cases have been reported. The majority of confirmed cases were from Europe and the Americas, with total no. and percentage at 2933, 86%, and 381, 11%, respectively (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON396). These statistics are expected to increase. Furthermore, Singapore and South Korea have reported imported cases of monkeypox, indicating a worsening of global spread by international travels. Laboratory diagnostic tests with high sensitivity and specificity are essential for early detection and case screening to reduce further MPXV spread.

Whereas transmission is thought to be through direct contact [2], recent reports had highlighted the unusual concentration among men who have sex with men (MSM), and among bisexual population, suggesting a unique transmission of the virus in these populations. The mechanism of transmission during sexual contact remains unknown. Although infectious MPXV has been found in semen, and MPXV DNA was detected in urine, feces, rectal swab, nasopharyngeal swab, and saliva in a recent report [3]. However, whether the virus can infect semen cells and reproduce in the genital tract remains unknown.

Monkeypox infection is a viral zoonosis. Infection results in characteristic overt lesions, similar to that of smallpox. Clinical presentations include fever and swollen lymph nodes, followed by a centrifugal evolving rash [2]. Interestingly, there are several unique clinical features of this current monkeypox outbreak, e.g., only few lesions appear, and at different stages of development, the distribution is either exclusively peri-genital and/or peri-anal, and the lesions appear to be ulcerated with umbilicated pustules [4].

In addition to clinical presentations, confirmatory diagnosis of monkeypox requires nucleic acid amplification tests (NAAT), i.e., PCR using various targets in the viral genome, to detect and to differentiate from other poxviruses. Several diagnostic assays had been well established from previous monkeypox outbreaks (Table 1). Real-time quantitative PCR (RT-qPCR) alone, or in combination with sequencing, was recommended by the WHO. We had critically examined with respect to their sensitivities three methods listed in the Interim Guidance by WHO [5] (Table 1).

These tests, with different target sites in the viral genome, have different sensitivities and limit of detection (LOD) for different strains of monkeypox virus, and for different Orthopoxviruses (OPV). As shown in Table 1, the RT-qPCR test developed by Li et al. has a limit of detection (LOD) for the "generic" monkeypox virus (MPXV) at 0.7 fg, which is equivalent to approximately 3.5 genomes; whereas for West African strain, the LOD is at 1.7 fg (~8.2 genome); and for the Congo Basin strain, at 9.46 fg (~40.4 genomes). On the other hand, the RT-qPCR test developed by Maksyutov et al. targets the viral F3L gene. This assay has a LOD of 20 copies/reaction. The test developed by Schroeder et al. for detecting OPV has a LOD of 22.08 DNA copies.

These above RT-qPCR testes had been deployed to monitor MPXV infection in several countries, and in combination with next-generation sequencing (NGS) technology, they provide accurate detection results and at the same time, expands genome sequence information.

A person with characteristic lesions associated with monkeypox is considered infectious until the lesions crusted over and fallen off. Rapid diagnostic tests are important to detect the early stages of infection. Accurate and sensitive testing reduces spreading of the virus by appropriate mitigation strategies, and provides evidence basis for treatments, such as use of antiviral drugs or vaccination. Of note, as more viral genome sequences are available, a larger number of nucleotide polymorphisms have been observed. A revised classification has been proposed, with three main clades, MPXV clades 1, 2 and 3. This new classification scheme or nomenclature avoids discrimination of infected persons, and stigmatizing [6].

Despite cumulating cases, the natural animal reservoir of MPXV remains unknown. However, previous studies have shown that MPXV can infect several species of rodents in Africa [7], suggesting that these animals could be potential reservoirs for monkeypox virus. Also, there may be more MPXV strains in other yet-to-be-identified reservoir hosts, and posing a potential threat to humans. Therefore, continuing surveillance of MPXV from their animal reservoir is important, and more viral clades may be unveiled, further broadening the diversity of MPXV [7]. Therefore, use of previous testing methods should be taken into account of this genetic diversity, as the sensitivity and specificity may be negatively impacted by the genetic diversity of the virus.

The advantages of RT-qPCR include high sensitivity, detection of virus at earlier stages (of infection), usefulness in the surveillance of the virus, including the wildlife, and its application in molecular epidemiology, etc. However, in remote locations or in rural areas of Africa, the infrastructure may not be feasible for these molecular testing. Reducing the cost of testing, increasing the sensitivity and specificity, and developing rapid tests for point-of care (POC) for rural areas will increase the monitoring of monkeypox infection. To control this global outbreak of monkeypox, we need similar strategies as used for mitigating the current COVID-19 pandemic, i.e., early detection, develop antivirals and vaccines, and to fill the knowledge gaps regarding the viral ecology and evolution of monkeypox virus.

Declaration of conflict of interest

The authors declare no conflict of interest.

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Table 1
List of assays for detecting monkeypox virus infections. The three assays by the WHO Interim guidance for detecting monkeypox virus infections are in bold.

Detection technique	Viruses detected	Target viral gene	Limit of detection	Reference	Specimen type
RT-qPCR	**West African strain**	**G2R**	**1.7 fg (~8.2 genome)**	https://linkinghub.elsevier.com/retrieve/pii/S0166-0934(10)00254-5	**Preference types**: the swabs of lesion surface or exudate, roofs from multiple lesion, or lesion crusts. **Additional types**: the swabs of semen, urine, rectal or genital, venous whole blood collected in EDTA.
	Congo Basin strain	**C3L**	**9.46 fg (~40.4 genomes)**		
	MPXV generic	**G2R**	**0.7 fg (~3.5 genomes)**		
RT-qPCR	**Variola virus**	**B12R**	**20 copies/reaction**	https://linkinghub.elsevier.com/retrieve/pii/S0166-0934(16)30067-2	
	Monkeypox virus	**F3L**	**20 copies/reaction**		
	Varicella-zoster virus	**ORF38**	**50 copies/reaction**		
RT-qPCR	**Orthopoxvirus**	**Viral core cysteine proteinase**	**22.08 copies/reaction**	https://linkinghub.elsevier.com/retrieve/pii/S0890-8508(09)00077-2	
	Molluscipoxvirus	**MC036R**	**9.7 copies/reaction**		
	Parapoxvirus	**Envelope protein**	**28.1 copies/reaction**		
Digital PCR	Nonvariola (NVAR) orthopoxviruses	E9L	9.697 copies/mL	https://wwwnc.cdc.gov/eid/article/28/9/22-0917_article#r9	
	Monkeypox virus	B7R	6.359 copies/mL		
	Monkeypox virus	Both targets combined	4.795 copies/mL		
RT-qPCR	Variola virus	A38R	20 copies/reaction	https://www.sciencedirect.com/science/article/pii/S0166093411001984?via%3Dihub	
	Monkeypox virus	B7R	20 copies/reaction		
	Cowpox virus	D11L	50 copies/reaction		
	Vaccinia virus	B10R	70 copies/reaction		
RPA	Monkeypox virus	G2R	16 DNA molecules/μL	https://www.sciencedirect.com/science/article/pii/S0732889318307466?via%3Dihub	
RT-qPCR	Monkeypox virus	F3L N3R	11-55 fg (50–250 copies of each gene)	https://www.nature.com/articles/3700143.pdf	
PCR	Monkeypox virus	ATI	undescribed	https://www.sciencedirect.com/science/article/pii/S0166093498000998?via%3Dihub	
RT-qPCR	Variola virus	A27L	0.7 fg (~4 copies/assay)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC404623/pdf/1612-03.pdf	
	Other orthopoxvirus	A27L	6 copies/assay		
RT-qPCR	Nonvariola (NVAR) orthopoxviruses	E9L	2.54 fg viral DNA (~12.5 genomes)	https://www.sciencedirect.com/science/article/pii/S1386653206001223	
	Monkeypox virus	B6R	~10 viral copies (2 fg)		
GeneXpert	Monkeypox virus	G2R	undescribed	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5303045/pdf/tropmed-96-405.pdf	
	Orthopoxvirus	E9L	undescribed		
LAMP	West African strain	ATI	10^3 copies/reaction	https://onlinelibrary.wiley.com/doi/epdf/10.1002/jmv.21494	
	Congo Basin strain	D14L	$10^{2.4}$ copies/reaction		
	Both Congo Basin and West African strain	ATI	10^2 copies/reaction		
RT-qPCR	Variola virus	14-kD protein gene	0.05 fg (25 copies/assay)	https://academic.oup.com/clinchem/article/53/4/606/5627648?login=true	
	Monkeypox, cowpox and vaccinia viruses	14-kD protein gene	0.05 fg (25 copies/assay)		
Microarray-based assay	Variola virus	C23L/B29R and B19R	undescribed	https://onlinelibrary.wiley.com/doi/10.1002/jmv.20698	
	Monkeypox virus	C23L/B29R and B19R			
	Ectromelia virus	C23L/B29R and B19R			
	Camelpox virus	C23L/B29R and B19R			
	Vaccinia virus	B19R			
	Cowpox virus	C23L/B29R and B19R			
FRET RT-qPCR	Cowpox virus Vaccinia virus Camelpox virus	HA	2.74–9.88 copies/PCR vial	https://academic.oup.com/clinchem/article/50/4/702/5639914?login=true	

(*continued on next page*)

Table 1 (*continued*)

Detection technique	Viruses detected	Target viral gene	Limit of detection	Reference	Specimen type
	Variola major virus Monkeypox virus				
RT-qPCR	Orthopoxvirus (camelpox, cowpox, monkeypox, virus vaccino, vaiolo)	CrmB	60 copies/assay	https://www.infezmed.it/index.php/article?Anno=2007&numero=1&ArticoloDaVisualizzare=Vol_15_1_2007_47	
	Herpes Simplex 1 virus	DNApol	990 copies/assay		
	Herpes Simplex 2 virus	DNApol	600 copies/assay		
	Varicella-zoster virus	ORF 29	160 copies/assay		
PCR	Vaccinia virus	F4L	20-30 PFU	https://www.sciencedirect.com/science/article/pii/S0890850804000726?via%3Dihub	
	Cowpox virus	B9R	20-30 PFU		
	Monkeypox virus	E5R	80-100 PFU		
	Variola virus	B11R–B12R	20-30 PFU		
	Vaccinia virus	C9L	20-30 PFU		
RT-qPCR	Variola virus	J7R	25 copies/assay (0.1fg)	https://www.sciencedirect.com/science/article/pii/S0166093408002541?via%3Dihub	
	Camelpox, cowpox, monkeypox and vaccinia virus	J7R	50 copies/assay (10 fg)		
Nested-multiplex PCR	Orthopoxvirus	VGF	1 ng	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2749831/	
	Parapoxvirus	B2L	1 ng		

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Zhiwen Jiang[1], Jiumeng Sun[1], Letian Zhang[1], Shiyu Yan, Dongyan Li, Chang Zhang
Jiangsu Engineering Laboratory of Animal Immunology, Institute of Immunology, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

Alexander Lai
School of Science, Technology, Engineering, and Mathematics, Kentucky State University, Frankfort, KY, USA

Shuo Su[*]
Jiangsu Engineering Laboratory of Animal Immunology, Institute of Immunology, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

[*] Corresponding author.
E-mail address: shuosu@njau.edu.cn (S. Su).

[1] Co-first authors