

**“COMPARISON OF NON STRUCTURAL PROTEIN-1 ANTIGEN
DETECTION BY IMMUNOCHROMATOGRAPHY TEST AND ENZYME
LINKED IMMUNO SORBENT ASSAY AND CORRELATION WITH
REAL-TIME POLYMERASE CHAIN REACTION FOR EARLY
DIAGNOSIS OF DENGUE WITH SEROTYPING AND CO-INFECTIONS
OF DENGUE IN AND AROUND ANANTHAPURAMU DISTRICT”**

ATHESIS

Submitted by

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BONAFIDE CERTIFICATE

Certified that this thesis titled **“COMPARISON OF NON STRUCTURAL PROTEIN-1 ANTIGEN DETECTION BY IMMUNO CHROMATOGRAPHY TEST AND ENZYME LINKED IMMUNO SORBENT ASSAY AND CORRELATION WITH REAL-TIME POLYMERASE CHAIN REACTION FOR EARLY DIAGNOSIS OF DENGUE WITH SEROTYPING AND CO INFECTIONS OF DENGUE IN AND AROUND ANATHAPURAMU DISTRICT”** is the bonafide work of **Mr. ADIREDDI PARADESI NAIDU (Reg.No.D16MS514)** who carried out the research under my supervision.

Certified further, that to the best of my knowledge the work reported herein and in the publications by the scholar does not form part of any other thesis or dissertation, on the basis of which a degree or award was conferred on an earlier occasion of thesis of any other candidate.

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DECLARATION

I ADIREDDI PARADESI NAIDU (D16MS514) declare that the thesis entitled **“COMPARISON OF NON STRUCTURAL PROTEIN-1ANTIGEN DETECTION BY IMMUNOCHROMATOGRAPHY TEST AND ENZYME LINKED IMMUNO SORBENT ASSAY AND CORRELATION WITH REAL-TIME POLYMERASE CHAIN REACTION FOR EARLY DIAGNOSIS OF DENGUE WITH SEROTYPING AND CO-INFECTIONS OF DENGUE IN AND AROUND ANANTHAPURAMU DISTRICT”** is an original independent research work done of my own in the partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy (Ph.D.,) and Submitted to the BIHER was carried under the guidance of **Dr. CHITRALEKHA SAIKUMAR MD., PROFESSOR AND HEAD,** Dept of Microbiology, Sree Balaji Medical College, Chrompet, Chennai, Tamilnadu.

I further declare that I have not submitted any of the results of the study for the award of any other degree, diploma, and Associate ship, Fellowship or any other similar title to any other university/institute prior to this date.

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ABSTRACT

Dengue is one of the most important mosquito-borne diseases in the world. The emergence and spread of four Dengue viruses (DENVs) (Serotypes) represent a global pandemic. The four distinct serotypes are, namely, DENV-1, DENV-2, DENV-3 and DENV-4. Early diagnosis of dengue fever is important for effective clinical care and vector control. Different serological tests based on the principle of immunochromatography and Enzyme-Linked Immunosorbent Assay (ELISA) is commonly used for detection of antigen and antibodies of dengue virus. The performance of these tests depends on the sensitivity and specificity. Hence, the study was undertaken to compare non structural protein-1 (NS1) antigen detection by rapid and ELISA with real-time polymerase chain reaction (RT-PCR) for diagnosis of dengue. Very few dengue serotyping studies have been reported from Andhra Pradesh. In this context, the present study focuses on the circulating serotypes of dengue in Ananthapuramu district, Andhra Pradesh. Infection with two agents might cause overlapping symptoms, confusing the treating physician. Dengue associated co-infections are rarely described and the evidence exists only in the form of few case reports and case series, that too individual co-infections dengue with either bacterial infections like UTI and blood stream infections, chikungunya, malaria, enteric fever, leptospirosis, scrub typhus, hepatitis B, or recently pandemic COVID-19. This co-infections study is important for public health point of view considering types of associated co-infections, severity of the diseases, management issues and prognosis in dengue patients in GGH Ananthapuramu.

This diagnostic Prospective study was done on 100 clinically suspected cases of Dengue between July-November 2021 at a tertiary care hospital (GGH) in Ananthapuramu, Andhra Pradesh, India. All the sera samples were collected and

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LIST OF ABBREVIATIONS, SYMBOLS

Ab	Antibody
ADCC	Antibody dependent cellular cytotoxicity
ADE	Antibody dependent enhancement
AFI	Acute febrile illness
Ag	Antigen
ALP	Alkaline phosphatase
ALT	Alanine transaminase
APT	Antigen processing transporter
ASO	Anti Streptolysin-o
AST	Aspartate transaminase
AST	Antibiotic Sensitivity Testing
BS	Bacillus sphaericus
BSI	Blood Stream Infection
C	Nucleo capsid or Coreprotein
CBP	Complete Blood Picture
CD	Cluster of differentiation
CDC	Centre for Disease Control
cDNA	Complementary deoxynucleicacid
CONS	Coagulase Negative Staphylococci
COVID-19	Coronavirus disease 2019
CFT	Complement fixation test
CHIKV	Chikungunya virus
CRP	C-Reactive Protein
CyT-TDV	Chimeric yellow fever-Tetra valent Dengue vaccine
DC	Dendriticcell

DF	Dengue Fever
DENV	Dengue virus
DENV-1	Dengue virus serotype1
DENV-2	Dengue virus serotype2
DENV-3	Dengue virus serotype3
DENV-4	Dengue virus serotype4
DHF	Dengue hemorrhagic fever
DHR	Department of Health Research
DNA	Deoxyribonucleic acid
DSS	Dengue shock syndrome
E	Envelope protein
EC	Endothelial cell
EDTA	Ethylene diamine tetraacetic acid
EIP	Extrinsic Incubation Period
ELISA	Enzyme linked immune sorbent assay
ER	Endoplasmic reticulum
GGH	Government General Hospital
GMC	Government Medical college
GPC	Gram Positive Cocci
GNB	Gram Negative Bacilli
H₂O₂	Hydrogen Peroxide
HBsAg	Hepatitis-B Surface Antigen
HCT	Haematocrit
HI	Haem agglutination inhibition
HLA	Human leucocyte antigen
HRP II	Histidine Rich Protein
IFN-γ	Interferon- γ

IC	Internal Control
ICMR	Indian Council of Medical Research
ICT	Immunochromatographic test
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-10	Interleukin-10
IMVIC	Indole, Methyl Red, Voges Proskauer, and Citrate test
ITMN	Insecticide Treated Mosquito Net
JEV	Japanese encephalitis
kDa	Kilo dalton
LA	Latex Agglutination
LAF	Laminar Air Flow
M	Membrane associated protein
MAC-ELISA	Monoclonal Antibody Capture-Enzyme linked immune sorbent assay
MALDI TOF MS	Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry
mAb	Monoclonal antibody
MIC	Minimum Inhibitory Concentration
MR	Methyl red Test
mL	Milli liter
mRNA	Messenger ribo nucleic acid

NAAT	Nucleic acid amplification tests
NASBA	Nucleic acid sequence bases amplification
NIV	National Institute of Virology
Non SEA	Non South East Asian
nm	Nanometre
NPV	Negative Predictive Value
NS1	Non structural 1
NS2A	Non structural 2A
NS2B	Non structural 2B
NS3	Non structural 3
NS4A	Non structural 4A
NS4B	Non structural 4B
NS5	Non structural 5
NT	Neutralisation test
NVBDCP	National Vector Borne Disease Control Programme
OD	Optical density
ORF	Open Reading Frame
PAF	Platelet activating factor
PCR	Polymerase Chain Reaction
pLDH	Parasite Lactate dehydrogenase
Pf	Plasmodium falcipuram
PPV	Positive Predictive Value
Pv	Plamodium vivax

prM	Precursor of Mprotein
RA	Rhuematoid Arthritis
RDT	Rapid Diagnostic Test
RNA	Ribo nucleicacid
RT-PCR	Reverse transcriptase polymerase chain reaction
SEA	South East Asian
TF	Tissue factor
TMB	Tetramethyl Benzidine
TNF	Tumor necrosis factor
TNF-α	Tumor Necrosis Factor alpha
TI	Theraputic Index
USA	United States of America
UTI	Urinary Tract Infection
VP	Voges - Proskauer Test
VRDL	Viral Research Diagnostic Laboratory
WBC	White blood cells
WHO	World Health Organization
WNV	West Nile Virus
YFV	Yellow Fever Virus
μm	micrometre
μ	Micron
$^{\circ}\text{C}$	Degree centigrade
$^{\circ}\text{F}$	Degree Fahrenheit