iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 11 **Issue:** X **Month of publication:** October 2023

DOI: https://doi.org/10.22214/ijraset.2023.56145

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 11 Issue X Oct 2023- Available at www.ijraset.com

The Era of Molecular Biology: Diagnostics for Nipah Virus

Kalpesh Khutade[1], Harshada Shah[2], Samiksha Patil[3], Hiren Patel[4]
[1, 2, 3, 4]*Department of Microbiology, Vedantaa Institute of Medical Sciences, Vedantaa Hospital and Research Centre, Dhundalwadi, Palghar, MUHS University, Nashik.*

Abstract: ***Nipah virus (NiV) is an emerging pathogen that, unlike other priority pathogens identified by the WHO, is endemic to Southeast Asia. It is most commonly transmitted through exposure to saliva or excrement from the Pteropus fruit bat or direct contact with intermediate animal hosts, such as pigs. NiV infection causes severe febrile encephalitic disease and/or respiratory disease; treatment options are limited to supportive care. The serological and nucleic acid amplification techniques have been developed for NiV and are used in laboratory settings, including some early multiplex panels for differentiation of NiV infection. The rural and remote nature of NiV outbreak settings, there remains a need for rapid diagnostic tests that can be implemented at the point of care. Additionally, more reliable assays for surveillance of communities and livestock will be vital to achieving a better understanding of the fruit bat host and transmission risk to other intermediate hosts, enabling the implementation to outbreak prevention and the management of this NiV. An improved understanding of NiV viral diversity and infection kinetics or dynamics will be central to the development of new diagnostics to enable effective validation and external quality assessments.***
Keywords: **Nipah virus (NiV); diagnosis; encephalitis; epidemiology**

I. INTRODUCTION

Nipah virus infection is an emerging bat-borne zoonotic disease transmitted to humans through infected animals (such as bats and pigs) or food contaminated with saliva, urine, and excreta of infected animals [1]. It can also be transmitted directly from person to person through close contact with an infected person (although this represents a less common transmission route). Nipah virus infection in humans causes a range of clinical presentations including acute respiratory infection and fatal encephalitis [2]. The case-fatality rates in outbreaks across Bangladesh, India, Malaysia, and Singapore typically range from 40% to 100%. Nipah virus (NiV) has been recognized as a highly pathogenic virus due to its high mortality in humans [2], [3].

A. Epidemiology (India)

India experienced five episodes of NiV outbreaks in West Bengal (2001, 2007) and Kerala (2018, 2019, 2021, and 2023). In India, From September 12 to 15, 2023, the Ministry of Health and Family Welfare, Government of India, reported six laboratory-confirmed Nipah virus cases, including two deaths, in Kozhikode district, Kerala (Fig.1) [4]. As of September 27, 2023, 1288 contacts from the confirmed cases were traced, including high-risk contacts and healthcare workers who are under quarantine and monitoring for 21 days. Since September 12, 387 samples have been tested, of which six cases were positive for Nipah virus infection, and all remaining samples tested negative. Since September 15, no new cases have been detected. All Indian outbreaks have seen person-to-person transmission. Though the epidemiology of NiV in India is similar to that in Bangladesh, since only three outbreaks have been reported so far, definitive evidence is unavailable [5]. There was a large outbreak (66 cases and 45 deaths) in Siliguri, West Bengal, in 2001, and another smaller outbreak (five cases, 100% fatality) in 2007 in Nadia district, West Bengal. In May 2018, an outbreak of NiV was declared in Kozhikode and Malappuram districts of Kerala, a southern state on the west coast that is geographically disconnected from previously affected areas [6]. There were 18 confirmed cases and 17 deaths as of June 1, 2018. All NiV cases belonged to the economically productive age group ([7], [8]-[10]).

B. Clinical indications

NiV causes severe encephalitis in humans, characterised by vasculitis and necrosis in the central nervous system (CNS). The incubation period of NiV is 4–14 days. NiV primarily affects the CNS via endothelial, vascular, and parenchymal cell infection, with high rates of viral replication in neuronal cells. At the early stage, NiV infection typically presents as febrile encephalitis, and can be difficult to distinguish from other febrile illnesses. Respiratory distress was a hallmark in approximately 20% of cases in the Malaysia–Singapore outbreak and 70% of cases in Bangladesh–India [11].

©IJRASET: All Rights are Reserved

The patients can present with fever, malaise, headache, myalgia, nausea, vomiting, vertigo and disorientation. The encephalitis cases with attending drowsiness and disorientation, which can rapidly progress to seizures and coma within 48 hours. Approximately 20% of encephalitis survivors sustain neurological dysfunction including persistent seizures, disabling fatigue and behavioural abnormalities [12], [13].

C. Disease Management and Prevention

Due to the high pathogenicity associated with henipa virus, NiV and HeV are classified as Biosafety Level-4 agents. Safe handling of specimens requires personal protection equipment, physical infrastructure, and strict operating procedures for both clinical and research operations [14]. As BSL-4 facilities may be limited in many endemic settings, BSL-3 and BSL-2 facilities may be sufficient if the virus can be inactivated following specimen collection. At present, there are no antiviral drugs or human vaccines available for NiV. Australia has developed the Hendra G protein-targeted vaccine, which can protect horses against NiV and HeV. Treatment is limited to supportive care. In preclinical animal models, NiV glycoprotein (G) and fusion (F) proteins are used in a number of vaccine candidates in development to stimulate a protective immune response [15].

This landscape analysis provides an overview of the current state of NiV diagnostics for screening, diagnosis and surveillance, highlighting further research and development needs.

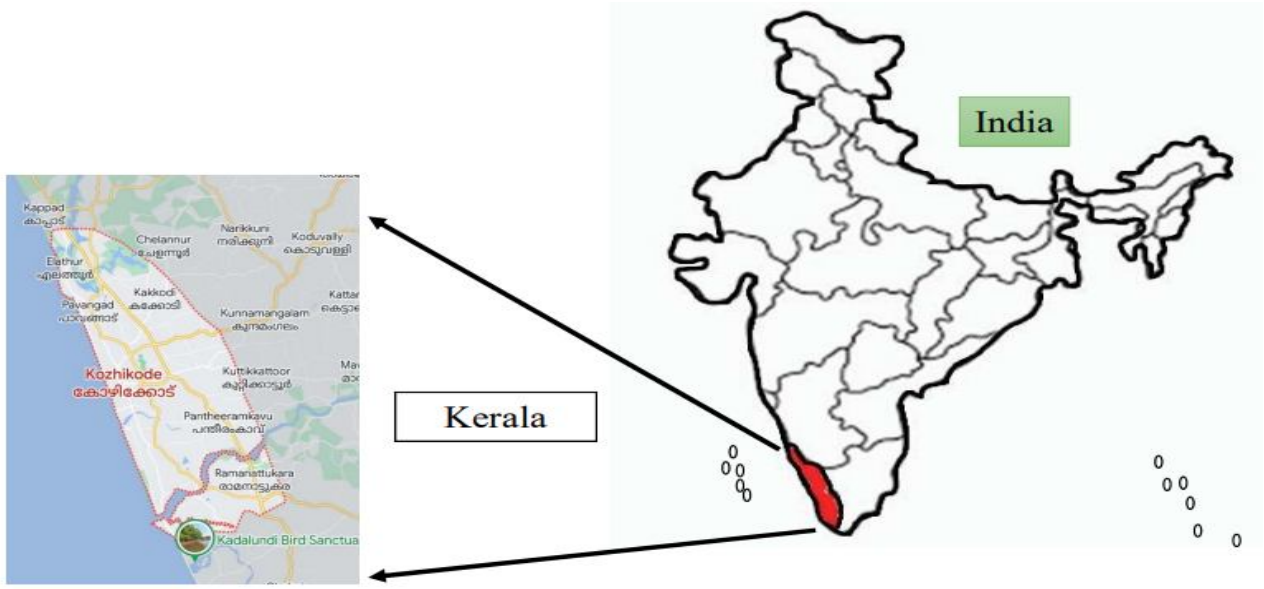


Fig. 1 Detection of Nipah virus in 2023 outbreak from Kozhikode district, Kerala, India.

II. NIPAH DETECTION AND DIAGNOSTIC TEST

The increasing awareness and threat of NiV have boosted the development and commercialization of diagnostics. Accurate diagnosis of NiV has traditionally relied on serological, molecular, or virological analyses, genome detection by PCR and quantitative PCR, and virus isolation. The NCDC, India, recommends with sterile nylon swabs in sterile 3ml viral transport medium contained in 15 ml conical tubes. Collected VTM samples were stored at -70^{0}C until viral RNA extraction. BSL 4 facility required for samples processing. However, virus inactivation by sample irradiation may be an effective technique to make the samples safe to use in a BSL-2 laboratory ([5]-[7], [9]-[12]). The main different methods to determine virus infection and immunity are:

A. Virus Isolation

Virus isolation should be performed for definitive diagnosis in an area with a newly suspected outbreak. NiV is detectable in oropharyngeal and nasal swabs as early as two days post-infection; experimentally infected animals continued to shed virus until three weeks post-infection [16]. African green monkey kidney or rabbit kidney cell lines are commonly used for virus isolation. A cytopathic effect (CPE) is usually seen within two to three days, but multiple passages of five days each are recommended before concluding that a sample is NiV negative [17].

B. NiV detection by real time RT-PCR

Nucleic acid amplification tests (NAATs), such as reverse-transcriptase PCR (RT-PCR), are preferred for detection of active viral infection [18]. Conventional Real Time PCR targeting the N (nucleocapsid protein) gene has been developed by the US Centers for Disease Control and Prevention. NiV RNA can be identified by Real Time PCR (RT-PCR) from respiratory secretions, urine or cerebro-spinal fluid [19]. These tests are highly sensitive and specific and are used commonly for diagnosis. A TaqMan probe-based assay developed in 2004 detects the N gene and has a very high sensitivity of ~1 pfu. A SYBR Green-based assay targeting a different region of the N gene has also been developed. It has lower sensitivity (~100 pfu) and detects HeV as well [20].

C. ELISA

Under such circumstances, the serological assays can provide appropriate alternative and rapid results. The main advantages of serological assays for NiV are low cost and easy-to use format which can be easily established in field their areas, remote settings and tertiary health care. NiV affected cases would be identified in a timely manner by serological test, which could help to curb further transmission. The availability of validated serological assays would also be useful for serosurveilance activities. As yet anti-Nipah ELISA reagents developed by the Centers for Disease Control and Prevention, United States of America, are used for serological confirmation of NiV in India [21]. Serological tests can directly detect NiV antigens, as well as IgM and IgG antibodies raised against NiV antigens, due to their high sensitivity, rapidity, ease, and safety of use. ELISAs for the detection of IgG and IgM developed by the CDC were used in the confirmation of diagnosis in India. The recombinant protein-based test has been developed using the more conserved N antigen. IgM antibodies have been found to be detectable in 50% of patients on day 1 of illness, while 100% of patients show IgG positivity after day 18. IgG positivity persists for several months ([6],[7], [13]-[16]).

D. Serum Neutralisation Test

This is the gold standard test, but it requires the use of a BSL-4 laboratory. In this test method, test sera are incubated with the virus and then allowed to infect Vero cells. Positive sera block the development of cytopathic effects, and tests can be read after 3 days. A modified neutralization test that can be read within 24 hours has been developed. Here, the virus-serum mixture is removed after a period of adsorption, and immunostaining is used for virus detection. Pseudo-typed viruses can be used to perform a surrogate neutralization test [22]. A pseudo-typed virus is an enveloped virus. They are one or more foreign envelope proteins. These viruses can be handled safely in the BSL-2 laboratory but contain NiV envelope proteins capable of being neutralized by positive sera [23]. The NCDC, India, has issued guidelines on the definitions of a suspected, probable, and confirmed case of NiV infection, which have been used effectively to control known outbreaks in India [21], [23].

III. CHALLENGES FOR NIV DIAGNOSTICS

The ideal NiV diagnostic RT-PCR tests were developed from Bangladesh-India (NiV-B) or Malaysia-Singapore (NiV-M) strains. Additionally, further information is needed on the viral and immune kinetics of NiV to identify the pathogenesis of NiV in blood and non-blood samples.

Understanding the course of NiV infection at different stages of the disease would help to identify the window for effective intervention and better monitor transmission and recovery [24].

For diagnostic test formulators, clinical samples are crucial to the confirmation process; still, confirmation for NiV tests has been limited due to the lack of NiV-positive sera available. While animal samples may be used to investigate the performance characteristics of a test, there's a threat that the antigens found in specific animal- sourced NiV infections aren't the same as those found in humans or other creatures.

As new individual tests are developed, these agencies could perform routine external quality assessment (EQA) monitoring of tests using up- to- date clinical specimen panels and reference norms. This part will be particularly important when test developers have little incitement to seek international nonsupervisory approval [25].

Given the often rural NiV outbreak settings and accurate diagnostics for NiV must be deployable under a range of circumstances, particularly at the point of care (POC). Existing POC and 'near-POC' NAAT platforms have lower infrastructure requirements than laboratory-based diagnostics, with automated sample preparation resulting in fewer training requirements for healthcare workers and cartridge-based formats allowing tests to be self-contained. They can therefore be more easily implemented in decentralised laboratories based settings. Current NiV PCR assays could potentially be easily converted to the POC format, considering the broad range of tests currently being developed for these commercial NAAT platforms [26].

Rapid diagnostic tests (RDTs) are ideal for field testing and low-infrastructure settings. RDTs have been developed to effectively screen. The pathogenicity of NiV makes specimen processing a challenge in a non-laboratory setting; thus, preliminary work in antigen ELISA development could serve as a starting point for an antigen NiV RDT. Education campaigns may be helpful to increase awareness of the risk of NiV infection in the community and promote appropriate care-seeking behavior, as well as to maintain vigilance for case identification by healthcare personnel. Early detection of NiV outbreaks should trigger a coordinated mobilization of resources for infection control, as well as patient isolation, care, and contact tracing ([21]-[24],[27], [28]).

IV. CONCLUSIONS

The diagnostic tests can enable a more nuanced understanding of the window of positivity and duration of infection, transmission risk, and risk factors for severity for NiV, one of the most widespread agents of febrile encephalitic disease. Target product profiles for NiV should be refined to include the need to identify all known lineages of NiV and the benefits of RDT POC diagnostics and syndromic panels. Diagnostics are a key element in achieving the goals of research and development. An improved understanding of NiV viral diversity and infection kinetics or dynamics will be central to the development of new diagnostics to enable effective validation and external quality assessments.

REFERENCES

[1] Alam AM. Nipah virus, an emerging zoonotic disease causing fatal encephalitis. Clinical Medicine. 2022;22(4):348. doi: 10.7861/clinmed.2022-0166
[2] Bae SE, Kim SS, Moon ST, Cho YD, Lee H, Lee JY, Shin HY, Lee HJ, Kim YB. Construction of the safe neutralizing assay system using pseudotyped Nipah virus and G protein-specific monoclonal antibody. Biochemical and biophysical research communications. 2019;513(4):781-6. https://doi.org/10.1016/j.bbrc.2019.03.212
[3] Banerjee S, Gupta N, Kodan P, Mittal A, Ray Y, Nischal N, Soneja M, Biswas A, Wig N. Nipah virus disease: A rare and intractable disease. Intractable & rare diseases research. 2019;8(1):1-8. https://doi.org/10.5582/irdr.2018.01130
[4] Bruno L, Nappo MA, Ferrari L, Di Lecce R, Guarnieri C, Cantoni AM, Corradi A. Nipah Virus Disease: Epidemiological, Clinical, Diagnostic and Legislative Aspects of This Unpredictable Emerging Zoonosis. Animals. 2022;13(1):159. https://doi.org/10.3390/ ani13010159
[5] Chattu VK, Kumar R, Kumary S, Kajal F, David JK. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. Journal of family medicine and primary care. 2018 Mar;7(2):275. doi: 10.4103/jfmpc.jfmpc_137_18
[6] Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipahand Hendra virus infections. Microbes and infection. 2001;3(4):289-95. https://doi.org/10.1016/S1286-4579(01)01382-X
[7] Garbuglia AR, Lapa D, Pauciullo S, Raoul H, Pannetier D. Nipah Virus: An Overview of the Current Status of Diagnostics and Their Role in Preparedness in Endemic Countries. Viruses. 2023 Oct 7;15(10):2062. https://doi.org/10.3390/v15102062
[8] Giangaspero M. Nipah virus. Trop Med Surg. 2013;1(129):2. http://dx.doi.org/10.4172/2329-9088.1000129
[9] Guillaume V, Lefeuvre A, Faure C, Marianneau P, Buckland R, Lam SK, Wild TF, Deubel V. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). Journal of virological methods. 2004;120(2):229-37. https://doi.org/10.1016/j.jviromet.2004.05.018
[10] Gurley ES, Spiropoulou CF, De Wit E. Twenty years of Nipah virus research: where do we go from here?. The Journal of infectious diseases. 2020;221:S359-62. https://doi.org/10.1093/infdis/jiaa078
[11] Harit AK, Ichhpujani RL, Gupta S, Gill KS. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. Indian Journal of Medical Research. 2006;123(4):553.
[12] Islam MR, Dhar PS, Rahman MM. Newly outbreak of Nipah virus: epidemiology, symptoms, transmission, diagnostic testing, treatment, and global health concern. International Journal of Surgery. 2023;109(3):507-8. DOI: 10.1097/JS9.0000000000000050
[13] Kulkarni DD, Venkatesh G, Tosh C, Patel P, Mashoria A, Gupta V, Gupta S. Development and evaluation of recombinant nucleocapsid protein based diagnostic ELISA for detection of Nipah virus infection in pigs. Journal of Immunoassay and Immunochemistry. 2016;37(2):154-66. https://doi.org/10.1080/15321819.2015.1074922
[14] Lim CT, Sitoh YY, Hui F, Lee KE, Ang BS, Lim E, Lim WE, Oh HM, Tambyah PA, Wong JS, Tan CB. Nipah viral encephalitis or Japanese encephalitis? MR findings in a new zoonotic disease. American journal of neuroradiology. 2000;21(3):455-61. http://www.ajnr.org/content/21/3/455
[15] Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. BMJ global health. 2019;4:e001118. http://dx.doi.org/10.1136/ bmjgh-2018-001118
[16] Ochani RK, Batra S, Shaikh A, Asad A. Nipah virus-the rising epidemic: a review. Infez Med. 2019 Jun 1;27(2):117-27.
[17] Pollak NM, Olsson M, Marsh GA, Macdonald J, McMillan D. Evaluation of three rapid low-resource molecular tests for Nipah virus. Frontiers in microbiology. 2023;13:5237. DOI 10.3389/fmicb.2022.1101914
[18] Ramphul K, Mejias SG, Agumadu VC, Sombans S, Sonaye R, Lohana P. The killer virus called Nipah: a review. Cureus. 2018;10(8). DOI: 10.7759/cureus.3168
[19] Sadanadan R, Arunkumar G, Laserson KF, Heretik KH, Singh S, Mourya DT, Gangakhedkar RR, Gupta N, Sharma R, Dhuria M, Jain SK. Towards global health security: Response to the May 2018 Nipah virus outbreak linked to Pteropus bats in Kerala, India. BMJ global health. 2018 Nov 1;3(6):e001086. http://dx.doi.org/10.1136/bmjgh-2018-001086
[20] Satter SM, Aquib WR, Sultana S, Sharif AR, Nazneen A, Alam MR, Siddika A, Akther Ema F, Chowdhury KI, Alam AN, Rahman M. Tackling a global epidemic threat: Nipah surveillance in Bangladesh, 2006–2021. PLOS Neglected Tropical Diseases. 2023;17(9):e0011617. https://doi.org/10.1371/journal.pntd.0011617

[21] Sayed A, Bottu A, Qaisar M, Mane MP, Acharya Y. Nipah virus: a narrative review of viral characteristics and epidemiological determinants. Public Health. 2019;173:97-104. https://doi.org/10.1016/j.puhe.2019.05.019

[22] Shariff M. Nipah virus infection: A review. Epidemiology & Infection. 2019;147:e95. https://doi.org/ 10.1017/S0950268819000086

[23] Shete AM, Jain R, Mohandas S, Pardeshi P, Yadav PD, Gupta N, Mourya D. Development of Nipah virus-specific IgM & IgG ELISA for screening human serum samples. The Indian Journal of Medical Research. 2022;156(3):429. DOI: 10.4103/ijmr.ijmr_2737_21

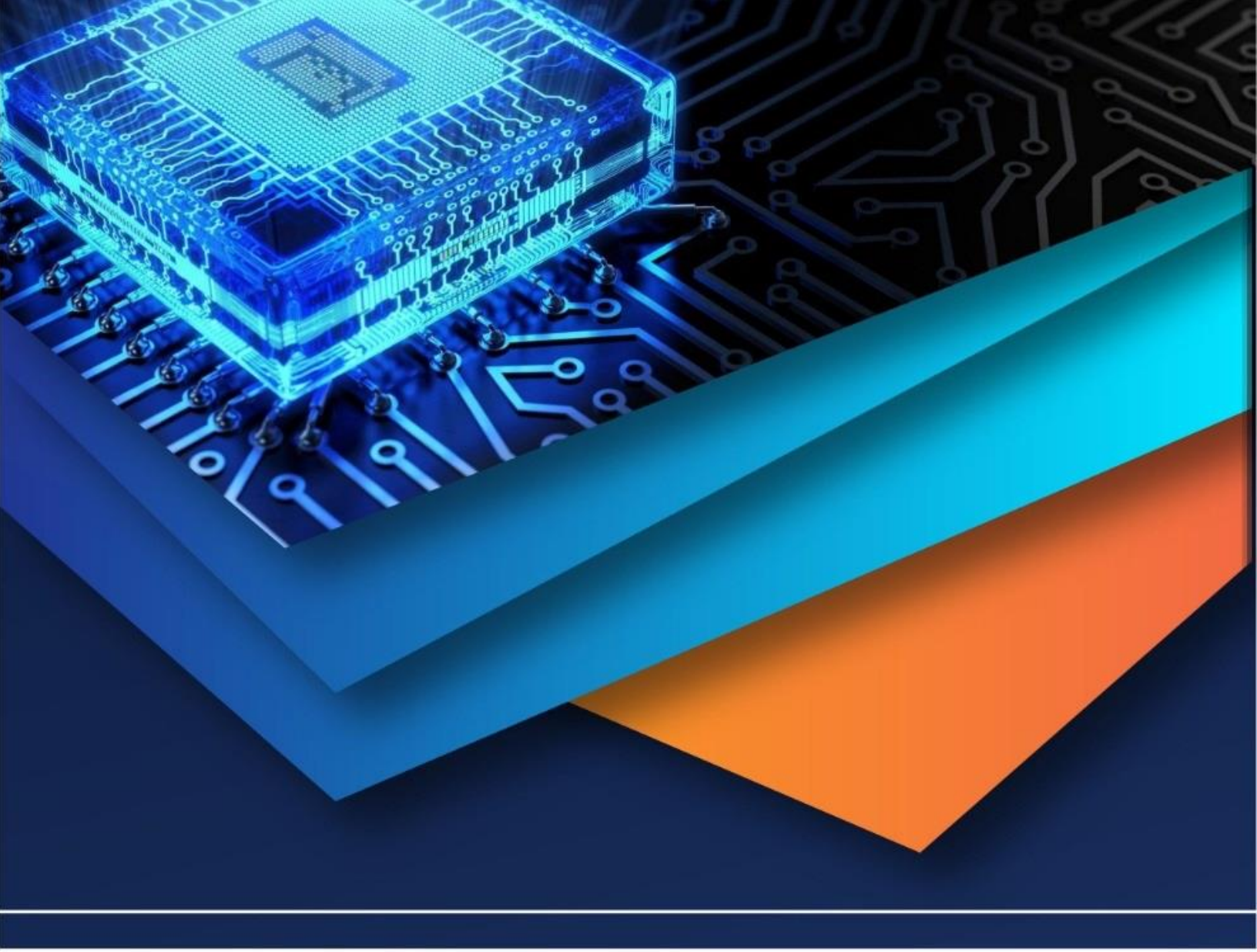
[24] Singh RK, Dhama K, Chakraborty S, Tiwari R, Natesan S, Khandia R, Munjal A, Vora KS, Latheef SK, Karthik K, Singh Malik Y. Nipah virus: epidemiology, pathology, immunobiology and advances in diagnosis, vaccine designing and control strategies–a comprehensive review. Veterinary Quarterly. 2019;39(1):26-55. https://doi.org/10.1080/01652176.2019.1580827

[25] Tamin A, Harcourt BH, Lo MK, Roth JA, Wolf MC, Lee B, Weingartl H, Audonnet JC, Bellini WJ, Rota PA. Development of a neutralization assay for Nipah virus using pseudotype particles. Journal of virological methods. 2009;160(1-2):1-6. https://doi.org/10.1016/j.jviromet.2009.02.025

[26] Wong KT, Shieh WJ, Zaki SR, Tan CT. Nipah virus infection, an emerging paramyxoviral zoonosis. In Springer Seminars in Immunopathology. 2002 (Vol. 24, pp. 215-228). https://doi.org/10.1007/s00281-002-0106-y

[27] Yadav PD, Majumdar T, Gupta N, Kumar MA, Shete A, Pardeshi P, Sultana S, Sahay RR, Manoj MN, Patil S, Floura S. Standardization & validation of Truenat™ point-of-care test for rapid diagnosis of Nipah. The Indian Journal of Medical Research. 2021 Oct;154(4):645. doi: 10.4103/ijmr.IJMR_4717_20

[28] Zhu W, Pickering B, Smith G, Pinette M, Truong T, Babiuk S, Kobasa. D, Banadyga L, Yang M. Development and laboratory evaluation of a competitive ELISA for serodiagnosis of Nipah and Hendra virus infection using recombinant Nipah glycoproteins and a monoclonal antibody. Frontiers in Veterinary Science. 2023;10:1120367. DOI: 10.3389/fvets.2023.1120367



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089 (24*7 Support on Whatsapp)