Current and Future Prospect of Aptamer in Monkeypox Diagnostics: A Scoping Review

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Abstract – Monkeypox is becoming a concern zoonotic orthopoxvirus of global health significance that affects not only the poorest region of the world but also developed parts of the globe. The recent reemergence of monkeypox in 2022 has caused more than 57,000 global confirmed clinical cases, meanly in regions that have not historically reported monkeypox. Early and efficient diagnosis of monkeypox plays a vital role in arresting the spread of this zoonosis. A significant problem in identifying certain types of orthopoxvirus is that the conventional diagnostic methods primarily target the most dangerous one, smallpox. As a result, these tools often overlook the less harmful orthopoxvirus species, leading to an underestimation of their prevalence. Our group conducted this current systemic review of original research work on detection of monkeypox with an emphasis on either antigen-based or protein-based detection techniques. Following screening of literature using CADIMA, we identified one original research article that suited this review. Monkeypox virus protein A29 (MPXV A29) with specific binding affinity to Monkeypox monoclonal antibody (mAb 69-126-3-7) was extensively studied. Sequence analysis of the amino acid residues of MPXV A29 revealed four different changes compared to other orthopoxvirus protein homologs. Immunoblot analysis demonstrated that mAb 69-126-3-7 produced antigenic reactivity with MPXV alone but not with other orthopoxvirus. Careful study of the nature of A29 protein will give insight into the design of MPXV specific protein (A29) binding aptamer, which will allow for differential diagnostic detection of MPXV amongst other orthopoxvirus infections. Current development in aptamer technology raises profound hopes for diagnostic detection of various pathogens. In conjunction with other high profile molecular diagnostic tools, it is certain that aptamer will play a significant role in detection of MPXV in a quicker, cheaper, and simpler manner.

Keywords *– Monkeypox, detection, diagnosis, antigen, protein*

1 INTRODUCTION

At a time when coronavirus disease 2019 have been declared as endemic in most parts of the globe, reemergence of monkeypox is becoming a serious cause for public health concern (1). Recent reemergence of monkeypox has caused more than 57,000 global confirmed clinical cases as of September 2022, with 99% of the total case occurring mainly in regions that have not historically reported monkeypox (CDC, 2022). The profound cause for public health concern is due to recurrent standups between high biotech equipped world powers, that could lead to a potential misuse of orthopoxvirus as bioweapons (2). Monkeypox is one of the four orthopoxvirus belonging to the Poxviridae family. Other members of this family include cowpox virus, variola virus and vaccinia virus. (3). Initially, isolated from monkey, this DNA virus coinhabit other hosts including the Gambian pouched rats, tree squirrels and dormice. The exact mode of transmission of monkeypox to humans is unclear but contact with infected animals or fluid from infected patients are the two possible ways the disease spreads.

Transmission to humans can be categorized into two, primary and secondary transmission. Primary transmission occurs from infected animal to human via contact or consumption of under cooked infected animal meat. While secondary transmission is from infected person to another person via contact with ill patient body fluids (4, 5).

A multiapproach smallpox prevention and control guidelines has been recommended (6). An integral aspect of this strategy is early detection of a smallpox case to achieved effective overall control. Manifestations of orthopoxvirus are clinically visible, however misdiagnosis often occurs. Misdiagnosis of infections presenting generalized body rash as chickenpox is not uncommon. Likewise cowpox is mistaken as anthrax when present physically on skin as large solitary (7).

Monkeypox infection is often misdiagnosed due to its profound similarity in it manifestation to smallpox (8). The main differential strategy towards distinguishing this infection is to carry out specific, quick, and sensitive lab-based diagnostic tests especially if smallpox is suspected. The latest state-of-the-art technique for diagnosing human monkeypox utilizes real-time polymerase chain reaction (PCR) analysis (9). This includes a multiplex PCR coupled with electrophoresis of DNA amplification product (7) and a multiplex real time TaqMan PCR assay (3). These advanced molecular methods enable highly accurate and timely detection of monkeypox viral DNA, enhancing the efficiency of diagnosis and monitoring of the disease. However, an antigen-based, or protein-based detection of monkeypox possess potential prospects in the future due to its simple, fast, and cost-effective nature to be used as point of care tool for preventing the spread of the monkeypox infection. This review discussed the current and future prospectus on monkeypox diagnosis.

2. MATERIALS AND METHOD

The present scoping review was carried out following the updated Preferred Reporting Items for systematic reviews and Meta-Analyses guidelines (10).

2.1. Search Strategy

The literature search was conducted in August 2022 through three databases (PROQUEST, EBSCO host (Medline) and Scopus) using lists of keywords referring to the Medical Subject Headings (MeSH) thesaurus. These keywords were combined using the Boolean operators as follows: [“monkeypox”] AND [“antigen OR protein”] AND [“detection OR diagnostic”]. An additional search was conducted by manually screening the references of the retrieved literature.

2.2. Selection of Studies

Articles were excluded if (i) the studies did not involve the detection of antigen and/or protein of monkeypox virus; (ii) the studies were published in languages other than English or Malay; (iii) the studies detected viruses other than monkeypox virus. The retrieved literature was downloaded into the CADIMA software (https://www.cadima.info/index.php) and duplicates were identified and removed. The references were distributed to three authors (A.W., K.M.F.M and F.Z.) who independently reviewed all the articles for title and abstract screening. Discrepancies between the authors were resolved through a discussion among authors. Full-text screening was performed in triplicate by three authors (A.W., K.M.F.M and F.Z) and their findings were summarized.

2.3. Questions for the Quality Assessment of Retrieved Studies

The studies included from PROQUEST, EBSCO host (Medline) and Scopus databases were analyzed according to their quality standards by eight questions defined by the Critical Appraisal Skills Programmed (CASP) qualitative checklist. For each retrieved article, the questions were answered by two authors (K.M.F.M and F.Z.) who filled in CADIMA software with the answers “no”, “yes”, “unclear” or “inapplicable”. Discrepancies between these authors were solved through a discussion with third author (A.W.). The questions are listed as follows: 1. Was there a clear statement of the aims of the research? 2. Is a qualitative methodology appropriate? 3. Was the research design appropriate to address the aims of the research? 4. Was the recruitment strategy appropriate to the aims of the research? 5. Was the data collected in a way that addressed the research issue? 6. Have ethical issues been taken into consideration? 7. Was the data analysis sufficiently rigorous? 8. Is there a clear statement of findings?

2.4. Data Extraction

The following data were extracted: type of biorecognition element, antigen and/or protein, sample, detection technique, assay time, limit of detection (LoD), sensitivity and specificity.

3. RESULTS

3.1. Search Results

A total of 479 studies were identified from the three databases and thirty-four (34) duplicates were removed. After screening the titles and abstracts, 436 studies that are not relevant were excluded. Based on the study criteria, eight (8) studies were excluded during full-text screening. The remaining one (1) study was included in the final review (Figure 1).

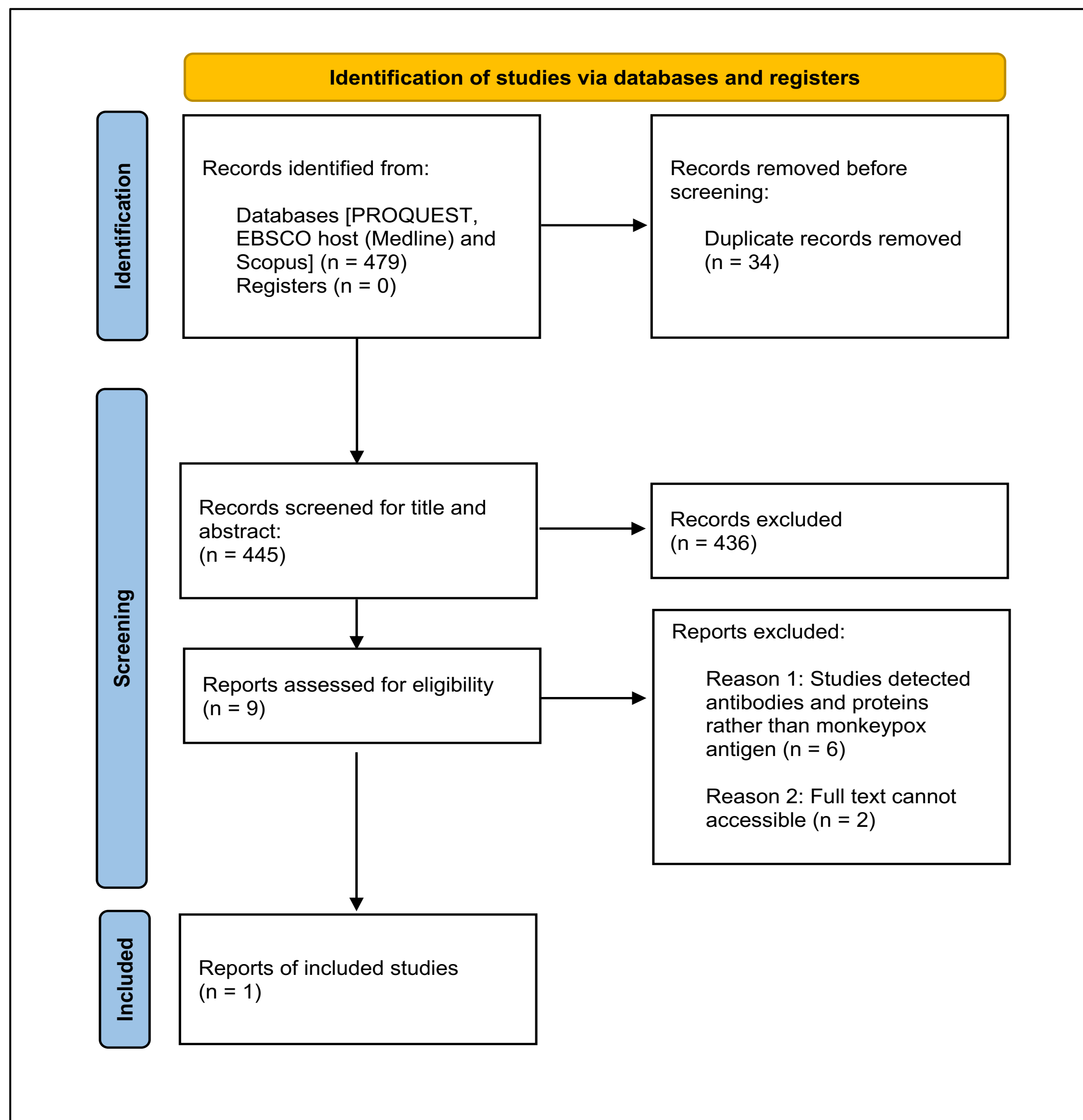

Figure 1. PRISMA flow diagram showing the process of selecting studies

3.2 Quality Assessment of Retrieved Studies

The result of the CASP Qualitative Checklist assessment showed a low risk of bias. Regarding the answers for each quality question, the included study has a clear statement of aims, appropriate qualitative methodology and research design, sufficient data collection and analysis and clear statement of finding. Besides that, ethical issues, and recruitment strategy are inapplicable for the included study.

The current scoping review was based on PRISMA (11). The strategy for literature search was broad and was via three databases (PROQUEST, EBSCO host (Medline) and

Scopus) with keywords referring to the Medical Subject Headings (MeSH) thesaurus and limited to study reported in English or Malay languages which produced minimal risk of bias according to the result of the CASP Qualitative Checklist assessment. Following application of exclusion criteria, only one study was included for thorough data extraction as shown in Table 1.

Table 1. Summary of the included study

No	Publication year	Type of Biorecognition Element	Antigen/ Protein Detected	Type of Sample	Assay Time	LOD	Sensiti -vity (%)	Specifi -city (%)	Ref
1	2014	Monoclonal antibody 69-126-3-7	MPXV A29	Synthesi-zed peptide	2 hours	NR	NR	100	(12)

MPXV: monkeypox virus; NR: not reported

4. DISCUSSION

The current review identified an original research work that revealed antigen/protein-based detection of species-specific monkeypox protein (MPXV A29) by highly specific biorecognition element in form of monoclonal antibody (mAb 69-126-3) against monkeypox virus in the midst of other orthorpox virus proteins (13). The author's approach, which involved conducting amino acid analysis through sequence alignment, was noteworthy. This approach aimed to identify a distinct factor that sets MPXV A29 apart from other OPXV homologs. Through this method, we can comprehend the unique aspect of MPXV A29, which involves four specific amino acid changes in the sequence. After translation, these changes lead to the creation of a distinctive peptide responsible for mediating the affinity binding and specificity for mAb 69-126-3. Binding kinetics assays have been profoundly utilized as a fundamental approach for establishing the relationship between biomolecules and play a key role in diagnostic and therapeutic (14, 15). Affinity of biomolecule to its ligand is a function of its specificity, high affinity binding leads to high specificity.

On the other hand, sensitivity of experimental technique is its ability to detect at minimal level the specific affinity binding of interacting molecules; which is determined by measuring the Limit of detection (LOD) (16). LOD is the minimum measurable amount of analyte that can be detected over blank (17, 18). Although, the author has established the specific binding of mAb 69-126-3 to MPXV A29 through both fluorescence labelled assay such as immunoblotting, ELISA and label-free technique such as Biolayer interferometry assay technique; the author did not report the sensitivity of these experiments toward detecting the molecular binding interactions occurring between MPXV A29 and mAb 69-126-3; and this represents the weakness of their work. Various rapid test methods for diagnostic detection of orthopoxvirus infection have been developed such as antigen-antibody based immune-filtration assays (19). Although this method detects orthopoxvirus, its inability to offer specific and differential detection of MPXV limits its application as a point of care tool for curtailing MPXV outbreak. Table 2 summarizes the conventional methods used for the detection of monkeypox along with their limitations.

Table 2. Conventional methods used for the detection of monkeypox along with their limitations

Detection Method	Principle	Advantages	Limitations
Virus Isolation	Culturing and identifying the virus from patient samples	Definitive diagnosis, virus characterization	Time-consuming, requires specialized facilities, risk to lab personnel
Polymerase Chain Reaction (PCR)	Amplifying viral DNA for detection	Rapid results, high sensitivity, and specificity	Requires well-equipped laboratories, expensive, requires trained personnel

Serological Assays	Detecting antibodies against monkeypox virus	Suitable for epidemiological studies	Limited sensitivity during early infection, cross-reactivity with related viruses
Immunofluorescence Assay (IFA)	Detecting viral antigens in patient samples	Rapid results, moderate sensitivity	Requires specialized equipment, skilled operators
Enzyme-Linked Immunosorbent Assay (ELISA)	Detecting monkeypox-specific antibodies or antigens	Quantitative results, high throughput	Cross-reactivity with other orthopoxvirus, limited sensitivity in early infection
Virus Neutralization Test (VNT)	Measuring the ability of patient serum to neutralize the virus	Confirmatory results	Labor-intensive, time-consuming, requires cell culture facilities
Hemagglutination Inhibition (HAI)	Detecting specific antibodies that inhibit hemagglutination	Suitable for serological studies	Requires fresh monkeypox virus, may not be widely available
Electron Microscopy	Direct visualization of virus particles	Identifies the virus morphology	Low sensitivity, cannot distinguish between poxviruses

Table 3. Representative aptamer developed against orthopoxviral proteins or virus

Method	Binding Site	Aptamer Sequence	Kd (nM)	Application	Ref
SELEX using vaccinia virus infected cells as a target	Glycosylated hemagglutinin expressed on the surface of vaccinia virus-infected cells	ATC CAG AGT GAC GCA GCA CGA GCC AGACAT CTC ACA CCT GTT GCA TAT ACA TTT TGC AT GGA CAC GGT GGC TTA GT	3.24 ± 0.63	Diagnostic detection of vaccinia infected cells	(36)
		ATC CAG AGT GAC GCA GCA TCG ACC TCT GTG CGA GCG GGT TGC ATA TAT ATT TTG CAT GGA CAC GGT GGC TTA GT	1.45 ± 0.30		
SELEX using VACV particles	VACV particles	TTGGACCGCGGTGGGTAGTCA GGTATACTCCAAAATGCTTTAT TTAGCACAAGG	27.7 ± 3.8	Diagnostic detection of orthopoxvirus	(35)
		TCCGTATAATAGTGCTGTACTA AGCAAATTTATAGTTCTCTAGA AAGTGCCCGC	73.6 ± 8.8		
		CTAGCGGTCAAATCATTGCACA CTTCCGGATATGCTCGGG	26.2 ± 2.8		
SELEX using dominant surface protein of orthopoxvirus (A27)	A27 proteins	CCAAAGATAGAAGCGGTGCGG GGGGGGGGAGCGTGGCGGAG AAACAAGTGAGTAG	24.65	Diagnostic detection of orthopoxvirus	(35)
		CCACACGCGACACAGAAAACA GAGTGAGGTCAGGGAGGGGC GGCAAAGCTGAGG	58.25		
		CCACTTGAGTCTGGAACGTGG TCGCAAGGTCCGGGAGTGGGG GGTGCCGGCTG	35.34		
		CCAGTGACATGCAAGGCAGGA GGAGGTACTAAGGAACGACGG TTGAGATGGGGG	37.97		

In response to the pressing demand for swift and precise diagnostics in the face of MPXV infections, there arises a compelling necessity for alternative tools that leverage different principles and recognition elements. Among these innovative approaches, aptamer technology stands out as a promising solution to combat the reemergence of MPXV, helping to mitigate the risk of a potential pandemic.

The term aptamer refers to a single-stranded, short sequence of nucleotide in either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) molecular form with ability to assume 3D structural conformation and bind a desired molecular target (20). Aptamers are generated from library pool of single-stranded nucleic acid through a process coined as systematic evolution of ligand by exponential enrichment (SELEX) (21-23). Aptamers possess added advantages over antibodies. They are readily synthesis through chemical synthesis, their physico-chemical characteristics can be enhanced through molecular modification, making them more cost effective than antibodies (24-26). Aptamer can be easily coupled with other cutting-edge technologies like nanopore, CRISPR/cas9 to achieved maximum performance (27). Additionally, aptamers have good reputation with regard to its application in therapy and diagnosis of pathogenic bacteria, viruses and parasite (28).

This promising feature of aptamer qualifies them to be an important candidate for MPXV diagnostic detection and therapy. The current review discussed the recent and future prospectus of protein-based detection method for MPXV with emphasis of utilizing aptamer technology. Available data on aptamer development against protein and /or particles of orthopoxvirus family are also briefed in this document. Up to now, there is no documented study that provides knowledge on an aptamer sequence developed against specific proteins of MPXV despite been fully characterized, however few aptamers have been developed against other orthopoxvirus.

4.1. Milestone Recorded by Aptamer Technology Against Zoonotic Viral Infections

The potential capability of aptamer to attached itself to any pathogenic organism and mediate the detection or destruction of such pathogen have paved the way for their utilization as a tool for counterattack strategy in the fight against various infectious causing agents including MPXV. Over the past two decades, the world has witnessed different zoonotic viral disease outbreak such as the recent MPXV in 2022 (29), Covid 19 in 2019 (30), MERS in 2012 (31), H1N1 in 2009 (32) and H5N1in 2004 (33). Various aptamers have been selected against most of these zoonotic pathogens especially Smallpox which is closely related to MPXV. Here we can vividly notice the potential of aptamer related technology in zoonotic viral infection diagnostic detection and therapeutics, thus indicating the profound potential of using this approach to MPXV disease.

4.2. Aptamer Developed Against Orthopoxvirus Infections

The battle against orthopoxvirus infections has received wide attention via different approaches including development of synthetic ligand to serve as a molecular entity for rapid and specific diagnosis of orthopoxvirus or their treatment. Aptamer developed against orthopoxvirus represents one example of such ligands. Because of their cross-reactivity and cross-protectivity, the chance of developing cross-specific and cross-selective synthetic ligand for all orthopoxvirus was considered most likely (34, 35). The fact that aptamer can be generated against micro-molecules such as proteins or macromolecule like viral particles, aptamer against VACV particles and aptamer against dominant surface protein of orthopoxvirus (A27) were developed using SELEX and characterized (35, 36) Table 3 gives the summary.

Aptamers developed against specific viral proteins, such as glycosylated hemagglutinin (HA) on vaccinia virus-infected cells (VAVC) (36), VAVC particles and A27 proteins (35), have significant potential in diagnosis of monkeypox. These aptamers carry immense potential to inspire researchers in the development of aptamer-based diagnostic tools for monkeypox such as Aptamer-Based Biosensor (ABB) and Enzyme-Linked Oligonucleotide Assay (ELONA).

4.2.1 Aptamer-Based Biosensor

Aptamer-based biosensors are analytical devices that use aptamers as recognition elements to selectively bind to specific target molecules. These biosensors are designed to detect and quantify the presence of these target molecules in various samples, such as blood, urine, or environmental samples. The binding of the aptamer to the target molecule triggers a detectable signal, enabling the

rapid and specific identification of the target of interest (37).

The glycosylated hemagglutinin (HA) on VAVC aptamer and A27 proteins aptamer, have significant potential in biosensor development for the point-of-care diagnosis of orthopoxvirus particularly, monkeypox. These aptamers can be immobilized on a sensor surface to capture target viral proteins rapidly, leading to a measurable signal, such as a change in electrical conductivity or fluorescence. This rapid detection capability ensures timely results, making these biosensors well-suited for point-of-care diagnostics.

Furthermore, the high specificity of aptamers is a significant advantage, enabling the detection of even low concentrations of the target viral proteins. This high sensitivity is crucial for early diagnosis and monitoring of monkeypox infections. Aptamer-based biosensors are typically compact and can be designed for portability, making them ideal for use in resource-limited settings, fieldwork, or mobile clinics. Additionally, real-time monitoring of viral protein levels is possible, which is valuable for tracking infection progression and assessing treatment effectiveness. Moreover, the use of multiple aptamers on a single biosensor allows for the simultaneous detection of various viral markers, a particularly beneficial feature for monkeypox, which may involve multiple viral proteins.

4.2.2 Enzyme-Linked Oligonucleotide Assay (ELONA)

Enzyme-Linked Oligonucleotide Assay (ELONA) is a laboratory technique used for the detection and quantification of specific nucleic acid sequences, often DNA or RNA, in a sample. It is a variation of traditional enzyme-linked immunosorbent assays (ELISA) but is tailored for nucleic acid analysis rather than protein detection (38). In addition to biosensors, aptamers find application in ELONA for the diagnosis of viral infections. ELONA using glycosylated hemagglutinin (HA) on vaccinia virus-infected cells, VAVC particles and A27 proteins aptamers will offer several advantages. Firstly, it will enable the quantitative measurement of orthopoxvirus particularly, monkeypox protein levels in a sample, which is critical for assessing infection severity and monitoring changes over time. The high specificity of aptamers ensures that ELONA results are less likely to be affected by cross-reactivity with other molecules in the sample.

ELONA can be automated, making it suitable for high-throughput screening of patient samples in clinical laboratories, thereby expediting the diagnostic process. Aptamers are highly reproducible, guaranteeing consistent and reliable results in ELONA assays. Their cost-effectiveness in comparison to antibodies is an added benefit for routine testing in healthcare facilities. Aptamers are stable molecules and can withstand the conditions required for ELONA procedures. Additionally, ELONA can be used to create standard curves through serial dilution assays, enabling the accurate quantification of the viral load.

5. CONCLUDING REMARKS

The amplification technique such PCR remains the main stay for diagnostic detection of MPXV. However, PCR is not economical, time consuming and requires sophisticated tools and skills making it unsuitable to be deployed as a countermeasure for curtailing MPXV outbreak in less developed countries. For efficient point of care testing (POCT), a rapid, simple, and sensitive testing modalities is needed, and aptamer-based detection technique can provide such. Various aptamer-based detection techniques have been in place with profound efficiency such as aptamer-based lateral flow assay (APTALFA), aptamer-based biosensor (aptasensor) and enzyme linked oligonucleotide assay (ELONA). The main advantage of aptamer-based methods over other methods such as protein biorecognition elements is that the former can be readily coupled to either electrochemical or optical sensing tools for portable, rapid, and ultra-sensitive detections.

The cross-selectivity and cross-reactivity between and amongst orthopoxvirus provides opportunity for developing universal aptamer exhibiting cross-sensitivity for rapid detection of individual orthopoxvirus. Further study needs to be done to investigate whether any of the selected aptamer against A27 and VACV particles could detect MPXV. Elucidation vividly the mode of aptamers interactions with dominant surface protein of orthopoxvirus (A27) or VACV particles will give insight into the design of MPXV specific protein (A29) binding aptamer which will allow for

differential diagnostic detection of MPXV amid other orthopoxvirus infection.

Current development in aptamer technology raises profound hopes for diagnostic detection of various pathogens. In conjunction with other high profile molecular diagnostic tools, it is certain that aptamer will play a significant role in detection of MPXV.

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List of Abbreviations

spp, Species; MPXV A29, Monkeypox virus protein A29; DNA, Deoxyribonucleic acid; PCR, Polymerase chain reaction; MeSH, Medical Subject Headings; A.W., Abdul Wahab; K.M.F.M , Khairul Mohd Fadzli Mustaffa; F Z, Fatihah Mohd Zaidi; CASP, Critical Appraisal Skills Programmed; LoD, Limit of detection; NR, Not reported; ELISA, Enzyme linked immunosorbent assay; SELEX, Systematic evolution of ligand by exponential enrichment; POCT, Point of care testing; APTALFA, Aptamer-based lateral flow assay; ELONA. Enzyme linked oligonucleotide assay; CRISP, clustered regularly interspaced short palindromic repeats; cas, CRISP-associated; VACV, Vaccinia virus; Covid 19, coronavirus disease 2019; MERS, Middle East Respiratory Syndrome; H1N1, Hemagglutinin Type 1 and Neuraminidase Type 1; H5N1, Hemagglutinin Type 5 and Neuraminidase Type 1.

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