Nitatpattana N, et al., J Virology Res 2022, 8: 020
DOI: 10.24966/VRV-0193/100020

HSOA Journal of Vaccines Research and Vaccination

Research Article

Preclinical Phase of the Inactivated Zika Vaccine Development in Thailand

Nitatpattana N[1]*, Duangkhae P[2], Rodpai E[1], Akkhawattanangkul Y[3], Nakgoi K[4], Poolam K[2], Treekarunasawad J[2], Juntarapornchai S[1], Palabodeewat S[1], Phadungchayap P[1], Wanlayaporn D[1], Chaiyo K[2], Wiriyarat W[3], Pesirikan N[2] and Gonzalez JP[5,6]

[1] *Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand*

[2] *Government Pharmaceutical Organization, Ministry of Public Health, Bangkok, Thailand*

[3] *Faculty of Veternary Science Mahidol University, Nakhon Pathom, Thailand*

[4] *Surat Thani Provincial Livestock Office Department of Livestock Development, Surat Thani Province, Thailand*

[5] *Georgetown Universities, School of Medicine, Washington D.C., USA*

[6] *ERP International LLC. Laurel, Maryland, USA*

Objective

Purpose: The Zika virus (ZIKV), a member of Flaviviridae family, is associated with serious congenital and neuropathological abnormalities in Thailand and abroad. Although several vaccines are being prepared by different agencies, there are no approved vaccines against ZIKV infection in human.

Methods: In this context, the need for vaccine development, our study was based on the development of the preclinical phase of an Inactivated Zika Vaccine using the Asian strain SV0010-15. Inactivated Zika vaccine was produced using WHO Vero cells (RCB 10-87) growth with formalin inactivated Serum Free Medium (VP-SFM) and following WHO recommendation and Quality controls. Pre-clinical models were developed using small animal for immune response, safety and dose formulation followed using no-human primate animal model for vaccine efficacy.

Results: Our results showed that an optimal and suitable dose formulation of Zika vaccine/Alum adjuvant (5 µg/250 µg) was obtained when performed with a use of two doses injections. Also, it showed to be safe, immunogenic and to have a protective effect against two ZIKV genotypes in Non-Human Primate. Ultimately, the first step of the development of this vaccine candidate has been successfully done accordingly to WHO guidelines and shown that the product could be developed in GMP pilot scale for a clinical stage at the request of health authorities.

Keywords: Inactivated vaccine; Thailand; vaccine; Zika vaccine; Zika virus

***Corresponding author:** Nitatpattana N, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand, Phone: +66 (0) 2441 9337; Fax: +66 (0) 2441 9336; Email: narong.nit@mahidol.ac.th

Citation: Nitatpattana N, Duangkhae P, Rodpai E, Akkhawattanangkul Y, Nakgoi K, et al. (2022) Preclinical Phase of the Inactivated Zika Vaccine Development in Thailand. J Vaccines Res Vaccin 8: 20.

Received: April 07, 2022; **Accepted:** April 14, 2022; **Published:** April 21, 2022

Introduction

The Zika Virus (ZIKV) is a mosquito-borne virus from the family Flaviviridae and genus Flavivirus which includes, among others, several human pathogenic flaviviruses such as Japanese Encephalitis Virus (JEV), Dengue Virus (DENV). ZIKV is an arbovirus transmitted principally by Aedes mosquito species including *Aedes aegypti* and *Aedes albopictus*. The geographical distribution of these mosquito species across tropical and subtropical regions has led to several outbreaks, including the recent pandemic in Brazil, followed by the Pacific islands and other areas of North and South America [1]. Today, ZIKV is globally widespread [2]. ZIKV was first identified and isolated from a sentinel monkey in a Ugandan forest in 1947 [3] and emerged as a global health threat in December 2015 [4]. For the past seven decades, ZIKV has had limited impact on public health systems worldwide, while few human cases were reported in Southeast Asia and Africa. It was not until 2007 that ZIKV caused large outbreaks and was first detected outside Asia and Africa. Furthermore, ZIKV emerged as a threat in Oceania with a large outbreak in French Polynesia in 2013-2014. Since 2015 ZIKV became endemic in Brazil, with increased pathogenicity impacting the nervous system with severe congenital malformations (microcephaly) as well as neurological as Guillain-Barré Syndrome (GBS) with acute inflammatory demyelinating neuropathy [5-7]. On February 1st, 2016, WHO declared the ZIKV epidemic in Brazil a public health emergency of international concern. In Thailand the first report of the possible presence of Zika virus was recorded in 1963 [8]. In early 2013, ZIKV was also detected among travelers and more recently several autochthonous cases were observed providing evidence that ZIKV is widespread throughout Thailand [9-10]. In 2018, United Kingdom health authorities (Public Health England (PHE) have classified Thailand as having a risk of Zika virus transmission).

Several ZIKV vaccine candidates have now been developed and tested in preclinical and clinical trials. These include nucleic acid vaccines (DNA and RNA vaccines), inactivated whole virus vaccines, live attenuated vaccines, viral vectored vaccines, protein antigen vaccines in the form of purified proteins from expression systems, or virus-like particles [11]. In mid-2016, WHO, UNICEF and a working group of independent subject matter experts have proposed a ZIKV vaccine Target Product Profile (TPP) for use in an emergency (i.e.,

urgent need during pregnancy in endemic areas), or in a future emerging outbreak scenario. The TPP suggested non-replicating platforms such as inactivated whole virion and subunit based and those that use alum as adjuvant. The proposed model of TPP was the inactivated Zika vaccine elicited ZIKV envelope specific neutralizing antibodies and protected Non-Human Primates (NHP) against challenge with the virus strains from Brazil and Puerto Rico [12]. In September 2017, the Center for Vaccine Development (CVD) Institute of Molecular Biosciences Mahidol University, Government Pharmaceutical Organization, (GPO) and National Vaccine Institute (NVI) Ministry of Public Health took the challenge and decided to develop an Inactivated Zika vaccine in Thailand. In September 2017, the three parties signed a Memorandum Of Understand (MOU) to develop such Inactivated Zika vaccine by using WHO Vero cell as recommended by the TPP. The present study report on the development of the pre-clinical phase of this ZIKV vaccine in Thailand.

Materials and Methods

Cell

WHO Vero cells RCB 10-87 were derived from GMP cell bank product that was prepared and extensively characterized at Government Pharmaceutical Organization (GPO).

Zika virus strains

The infectious strain of ZIKV SV0010/15 was used to produce the inactivated vaccine candidate (GenBank: KX051562.1). ZIKV SV0010/15 was generously provided by the Epidemiology Department of Disease Control, Ministry of Public Health, and Thailand. ZIKV SV0010/15 was amplified in WHO Vero cell using Hyper-flask (Corning, Corning, NY) and harvested on day 4 and 6 after inoculation. The virus titer tested 7 $\log_{10}$ plaque forming unit/ml and confirmed free of mycoplasma. The identity of Zika virus was confirmed by genomic RNA sequencing from Direct PCR amplicon. ZIKV strain MR766 (African strain) was used for the heterologous ZIKV challenge in primate model. Inactivation of ZIKV candidate vaccine strain SV0010/15 was produced inside the virus vaccine clean room facility at Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University. WHO Vero cell was scale up in Hyper-flask (Corning;). ZIKV inoculation in Vero cells was Performed using serum Free Medium (VP-SFM, Gibco) and carried out at a standardized MOI of 0.01 PFU/cell. The virus was harvested at 4 and 6 days. Pooled virus harvest was clarified by Refrigerated Centrifugation 5,000 rpm about 10 minute and filter 0.45 micron using Sartopure 0.45 µ PP3 (Sartorius), concentrated by Tangential Flow Filtration (TFF) Pellicon 2 Mini Filter Ultrafiltration 500 Kda (Merck millipore). Residual Vero cellular DNA was removed by using nuclease digestion (Benzonase endonuclease, Merck), and the viral suspension purified on Capto Core 700 (GE Healthcare Life Sciences, Pittsburg, USA) column. ZIKV was inactivated with 0.05% of formalin for 7 days at 22 °C following the previously described method [13]. Formalin was removed by diafiltration 100 Kda (Merck millipore). Ultimately, the antigen concentration was adjusted with a 6% sucrose, PBS pH 7.4 buffer. Inactivation was considered as complete when no infectious particle could be detected by Indirect Fluorescent after three serial amplifications of the sample in vitro in C6/36 for 7 days. Quality controls were applied accordingly to the Requirements for Japanese Encephalitis Vaccine (Inactivated) for Human Use as previously described [14].

Preclinical Immunogenicity in Small mammal

The preclinical phase on the immune response was carried out in Balb/c mice at the animal facility in Faculty of Veterinary Science, Mahidol University. Balb/c female mice 6–8 weeks of age were purchased from M-CLEA Bioresource Company, Thailand. The animals (n = 6 specimen by group) were allocated randomly to different groups. Mice were vaccinated with booster doses (day 0 and day 14) of 5µg vaccines and 10µg adjuvant (2% Alhydrogel, Invivogen) by IM routes in a 100µl volume. All of mice blood samples were collected on day 35 after the second dose, by cardiac puncture [15].

Preclinical Immunogenicity in Primates

Two groups of nine (9) 4-5 years old cynomolgus macaque *(Macaca fascicularis*), from the animal facility of the National Primate Center, Chulalongkorn University at Saraburi Province Thailand, were vaccinated including: A first group (n=6) that received a booster dose (day 0, 14) of 5 µg of inactivated Zika vaccine and adjuvant (2% Alhydrogel, Invivogen); A second group (n=3) serves as negative control. All specimen received only adjuvant and PBS7.4 and were randomly injected by IM routes in a 100 µl volume vaccine. Also, blood samples were collected on day 0, 7, 14, 21, 30, 60 after the first injection.

Ethics

All animal experiment protocols for small mammal models were approved by the Faculty of Veterinary Science, Mahidol University-Institute Animal Care and Use Committee (COA. No: MUVS-2017-12-56). The animal experiment protocol for the Pre-clinical Immunogenicity in Primates was approved by the Chulalongkorn University Animal Care and Use Committee (COA. No: 2075002).

Challenge test in Monkeys

On day 180, the first group of cynomolgus macaque was separated in two subgroups: The subgroup I (1/1 to 1/3) challenged by the ZIKV strain SV0010/15 (Asian strain, titer 5.7 log10) and subgroup II (1/4 to 1/6) challenged by ZIKV strain MR766 (African strain, titer 5.7 log10). Both of them will be injected about 0.5 ml by Sub Cutaneous (SC) route.

The second group was positive control challenged (group III) (2/1 to 2/3). It was challenge by ZIKV strain SV0010/15 with the same dose (0.5 ml of 5.7 log10 titer). Blood samples were collected on a daily base from day 180 to day 187 following a previously establish protocol [12].

ELISA (IgG) serology test

Briefly, we used the FBS-depleted ZIKV antigen strain MR766 which derived from Vero cells as previously described, has been slightly adapted from the described technique using Flavivirus mouse brain antigen and replaced by the use of Zika virus cell culture antigen [16].

ELISA (IgM) serology test

ZIKV specific capture IgM were by a modified ELISA as previously described [17-18].

ELISA optical reading value

For each sample the ratio (P/N) in Optical Density reading (OD) value was positive control serum including sample well value divided

by the OD value of the negative control serum well. It was used for both IgG and IgM ELISA tests. Thus, The sample was considered positive when the P/N value ≥ 2 or sample OD higher than the negative control OD about 2 times [18].

Plaque Reduction Neutralization Test (PRNT50)

Briefly, we used the ZIKV strain SV0010/15 (Asian strain) and MR 766 (African strain) for neutralizing homologous and heterologous antibody respectively following the protocol as previously described [19].

RT-PCR

Following [20] we used the outer primer pairs of Uni for (5′ $_{1171}$ TGGGGNAAYSRNTGYGGNYTNTTYGG $_{1197}$ 3′) and Unirev (5′ $_{2178}$ CCNCCHRNNGANCCRAARTCCCA $_{2155}$ 3′) and, followed by the inner pairs of Mounifor2 (5′ $_{1209}$ GGRDRMDTBKWSAYVTGYGCNAWRTT $_{1235}$ 3′) and Mounirev2 (5′ $_{2094}$ CCNATNSWRCTHCCHKHYYTRWRCCA $_{2068}$ 3′). Amplification was performed using the following procedure: 1 cycle at 50◦C for 30 min and 95◦C for 2 min, and 35 cycles at 95◦C for 20s, 55◦C for 20s, and 68◦C for 30s.

Virus Isolation

C6/36 cells were used for virus isolation and identification by Indirect Fluorescent (IFA) as previously described [21].

Plaque assay

Vero cells monolayer was used for virus titration by plaque assay. The plaques were counted, and the viral titer was calculated and expressed as PFU/ml [19].

Results

Inactivated Zika vaccine strain SV0010/15 demonstrated to be safe and immunogenic in Balb/c mice. Also, the dose formulation of Zika vaccine/alum at (5 µg /250 µg) produced a variable immunogenicity for ZIKV genotypes (Table 1).

Group (1)	Vaccine/Alum (Formulation)	Geometric Mean Titer (GMT) (2)	
		Day 0	Day 35
A	5/100	<10(3)/<10(4)	205/131
B	5/250	<10/<10	638/923

Table 1: Immunogenicity of Inactivated Zika vaccine strain SV0010/15 in Balb/C mice by Plaque Reduction Neutralization Test 50 % (PRNT50).

Mice group;

Geometric Mean Titer (GMT) was mean calculated from six mice neutralizing antibody;

Neutralized by Zika virus strain SV0010/15 (homologous strain);

Neutralized by Zika virus strain MR766 (heterologous strain)

From the Six cynomolgus macaques immunized with two doses of Zika vaccine/alum no local or systemic reactions were observed after injection. Most of the monkeys showed high positive ELISA of both IgM and IgG on day 14 (Figure 1). Also, the neutralizing antibody were observed on day 60 and day 180 against ZIKV strain SV0010/15 (homologous strain) and ZIKV strain MR766 strain (heterologous strain) with about 31, 48 and 14, 34 respectively (Figure 2).

Ultimately, all vaccinated monkeys were completely protected against ZIKV challenge by two strains of ZIKV, as demonstrated by the lack of detection of viral RNA in the serum samples but excepted the sham control macaques (Table 2).

Group (1)	Monkey code	Virus detection (2)	Virus isolation (Virus titration) (3)	Viremia (day)
I	1/1	-	-	-
	1/2	-	-	-
	1/3	-	-	-
II	1/4	-	-	-
	1/5	-	-	-
	1/6	-	-	-
III (4)	2/1	+	+ (2.1 log10)	183
	2/2	+	+ (1.8 log10)	184
	2/3	+	+ (2.3 log10)	183

Table : 2 Virus detection, isolation and titration after Challenging test in Cynomolgus monkeys.

Group I and III were respectively challenged with ZIKV strain SV0010/15 (homologous strain) while Group II specimens were challenged by with ZIKV strain MR766 (heterologous strain);

Virus detection by RT PCR;

Virus isolation by C6/36, IFA and Virus titration by Plaque assay;

Positive control challenge group;

Discussion

These preclinical results in small animal model showed the safety (dose) and immunogenicity of the inactivated Zika vaccine. The experiments in macaques confirmed the prior results in mice and could be extend them to Non-Human Primate model using vaccine doses and an administration route applicable to humans. Also, the optimized Zika vaccine adjuvanted with AlOOH provided complete 100% protection against two strains of Zika virus of Cynomolgus macaques 6 months after immunization. No specific adverse effects related to the vaccine was reported based on local and systemic observations [22]. Seroconversion was shown in all macaques including IgG/IgM while neutralizing antibody responses remained detectable at 6 months post immunization. This candidate vaccine is the first-generation Zika vaccine in Thailand. Also, it is compliant and deliver a high-quality vaccine.

Conclusion

This new inactivated ZIKV vaccine quality candidate has a potential to be developed up to GMP pilot scale. Combined with its excellent performance in animal models this indicates that the vaccine would be appropriate for an accelerated development based on public demand.

Acknowledgements

We would like to thank Doctor Rome Buathong Burean of Epidemiology Department of Disease Control, Ministry of Public Health who provide Zika virus strain SV0010/15 and the Government Pharmaceutical Organization (GPO) who provide WHO Vero cell, some supported and staffs. Also, the authors thank the staffs of the Center

Time (day post inoculation)	Monkey																	
	Immunized group (1)												Negative control group (2)					
	1/1		1/2		1/3		1/4		1/5		1/6		2/1		2/2		2/3	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgG	IgG	IgG	IgG
0	0(3)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	2.3	0	2.4	0	0	0	3.4	0	2.7	0	0	0	0	0	0	0
14	2.5	2.5	11.6	3.7	18.3	5.1	5.0	4.8	30	3.4	22.2	3.1	0	0	0	0	0	0
30	0	2.2	5.8	3.1	9.7	5.0	2.6	5.3	18.1	3.3	16.8	3.9	0	0	0	0	0	0

Figure : 1 ELISA Reacting antibody from immunized cynomolgus macaque with inactivated Zika vaccine strain ZIKV SV0010/15.

Time (day post inoculation)	Monkey																	
	Immunized group (1)												Negative control group (2)					
	1/1		1/2		1/3		1/4		1/5		1/6		2/1		2/2		2/3	
	Z1(3)	Z2(4)	Z1	Z2	Z1	Z2	Z1	Z2	Z1	Z2	Z1	Z2	Z1	Z2	Z1	Z2	Z1	Z2
0	<10(5)	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
60	11	18	273	320	12	20	91	149	26	33	10	21	<10	<10	<10	<10	<10	<10
180	16	<10	244	172	18	<10	87	120	12	24	20	13	<10	<10	<10	<10	<10	<10

Figure : 2 Immunogenicity of Inactivated Zika vaccine candidate (ZIKV strain SV0010/15) in Monkey by Plaque Reduction Neutralization Test 50 % (PRNT50).

They had injected by Inactivated ZIKV vaccine strain SV0010/15;

They had injected by adjuvant and PBS7.4;

Neutralized by ZIKV strain SV0010/15 (homologous strain);

Neutralized by ZIKV MR766 strain (heterologous strain);

PRNT value

for Vaccine Development Institute of Molecular Biosciences and Faculty of Veterinary Science Mahidol University who helped on this project. This project is supported by National Vaccine Institute (NVI) Ministry of Public Health. Dr Gonzalez was supported by ERP International LLC.

References

1. Tham HW, Balasubramaniam V, Ooi MK, Chew MF (2018) Viral Determinants and Vector Competence of Zika Virus Transmission. Front Microbiol 9: 1040.
2. Calvet GA, Santos FB, Sequeira PC (2016) Zika virus infection: Epidemiology, clinical manifestations and diagnosis. Curr Opin Infect Dis 29: 459-466.
3. Dick GW, Kitchen SF, Haddow AJ (1952) Zika virus Isolations and serological specificity.Trans R Soc Trop Med Hyg 46: 509–520.
4. Gulland A (2016) Zika virus is a global public health emergency, declares WHO. BMJ: 352-657.
5. Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, et al. (2016) Zika virus associated with microcephaly.N Engl J Med 374: 951-958.
6. Costello A, Dua T, Duran P, Gülmezoglu M, Oladapo OT, et al. (2016) Defining the syndrome associated with congenital Zika virus infection. Bull World Health Organ 94: 406-406A.
7. Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, González-Manrique G, et al. (2016) Guillain–Barré Syndrome Associated with Zika Virus Infection in Colombia. N Engl J Med 375: 1513-1523.
8. Pond WL (1963) Arthropod-borne virus antibodies in sera from residents of South-East Asia. Trans R Soc Trop Med Hyg 57: 364-371.
9. Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, et al. (2014) First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg 91: 1035–1038.
10. Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, et al. (2015) Detection of Zika Virus Infection in Thailand, 2012-2014. Am J Trop Med Hyg 93: 380-383.
11. Pattnaik A, Sahoo BR, Pattnaik AK (2020) Current Status of Zika Virus Vaccines: Successes and Challenges. Vaccines (Basel) 8: 266.
12. Abbink P, Larocca RA, De La Barrera RA, Bricault CA, Moseley ET, et al. (2016) Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. Science 353: 1129-1132.
13. Larocca RA, Abbink P, Peron JP, Zanotto PM, Iampietro MJ, et al. (2016) Vaccine protection against Zika virus from Brazil. Nature 536: 474-478.
14. World Health Organization. Annex 6: Requirements for Japanese Encephalitis Vaccine (Inactivated) for Human Use. World Health Organization Technical Report Series, Vo.771, 1988.
15. Sumathy K, Kulkarni B, Gondu RK, Ponnuru SK, Bonguram N, et al. (2017) Protective efficacy of Zika vaccine in AG129 mouse model. Sci Rep 7: 46375.
16. Tio PH, Malasit P (1995) Anti-dengue IgG detection by an indirect ELISA. Southeast Asian J Trop Med Public Health 26: 673-676.

17. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, et al. (1989) An enzyme-linked immunosorbent assay to characterize dengue infection where dengue and Japanese encephalitis co-circulate. Am J Trop Med. Hyg 40: 418-427.

18. Shu, PY, Chen, LK., Chang SF, Yueh, YY, Chow L, et al. (2003) Comparison of capture immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) and nonstructural protein NS1 serotype-specific IgG ELISA for differentiation of primary and secondary dengue virus infections. Clin Diagn Lab Immunol 10: 622-630.

19. Russell PK, Nisalak A, Sukhavachana P, Vivona S (1967) A plaque reduction test for dengue virus neutralizing antibodies. J Immunol 99: 285-290.

20. Faye O, Dupressoir A, Weidmann M, Ndiaye M, Alpha Sall A, et al (2008) One-step RT-PCR for detection of Zika virus. J ClinVirol 43: 96-101.

21. Henchal EA, McCown JM, Seguin MC, Gentry MK, Brandt WE (1983) Rapid identification of dengue virus isolates by using monoclonal antibodies in an indirect immunofluorescence assay. Am J Trop Med Hyg 32: 164-169.

22. Lecouturier V, Pavot V, Berry C, Donadieu A, de Montfort A, et al. (2020) An optimized purified inactivated Zika vaccine provides sustained immunogenicity and protection in cynomolgus macaques. NPJ Vaccines 5: 19.

Advances In Industrial Biotechnology | ISSN: 2639-5665

Advances In Microbiology Research | ISSN: 2689-694X

Archives Of Surgery And Surgical Education | ISSN: 2689-3126

Archives Of Urology

Archives Of Zoological Studies | ISSN: 2640-7779

Current Trends Medical And Biological Engineering

International Journal Of Case Reports And Therapeutic Studies | ISSN: 2689-310X

Journal Of Addiction & Addictive Disorders | ISSN: 2578-7276

Journal Of Agronomy & Agricultural Science | ISSN: 2689-8292

Journal Of AIDS Clinical Research & STDs | ISSN: 2572-7370

Journal Of Alcoholism Drug Abuse & Substance Dependence | ISSN: 2572-9594

Journal Of Allergy Disorders & Therapy | ISSN: 2470-749X

Journal Of Alternative Complementary & Integrative Medicine | ISSN: 2470-7562

Journal Of Alzheimers & Neurodegenerative Diseases | ISSN: 2572-9608

Journal Of Anesthesia & Clinical Care | ISSN: 2378-8879

Journal Of Angiology & Vascular Surgery | ISSN: 2572-7397

Journal Of Animal Research & Veterinary Science | ISSN: 2639-3751

Journal Of Aquaculture & Fisheries | ISSN: 2576-5523

Journal Of Atmospheric & Earth Sciences | ISSN: 2689-8780

Journal Of Biotech Research & Biochemistry

Journal Of Brain & Neuroscience Research

Journal Of Cancer Biology & Treatment | ISSN: 2470-7546

Journal Of Cardiology Study & Research | ISSN: 2640-768X

Journal Of Cell Biology & Cell Metabolism | ISSN: 2381-1943

Journal Of Clinical Dermatology & Therapy | ISSN: 2378-8771

Journal Of Clinical Immunology & Immunotherapy | ISSN: 2378-8844

Journal Of Clinical Studies & Medical Case Reports | ISSN: 2378-8801

Journal Of Community Medicine & Public Health Care | ISSN: 2381-1978

Journal Of Cytology & Tissue Biology | ISSN: 2378-9107

Journal Of Dairy Research & Technology | ISSN: 2688-9315

Journal Of Dentistry Oral Health & Cosmesis | ISSN: 2473-6783

Journal Of Diabetes & Metabolic Disorders | ISSN: 2381-201X

Journal Of Emergency Medicine Trauma & Surgical Care | ISSN: 2378-8798

Journal Of Environmental Science Current Research | ISSN: 2643-5020

Journal Of Food Science & Nutrition | ISSN: 2470-1076

Journal Of Forensic Legal & Investigative Sciences | ISSN: 2473-733X

Journal Of Gastroenterology & Hepatology Research | ISSN: 2574-2566

Journal Of Genetics & Genomic Sciences | ISSN: 2574-2485

Journal Of Gerontology & Geriatric Medicine | ISSN: 2381-8662

Journal Of Hematology Blood Transfusion & Disorders | ISSN: 2572-2999

Journal Of Hospice & Palliative Medical Care

Journal Of Human Endocrinology | ISSN: 2572-9640

Journal Of Infectious & Non Infectious Diseases | ISSN: 2381-8654

Journal Of Internal Medicine & Primary Healthcare | ISSN: 2574-2493

Journal Of Light & Laser Current Trends

Journal Of Medicine Study & Research | ISSN: 2639-5657

Journal Of Modern Chemical Sciences

Journal Of Nanotechnology Nanomedicine & Nanobiotechnology | ISSN: 2381-2044

Journal Of Neonatology & Clinical Pediatrics | ISSN: 2378-878X

Journal Of Nephrology & Renal Therapy | ISSN: 2473-7313

Journal Of Non Invasive Vascular Investigation | ISSN: 2572-7400

Journal Of Nuclear Medicine Radiology & Radiation Therapy | ISSN: 2572-7419

Journal Of Obesity & Weight Loss | ISSN: 2473-7372

Journal Of Ophthalmology & Clinical Research | ISSN: 2378-8887

Journal Of Orthopedic Research & Physiotherapy | ISSN: 2381-2052

Journal Of Otolaryngology Head & Neck Surgery | ISSN: 2573-010X

Journal Of Pathology Clinical & Medical Research

Journal Of Pharmacology Pharmaceutics & Pharmacovigilance | ISSN: 2639-5649

Journal Of Physical Medicine Rehabilitation & Disabilities | ISSN: 2381-8670

Journal Of Plant Science Current Research | ISSN: 2639-3743

Journal Of Practical & Professional Nursing | ISSN: 2639-5681

Journal Of Protein Research & Bioinformatics

Journal Of Psychiatry Depression & Anxiety | ISSN: 2573-0150

Journal Of Pulmonary Medicine & Respiratory Research | ISSN: 2573-0177

Journal Of Reproductive Medicine Gynaecology & Obstetrics | ISSN: 2574-2574

Journal Of Stem Cells Research Development & Therapy | ISSN: 2381-2060

Journal Of Surgery Current Trends & Innovations | ISSN: 2578-7284

Journal Of Toxicology Current Research | ISSN: 2639-3735

Journal Of Translational Science And Research

Journal Of Vaccines Research & Vaccination | ISSN: 2573-0193

Journal Of Virology & Antivirals

Sports Medicine And Injury Care Journal | ISSN: 2689-8829

Trends In Anatomy & Physiology | ISSN: 2640-7752

Herald Scholarly Open Access, 2561 Cornelia Rd, #205, Herndon, VA 20171, USA.
Tel: +1 202-499-9679; E-mail: info@heraldsopenaccess.us
http://www.heraldopenaccess.us/