

**DEVELOPMENT OF A MICROFLUIDIC
DEVICE FOR BIOMARKER
QUANTIFICATION AND ENUMERATION
OF BLOOD CELLS**

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**SCHOOL OF INTERDISCIPLINARY RESEARCH
INDIAN INSTITUTE OF TECHNOLOGY DELHI
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by

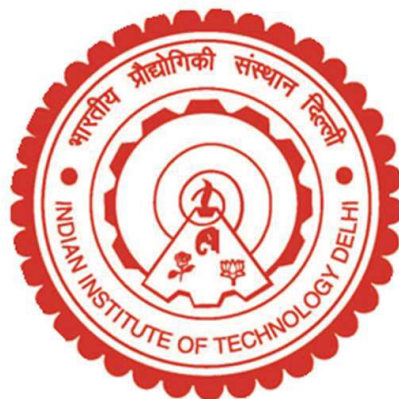
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Submitted

in partial fulfilment of the requirements of the degree of Doctor of Philosophy

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Dedicated to my Family

Certificate

This is to certify that the thesis entitled ‘Development of a microfluidic device for biomarker quantification and enumeration of blood cells’, submitted by Neha Khaware to the Indian Institute of Technology Delhi, for the award of the degree of Doctor of Philosophy in August 2026, is a record of the original, bona fide research work carried out by her under the supervision and guidance of Dr. Ravikrishnan Elangovan and Dr. Vivekanandan Perumal. The thesis has reached the standards fulfilling the requirements of the regulations related to the award of the degree.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma to the best of my knowledge.

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Prof. Vivekanandan Perumal
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Abstract

Developing a rapid and accurate diagnostic method for bacterial infections and their differentiation from viral infections remains a critical challenge and needs immediate attention. Bacterial and viral infections have overlapping symptoms that often confuse viral infections with bacterial infections, and patients are prescribed antibiotics without performing the standard diagnostic protocol, leading to unnecessary consumption. Misuse and overuse of antimicrobials are the significant drivers behind antimicrobial resistance development. The traditional diagnostic method for infection is a microbiological culture, which can take more than 48 hours to produce results, posing a significant challenge in managing critically ill patients. Hence, there is an invoking need for a rapid, accurate, and sensitive point-of-care test that can differentiate between bacterial and viral infections.

Blood cells have been explored as a non-invasive source of biomarkers for detecting infection. White blood cells are one of the early responders to infection, and their surface protein expression varies depending on the type of infection. For instance, type 1 interferons are rapidly induced in viral infections, activating the innate and adaptive immune cells. Similarly, IFN- γ is crucial for inducing an immune response against bacterial infections. Myeloid cells, particularly neutrophils and monocytes, express surface proteins crucial for their response against bacterial and viral infections.

In this work, I have developed two assays, one for detecting bacterial infections and the other for differentiation between bacterial and viral infections. For the first case, a rapid, sensitive, and easily deployable microfluidic device called mCytoCounter has been developed to detect bovine mastitis using milk samples. Mastitis is a bacterial infection characterized by an increase in the somatic cells in milk. Somatic cells mainly comprise leukocytes, and their

number is elevated in the case of mastitis. mCytoCounter consists of a microfluidic cartridge to capture and enrich the somatic cells on a membrane and an optical reader to quantify these cells and produce results on the screen that are easily readable by the users with a turnaround time of 20 minutes.

In the work's second part, CD64 and CD169 biomarkers expressed on leukocytes have been explored to develop an assay for distinguishing bacterial from viral infections. CD64 expression amplifies in bacterial infection and is an established biomarker for sepsis. CD64 is used in combination with CD169, which is a specific biomarker for viral infection. An assay for rapid and accurate quantification of these markers is developed and integrated into a microfluidic device called AB_xSure. Along with these biomarkers, total white blood cells are also quantified in AB_xSure to provide an all-in-one infection diagnosis system. AB_xSure consists of a microfluidic cartridge for sample processing and a device with an in-built optical reader for liquid maneuvering. The blood cells are lysed and labeled with fluorophore-conjugated antibodies to quantify CD64 and CD169 and a nucleic acid binding fluorescence dye for total leukocyte count.

The central focus of this thesis work has been to develop biological assays and systems that are rapid, easy to use, and more accessible in low-resource settings. Available somatic cell counters are bulky and expensive, and their incorporation into local dairy farms is challenging. mCytoCounter will provide an economical and easily deployable setup for monitoring mastitis to prevent the overuse of antibiotics and provide the required treatment to dairy animals. AB_xSure is a device that has the capability to differentiate bacterial from viral infections. This preliminary test can ensure that patients receive antibiotics only when required, thereby controlling overprescription and, eventually, antimicrobial resistance.

सार

बैक्टीरिया (जीवाणु) के संक्रमणों के लिए एक तीव्र और सटीक नैदानिक विधि विकसित करना और उन्हें वायरल (विषाणु) संक्रमणों से अलग करना एक महत्वपूर्ण चुनौती बनी हुई है, जिस पर तत्काल ध्यान देने की आवश्यकता है। बैक्टीरिया और वायरल संक्रमणों के लक्षण आपस में मिलते-जुलते (overlapping) होते हैं, जिसके कारण अक्सर वायरल संक्रमण को बैक्टीरिया का संक्रमण समझ लिया जाता है। इसके परिणामस्वरूप, मानक नैदानिक प्रोटोकॉल (standard diagnostic protocol) का पालन किए बिना ही रोगियों को एंटीबायोटिक दवाएं दे दी जाती हैं, जिससे उनका अनावश्यक सेवन बढ़ता है। रोगाणुरोधी दवाओं (antimicrobials) का दुरुपयोग और अत्यधिक उपयोग, रोगाणुरोधी प्रतिरोध (antimicrobial resistance) के विकसित होने के पीछे प्रमुख कारण हैं। संक्रमण की पहचान की पारंपरिक नैदानिक विधि 'माइक्रोबायोलॉजिकल कल्चर' है, जिसके परिणाम आने में 48 घंटे से अधिक का समय लग सकता है। यह गंभीर रूप से बीमार रोगियों के इलाज और प्रबंधन में एक बड़ी चुनौती पेश करता है। इसलिए, एक तीव्र, सटीक और संवेदनशील 'पॉइंट-ऑफ-केयर' (point-of-care) परीक्षण की अत्यधिक आवश्यकता है जो बैक्टीरिया और वायरल संक्रमणों के बीच स्पष्ट रूप से अंतर कर सके।

रक्त कोशिकाओं (blood cells) का उपयोग संक्रमण की पहचान के लिए बायोमार्कर (biomarkers) के एक गैर-आक्रामक (non-invasive) स्रोत के रूप में किया गया है। श्वेत रक्त कोशिकाएं (white blood cells) संक्रमण के प्रति शुरुआती प्रतिक्रिया देने वाली कोशिकाओं में से एक हैं, और उनकी सतह पर प्रोटीन की अभिव्यक्ति (expression) संक्रमण के प्रकार के आधार पर भिन्न होती है। उदाहरण के लिए, वायरल संक्रमणों में टाइप 1 इंटरफेरॉन (type 1 interferons) तेजी से सक्रिय होते हैं, जो जन्मजात (innate) और अनुकूलन योग्य (adaptive) प्रतिरक्षा कोशिकाओं को प्रेरित करते हैं। इसी प्रकार,

बैक्टीरिया के संक्रमण के विरुद्ध प्रतिरक्षा प्रतिक्रिया उत्पन्न करने में 'IFN- γ ' की महत्वपूर्ण भूमिका होती है। मायलॉइड कोशिकाएं (myeloid cells), विशेष रूप से न्यूट्रोफिल और मोनोसाइट्स, ऐसी सतही प्रोटीन (surface proteins) व्यक्त करती हैं जो बैक्टीरिया और वायरल संक्रमणों के विरुद्ध उनकी प्रतिक्रिया के लिए अत्यंत महत्वपूर्ण हैं।

इस शोध कार्य में, मैंने दो एसे (assays) विकसित किए हैं, एक बैक्टीरियल संक्रमणों का पता लगाने के लिए और दूसरा बैक्टीरियल एवं वायरल संक्रमणों के बीच अंतर करने के लिए। पहले मामले के लिए, दूध के नमूनों का उपयोग करके बोवाइन मैस्टाइटिस (bovine mastitis) का पता लगाने के लिए mCytoCounter नामक एक तीव्र, संवेदनशील और सुगमता से तैनात (deployable) किए जाने योग्य माइक्रोफ्लुइडिक उपकरण विकसित किया गया है। मैस्टाइटिस एक बैक्टीरियल संक्रमण है जिसकी पहचान दूध में सोमैटिक कोशिकाओं (somatic cells) की वृद्धि से होती है। सोमैटिक कोशिकाओं में मुख्य रूप से ल्यूकोसाइट्स (leukocytes) होते हैं, और मैस्टाइटिस की स्थिति में उनकी संख्या बढ़ जाती है। mCytoCounter में एक झिल्ली (membrane) पर सोमैटिक कोशिकाओं को कैचर और समृद्ध (enrich) करने के लिए एक माइक्रोफ्लुइडिक कार्ट्रिज और इन कोशिकाओं की गणना (quantify) करने के लिए एक ऑप्टिकल रीडर होता है, जो स्क्रीन पर ऐसे परिणाम प्रदर्शित करता है जिन्हें उपयोगकर्ता आसानी से पढ़ सकते हैं; इसकी पूरी प्रक्रिया (turnaround time) मात्र 20 मिनट की है।

इस कार्य के दूसरे भाग में, ल्यूकोसाइट्स (leukocytes) पर व्यक्त होने वाले CD64 और CD169 बायोमार्कर के माध्यम से बैक्टीरियल और वायरल संक्रमणों के बीच अंतर करने के लिए एक 'एसे' (assay) विकसित करने का अन्वेषण किया गया है। बैक्टीरियल संक्रमण में CD64 की अभिव्यक्ति (expression) बढ़ जाती है और यह सेप्सिस (sepsis) के लिए एक स्थापित बायोमार्कर है। CD64 का उपयोग CD169 के साथ किया जाता है, जो वायरल संक्रमण के लिए एक विशिष्ट बायोमार्कर है। इन मार्करों के तीव्र और सटीक मात्रा निर्धारण (quantification) के लिए एक 'एसे' विकसित किया गया है और इसे AB_xSure

नामक माइक्रोफ्लुइडिक उपकरण में एकीकृत (integrate) किया गया है। इन बायोमार्कर के साथ-साथ, 'ऑल-इन-वन' संक्रमण निदान प्रणाली प्रदान करने के लिए AB_xSure में कुल श्वेत रक्त कोशिकाओं (total white blood cells) की गणना भी की जाती है। AB_xSure में नमूना प्रसंस्करण (sample processing) के लिए एक माइक्रोफ्लुइडिक कार्ट्रिज और तरल संचालन (liquid maneuvering) के लिए एक इन-बिल्ट ऑप्टिकल रीडर वाला उपकरण शामिल है। रक्त कोशिकाओं को अपघटित (lysed) किया जाता है और CD64 एवं CD169 की गणना के लिए फ्लोरोफोर-संयुग्मित एंटीबॉडी (fluorophore-conjugated antibodies) के साथ, तथा कुल ल्यूकोसाइट गणना के लिए एक न्यूक्लिक एसिड बाइंडिंग फ्लोरोसेंस डाई (nucleic acid binding fluorescence dye) के साथ लेबल (label) किया जाता है।

स शोध कार्य का मुख्य केंद्र उन जैविक परीक्षणों (biological assays) और प्रणालियों को विकसित करना रहा है जो तीव्र, उपयोग में आसान और कम संसाधन वाले क्षेत्रों (low-resource settings) में अधिक सुलभ हों। वर्तमान में उपलब्ध 'सोमैटिक सेल काउंटर्स' भारी और महंगे हैं, और स्थानीय डेयरी फार्मों में उनका समावेशन चुनौतीपूर्ण है। mCytoCounter मैस्टाइटिस की निगरानी के लिए एक किफायती और आसानी से तैनात (deployable) करने योग्य व्यवस्था प्रदान करेगा ताकि एंटीबायोटिक दवाओं के अत्यधिक उपयोग को रोका जा सके और डेयरी पशुओं को आवश्यक उपचार प्रदान किया जा सके। AB_xSure एक ऐसा उपकरण है जिसमें बैक्टीरियल और वायरल संक्रमणों के बीच अंतर करने की क्षमता है। यह प्रारंभिक परीक्षण यह सुनिश्चित कर सकता है कि रोगियों को केवल आवश्यकता पड़ने पर ही एंटीबायोटिक दवाएं मिलें, जिससे दवाओं के अत्यधिक नुस्खे (overprescription) और अंततः रोगाणुरोधी प्रतिरोध (antimicrobial resistance) को नियंत्रित किया जा सके।

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Abbreviations

3D Three Dimensional

ADC Analog Digital Converter

AI Artificial Intelligence

AMR Anti-Microbial Resistance

APC Antigen Presenting Cells

AUC Area Under Curve

BSA Bovine Serum Albumin

CCD Charge- Coupled Device

CD11b Cluster of Differentiation 11b

CD14 Cluster of Differentiation 14

CD169 Cluster of Differentiation 169

CD64 Cluster of Differentiation 64

CDs Carbon Dots

CFSE 5-(and-6)-Carboxyfluorescein Diacetate Succinimidyl Ester

CMOS Complementary Metal-Oxide Semiconductor

CMT California Mastitis Test

CRP C-reactive protein

CXCR4 CXC chemokine receptor- 4

DAMPs Danger-Associated Molecular Patterns

DC Dendritic Cells

DLL1 Delta-Like Canonical Notch Ligand 1

DMSO Dimethyl Sulphoxide

DNA Deoxyribonucleic Acid

EBV Epstein - Barr virus

EDC N-(3-Dimethylaminopropyl)-N'-EthylCarbodiimide hydrochloride

EDTA Ethylenediaminetetraacetic acid disodium salt

ELISA Enzyme-linked immunosorbent assay

ERK Extracellular Signal-Regulated Kinase

ESR Erythrocyte Sedimentation Rate

FACS Fluorescence-Activated Cell Sorting

FcγRI Fragment of Crystallization Gamma Receptor 1

FDA Fluorescein Diacetate

G-CSF Granulocyte Colony-Stimulating Factor

GF/B Glass Fibre- B

GF/CP Glass Fibre- CP

GF/D Glass Fibre- D

GF/F Glass Fibre- F

GUI Graphical User Interface

HHV Human HerpesVirus

HIV Human Immunodeficiency Virus

HL60 Human Leukemia 60

HRP Horse Radish Peroxidase

HuCMV Human Cytomegalovirus

ICAM Intercellular Adhesion Molecule

IFN- α Interferon Alpha

IFN- γ Interferon Gamma

IgG Immunoglobulin G

IL-3 Interleukin-3

IL-6 Interleukin-6

INAA Isothermal Nucleic Acid Amplification

IP-10 interferon-gamma inducible protein-10

IRF-8 Interferon Regulatory Factor- 8

JAK-STAT Janus Kinase-signal transducer and activator of transcription

LAMP Lysosomal Associated Membrane Proteins

LED Light Emission Diode

LMIC Low And Middle-Income Countries

LOAD Lab-on-a-Disc

LOC Lab-on-a-Chip

LPS Lipopolysaccharide

MACS Magnetic-Activated Cell Sorting

MAPK Mitogen-Activated Protein Kinase

MCP-1 Monocyte Chemoattractant Protein-1

MDP Macrophage- DC Precursor

MEMS micro-electro-mechanical systems

MHC Major Histocompatibility Complex

miRNA microRNA

MNP Magnetic Nanoparticles

MPO Myeloperoxidase

MRSA Methicillin-resistant Staphylococcus aureus

NFκB Nuclear Factor kappa B

NHS N-Hydroxysuccinimide

NK cells Natural Killer Cells

PAMP Pathogen-Associated Molecular Pattern

PBS Phosphate Buffer

PC Polycarbonate

PCR Polymerase Chain Reaction

PCT Procalcitonin

PCTE Polycarbonate Track-Etched

pDCs Plasmacytoid DCs

PDMS Polydimethylsiloxane

PE Phycoerythrin

PHCs Primary Healthcare Centres

PKC Protein Kinase C

PLC Phospholipase C

PMA Phorbol 12-Myristate 13-Acetate

POC Point-of-Care

PP Polypropylene

PRR Pattern Recognition Receptors

PSGL-1 P- selectin glycoprotein ligand- 1

PU Polyurethane

QDs Quantum Dots

RBC Red Blood Cells

RFU Relative Fluorescence Units

RNS Reactive Nitrogen Species

ROC curve Receiver Operating Characteristic Curve

ROS Reactive Oxygen Species

RSV Rous-Sarcoma Virus

RT-LAMP Reverse Transcription- Loop-Mediated Isothermal Amplification

RT-PCR Reverse Transcription Polymerase Chain Reaction

SAMs Self-Assembled Monolayers

SARS-CoV Severe Acute Respiratory Syndrome Coronavirus

SCC Somatic Cell Count

SDF-1 Stromal-Derived Factor-1

SERS Surface-Enhanced Raman Scattering

SIGLEC Sialic Acid Binding Immunoglobulin-Like Lectins

SLA Stereolithography

STAT Signal Transducer And Activator Of Transcription

TFT Thin-film transistor

TLR Toll-Like Receptors

TNF- α Tumor Necrosis Factor α

TRAIL Tumor Necrosis Factor-related Apoptosis Inducing Ligand

T_{reg} cells Regulatory T cells

UCNPs Upconversion Nanoparticles

UV Ultraviolet

VDR Vitamin D Receptor

VEGF Vascular Endothelial Growth Factor

VZV Varicella-Zoster virus

WBC White Blood Cells

WMT Wisconsin Mastitis Test

μPADs Microfluidic Paper-Based Analytical Devices