National Institute of Pathology
(Indian Council of Medical Research)

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LAMP ASSAY A PROMISING DIAGNOSTIC TOOL FOR INFECTIOUS DISEASES

- Keerti Kaumudee Dixit

Nowadays in routine diagnostics of infectious diseases, techniques based on nucleic acid amplification are used. Polymerase chain reaction methods such as nested PCR, multiplex PCR, reverse transcription PCR or real-time PCR are commonly applied due to their high sensitivity, specificity and rapidity.

In spite of PCR being a powerful tool in diagnosis with high sensitivity, it requires a well-established lab and sophisticated thermal cycler for detection and analysis of amplification. The overall PCR running along with post-PCR steps are time consuming. Quantitative PCR is quite rapid and accurate in parasite quantification but it also requires expensive instrument, reagents and a complex analysis.

Loop mediated isothermal amplification (LAMP) is anticipated to be an innovative and novel technique to amplify DNA with high specificity and rapidity under isothermal conditions (Nagamine et al., 2002; Notomi et al., 2000, Takagi et al., 2009). LAMP method was developed by Eiken Chemical Company (Japan). It does not require any expensive instruments and complicated analysis. The technique is extremely sensitive, specific and rapid and most importantly does not require expensive instruments and complicated methods of detection.

LAMP has a wide range of applications from being used in food industry, agriculture, fisheries and more recently being used in diagnosis of various infectious diseases.In the initial phase of development, LAMP has been applied to many kinds of pathogens causing food-borne diseases.LAMP kits for detecting *Salmonella*, *Legionella*, *Listeria*, verotoxin-producing *Escherichia coli*, and *Campylobacter* have been commercialized. For eg. Utility of LAMP in detection of *Salmonella* in artificially contaminated milk products. It has also found its potential in detection of various aquatic pathogens in fisheries like *Tetracapsuloides bryosalmonae* etc.

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The Foundation for Innovative New Diagnostics (FIND) has partnered with Eiken Chemical Company (Eiken), Tokyo, Japan, to develop molecular assays for the detection of several infectious diseases (including TB, malaria, sleeping sickness and leishmaniasis) using their loop-mediated isothermal amplification platform (LAMP). This molecular method was chosen because of specific features, which favor its use in simplified testing systems that might be appropriate in resource-limited settings.

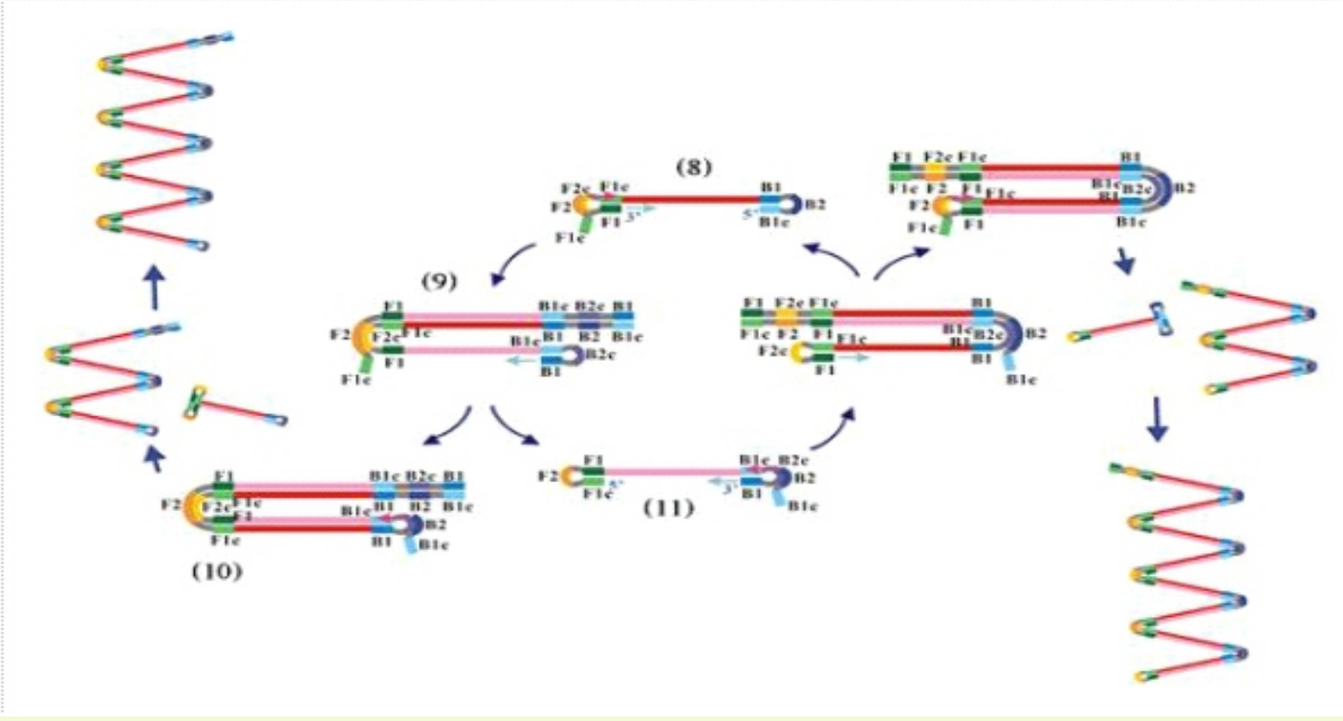
LAMP PRINCIPLE

The method requires 4-6 primers designed specifically for spanning 6-8 gene sequences. DNA amplification is carried out in presence of DNA polymerases (*Bst* polymerases) with strand displacing activity. Owing to this feature there is no need of denaturation step to obtain single stranded DNA. Thus the strand displacing activity permits isothermal amplification, unlike conventional PCR requiring denaturation step.This assay is based on generation of artificial stem loop structures flanking target sequences. This cyclic strand displacement is performed at a constant temperature of 65C at which double stranded DNA remains in a dynamic equilibrium.

1: The differential diagnosis is vast in most scenarios. For example, the differential diagnosis for a lung nodule detected on CT includes primary lung malignancies, metastasis from distant sites, benign neoplasms and nonneoplastic entities, such as nodular organizing pneumonia. It would be difficult for IVM to achieve the required sensitivity and specificity to reliably differentiate among all these potential entities.

2: Generally, high-resolution imaging techniques require endoscopic access (i.e., endoscopy, bronchoscopy, cystoscopy) to image tissues because of penetration depth limitations. If a patient

must undergo an endoscopic procedure to access the tissue for imaging, it is likely that the physician will continue to acquire physical tissue biopsy specimens to accompany optical imaging.



BASIC LAMP PRINCIPLE

(**Available at: http://loopamp.eiken.co.jp/e/lamp/principle.html**)

The overall LAMP process is divided into two main steps NON-CYCLIC and CYCLIC

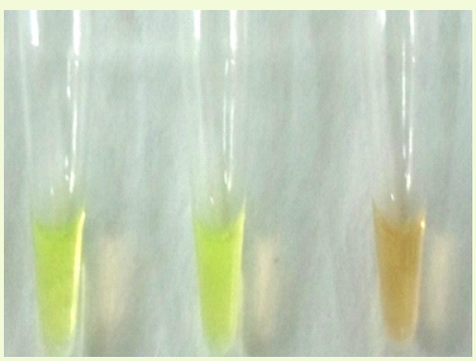
NON- CYCLIC

At the end of first step artificial stem-loops are generated which are to be used in further steps. Initially the forward inner primer (FIP primer) binds to the target sequence and initiates the polymerization. Next, the forward outer primer (F3 primer) binds to the product formed and displaces it with a single artificial stem-loop affixed to the target. The single stranded DNA now will serve as the template for BIP (BIP primer). Then, the reverse outer primer (B3 primer) binds to the product and displaces the product with two artificial stem loops flanking the target sequence. This structure will now be the starting point for next, cyclic amplification step.

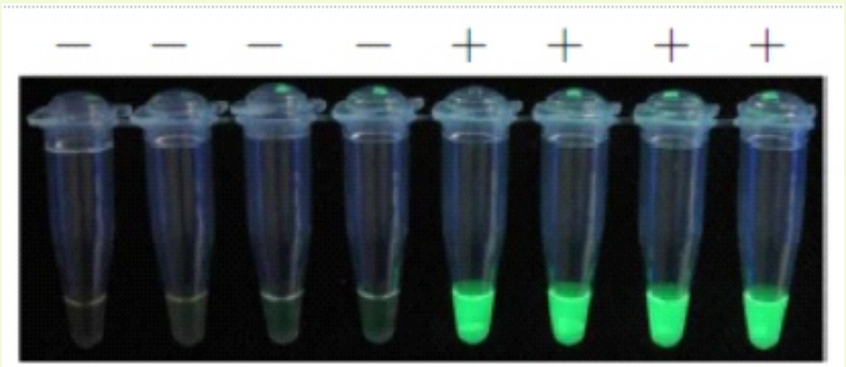
CYCLIC

This step begins with the FIP primer hybridization to the loop on the product formed in non-cyclic step. The displacing activity of polymerases leads to the generation of DNA with new stem loop structure at one end and an additional target sequence. These structures are used as templates in further DNA synthesis with the use of internal primers.At the end there is generation of various-sized structures possessing the alternately inverted repeats of the target sequence on the same strand. The addition of loop primers, which contain sequences complementary to the single stranded loop region on the 5' end of the hairpin structure, speeds the reaction by providing a greater number of starting points for DNA synthesis. Using loop primers, amplification by 10^9 - 10^{10} times can be achieved within 15-30 minutes.

The LAMP assay has several advantages which includes its sensitivity, specificity, high efficiency, rapidity and simplicity than any routine nucleic acid amplification assays. High specificity is obtained by the use of 4-6 primers spanning 6-8 target sequences of a gene. The amplification is performed at isothermal conditions using basic equipment such as heat block or water bath. Both amplification and detection of result can be carried out in same tube. Moreover the sensitivity of LAMP is less affected by the inhibitory components in DNA samples and DNA by-products (pyrophosphate salts), thus the reaction proceeds until large amounts of amplicon are generated. This feature makes visible detection of successful amplification possible by using ds DNA-binding dyes such as SYBR green, by detecting turbidity caused by precipitating magnesium pyrophosphate, or by using a non-inhibitory fluorescing reagent that is quenched in the presence of divalent cations.



Naked eye visual detection of LAMP amplified product using SYBR green



Visual read out of LAMP results on application of ultraviolet light

LAMP IN DIAGNOSTICS

The technique has been successfully applied in diagnosis of various diseases using different targets which are enlisted below

S.NO.	PATHOGEN	DISEASE	TARGET SEQUENCE	METHOD OF DETECTION	REFERENCE
1.	Coronavirus	SARS	1bRep gene	Analysis by recording optical density with real time turbidimeter	Hong et al.,2004
2.	*Mycobacterium tuberculosis, M. avium, M.intracellulare*	Mycobacteriosis	*gyrB* gene	Visualization by addition of SYBR green I to the reaction tube	Iwamoto et al., 2003
3.	*Plasmodium falciparum*	Malaria	18s ribosomal RNA gene	Analysis by turbidimeter in real-time or visually at the end of LAMP.	Poon et al, 2006
4.	*Staphylococcus aureus*	Sepsis	16s ribosomal RNA gene	Visually distinguished by turbidity	Zhang et al., 2013
5.	Human immunodeficiency virus	AIDS	Highly conserved sequences present within the protease & p24 gene regions	Identification by agarose gel electrophoresis and Visually by addition of fluorescent nucleic acid stain	Curtis et al, 2008,2009,2012
6.	*Staphylococcus pneumoniae*	Pneumonia	*LytA* gene	Analysis by agarose gel electrophoresis and visually by precipitation or turbidity,	Seki et al., 2005
7.	*Leishmania donovani*	Leishmaniasis	Kinetoplast minicircle DNA (kDNA)	Naked eye visual detection by addition of SYBR green	Verma et al, 2013
8.	*Salmonella enterica*	Enteridis	*Sdf1* sequence	Detection by SERS (Surface enhanced Raman spectroscopy)	Draz et al., 2016

The greatest challenge for using molecular assays in resource-limited settings is the complexity of sample preparation and DNA extraction. The lack of need for an initial denaturation step to form single-stranded DNA, the isothermal amplification conditions, the high amplification speed and efficiency, the requirement for only one inexpensive enzyme, the robustness to suboptimal reaction conditions or presence of inhibitors, and the generation of a visually detectable readout are all specific advantages that might allow the
development of simple and inexpensive molecular tests. These features make LAMP a promising platform for the development of nucleic acid amplification tests suitable for diagnosis of infectious diseases in developing countries.
Following references can be accessed for further details

1.Curtis, S. M., K. A. Rudolph and D. L. Owen (2008), 'Rapid detection of HIV-1 by reverse-transcription, loop-mediated isothermal amplification (rt-lamp)', *J Virol Methods* **2**, 264-270.

2.Curtis, S. M., K. A. Rudolph and D. L. Owen (2009), 'Sequence-specific detection method for reverse transcription,loop-mediated isothermal amplification of HIV-1',*J Med Virol* **81**, 966-972.

3.Curtis, S. M., K. A. Rudoplh, I. Nejad, J. Sinleton, A. Beddoe, B. Weigl, P. La Barre and S. M. Owen (2012), 'Isothermal amplification using a chemical heating device for point-of-care detection of HIV-1', *PLoS One* **7**, e31432

4.Hong, T. C., Q. L. Mai, D. V. Cuong, M. Parida, H. Minekawa and T. Notomi (2004),'Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus', *J Clin Microbiol* **42**, 1956-1961.

5.Iwamoto, T., T. Sonobe and K. Hayashi (2003), 'Loop-mediated isothermal amplification for direct detection of mycobacterium tuberculosis complex, *M. avium,* and M. intracellulare in sputum samples', *J Clin Microbiol* **41**, 2616-2622.

6.Notomi, T., H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe and N. Amino (2000), 'Loop-mediated isothermal amplification of DNA', *Nucleic Acids Re* **28**, e63.

7.Verma S, Avishek K, Sharma V, Negi NS, Ramesh V, Salotra P. Application of loop mediated isothermal amplification assay for the sensitive and rapid diagnosis of visceral leishmaniasis and post-kala-azar dermal leishmaniasis. Diagn Microbiol Infect Dis. 2013 Apr;75(4):390-5. doi: 10.1016/j.diagmicrobio.2013.01.011. Epub 2013 Feb 19

8.Seki, M., Y. Yamashita, H. Torigoe, H. Tsuda, S. Sato and M. Maeno (2005), 'Loop mediated isothermal amplification method targeting the lyta gene for detection of *Streptococcus pneumoniae'*, *J Clin Microbiol* **43**, 1581-158.

**** The views expressed are purely that of the author.***

Very Nice Definition of Time

Time is slow when you wait!

Time is fast when you are late!

Time is deadly when you are sad!

Time is short when you are happy!

Time is endless when you are in pain!

Time is long when you feel bored!

Every time, time is determined by your

Feelings and your psychological conditions

and not by clocks. So have a nice time Always!

Journals Club

•"HLA-C and TNF gene polymorphisms are associated with psoriasis in Brazilian patients" by Ms. Sachita Singh On 14-1-2016.

• "CNS manifestation of HIV infection" by Dr. Chandra Bhushan Rai on 19-1-2016.

•"Cytoplasmic levels of cFLIP determine a broad susceptibility of breast cancer stem/progenitor-like cells to TRAIL" by Ms.Renu Yadav on 19-1-2016.

•"Vasculitis" by Dr. Chandra Bhushan Rai on 9-2-2016.

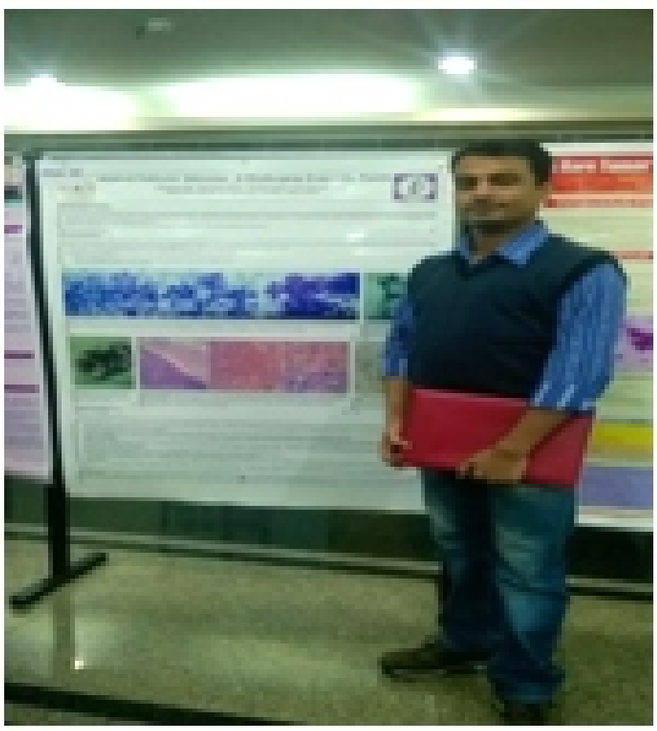
•"Mucosal vaccination with attenuated *Mycobacterium tuberculosis* induces strong central memory responses and protects against tuberculosis" by Dr. Javed Ahmad Sheikh on 09-02-2016.

•"The Rise of CRISPR/Cas for for Genome Editing Bacterial immune system to bacteriophages" by Ms. Vanila Sharma on 01-03-2016.

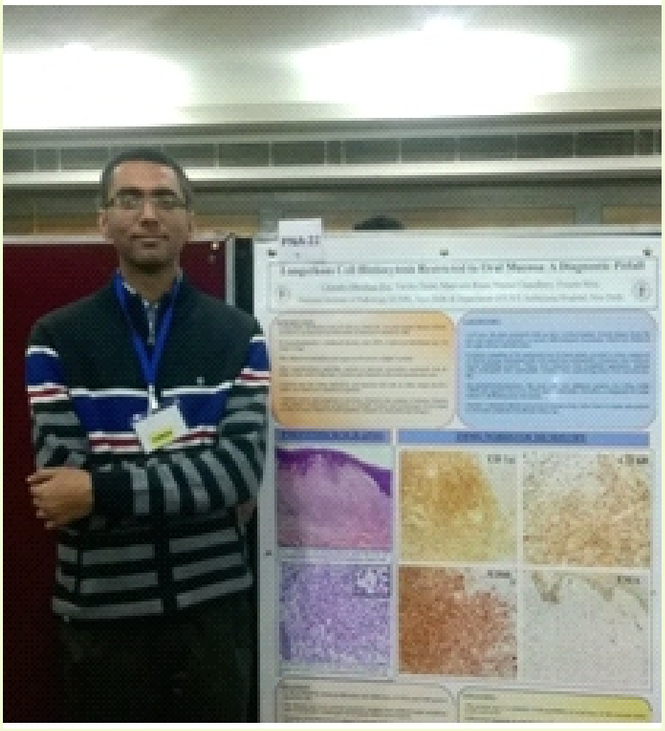
•"Mediastinal masses" by Dr. Sadia Khanam on 12/4/2016.

•"Pathology Informatics" by Dr. Varsha Dalal on 17/5/2016

DAPCON 2016 : Poster Presentation

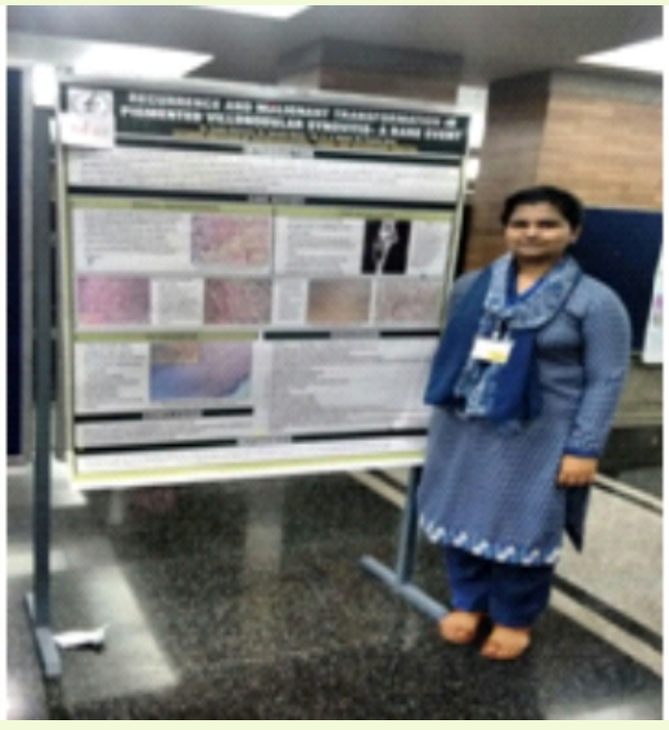


Dr. Kaushik Kar: Atypical follicular adenoma of thyroid : A diagnostic dilemma



Dr. Chandra Bhushan Rai: Solitary Langerhans cell histiocytosis of Hard Palate: A diagnostic pitfall

Dr. Sonam Jain: Expression and clinicopathologic significance of the androgen receptor in triple negative breast cancer.



Dr. Sadia Khanam: Malignant transformation of pigmented villonodular synovitis: A Rare Event

CONFERENCES/PROCEEDINGS/SEMINARS/TRAINING COURSES ORGANISED/ATTENDED

Dr. Sunita Saxena

1. Dr. Sunita Saxena, Director attended a meeting on CHAMPS " Child Health and Mortality Prevention Surveillance" at NIOP on 11.1.2016.
2. Dr. Sunita Saxena, Director attended MRHRU meeting at DHR on 19.1.2016.
3. Dr. Sunita Saxena, Director attended Advisory Membership of "University Research Advisory Board at Swami Vivekanand Subharti University", Meerut on 22/1/16.
4. Dr. Sunita Saxena, Director attended " Understanding and Applications of cancer genomics at Onco- Pathology workshop" organised by VMMC,SJH and NIP on 29/1/2016.
5. Dr. Sunita Saxena, Director attended Review Committee Meeting Univ. Status for ICMR at NIP on 3/2/2016.
6. Dr. Sunita Saxena, Director attended Ethics Committee Meeting at S.J. Hospital on 9/2/2016.
7. Dr. Sunita Saxena, Director attended International Symposium on "Role of Herbal in Cancer Chemoprevention and Treatment' at JNU on 9/2/16 to 10/2/16.
8. Dr. Sunita Saxena, Director attended Annual Medical students conference at S.J.Hospital on 29/2/2016.
9. Dr. Sunita Saxena, Director attended SAC meeting at ICPO NOIDA on 11/3/2016.
10. Dr. Sunita Saxena, Director attended a Workshop on NGS and Bioinformatics organised at NIP on 14/3/2016.
11. Dr. Sunita Saxena, Director attended Selection Committee Meeting for the Award of PDF at ICMR on 22/3/2016.
12. Dr. Sunita Saxena, Director attended National Workshop to prepare guidelines on use of anti Cancer Drugs at AIIMS on 21/3/2016.
13. Dr. Sunita Saxena, Director appointed as Appraisal to appraise the conduct of DNB-CET June 2016 at OJAS Institute of Managemant on 30/6/2016.
14. Dr. Sunita Saxena, Director appointed as Appraisal for Foreign Medical Graduation examinationa at OJAS Institute on 29/6/16.
15. Dr. Sunita Saxena, Director appointed as Appraisal for DNB Post Diploma CET test on 28/6/2016.

Dr Poonam Salotra

1. Attended and Presented work on "Diagnostics for VL and PKDL" at "Brainstorming meeting of Vector Borne Diseases Science Forum" held at ICMR, Delhi in Feb, 2016.
2. Member of Scientific Advisory Committee (SAC) of RMRI Patna in Jan, 2016
3. Meeting on "Monitoring drug susceptibility in VL cases" with DNDi and Kalacore at PHD house, New Delhi Jan 15.
4. Invited participant at Consultative meeting of ICMR and DNDi, PHD house, Delhi Dec 2015.
5. Member of Technical Advisory Committee Meeting at Hyderabad for BIRAC project Dec 2015.
6. Attended 2nd National Workshop on Next generation sequencing in disease diagnosis and therapeutic target discoveryorganized by BIC,National Instiute of Pathology,ICMR,New Delhi from 14th to 18th March,2016

Dr. Avninder P. Singh

1. Awarded 'Dr. Bishnupriya Devi Award' for best original article published in IJDVL for year 2015 at Dermacon-2016, Coimbatore on 6th Jan.2016.

Dr. Ruchi Singh

1. Participated and present work in presented the research work on miltefosine resistance in *Leishmania donovani* in "56th Annual Conference of Association of Microbiologists of India" to be organized by School of Life Sciences, Jawaharlal Nehru University, New Delhi from 7-10 December 2015.
2. Participated and received BILL & MILINDA GATES foundation award for Young Investigator from India and Southeast Asia presented by the International Society for Infectious Diseases at 17th International Congress on Infectious Diseases, Hyderabad, India March 2-5 2016.
3. Participated in "Women of worth conclave" Organised by Loreal and NDTV at Taj Palace, New Delhi on March 8, 2016.

INSTITUTIONAL ACTIVITIES

•NIP organized 2nd National Workshop on "Next Generation Sequencing in Disease Diagnosis and Therapeutics Target Discovery" 14-18 March 2016.

•On 23rd May 2016 a National Swacchta Diwas was celebrated and an Oath was taken by all the staff of NIP.

•Poster Presented in Clinico-Pathological Conference on Gastro-Hepato-pathology :A Case Based Discussion. organized By SRL Ltd., Fortis Escorts Heart Institute, Okhla Road on 21st February, 2016.The details of poster presenter are given below:

a. Dr. Chandra Bhushan Rai: Mesenteric Leiomyosarcoma: An Unusual Location of a Rare tumor.

b. Dr. Sonam Jain: Collision tumor of the Rectum.

c.Dr. Koushik Kar: Synchronous colonic adenocarcinoma and renal transitional cell carcinoma.

16th Smt. Pushpa Sriramachari foundation day Oration was organised by NIP and the guest oration was delivered by Prof. P.N. Tandon, National Prof. & President NBRC, Society on Biomedical Research in India: Opportunities and Challenges on May 2nd 2016.

On completion of 25 year of service a Momento was given to NIP Employees

by

DR. SOUMYA SWAMINATHAN

SECRETARY DHR & DIRECTOR GENERAL ICMR

Mr. Raja Ram

Mr. Shiv Prakash

Mr. Subhash Babu

Mrs. Sushma Ralhan

Mr. Jagdish Pant

Mr. Satya Pal

Mr. Shyam Sunder

Mr. Puran Singh

Dr. S. Sriramachari
Young Scientist Award 2016

Dr. Sonam Jain (Medical)

Mr. Javed (Biomedical)

स्वच्छ भारत अभियान

सुभाष बाबू

माननीय प्रधानमंत्री श्री नरेन्द्र मोदी जी ने महात्मा गांधीजी के जन्मदिन 2 अक्टूबर 2014 को स्वयं अपने हाथों में झाड़ू लेकर सफाई अभियान की शुरुआत की थी क्योंकि महात्मा गांधीजी जी ने अपने जीवन काल में सफाई पर बहुत जोर दिया था उनका कहना था कि स्वच्छता आजादी से ज्यादा जरूरी है। अतः एव श्री नरेन्द्र मोदी जी ने महात्मा गांधीजी के सपनों को साकार करने के लिए वर्श 2019 महात्मा गांधीजी की 150 वीं जयंती हैं इसलिए स्वच्छ भारत अभियान को पूरी तरह आपस में मिलजुल कर पूरा करेंगे तो यही महात्मा गांधीजी के लिए सच्ची श्रद्धांजली होगी।

इसके बावजूद श्री नरेन्द्र मोदी जी के सपनों का साकार करने के लिए अनेक दिग्गज हस्तियों ने भी इस मिशन में शामिल होकर सहयोग दिया है उनके नाम इस प्रकार है जैसे सचिन तेन्दुलकर, अनिल अंबानी,अमिताभ बच्चन,आमिर खान, प्रियंका चोपडा,सलमान खान, एवं बाबा रामदेव आदि हमें भी अपनी सोच बदलकर इस अभियान को सफल बनाना है इस अभियान को सफल बनाना है इस अभियान का लक्ष्य खुले में शौच की समस्या को रोकना हर घर गाँव में शौचालयों का निर्माण करवाना सड़को, गली मोहल्लों की सफाई करना ही नहीं है बल्कि आम लोगों को स्वच्छता के प्रति जागरूक करना भी शामिल हैं।

हमें भारत में आने वाले उन विदेशी पर्यटकों से भी सबक लेना चाहिए, जो खाने पीने की वस्तुओं को खाने के बाद बचे खुचे छिलकों, खाली बोतलो आदि को तब तक लेकर घूमते रहते है, जब तक कि उन्हें फेंकने के लिए उचित स्थान न मिल जायें इसी प्रकार की धारणा हमें भी अपनानी चाहिए और इस अभियान से जुड़कर एक दूसरे को जागरूक करके एक से भले दो और दो से भले चार वाली कहावत को चरितार्थ करना है इस तरह कड़ी से कड़ी जोड़कर श्रंखला बनानी होगी तभी यह अभियान सफल हो सकता हें।

स्वच्छ भारत अभियान के सपनों को साकार करने का काम इतना आसान नहीं है,जितना कि लोग समझते है एक दिन हाथों में झाड़ू लेकर 10,15 मिनट के लिए मीडिया के समक्ष अखबारों की सुर्खियां बनना और वाह वाही लूटना,उसके बाद किसी का भी दिखाई न देना तो फिर आप जनता से ये उम्मीद करेंगे कि जनता इस अभियान से जुड़ी रहेगी..... नहीं। जनता भी इसी तरह का ढोंग करेगी क्योंकि भारतेन्दु हरिशचंद्र ने कहा है कि हमारे भारतवासी रेल के डिब्बे है, जब तक उनमें इंजन नहीं लगेगा और वो उनको नहीं खिंचेगा तब तक वे नहीं चलेंगें।

इसलिए, इस अभियान को सफल बनाने के लिए हम सबको जागरूक होना पड़ेगा और हर गाँव,हर शहर में,गली मोहल्लों में अलग अलग समितियाँ बनानी चाहिए और समिति का इंजन रूपी अध्यक्ष होना चाहिए, जिसकी अध्यक्षता में हम रेल के डिब्बे रूपी जनता हो इस अभियान से जोड़ सकें और स्वच्छता पर विशेश ध्यान दें सकें इसके बावजूद रेलगाड़ी को चलाने के लिए चालक की भी आवश्यकता होती है तो उसकी कमान ग्रम प्रधान,चैयरमैन,मेयर आदि के हाथों में होनी चाहिए और भारत सरकार को अभियानसे जुड़ी मासिक त्रैमासिक रिपोर्ट भेजनी चाहिए, अगर स्वच्छ भारत अभियान को सफल बनाना है तो सरकार को इस अभियान से जुड़े कार्यकताओं को समय समय पर पुरस्कृत भी करना चाहिए ताकि ज्यादा से ज्यादा लोग इस मिशन में भाग ले सकें, तभी ये अभियान सफल हो सकता है।

जय हिन्द