

**NON-INVASIVE TRANSLATIONAL DIAGNOSTIC
TECHNOLOGIES USING CONSERVED AND EPIGENETIC
BIOMARKERS FOR PAN-CANCER AND VIRAL DETECTION**

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Non-invasive Translational Diagnostic Technologies using Conserved and Epigenetic Biomarkers for Pan-cancer and Viral Detection

by

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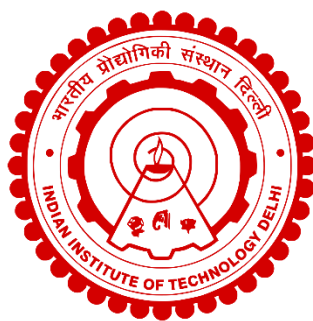
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**I dedicate this thesis to my parents, Mr Siddharth and Mrs Asha
Dube.**

Certificate

This is to certify that the thesis titled “Non-invasive Translational Diagnostic Technologies using Conserved and Epigenetic Biomarkers for Pan-cancer and Viral Detection” being submitted by Miss. Tejal Siddharth Dube, in the Department of Chemical Engineering, Indian Institute of Technology Delhi, for the award of the degree of Doctor of Philosophy in Chemical Engineering, is a record of the original, bona fide research work carried out by her under my guidance and supervision. In my opinion, the thesis has reached the standards fulfilling the requirements of the regulations relating to the degree. The results contained in this thesis have not been submitted for the award of any other degree, associateship or similar title of any university or institution.

Prof. Manojkumar Ramteke,
Department of Chemical
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Abstract

The global burden of cancer and infectious diseases has necessitated the development of diagnostic strategies that are not only accurate and sensitive but also rapid, scalable, and accessible to populations with limited healthcare infrastructure. Traditional diagnostic techniques, such as imaging, PCR, and tissue biopsies, though effective, often fall short in providing early detection, especially in decentralized and resource-constrained settings. This thesis addresses these challenges by presenting four interlinked diagnostic innovations that leverage biosensing, artificial intelligence (AI), epigenetic computation, and nanotechnology.

The first objective focuses on the development of a novel label-free biosensor platform Asima™ Rev, for the early detection of cancer using circulating cell-free DNA (cfDNA). This system employs impedance spectroscopy to detect changes in the electrochemical properties of cfDNA associated with global hypomethylation, a hallmark of early-stage malignancies. The sensor eliminates the need for complex sample processing or optical labelling, thereby making cancer screening more affordable and field-deployable.

Building upon this, the second objective introduces PanCHealth, a machine learning (ML) framework that integrates impedance data, cfDNA concentration, and global methylation percentage to accurately predict cancer stage. Using real-world clinical data and model explainability tools such as SHAP (SHapley Additive exPlanations), this framework demonstrates high diagnostic accuracy and potential for clinical translation, particularly for cancer risk stratification.

The third objective explores the construction of DNA-based logic gates capable of executing Boolean operations based on the methylation state of DNA oligonucleotides. By leveraging impedance-based readouts, these gates function as molecular computing systems that can process diagnostic inputs without relying on optical or enzymatic outputs. This work not only expands the diagnostic utility of methylation biomarkers but also opens up avenues for biosensors that combine sensing with decision-making at the molecular level.

The fourth objective extends these innovations into the domain of infectious diseases through the development of a vertical flow assay for SARS-CoV-2 detection. This system utilizes gold nanoparticles conjugated with ACE2 receptors to detect virus-like particles (VLPs) in saliva samples. The colorimetric response is rapid and visible to the naked eye, eliminating the need for instrumentation. Compared to lateral flow kits, this assay demonstrates higher sensitivity

and is effective against multiple viral variants, emphasizing its utility for rapid field diagnostics.

Together, these four components offer a unified framework for point-of-care diagnostics that is applicable across oncology and virology. Each innovation targets a specific gap, whether it be early detection, staging, computational interpretation, or rapid infectious screening, yet they collectively represent a cohesive, modular, and scalable approach to 21st-century diagnostics. This thesis thus provides both technological solutions and conceptual advancements toward the future of accessible precision medicine.

Hindi Abstract

कैंसर और संक्रामक रोगों का वैश्विक बोझ ऐसे निदान रणनीतियों के विकास की आवश्यकता को दर्शाता है जो न केवल सटीक और संवेदनशील हों, बल्कि तेज़, स्केलेबल और सीमित स्वास्थ्य संसाधनों वाले क्षेत्रों में भी आसानी से उपलब्ध हो सकें। पारंपरिक तकनीकों जैसे इमेजिंग, पीसीआर और ऊतक बायोप्सी, यद्यपि प्रभावी हैं, प्रारंभिक चरण में रोग पहचान हेतु अक्सर असफल रहती हैं, विशेषकर विकेंद्रीकृत और संसाधन-सीमित सेटअप में। यह शोध चार परस्पर जुड़े हुए निदान नवाचार प्रस्तुत करता है जो बायोसेंसिंग, कृत्रिम बुद्धिमत्ता (AI), एपिजेनेटिक कंप्यूटेशन और नैनोप्रौद्योगिकी का उपयोग करते हैं।

प्रथम उद्देश्य में Asima™ Rev नामक एक नवीन, लेबल-फ्री बायोसेंसर प्लेटफ़ॉर्म का विकास किया गया है जो परिपथीय सेल-फ्री डीएनए के विद्युत-रासायनिक गुणों में बदलाव के माध्यम से कैंसर की शीघ्र पहचान करता है।

द्वितीय उद्देश्य PanCHHealth नामक मशीन लर्निंग ढांचे को प्रस्तुत करता है जो cfDNA की सांद्रता, इम्पीडेंस डेटा और मिथाइलेशन प्रतिशत को एकीकृत कर कैंसर स्टेज की भविष्यवाणी करता है। यह मॉडल व्याख्यात्मकता टूल्स जैसे SHAP का उपयोग करता है।

तृतीय उद्देश्य डीएनए के मिथाइलेशन स्थिति के आधार पर लॉजिक गेट्स बनाता है जो ऑप्टिकल या एंजाइमेटिक निर्भरता के बिना डायग्नोस्टिक इनपुट्स को प्रोसेस कर सकते हैं।

चतुर्थ उद्देश्य ACE2-संयुग्मित गोल्ड नैनोपार्टिकल्स के माध्यम से लार में SARS-CoV-2 वायरस-जैसे कणों की पहचान हेतु एक वर्टिकल फ्लो असे का विकास करता है। यह कलर-आधारित टेस्ट उपकरण-मुक्त है और कई वेरिएंट्स के विरुद्ध प्रभावी है।

ये चारों नवाचार मिलकर कैंसर और संक्रामक रोगों के लिए एकीकृत, मॉड्यूलर और भविष्य-उन्मुख डायग्नोस्टिक समाधान प्रस्तुत करते हैं जो सटीक, सुलभ और अनुकूलनीय हैं।

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Nomenclature

Abbreviation	Full Form
DNA	Deoxyribonucleic Acid
cfDNA	Circulating Cell-Free DNA
ctDNA	Circulating Tumour DNA
AI	Artificial Intelligence
PCR	Polymerase Chain Reaction
ML	Machine Learning
SHAP	SHapley Additive exPlanations
ACE2	Angiotensin-Converting Enzyme 2
VLPs	Virus-Like Particles
% w/v	Percentage Weight/Volume
°C	Degree Celcius
∧	AND
¬	NOT
∨	OR
C	Cytosine
sigma	Variance
bp	Base pair
dsDNA	Double-stranded DNA
ssDNA	Single-stranded DNA
RT	Room Temperature
GC Content	Amount of Guanine and Cytosine present in the DNA sequence
N	Number of samples
min	Time in Minutes
EGFR	Epidermal Growth Factor Receptor
KRAS	Kirsten rat sarcoma viral oncogene
TB	Tuberculosis
MERS	Middle East Respiratory Syndrome
RSV	Respiratory syncytial virus

Abbreviation	Full Form
HIV	Human
RNA	Ribonucleic Acid
cfRNA	Cell Free RNA
5hmC	5-hydroxymethylcytosine
PSA	Prostate-Specific Antigen
CRP	C-Reactive protein
CA-125	Cancer antigen 125
LC-MS	Liquid Chromatography-Mass Spectrometry
NMR	Nuclear Magnetic Resonance
TEM	Transmission Electron Microscopy
AuNP	Gold Nanoparticle
WGS	Whole Genome Sequence
VFA	Vertical Flow Assay
LFA	Lateral Flow Assay
AUC	Area Under Curve
ROC	Receiver Operating Characteristic
RT-qPCR	Reverse Transcription quantitative PCR
LAMP	Loop-mediated isothermal amplification
RT-LAMP	Reverse-transcription
DPV	differential pulse voltammetry
S1	Spike protein
PDMS	Polydimethylsiloxane
LB	Liquid Biopsy
%v/v	Percentage Volume/Volume
EDTA	ethylenediamine tetra-acetic acid
IDE	Interdigitated Electrode
Ω	Ohms
ΔZ	Delta Impedance
Ztest	Cancer/Healthy sample Impedance
Zref	Blank sample Impedance
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
CV	Coefficient of Variation

Abbreviation	Full Form
CpG	Cite of Cytosine
LOD	Limit of Detection
θ	Phase angle
gDNA	Genomic DNA
S/N	Signal-to-Noise ratio
hTERT	human Telomerase Reverse Transcriptase
DTC	Decision Tree Classifier
KNN	K-Nearest Neighbors Classifier
RFC	Random Forest Classifier
GBM	Gradient Boosting Machine
NB	Naive Bayes
XGB	Extreme Gradient Boosting
LGBM	Light Gradient Boosting Machine
GNB	Gaussian Naïve Bayes
SVM	Support Vector Machine
LR	Logistic Regression
SAT	Satis Problem
BSA	Bovine Serum Albumin
AuNP-ACE2-1	ACE2 functionalized 16nm diameter gold nanoparticle
AuNP-ACE2-2	ACE2 functionalized 40nm diameter gold nanoparticle
SD	Standard Deviation
GM	Global Methylation
PCC	Pearson Correlation Coefficient