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Ministry of Science & Technology

Tropical Zoonosis: Emerging and Re-emerging Diseases Development of Diagnostic kits for Resource limited or Point of care areas

Rathnagiri Polavarapu, Ph.D.,
President /CEO

GENOMIX Biotech Inc. (USA)

GENOMIX Molecular Diagnostics Pvt. Ltd

GENOMIX CARL Pvt. Ltd. (India)

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World pathogen traffic

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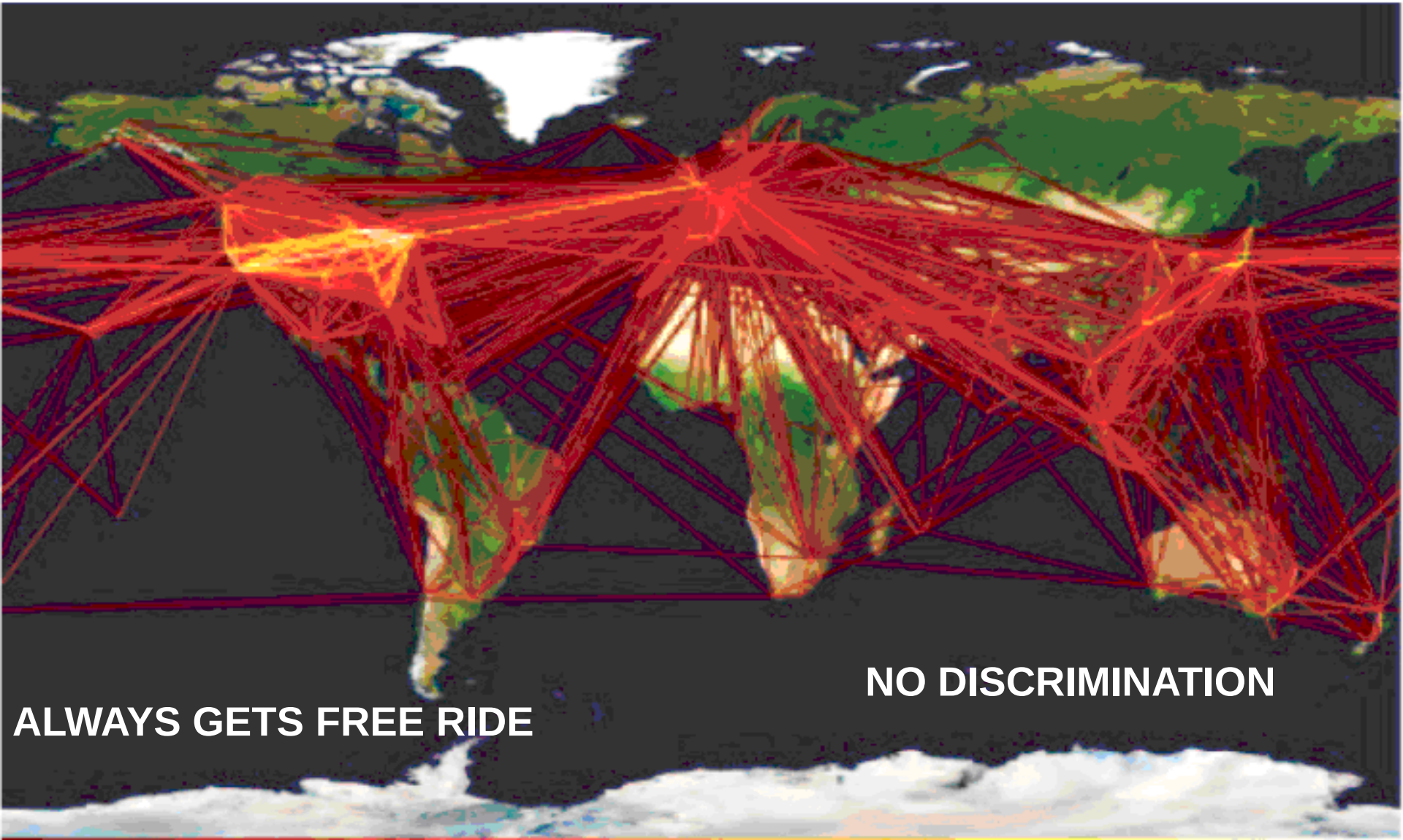
NO VISA

NO SECURITY CHECK

NO TIME LINE



NO DISCRIMINATION

ALWAYS GETS FREE RIDE

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Zoonosis

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- HUMAN to Animals

- ANIMALS to Human
- **TROPICAL ZOONOSIS**: Most of the time confined to tropical fauna
- More than Trillion Dollar Burden on the world economy and On human/animal Healthcare
- Best Temp & atmosphere

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Major Tropical Zoonotic Diseases

Highly neglected, Dejected, Under reported diseases

- Brucellosis: Brucella sps
- Leptospirosis: Leptospira sps
- Glanders/Melioidosis: Burkholderia sps
- Mycobacterium Complex
- (Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium subspecies paratuberculosis-Johnes disease)
- Food born pathogens: Listeria, Campellobacter, Salmonella sps

IVD (*In Vitro* Diagnostics)

Technology Impact and Penetration

4.9% → R&D

Ex: Sequencers, Real Time PCR, Mass Specs

Microarrays

Thousands of IPs & Patents

95%

0.1% → Diagnostics

Diagnostics

Spe. Labs

Spec.
PCR
ELISA

Primary Health Care

Rapid kits

Chemical tests

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IVD for Point of care areas or Resource limited areas

- Serological: RDT (Rapid Diagnostic Test)

  or LFA (Lateral Flow Assay)

  Hand-held ELISA (Enzyme Linked Immunosorbant Assay)- Indirect or Competitive
- Antigen test: Identify antigens in test samples by RDT or ELISA
- Molecular tests: PCR or LAMP for field

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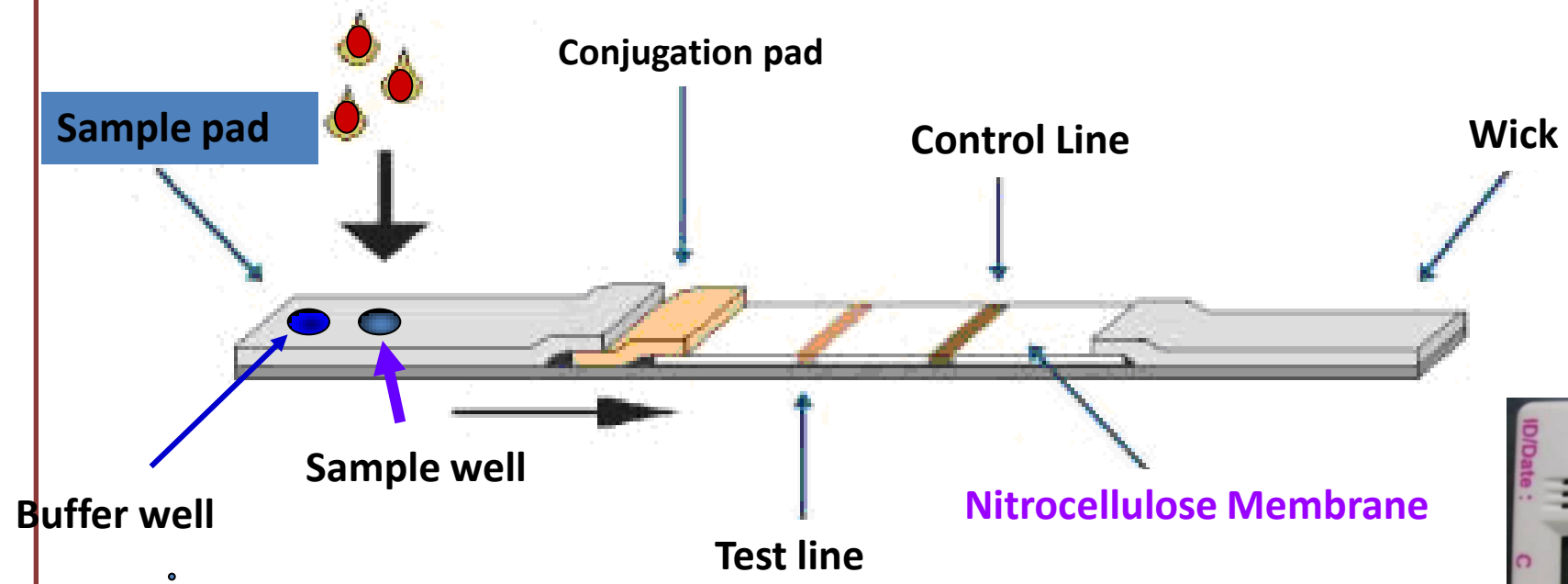
Lateral Flow Assay

Typical Lateral-Flow Assay Format



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Hand held ELISA reader for POC
And for Penside

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Less than 400 grams
Exchange filters
Can read cuvettes
Strips
Battery operated
Got CE mark
Smart phone usage for data sharing

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Membrane Filters for remote areas

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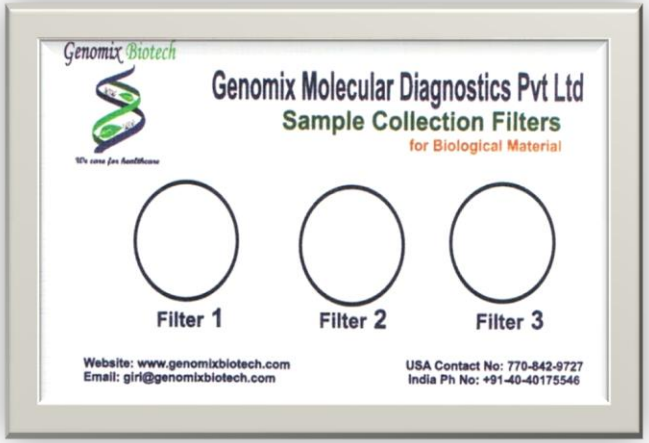
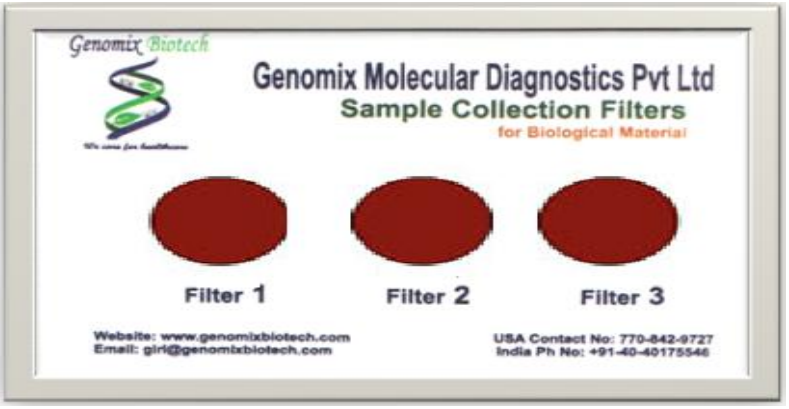


100 ul of Specimen spotted on Genometrix Sample Collection Filter.



Store or transport for further testing

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LAMP
(Loop Mediated Isothermal Amplification Assay)

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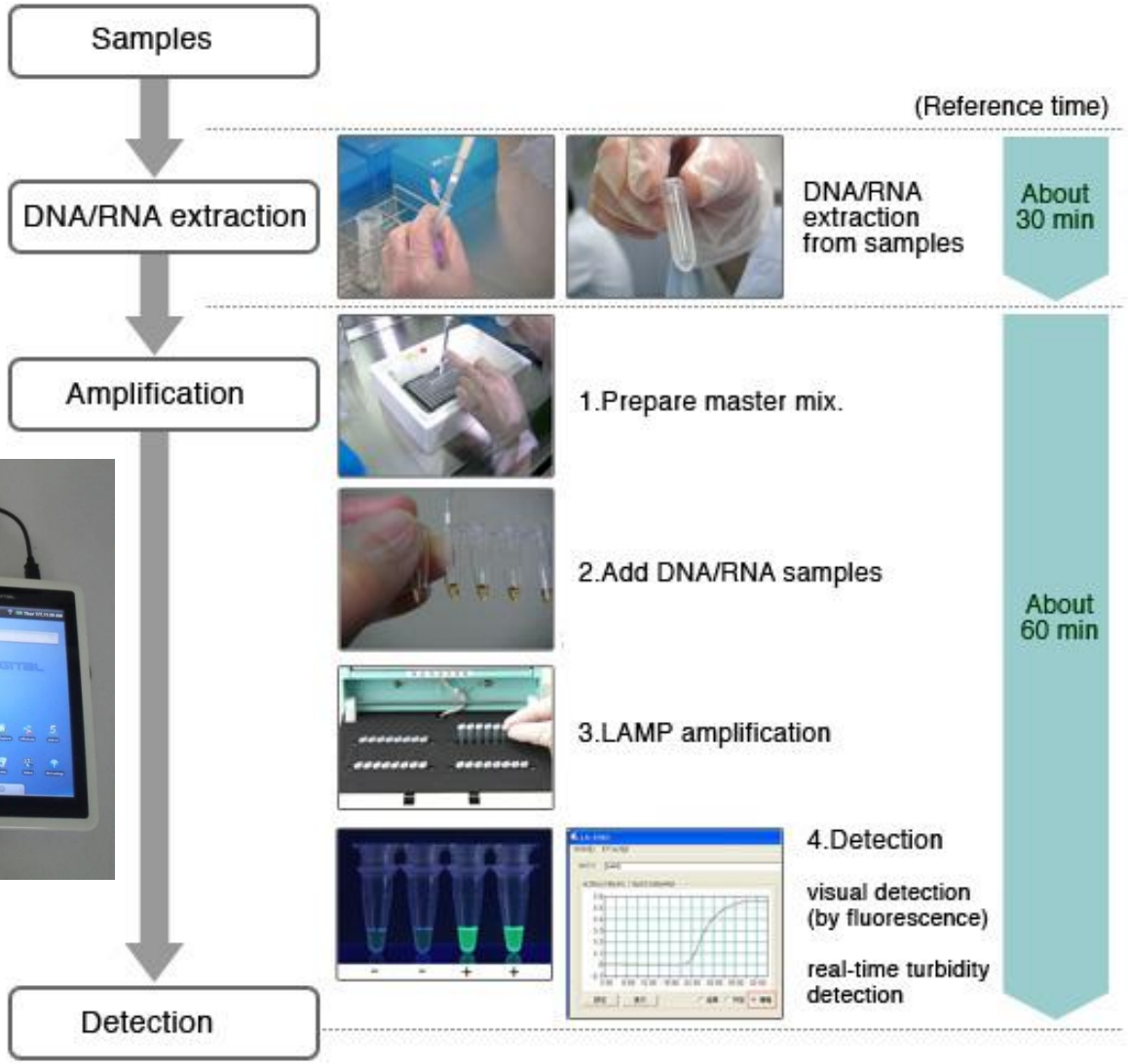
900 grams weight



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LAMP coupled with LFA Device

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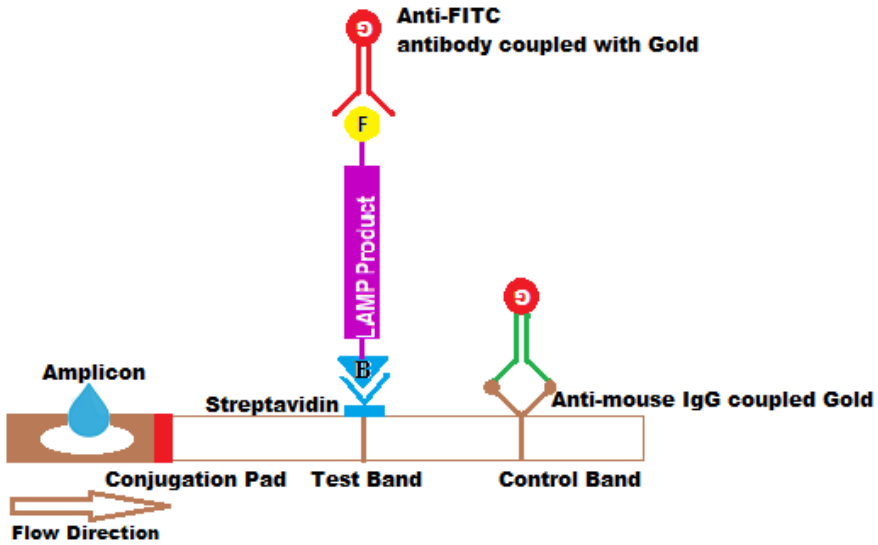
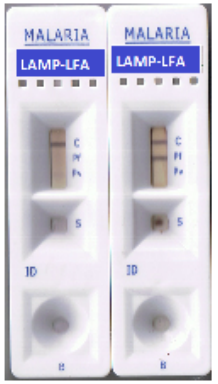
1.

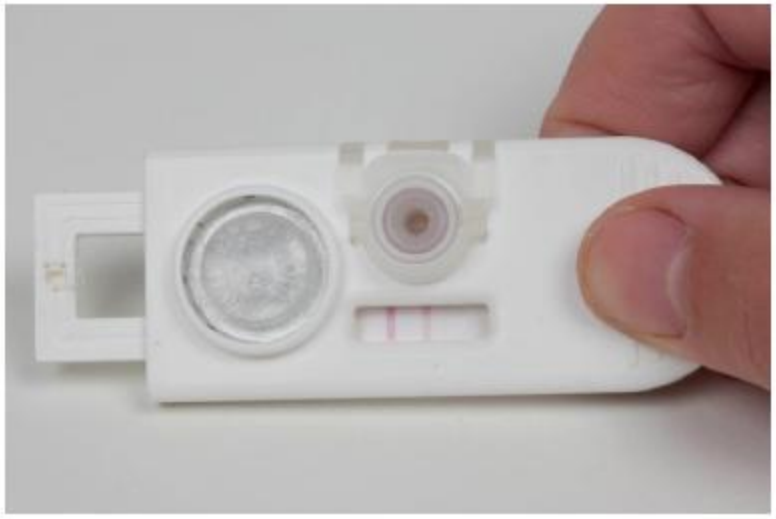
2.



3.



Novel Method of LAMP coupled Lateral Flow assays for point of care or resource limited areas



A novel, rapid, easy to use device for molecular diagnostics

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BRUCELLOSIS
A Dreadful Zoonotic Disease

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Highly Neglected Dejected Under reported Disease

ECONOMICAL BURDEN: 28,000 CRORES RUPEES PER ANNUM IN INDIA

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PREMATURELY ABORTED FETUS

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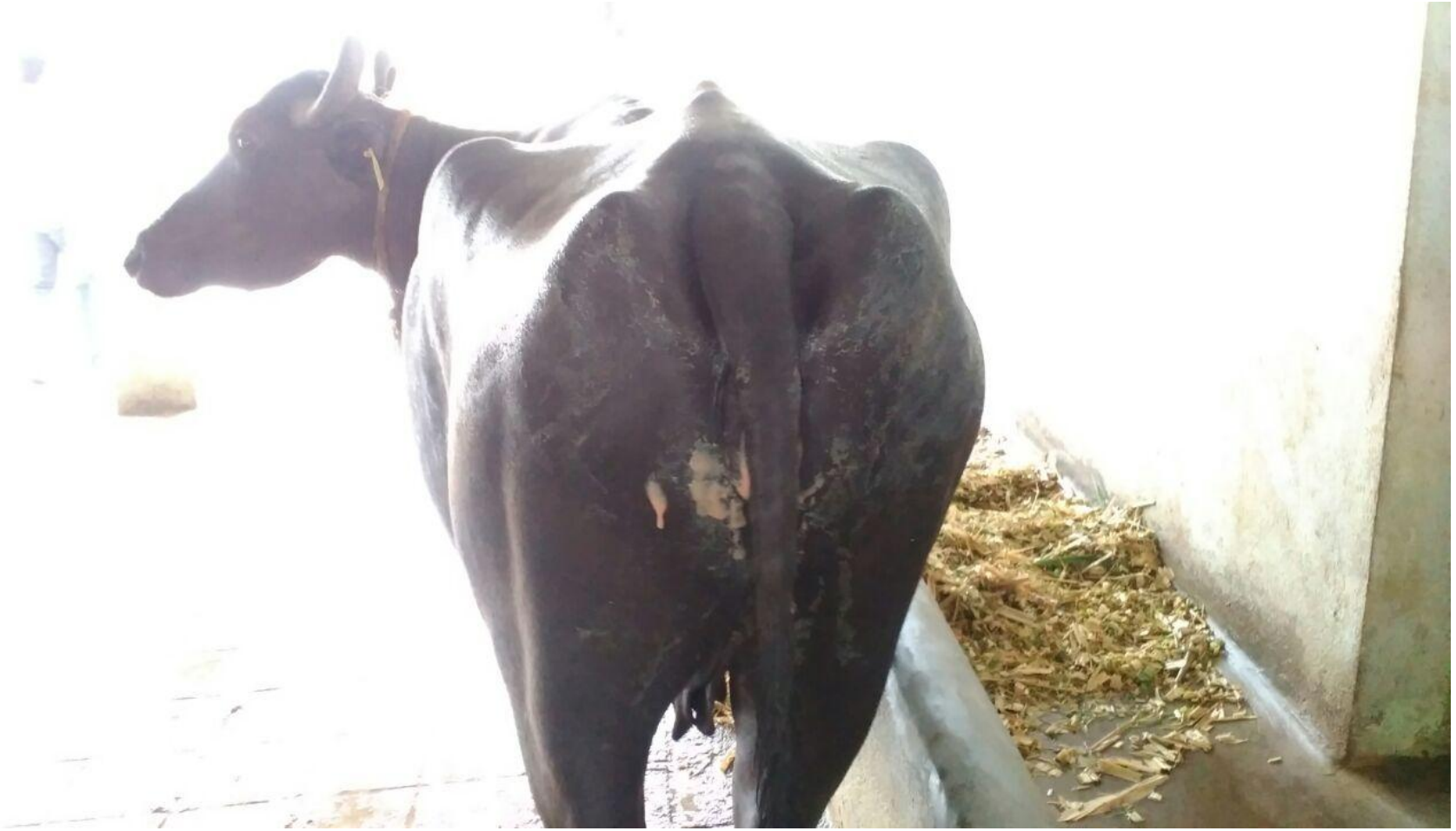
Brucella Infected Buffalo after Abortion



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Brucellosis-
A zoonotic disease

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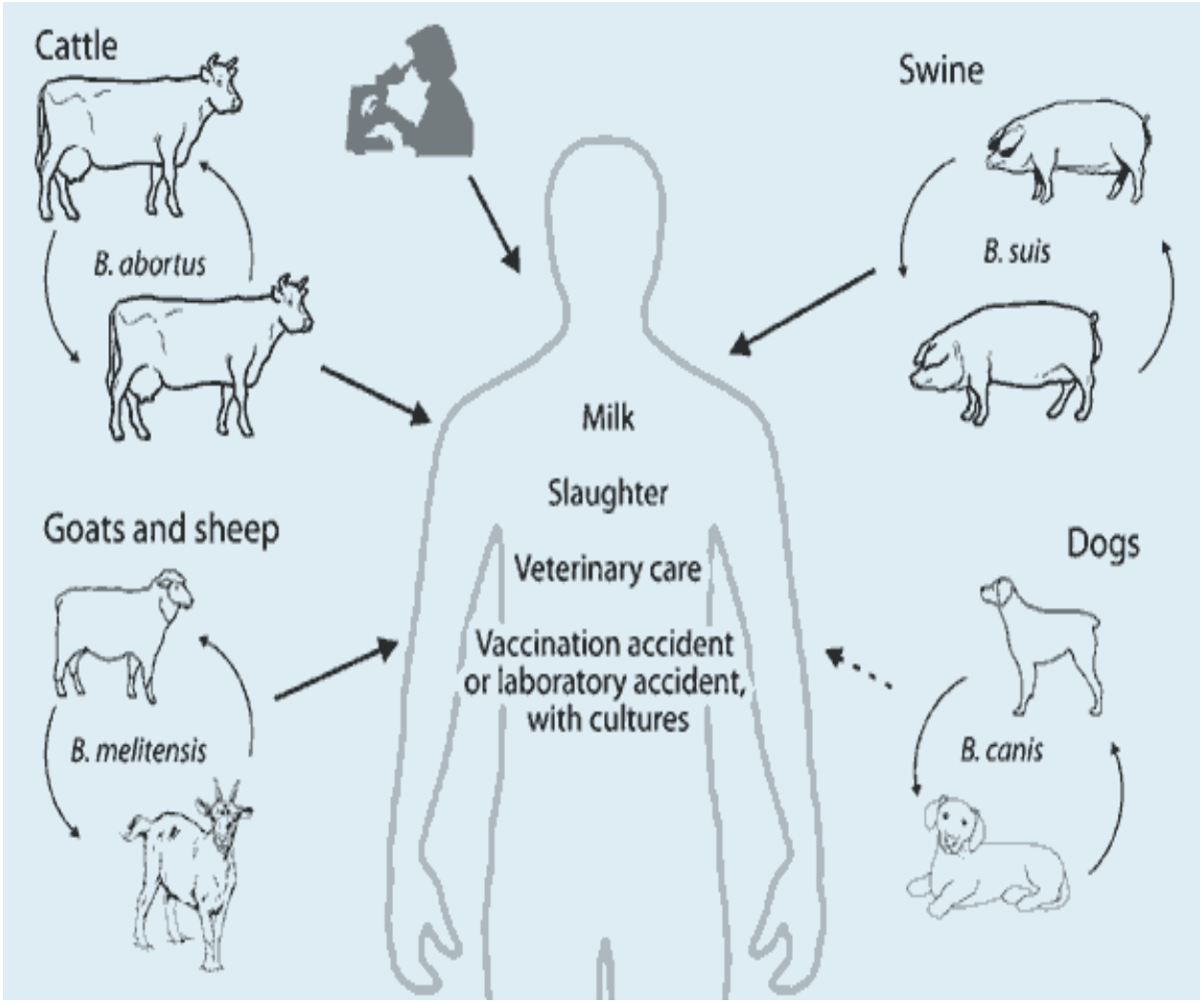

Importance of Brucellosis

Endemic, zoonotic disease

• **Economic loss**: US $ 3.4 billion (Rs. 28,000 Crore) per year in India (Singh *et al.* 2015. Economic losses occurring due to brucellosis in Indian livestock populations.)

• **Who is at risk?**

❖ **Occupational Disease**: Dairy farmers, Veterinary practitioners, Abattoir workers, meat inspectors, Lab workers

❖ **Non occupational infection** results from consumption of fresh unpasteurized raw milk, milk products and meat.

Seroprevalence of Brucellosis

Species	Total Samples collected	Number positive by RBPT	% positive by RBPT
Cattle	6626	981	14.8
Buffalo	1068	145	13.6
Sheep	758	81	10.68
Goat	1638	63	3.84
Pigs	1079	11	1
Camel	231	47	20.35
Dogs	69	48	69.5?
Total	11478	1377	12

Source: DBT Network Project on Brucellosis

Brucella species

- Four species in India
- Brucella abortus
- Brucella melitensis
- Brucella suis
- Brucella canis
- Gram Negative intra cellular
- Zoonotic bacteria
- Infects human and other animals

BRUCELLOSIS
A Dreadful Zoonotic Disease

ECONOMIC LOSSES OCCURRING DUE TO BRUCELLOSIS IN INDIAN LIVESTOCK POPULATIONS

B.B. SINGH ET AL. ;

PREVENTIVE VETERINARY MEDICINE 119 (2015) 211–215

$4.2 BILLION PER ANNUM (28,000 CRORE RUPEES)

$6.8 USD PER CATTLE

$18.2 PER BUFFALO

$0.7 PER SHEEP

$0.5 PER GOAT

$0.6 PER PIG

20% OF BUTCHERS AND ANIMAL HANDLERS AND VETS ARE INFECTED

Diagnostics for Brucellosis

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Recombinant proteins from Brucella species and their suitability for sero-Diagnostics

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S.No.	Recombinant protein	Molecular weight	Source/Received From
1.	BP26	26KDa	Dr. Batnagar, JNU, Delhi.
2.	BCSP 31	31 KDa	DFRL, Mysore. (Dr. Batra Team)
3.	Chimeric protein(19+P39)	60KDa	DFRL, Mysore.
4.	P 39	41KDa	DFRL, Mysore.
5.	P19	17KDA	DFRL, Mysore.
6.	OP Protein	------	DFRL, Mysore.
7.	25 E	25 KDa	DFRL, Mysore.
8.	L7-25 E	37 KDa	DFRL, Mysore.
9.	BLS	18 KDa	Dr. Nagalingam, NIVEDI.
10.	V Protein	18 KDa	Dr. Nagalingam, NIVEDI.
11.	OMP19	19 KDa	Genomix
12.	OMP 28	28 KDa	Genomix

Results:
None of the Recombinant proteins yielded strong signal either by RDT or by ELISA in our hands

Acknowledgements: In collaboration with Prof.. Batnagar, Drs. Batra, Bala Krishna, DFRL, Dr. Nagalingam NIVEDI

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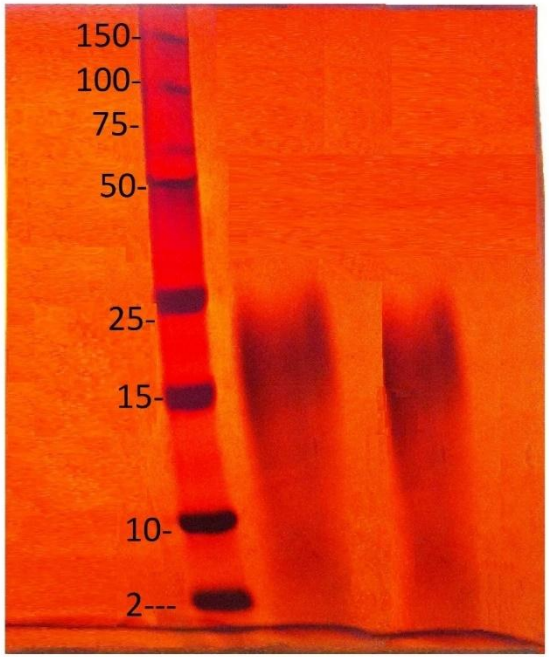
sLPS purification from S99 strain and silver staining

- sLPS was Purified as per OIE guidelines With Minor modifications
- Purified sPLS was tested for its purity using SDS PAGE coupled with silver staining
- sLPS was used to develop RDT
- and iELISA kits

12% SDS PAGE with Silver staining



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All in One Single RDT Kit for Brucella Ab detection

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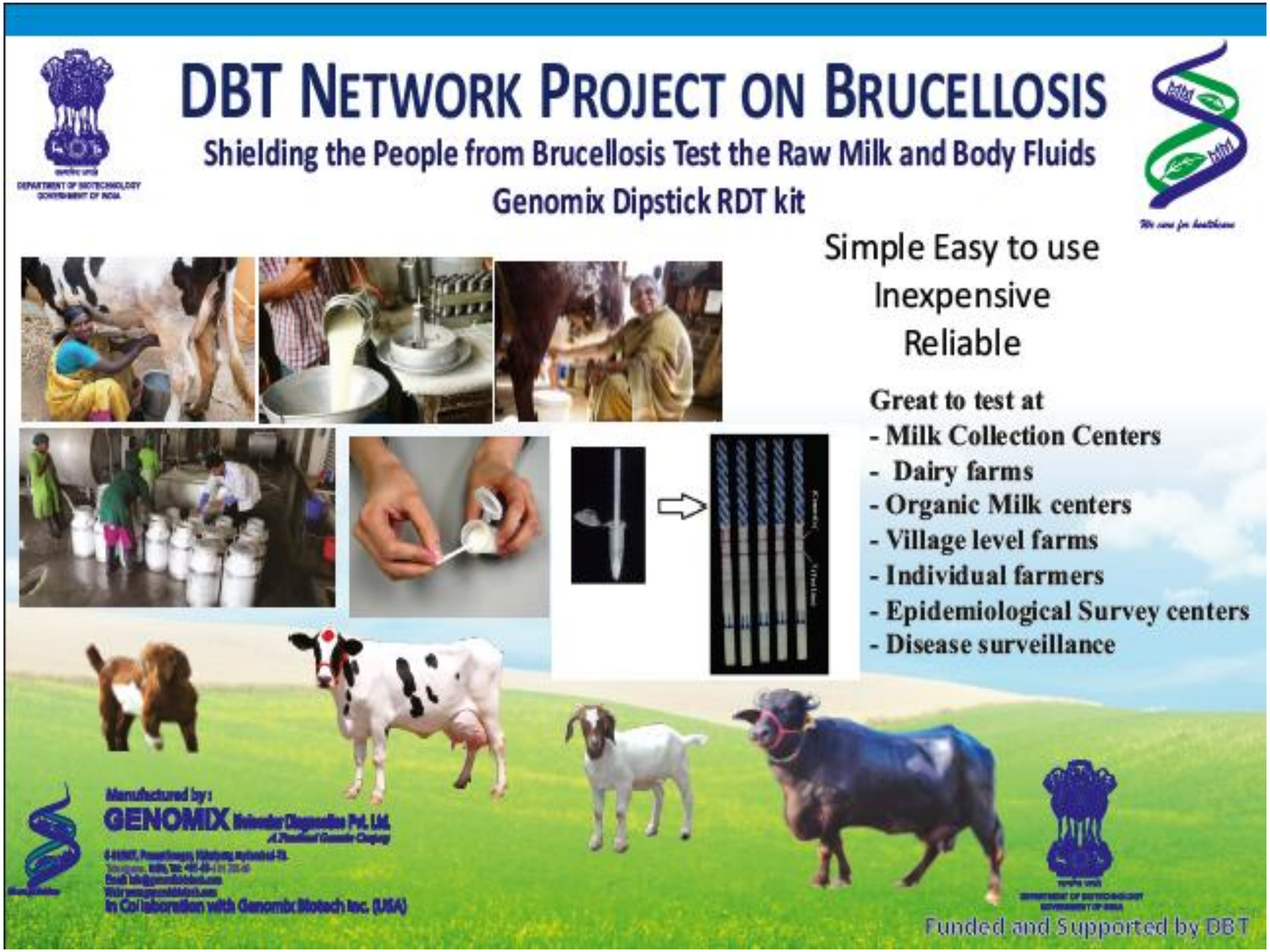
1.Gloves
2. Lancet
3. Buffer
4. Dropper
5. Alcohol Swab
5. Pouch with Cassette, Desiccant Dropper
7. Instructions


DBT NETWORK PROJECT ON BRUCELLOSIS
Shielding the People from Brucellosis Test the Raw Milk and Body Fluids
Genomix Dipstick RDT kit
Simple Easy to use
Inexpensive
Reliable
Great to test at
- Milk Collection Centers
- Dairy farms
- Organic Milk centers
- Village level farms
- Individual farmers
- Epidemiological Survey centers
- Disease surveillance
Manufactured by:
GENOMIX
In Collaboration with Genomix Biotech Inc. (USA)
Funded and Supported by DBT

DBT Network Project on Brucellosis

Genomix Bio-sample collection Cards






Collect fresh samples
(suspected blood, serum, milk & any body fluid)
absorb to filters and carry at room temperature
Biological material stable at room temperature
Easy to transport
No Spills
Low Degradation
Field to Lab-Lab to Field

Manufactured by :
GENOMIX Molecular Diagnostics Pvt. Ltd.
A Parallel Genetic Group

In Collaboration with Genomix Biotech Inc. (USA)

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DBT Network Project on Brucellosis

Genomix Brucellosis iELISA Antibody test kit Screening

Livestock for Brucellosis infection

Easy to test for Brucellosis Antibodies at

- POC
- Field
- Farm
- Lab

It is Simple

- Easy to Use
- Penside
- In Expensive
- Reliable
- Specific
- Sensitive

Funded & Supported By DBT

Test the Animal Before Buying-It is worth it

Make Brucella free country

Manufactured by

GENOMIX Molecular Diagnostics Pvt. Ltd.

In Collaboration with Genomix Biotech Inc. (USA)

Funded and Supported by DBT

DBT Network Project on Brucellosis

Genomix Brucella Ab Rapid kit
Single Kit

Easy to test for Brucellosis Antibodies at

- POC
- Field
- Farm
- Lab

It is Simple

- Easy to Use
- Penside
- In Expensive
- Reliable
- Specific
- Sensitive

Manufactured by :
GENOMIX Molecular Diagnostics Pvt. Ltd.
A Franchised Genomix Company
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Make Brucella free country

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Kits developed through DBT Network Project

Steps wise procedure

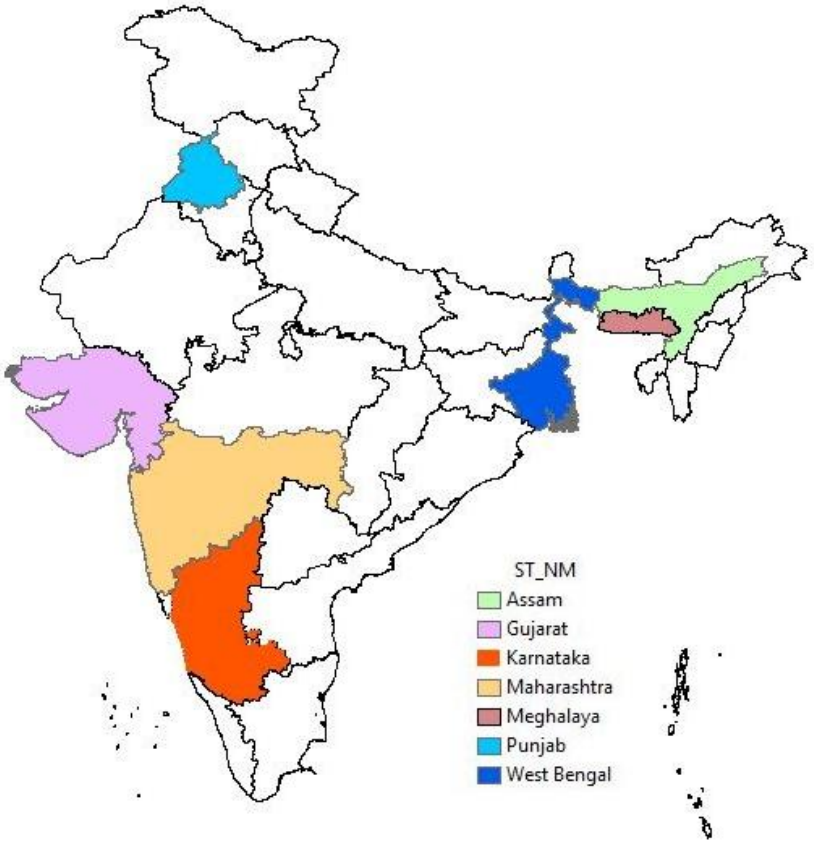
Second round Validation data from NIVEDI And YRPVB/TANUVAS

88% Sensitivity RDT
100% with ELISA

Acknowledgements

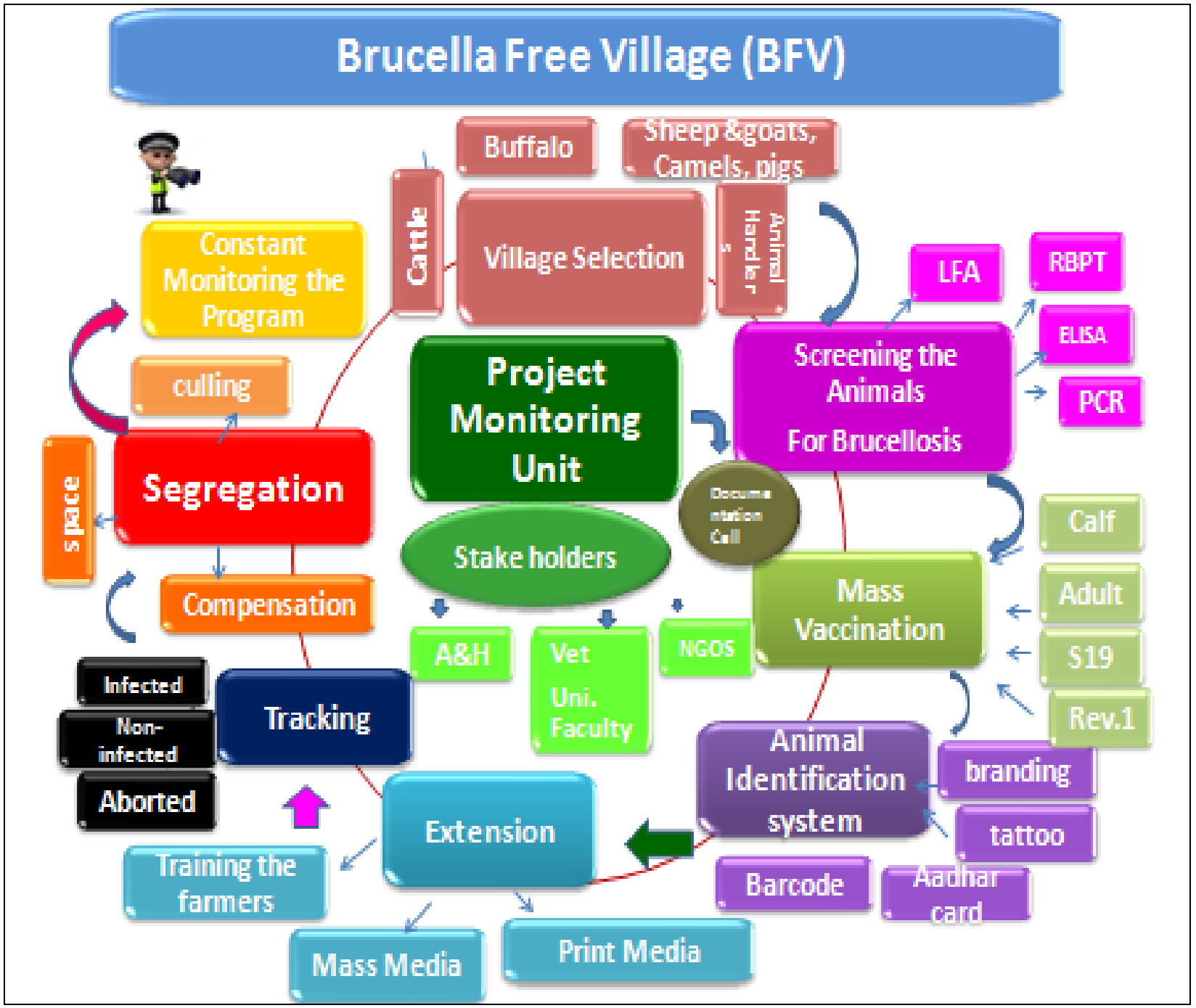
The validation of LFA and ELISA were performed By Dr. R. Shome ,NIVEDI & TRPVB using OIE reference sera panel

DBT Network project on brucellosis: sero-epidemiology prevalence



Species	No. tested	No. positives	% positive
Cattle	25684	4159	16.19
Buffaloe	3682	551	14.96
Sheep	2857	446	15.61
Goat	5195	272	5.23
Pig	4016	34	0.84
Horse	5	1	20
Camel	658	131	19.901
Total	**42097**	**5594**	**13.28**

Species	No. tested	No. positive	% positive
Human	8926	1421	15.91
Livestock	42097	5594	13.28
	51023		


Brucella Free Village (BFV)
Buffalo
Sheep &goats, Camels, pigs
Cattle
Village Selection
Animal Handler
Constant Monitoring the Program
LFA
RBPT
ELISA
PCR
Screening the Animals For Brucellosis
culling
Project Monitoring Unit
Segregation
space
Documentation Cell
Stake holders
Calf
Adult
S19
Rev.1
Mass Vaccination
Compensation
A&H
Vet Uni. Faculty
NGOS
Infected
Non-infected
Aborted
Tracking
Animal Identification system
branding
tattoo
Barcode
Aadhar card
Extension
Training the farmers
Mass Media
Print Media

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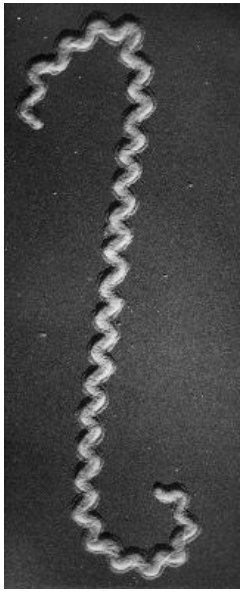
Leptospirosis

LEPTOSPIROSIS

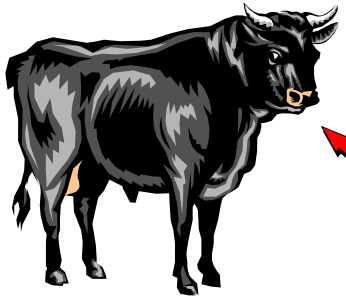


Domine: Bacteria
Phylum: Spirochaetes
Order : Spirochaetes
Class: Leptospirales
Family: Leptospiraceae
Genus: Leptospira
Species: *L. interrogans*
L. Biflexa, Etc,.

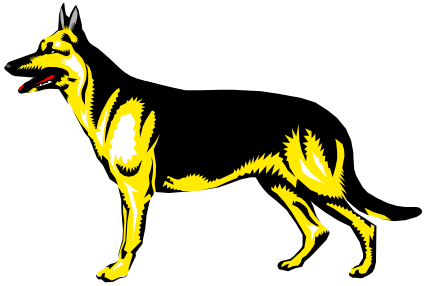
- *Leptospira* are spiral-shaped bacteria that are 6-20 µm long and 0.1 µm in diameter with a wavelength of about 0.5 µm. live *Leptospira* are best observed by dark field microscopy.

- Aerobic with hooked ends, mobile, endo flagelleted Gram negative.

Leptospirosis:Transmission Cycle

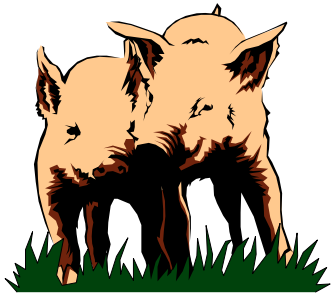


hardjo



canicola

Urine
Soil and Water
Tissues
Contaminated
with
Leptospires



Pomona
cattle, skunks, opossums

icterohemorrhagica

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The genera Leptospira contains three species, namely *L interrogans*, *L biflexa* and *L parva*.

Serovars having antigenic similarities are formed into serogroups, and all serovars have been divided into 25 serogroups and more than 250 serovars and is the principal cause of leptospirosis in humans and animals. most common being *L. canicola, L. hardjo* and *L. hebdomadis.*

The etiologic agent of leptospirosis is *Leptospira interrogans*.

It is a thin spiral organism 0.1mm x 6 - 20mm, with tightly set coils

It is characterized by very active motility, by rotating ("spinning") and bending. Usually one or both ends of this single-cell organism are bent or hooked.

Because of their narrow diameter, the leptospires are best visualized by dark-field illumination or phase contrast microscopy and they do not stain readily with aniline dyes.

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Reservoirs

Wild and domestic animals rodents, livestock (cattle, horses, sheep, goats, swine), canines, and wild mammals are the reservoir for leptospirosis.

Many animals have prolonged leptospirosis without suffering from the disease themselves.

Leptospira 19 serovars : only in Indian subcontinent

Species	Serovar	Strain	Serogroup
L. interrogans	Australis	Ballico	Australis
L. interrogans	Bankinang	Bankinang 1	Autumnalis
L. interrogans	Canicola	Hond Utrech IV	Canicola
L. interrogans	Hardjo	Hardjo prajitno	Sejroe
L. interrogans	Hebdomadis	Hebdomadis	Hebdomadis
L. interrogans	Pyrogenes	Salinem	Pyrogenes
L. borgpetersenii	Tarassovi	Perepelicin	Tarassovi
L. interrogans	Icterohaemorrhagiae	RGA(ATCC443642)	Icterohaemorrhagiae
L. interrogans	Pomona	Pomona	Pomona
L. biflexia	Patoc	Patoc 1	Semaranga
L. Santarosai	Shermani	1342 K	Shermani
L. inadai	Kaup	LT 64 - 68	Tarassovi
L. kirschneri	Grippotyphosa	MoskvaV	Grippotyphosa
L. fainei	Hurstbridge	BUT 6	Hurstbridge
L. borgpetersenii	Javanica	Poi	Javanica
L. noguchii	Panama	CZ 214 K	Panama
L. interrogan	Djasiman	Djasiman	Djasiman
L. interrogan	Copenhageni	M 20	Icterohaemorrhagiae
L. interrogan	Bataviae	Swart	Bataviae

Serovars causing Canine leptospirosis

Australis
Automonalis
Canicola
Pyrogenis
Pomona
Icterohaemorrhagiae
Grippotyphosa
Javanica (Ja),
Potoc

IDENTIFICATION OF ANTIGENIC/IMMUNOGENIC EPITOPES OF LEPTOSPIRA

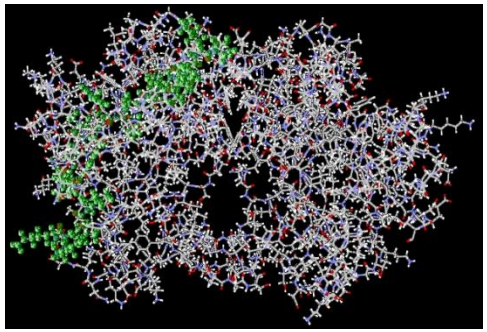
Lepto Diagnostics

Is it a serious Issue?

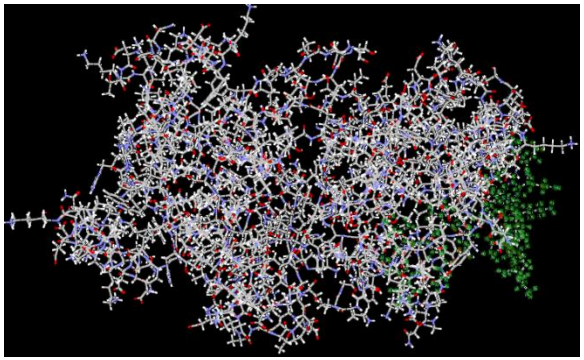
OMPL1 - MYIGVAPRKAIPAGGGGSSIVIPAAVGIKLNVTEDA
LIPL21 - ASDVVKKMVGETVESAGGGGDALVAKAQEVS
LIPL41 - KKAVVSSPAKIFNSVGGGGIDAVAAGLKVAGF
LIPL32 - KKLLVRGLYRISFTTYKPGGGGWIRVERMSAIMPDQIAKAAKAKPVQKL

PROTEIN MODELING USING BIO-INFORMATICS

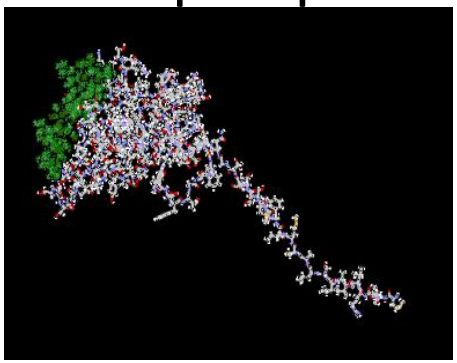
Design and Synthesis of multiepitope antigen of leptospira



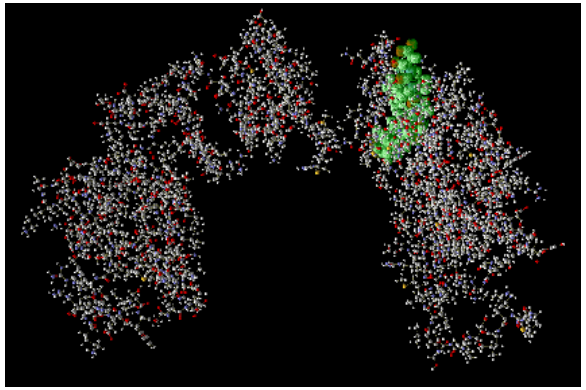
LipL32
KKLLVRGLYRISFTTYKP



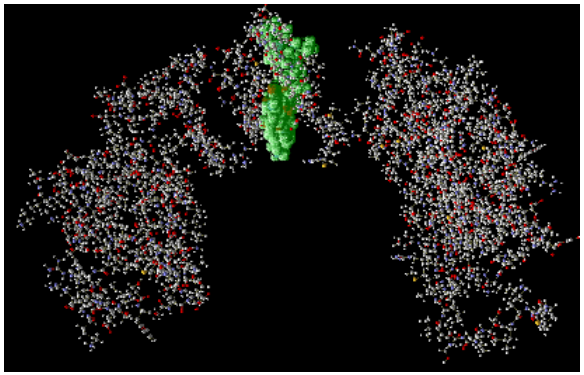
LipL32
WIRVERMSAIMPDQIAKAAKAKPVQKL



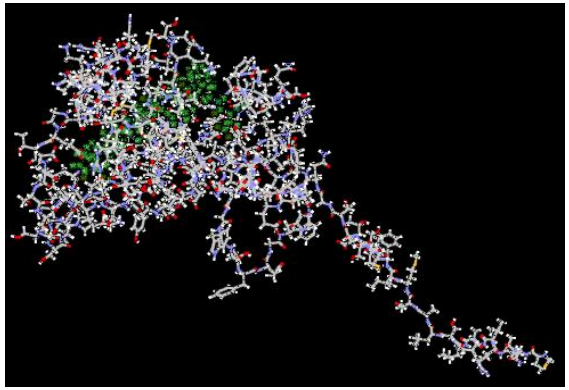
LipL21
DALVAKAQEVS



LipL41
IDAVAAGLKVAGF



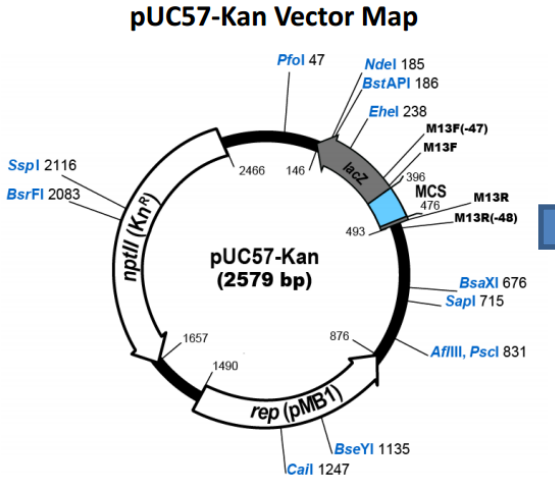
LipL41
KKAVVSSPAKIFNSV

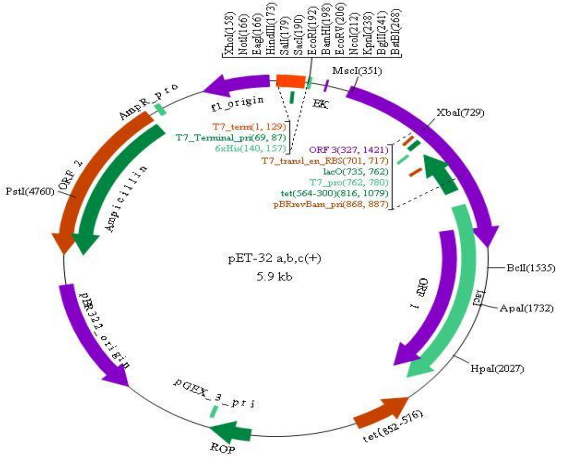


LipL21
ASDVVKKMVGETVESA

OmpL1 : **MYIGVAPRKAIPA** **SSIVIPAAVGIKLNVTEDA**

CLONING AND EXPRESSION

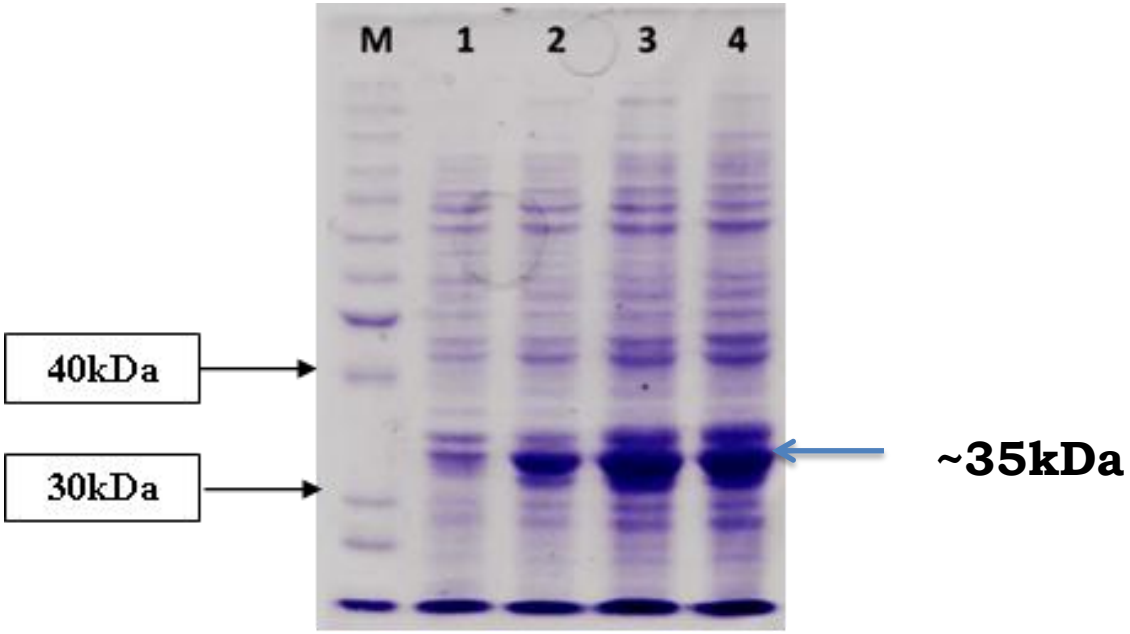




Transformed in DH5α cells

Expressed in BL21 cells

EXPRESSION PROFILING OF PROTEIN EXPRESSED IN BL21 CELLS



M	Marker
1	Before induction
2	2 hours after induction
3	4 hours after induction
4	6 hours after induction

OPTIMIZED PROCESS FLOW FOR rec-LEP-MEG protein preparation

DAY- 1

- Inoculate 0.1 mL of cell suspension 500 mL media
- Incubate for 5.5 to 7.5 hours at 37±1°C, 250 RPM

Day-2

- Inoculate 1% of the seed to 500mL LB media
- Incubated at 37°C at 220rpm
- Once OD reaches 0.6 to 0.8
- Induction using 1mM IPTG
- Harvest the cells after 6hrs by centrifuging
- Store pellet at -70°C

Day-3

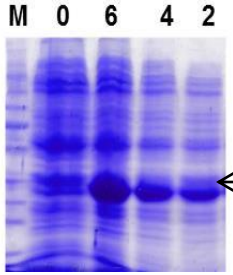
- Pellet Lysed by sonication and Lysis buffer
- Centrifuge at high speed for 30min
- Purification sing Ni-NTA column
- Analyse Elution fractions

Day-4

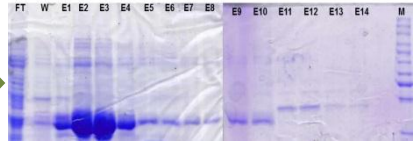
- Pool high concentration fraction
- Dialyse the Protein against PBS
- Check purity, conc. and specificity

In-process Quality Control (QC)

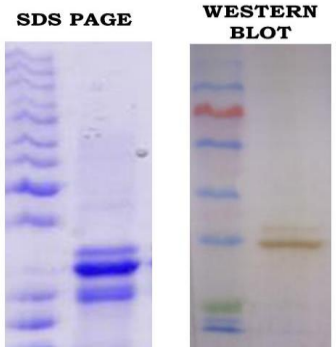
Check culture fraction collected at different time in hours point



Check purification fraction collected at different time in hours point

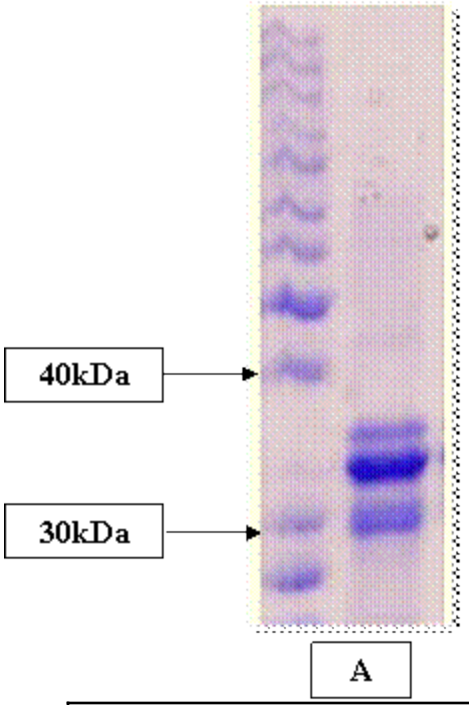


SDS, Western & BCA, RP-HPLC

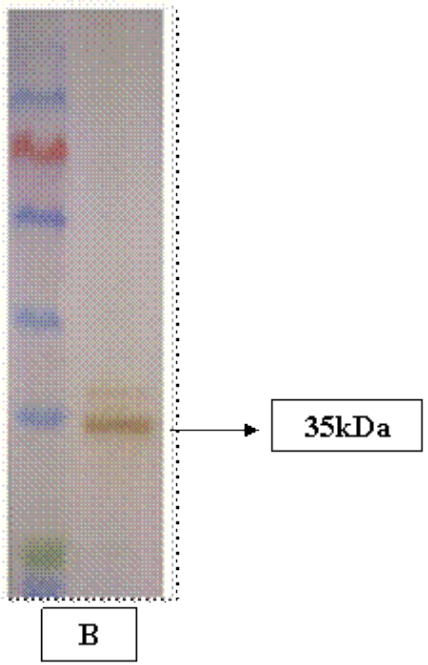


PROTEIN CHARACTERIZATION

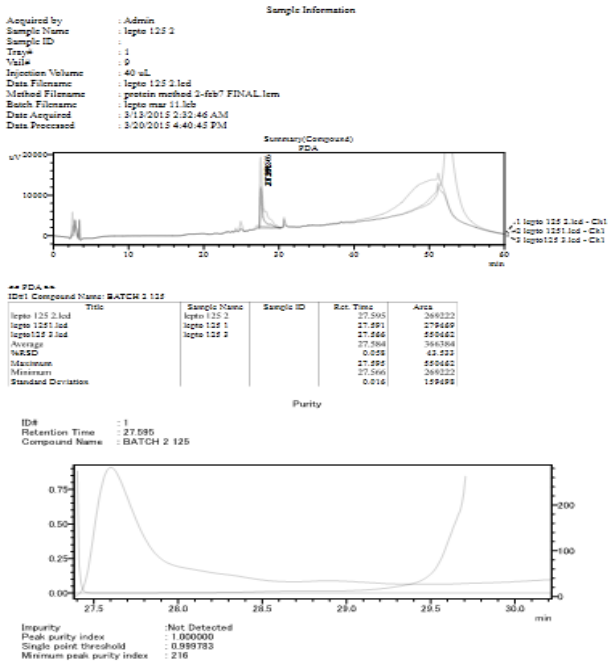
SDS- PAGE



WESTERN BLOT

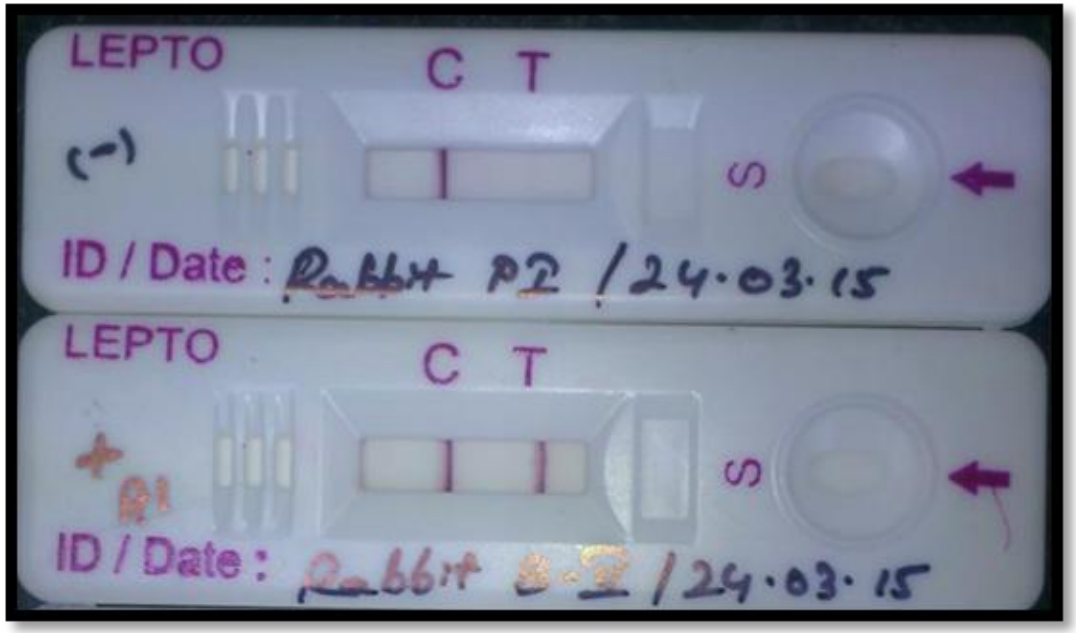


RP- HPLC



Protein Batch	Protein Yield	Purity by RP-HPLC
1	5.26mg	0.99
2	5.95mg	0.86
3	8mg	0.99

Development of LFD Kit



Lateral flow test kit made using rLEP-MEP and tested using hyper immune sera raised in rabbits.

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Glanders/Melioidosis

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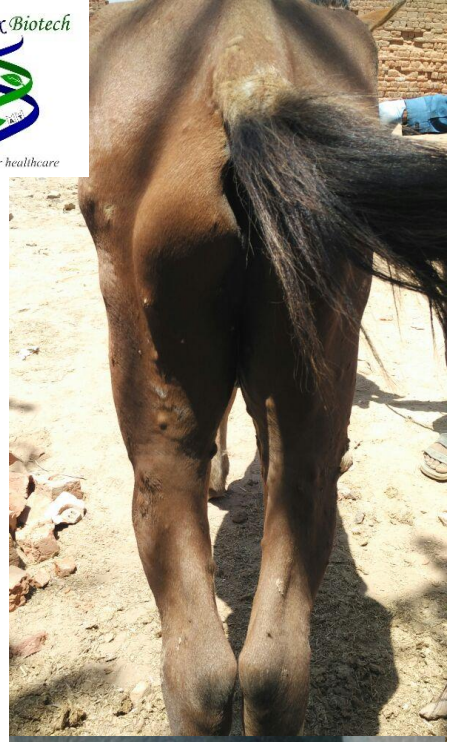
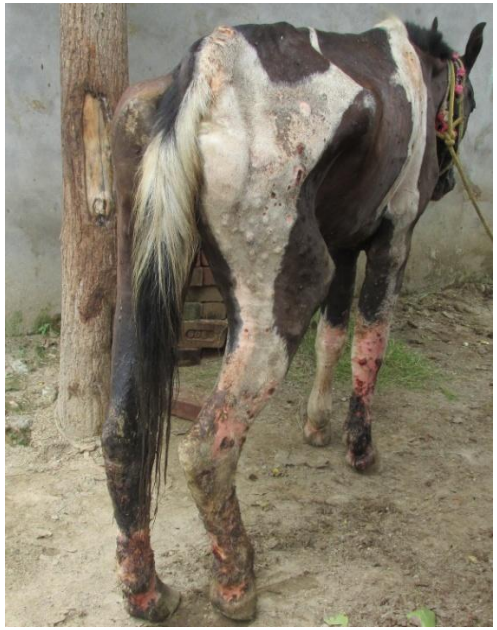
Burkholderia mallei

Burkholderia pseudomallei

Glanders Clinical Signs





Cutaneous form

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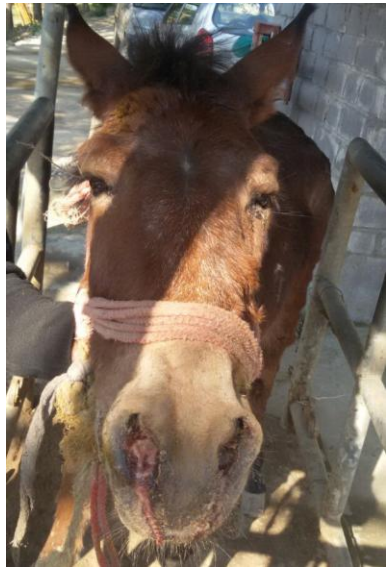
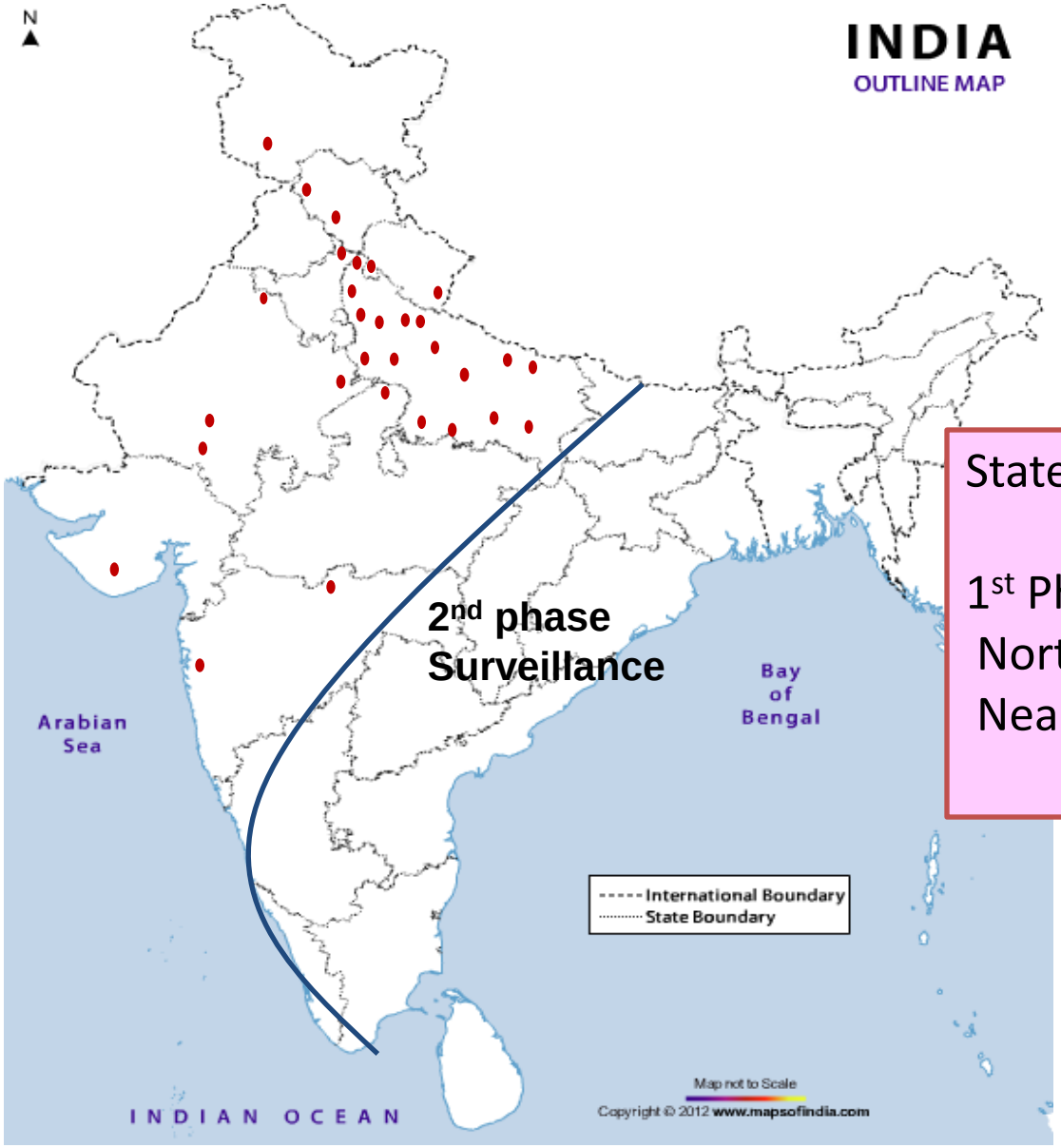


Nasal form

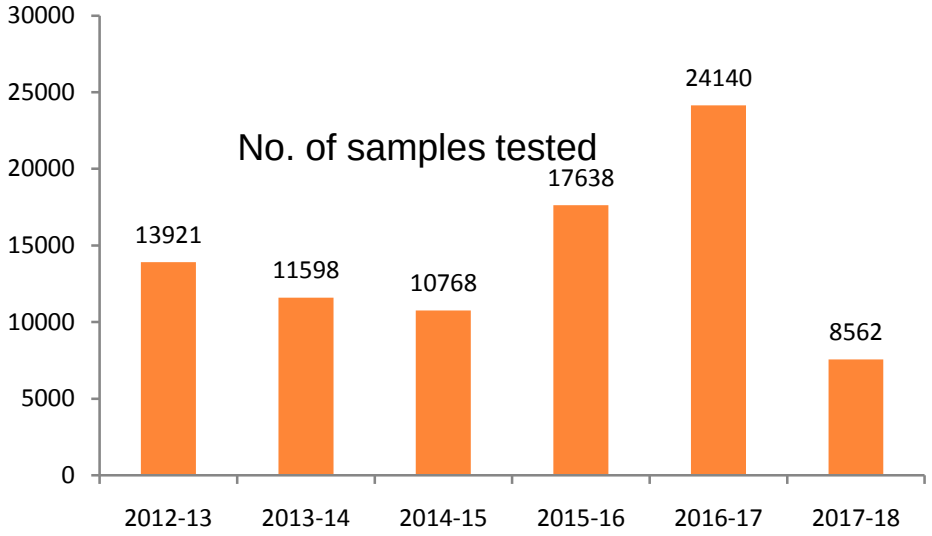
Present outbreak & surveillance status



State wide surveillance programme

1st Phase :
Northern & North-Western India
Nearly 0.8 million equines

Glanders status report- 5 Years



No. of positive cases

2011-12	2012-13	2013-14-	2014-15	2015-16	2016-17	2017-18 (Apr- Sept)
6	7	12	13	87	143	**215**

UP, HP, UK

+ GJ, J&K, HR, MP, RJ, MH

Glanders Recombinant Proteins

B. mallei Type 6 secretion (TSS) protein

-Hcp1, TssA, TssB
-haemagglutinin family protein
-highly immunogenic
-secreted in vivo during infection
- help in detecting active infection

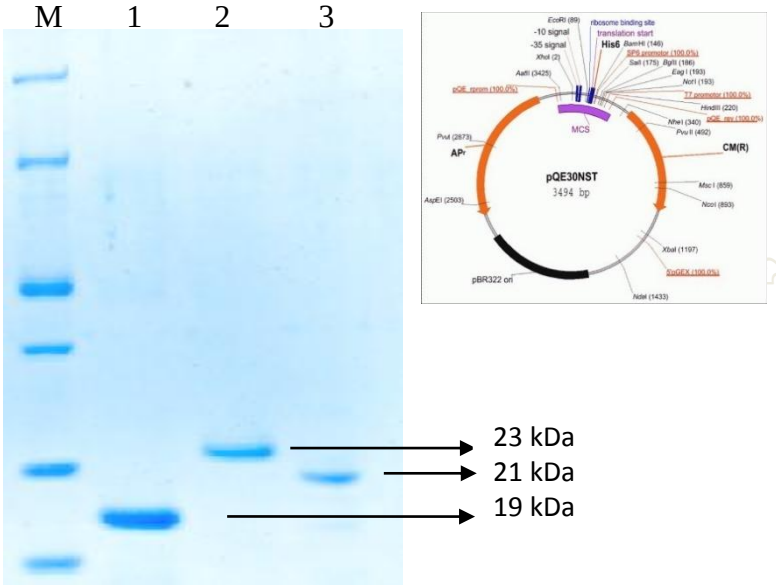


Fig. 1: Purified recombinant *B. mallei* protein
Lane M: Protein molecular weight marker,
Lane 1: Hcp1, Lane 2: TssA, Lane 3: TssB

Hcp1- locus BMAA0742: full length
TssB- locus BMAA0743: truncated N-terminal 200 aa
TssA- locus BMAA0744: full length

Sensitivity & Specificity of ELISAs

	Hcp1	TssA	TssB
No of samples	21873	1162	2235
Sensitivity (%)	97.3	96.5	98
Specificity (%)	98.2	97.7	96.1
Cross reactivity			
Melioidosis	Yes	-	-
Malleinized serum	-	-	-
Strangles	-	-	-

Validation

- ✓Internal-3 Labs
- ✓External - 4 labs (IVRI, LUVAS, DRDE, CMVL)
  - 100% agreement
- ✓International - in progress at OIERL, Germany

Singha *et al* 2014

Rationale for undertaking the work

- **Novelty/Inventive component:**

  ELISA and rapid pen side kits using homogenous preparation of recombinant immono-dominant proteins (Hcp1, TssA, TssB and p26)

- **Scientific merit /Technical highlights:**

  All the recombinant proteins have been validated and evaluated using large no of samples. Presently Glanders ELISAs are being validated at OIE Referral Lab, Germany.

- **Societal Relevance:**

  - **Glanders is a zoonotic disease and is fatal for human and animals**
  - **Has been identified as a select agent or bioterroristic agent**
  - **1.15 million indigenous equines belong to rural area are at high risk**
  - **substantial source of livelihood to the socio-economically backward community**
  - **hidden risk of invading the infection to highly organized equine sector**

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Analysis of first batch of Rapid and ELISA Kit

Combo	Hcp1

Negative sample

Combo	Hcp1

Positive sample

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Zoonotic Malaria

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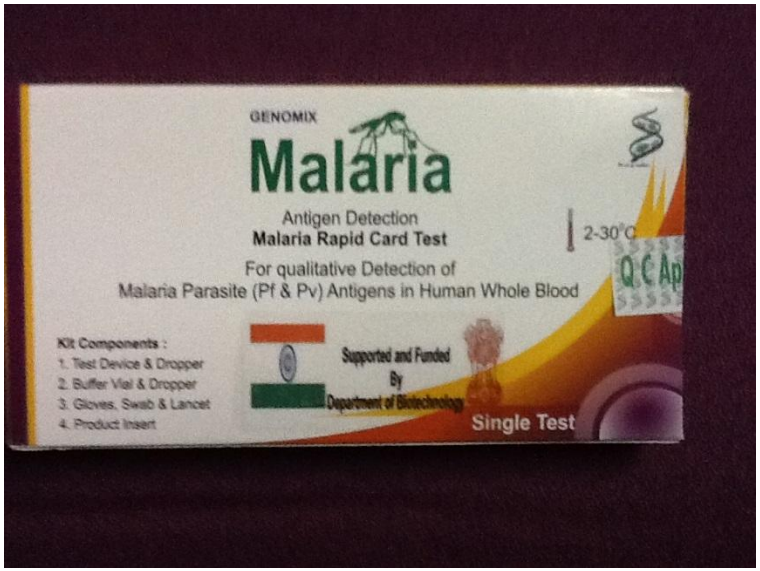
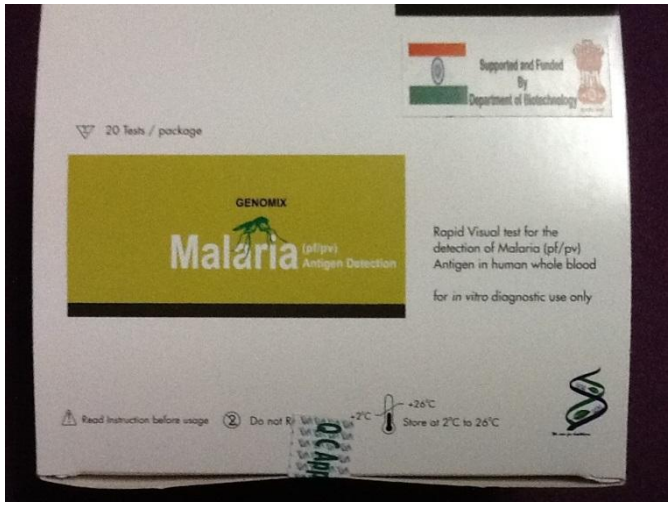
Ministry of Science & Technology

– Plasmodium knowlesi

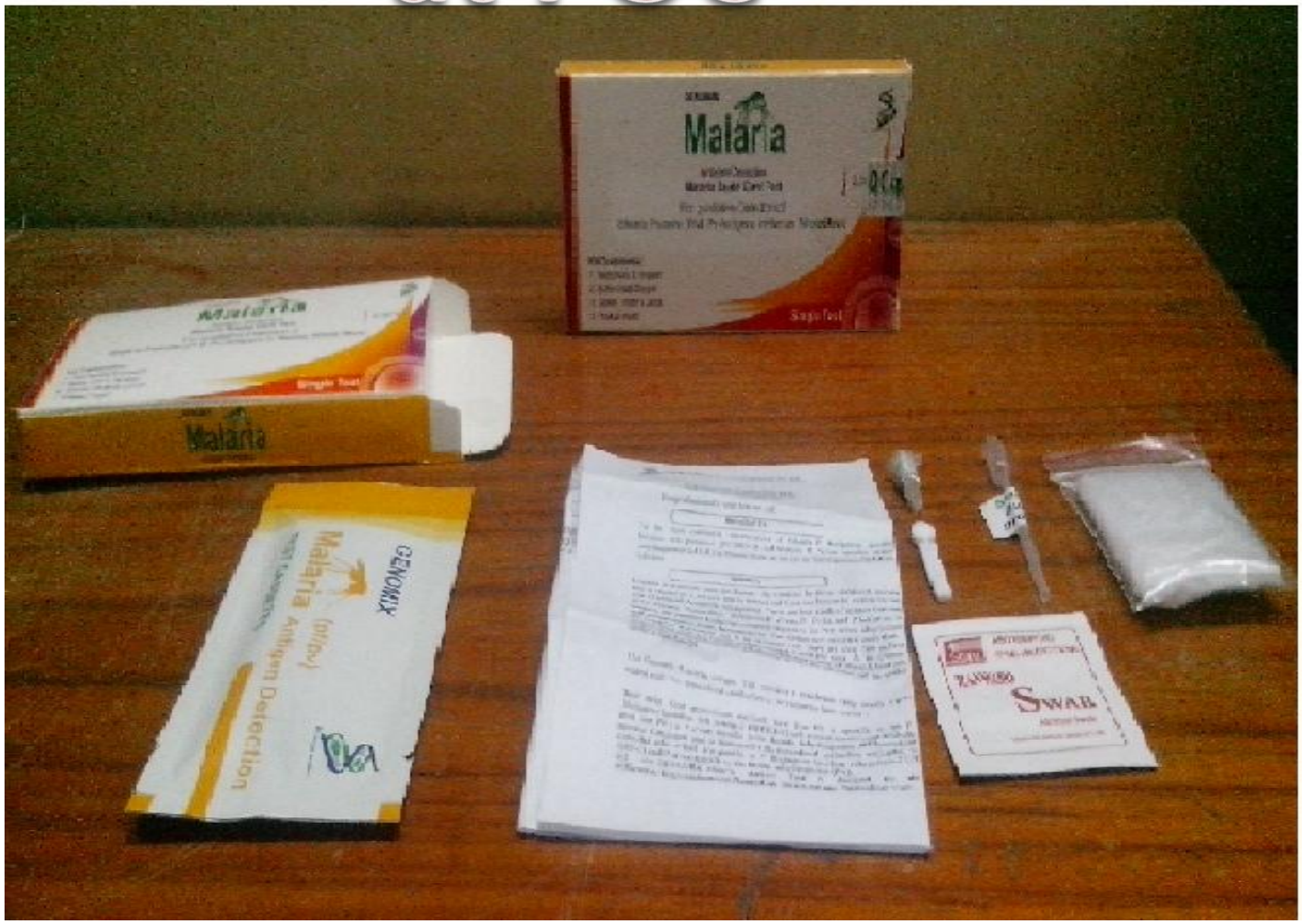
Outcome from DBT project

Genomix
Malaria Pf/Pv Rapid kits





Malaria Rapid kit to use at POC



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Department of Biotechnology
Ministry of Science & Technology

Genomix Molecular Diagnostics Pvt.Ltd Malaria Pf (HRPII)/ PV (PLDH) Antigen Detection Test Device (GM006)

WHO MALARIA RDT PRODUCT TESTING ROUND 5

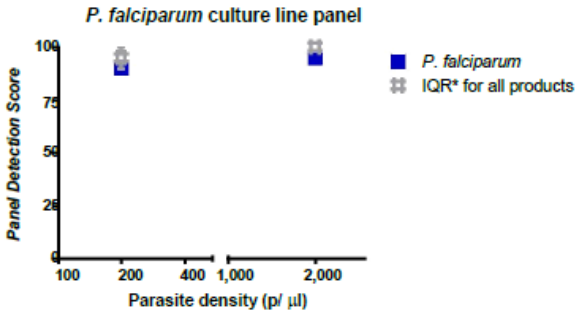
MANUFACTURER' S REPORT

World Health Organization

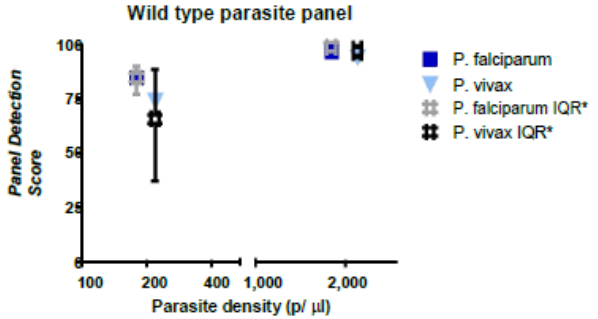
FIND
foundation for innovative new diagnostics

Genomix RDT kits performance At WHO round 5

95% Sensitive and 100% specific



* IQR: interquartile range with median (square) for all products tested



* IQR: interquartile range with median (square) for all products tested

Panel Detection Score (PDS) and Sample Size (n)

		200 p/ µl	2000 p/ µl
All Products (PDS)	Q1 (25th %ile)	90.0	100.0
	Median	95.0	100.0
	Q3 (75th %ile)	100.0	100.0
	Your product scored:	90.0	95.0
	n	20.0	20.0

Panel Detection Score (PDS) and Sample Size (n)

			200 p/ µl	2000 p/ µl
All Products (PDS)	P. falciparum	Q1 (25th %ile)	77.0	97.0
		Median	85.0	99.0
		Q3 (75th %ile)	90.0	100.0
		Your product scored:	85.0	97.0
		n	100.0	100.0
All Products (PDS)	P. vivax	Q1 (25th %ile)	37.1	94.3
		Median	65.7	97.1
		Q3 (75th %ile)	88.6	100.0
		Your product scored:	74.3	94.3
		n	35.0	35.0

LAMP coupled lateral flow assay

Am. J. Trop. Med. Hyg., 00(00), 2018, pp. 1–5
doi:10.4269/ajtmh.18-0177

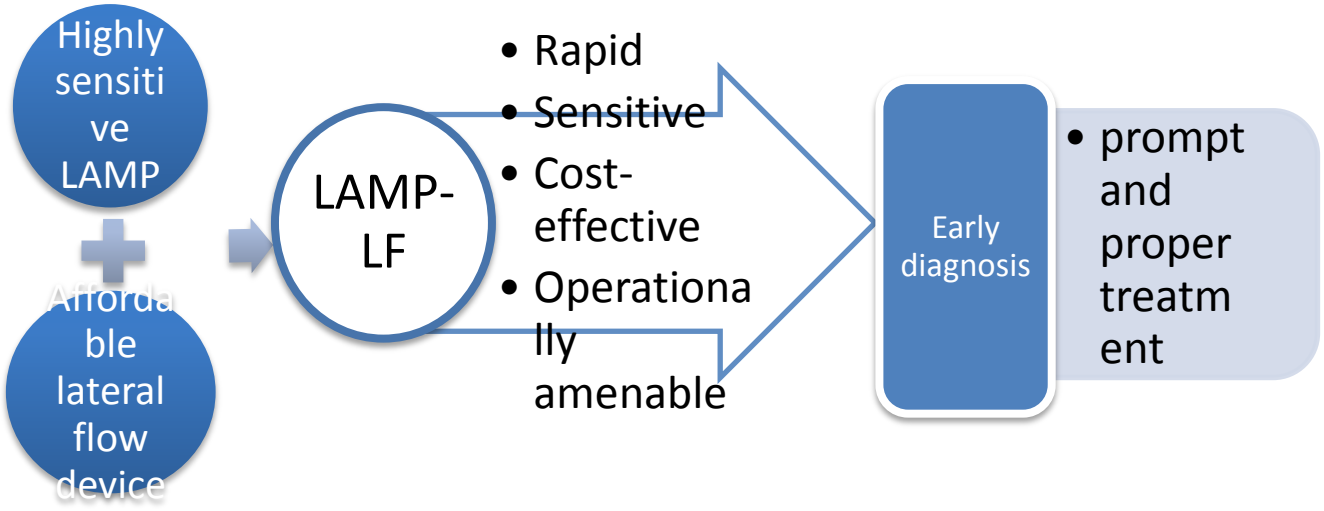
Development of Loop-Mediated Isothermal Amplification–Based Lateral Flow Device Method for the Detection of Malaria

Prudhvi Chand Mallepaddi,[1,2]† Meng-Yee Lai,[3]† Sudhakar Podha,[2] Choo-Huck Ooi,[4] Jonathan Wee-Kent Liew,[3] Rathnagiri Polavarapu,[1,2] and Yee-Ling Lau[3]*

[1]*Genomix Molecular Diagnostics Pvt. Ltd., Hyderabad, India;* [2]*Department of Biotechnology, Acharya Nagarjuna University, Guntur, India;* [3]*Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia;* [4]*Sarawak State Health Department, Jalan Diplomatik, Kuching, Malaysia*

Abstract. The present study aims to develop a method for rapid diagnosis of malaria using loop-mediated isothermal amplification (LAMP) combined with a lateral flow device (LFD). By adding the biotin-labeled and FAM-labeled loop primers to the LAMP reaction solution, the end product can be visualized on a lateral flow dipstick. The entire proce[illegible]e takes approximately 42 minutes to complete, LAMP assay exhibited high sensit[illegible] as the detection limit was 0.01 pg/μL for all five *Plasmodium* species. It was demonstrated that all *Plasmodium knowlesi* ($N = 90$) and *Plasmodium vivax* ($N = 56$) were positively amplified by LAMP-LFD assay, whereas healthy donor samples ($N = 8$) were negative. However, not all mixed infections were positive, and other infected nonmalaria samples were negative. Loop-mediated isothermal amplification-LFD represents a robust approach with potential suitability for use in resource-constrained laboratories. We believe that LAMP-LFD has a potential to be developed as point-of-care diagnostic tool in future.

LAMP coupled LFA for Malaria



In collaboration with Dr. Lau Yee Ling

University of Malaya

Methodology:

Setting-up of LAMP reaction

Incubation in heating block 65˚C, 30 min

Loading of LAMP product onto LF device

Results in <1 hour

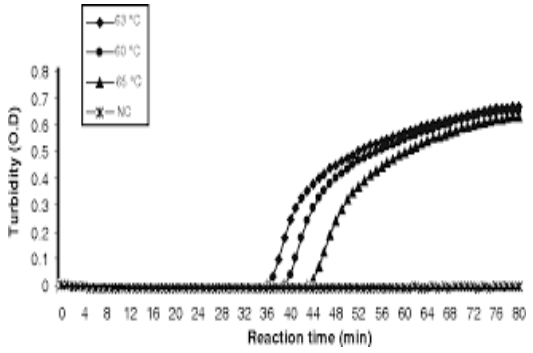
LAMP coupled lateral flow assay

TABLE 3

Sensitivity and specificity of LAMP, microscopy, and nested PCR for all five human malaria species detection

Plasmodium species	Method	Specificity (%)	Sensitivity (%)
Plasmodium falciparum	LAMP	100	100
	Microscopy	100	96.7
	Nested PCR	100	96.7
Plasmodium vivax	LAMP	100	100
	Microscopy	100	100
	Nested PCR	100	100
Plasmodium knowlesi	LAMP	100	100
	Microscopy	100	98.2
	Nested PCR	100	98.2
Plasmodium malariae	LAMP	100	100
	Microscopy	100	100
	Nested PCR	100	100
Plasmodium ovale	LAMP	100	100
	Microscopy	100	100
	Nested PCR	100	100
Mixed infection	LAMP	100	100
	Microscopy	100	100
	Nested PCR	100	50

LAMP = loop-mediated isothermal amplification; PCR = polymerase chain reaction.
A composite diagnosis for each sample based on two out of three methods giving the same result was used as reference.



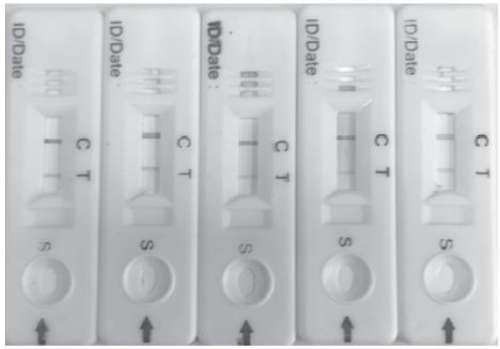


FIGURE 2. Representative image of determination for the detection limit of loop-mediated isothermal amplification-lateral flow device (LAMP-LFD) assay. A 10-fold serial dilution of the stock (100 to 0.01 pg/μL *Plasmodium knowlesi* plasmid) was performed with sterile distilled water. Cassettes 1, 2, 3, 4, and 5 indicate LAMP-LFD assay using 100, 10, 1, 0.1, and 0.01 pg/μL of plasmid as template. T: *Plasmodium* genus–specific or *Plasmodium* species-specific *18S rRNA* gene detection; C: Control line. This figure appears in color at www.ajtmh.org.

In this study, 290 samples were tested using the genus- and species-specific LAMP method. These samples included from 90 *P. knowlesi*, 49 *P. falciparum*, 56 *P. vivax*, 15 mixed infections of *P. falciparum* and *P. vivax*, 60 nonmalaria infected human samples, one *Toxoplasma gondii*, one *Sarcocystis* spp., and eight healthy donor samples. The results indicate that all 90 *P. knowlesi* and *P. vivax* samples were positively amplified by LAMP-LFD assay. However, one *P. falciparum* and one mixed-infection samples was not amplified, possibly because of low parasitemia or DNA degradation. Although LAMP-LFD was negative for the two cases of *P. falciparum* infections, its sensitivity for *P. vivax* remains high. Of 60 nonmalaria-infected human samples, two samples were detected to be positive for *P. vivax*. The remaining eight healthy donor samples were negatively amplified.

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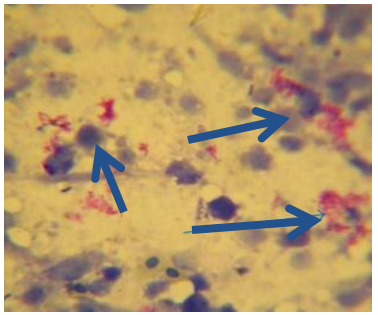
Mycobacterium Complex

TB is one of those diseases
that looks easy when you don't know
very much but the problems
look harder and harder the more you know!

300 Billion $ Burden Annually
World wide

Diagnosis of Bovine Tuberculosis:
Staining & Cultures

1. **Identification of the Organism:**



a. Microscopy:

- ✓ Classical Ziehl-Neelsen staining
- ~ Fluorescent acid fast staining
- ~ Histology

b. Culture:

M. bovis on LJ Slant

Solid egg based media (contain either pyruvate or pyruvate and glycerol)

- ✓ Lowenstein – Jensen medium (with Pyruvate)
- ~ Coletsos base or Stonebrinks

Agar-based media

- ✓ Middlebrook 7H11 (supplemented with OADC)
- ~ Blood based agar medium (B83) (with sodium pyruvate & L-Aspergine)

Liquid Media

- ~ Middlebrook 7H10 (supplemented with OADC)
- ✓ Liquid culture systems (growth measured by fluorometric / radiometric means)

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Different Diagnostic Methods

Mycobacterial Cell culture- Gold standard

Early Stage

Late stage Infection

Post Mortem

Cellular Immunity

Humoral Immunity

Pathological Evaluation

Tuberculin Test

TB IGRA

ELISA

RDT

PCR

LAMP - yet to be validated

What are the clinical signs

The usual clinical signs include:

- weakness
- loss of appetite
- weight-loss
- fluctuating fever
- intermittent hacking cough
- diarrhoea
- large prominent lymph nodes

However, the bacteria can also lie dormant in the host without causing disease

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Bovine MBP 83:70 clone commercially obtained from TRPVB/TANUVAS

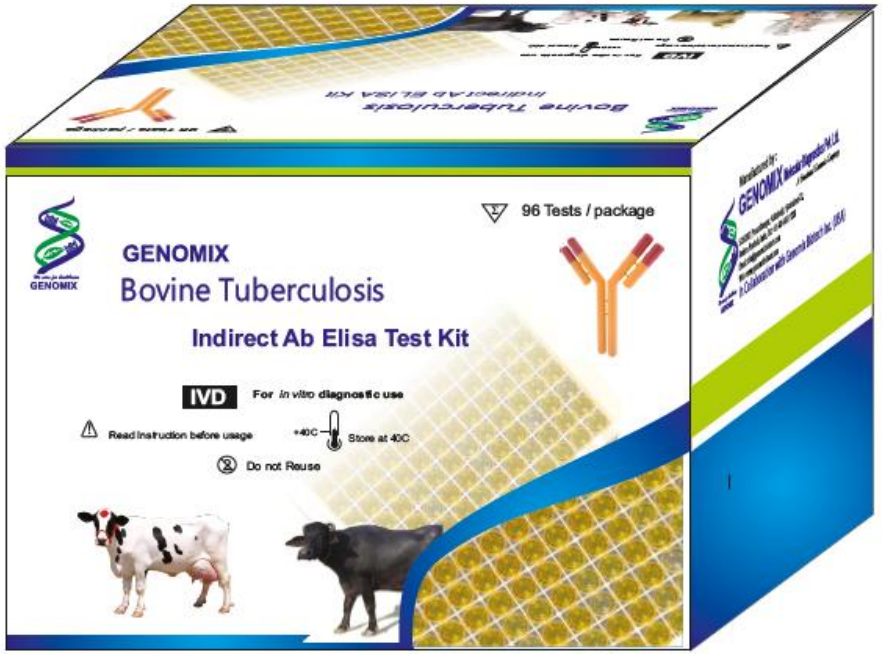
TRPVB

The synthetic construct was prepared by cloning the 1301bp sized fusion protein gene(MPB83:70) (Carried out by TRPVB/TANUVAS) Into the pET28a vector between the *NcoI*(5') and Xho I (3') sites.

These synthetic construct transformed into expression host E coli BL21 (DE3).Then the expression was confirmed by SDS-PAGE analysis, Western blotting. These 48.2KDa sized recombinant protein purified by using Ni-NTA Column.

The Recombinant protein was used for developing RDT and ELISA & Bovine IGRA

Lateral Flow Assay for bTB



Indirect ELISA For bTB

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Wild Animals & Bovine TB (tuberculosis)

These kits were validated at TRPVB, TANUVAS and National Zoo

In Collaboration with TRPVB

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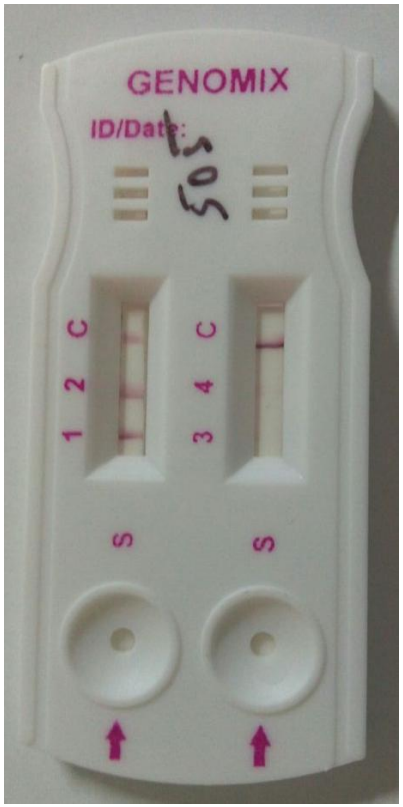
Lateral Flow Assay for Bovine TB & Human TB



1. MBP 83-70
2. Bovine PPD
3. ESAT 6 &CFP10
4. Avian PPD

C. Control line

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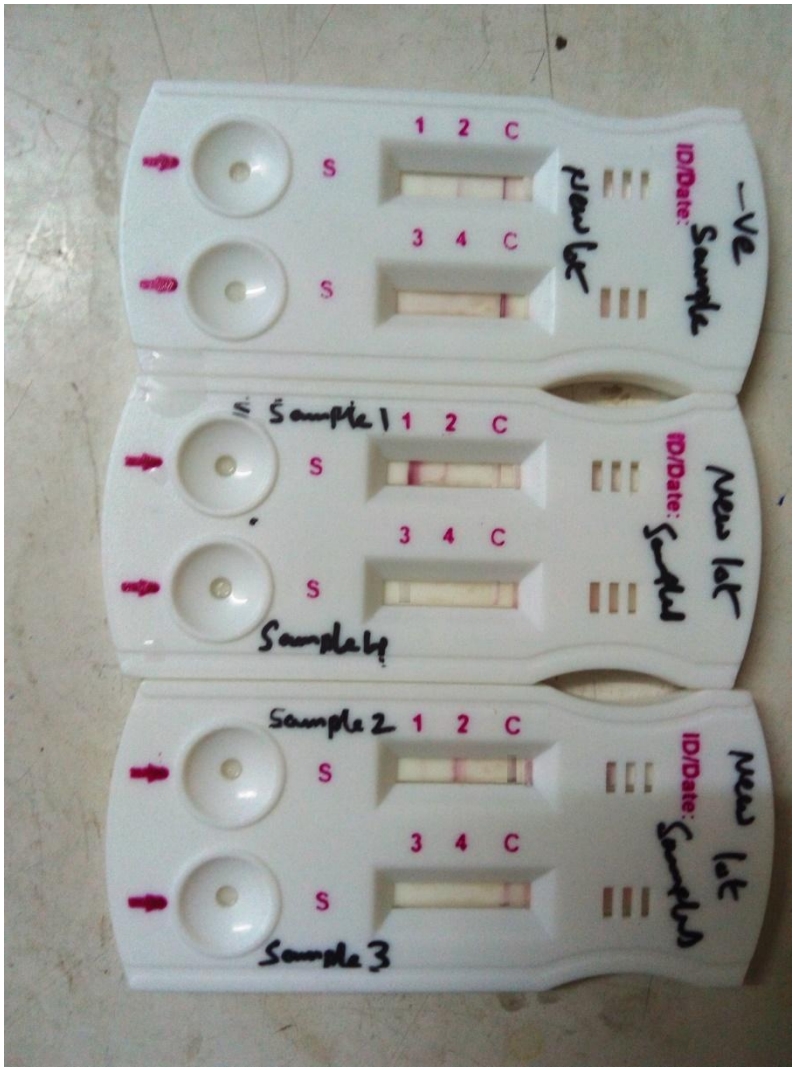
LFA to test MTB complex



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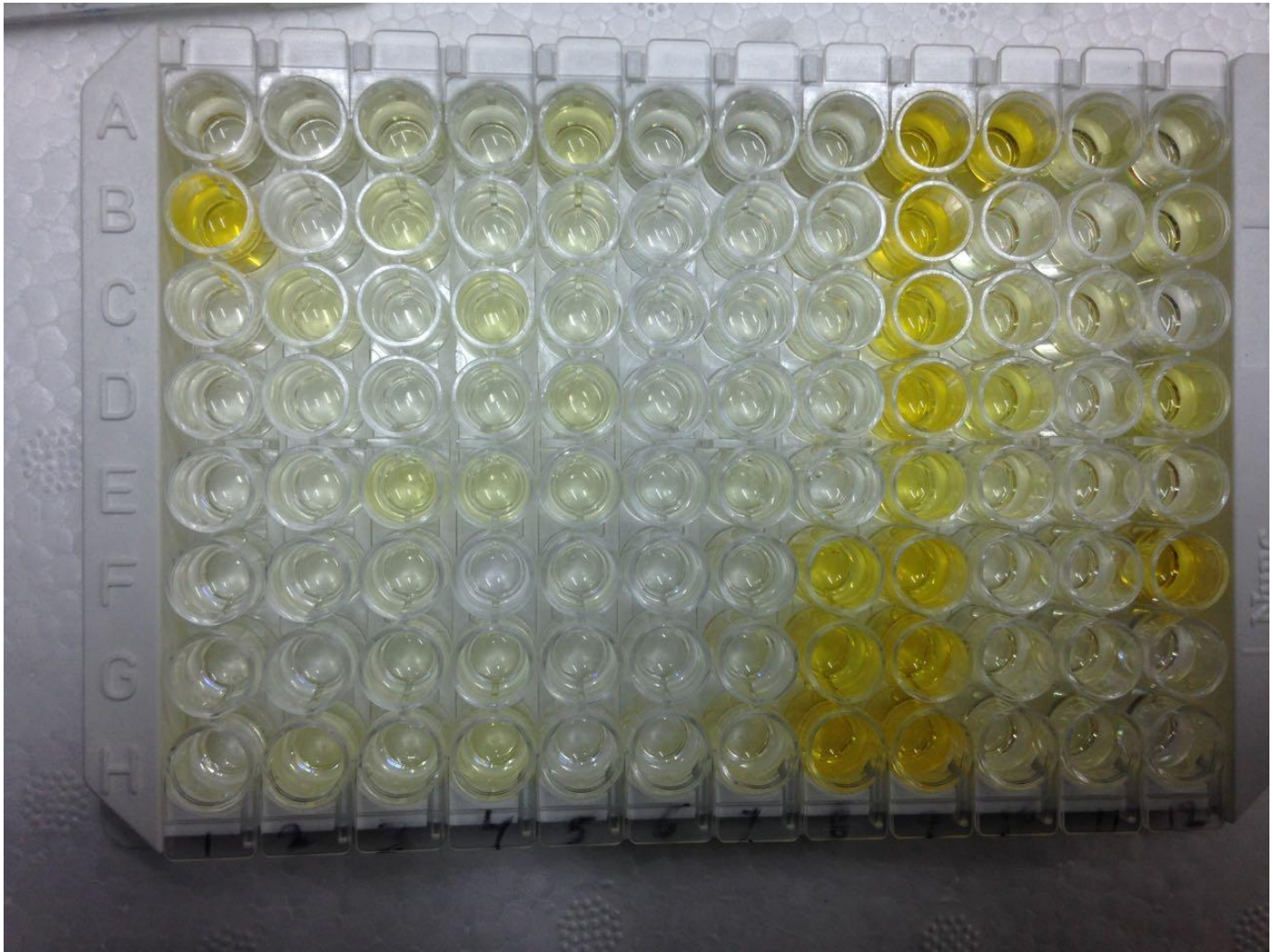
ELISA for Bovine TB using MBP 83-70



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Clinical Cases of Paratuberculosis

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Diagnostic Tests to Detect Para TB Mycobacterium avium sub species para tuberculosis (Johnes Disease)

Collaborated with

Dr. J. Sohal

Amity University

Jaipur

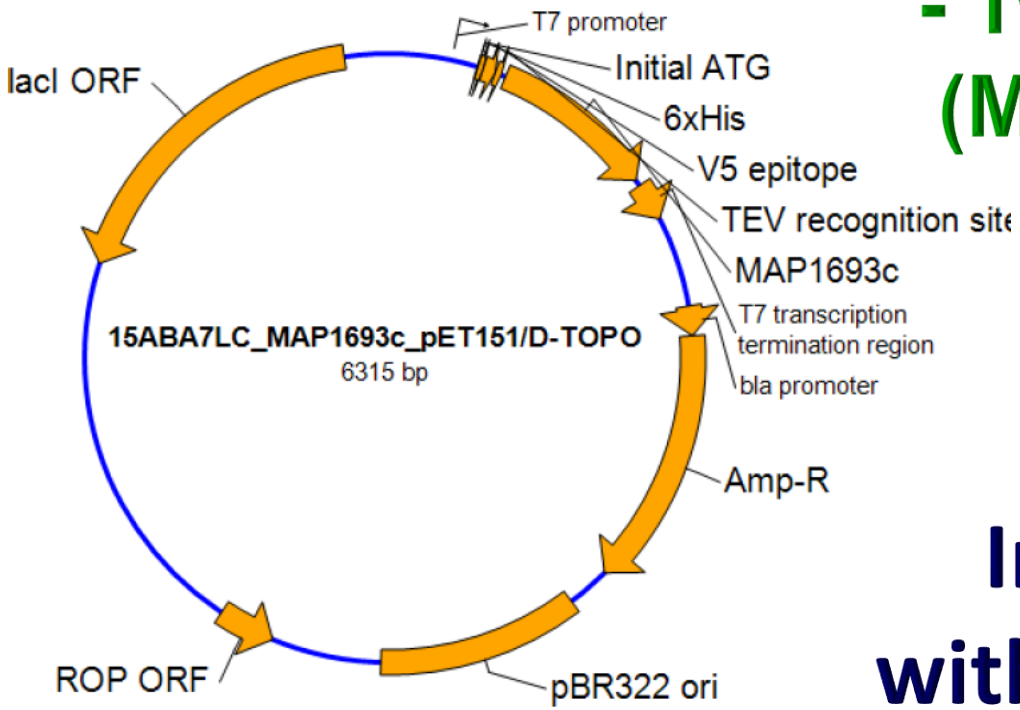
Maintanance of Paratuberculosis Bacilli Isolates at Amity University

- Amity University is maintaining 19 isolates of paratuberculosis bacilli recovered from clinical cases of difference species
- Molecular identity of these isolates has been confirmed by NJIL&OMD, Agra
- Amity University & Genomix are developing joint TB-JD vaccine using these isolates
- OIE suggests vaccination is the most suited method to control paratuberculosis, but DIVA is required
- Amity University has developed DIVA and technology is transferred to Genomix

Cloning & Expression of specific proteins (secretory & surface)

- Six Secretory Proteins
- (MAP1693, MAP2677, MAP4308, MAP3547, MAP2168)

- Two Surface Proteins (MAP0862 & MAP065)



In collaboration with Amity University

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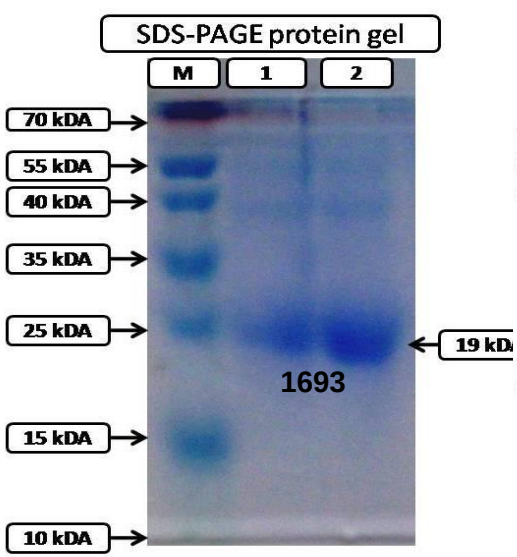
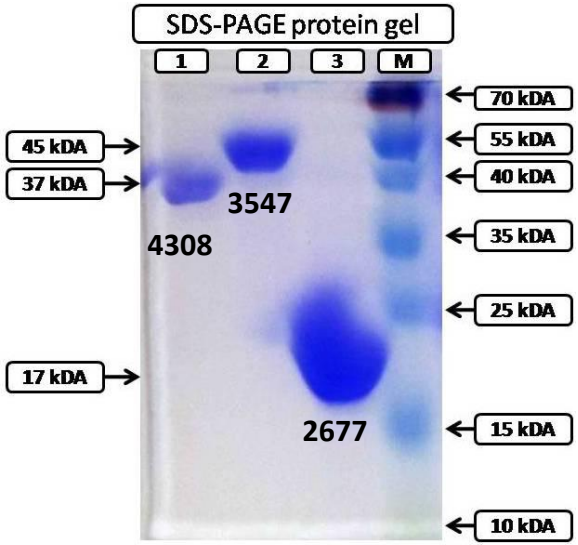
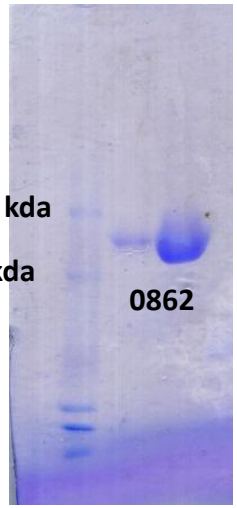
Recombinant proteins were purified to homogeneity and used for ELISA, RDT kits preparation

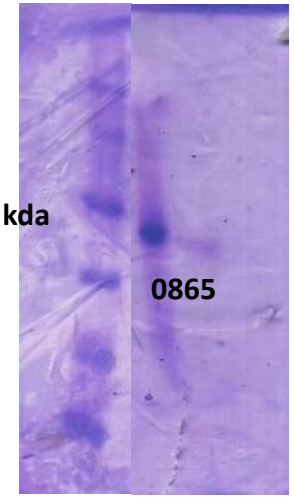




SDS PAGE



SDS PAGE



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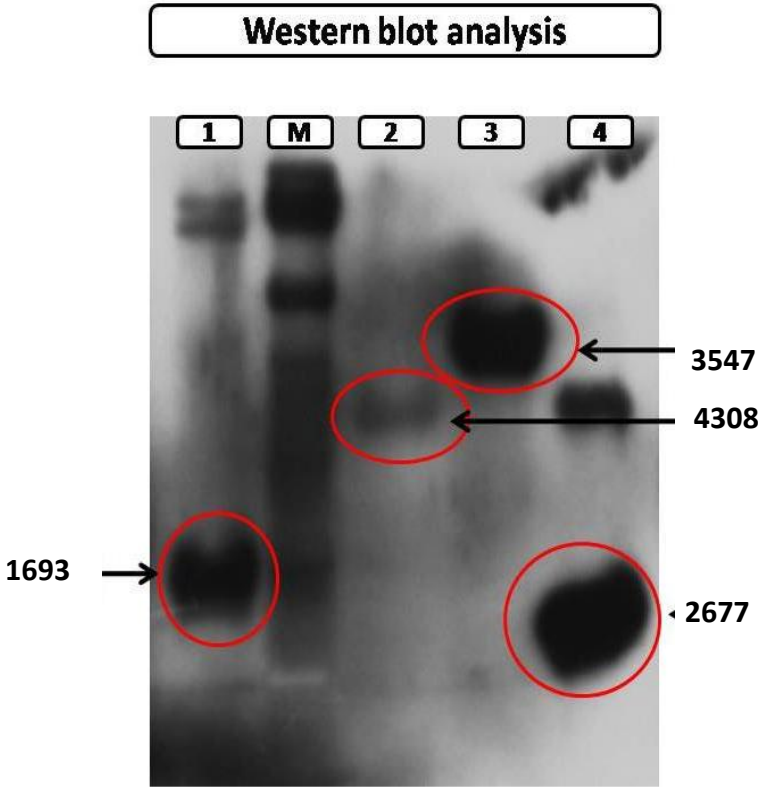
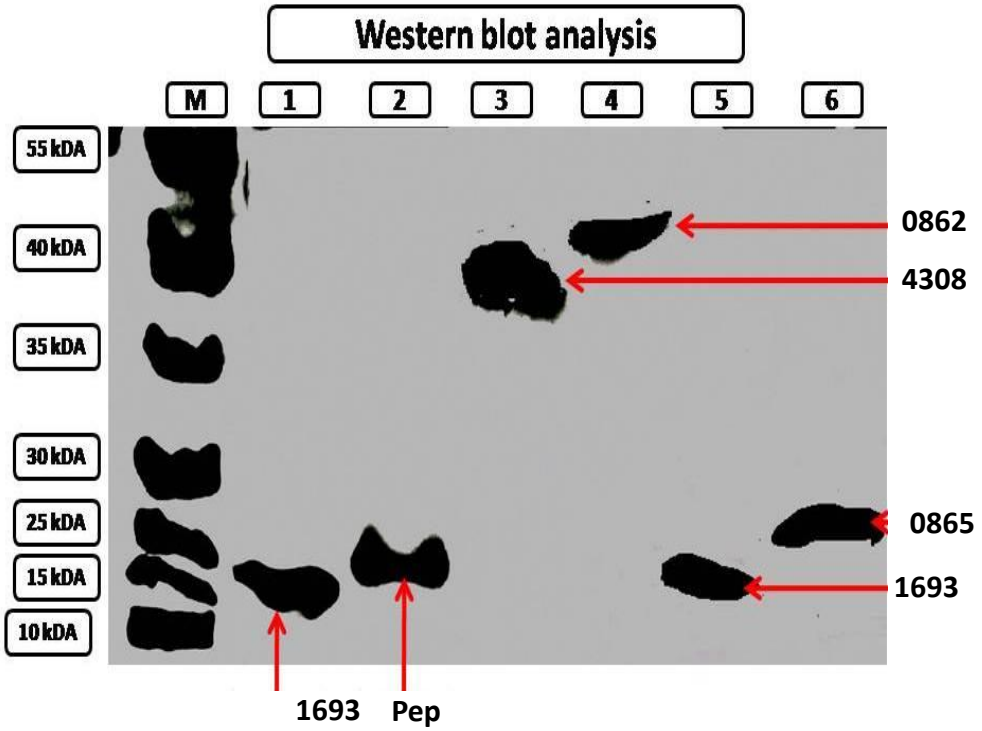
All expressed proteins were found immuno-dominant in ruminant species





Paratuberclusos Alert Rapid Antibody Detection Kit (Penside)

- **Based on specific proteins of paratuberculosis bacilli absent from *M. tuberculosis* complex, onsite test**
- **Five minute onsite diagnostic test without need of any trained personal or instrument**
- **Number of animals tested- over 500 so far**
- **Sensitivity- 77.7% and Specificity- 97.5%**
- **OIE recommends ELISA based testing for prevalence studies and also suggests ELISA test for Population freedom from infection**

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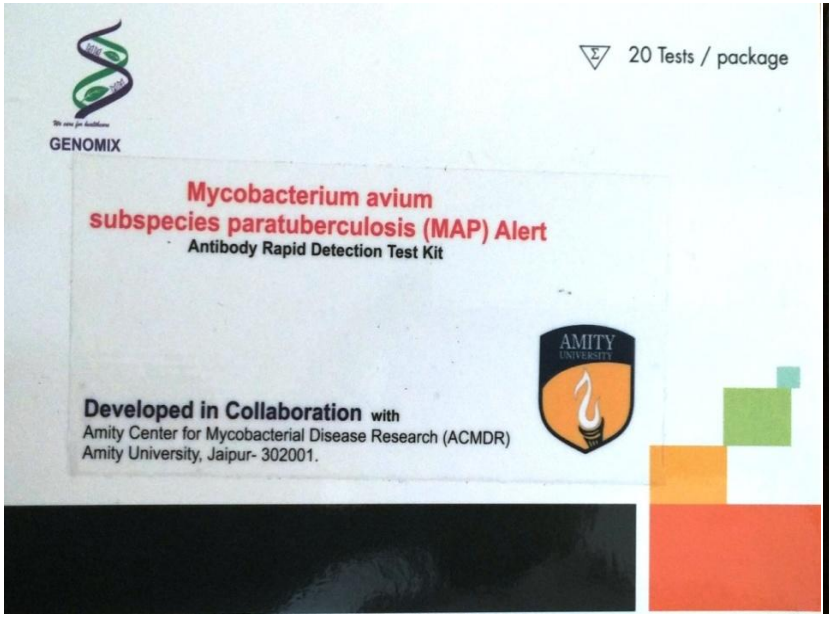
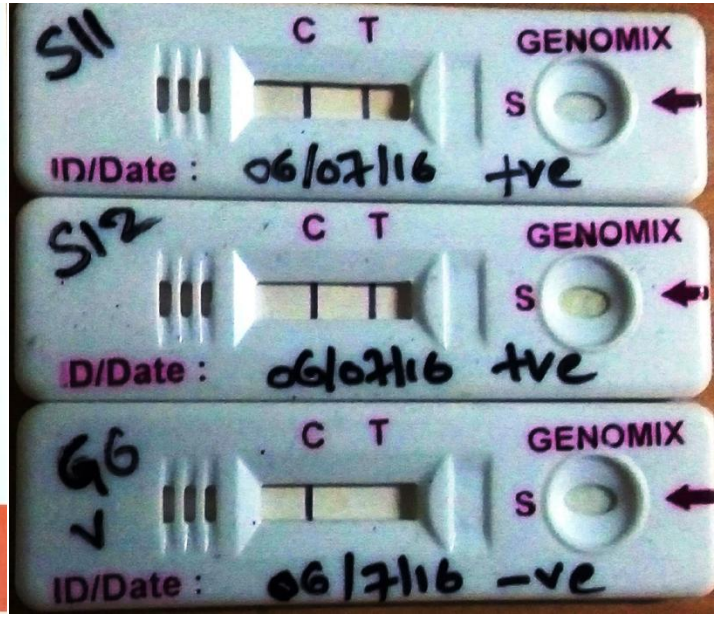
Simple **Easy to use**

Inexpensive

Use at Resource limited areas **Point of care areas**





Positive Animals

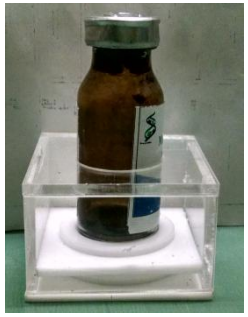
Negative Animals

Collect the Faecal Sample from goats

Mix the collected sample in diluent

Inject the diluent with sample in to Magnetic nano particle container

Incubate the Particle chamber with Sample at RT



Concentrate on Magnetic Chamber

Remove the unbound solution

Recollect the Concentrated Ag from Magnetic Nano particle using Elution Buffer

Concentration of MAP bacteria/ Antigen/nucleic acids

A novel, rapid, easy to use device for molecular diagnostics

Place swab in sample prep fluid

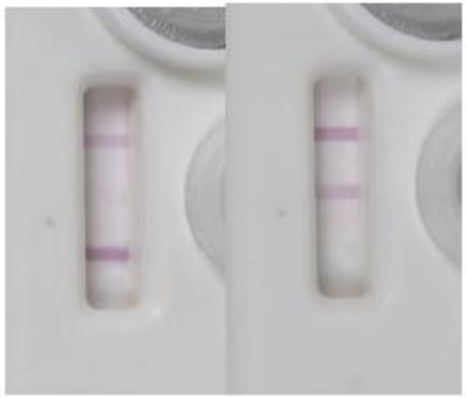
Heat to 95°C for 2 min

1 or 2 drops added to AMPlite

Place AMPlite in heater unit

Prototype single channel heater instrument

30min



Positive Negative

***Under Development**

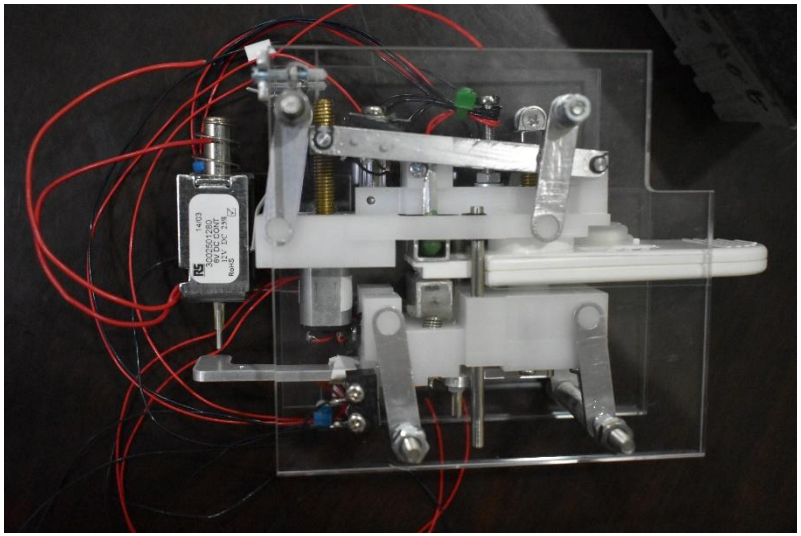
LAMP- LFA;

(1) LAMP assay amplicons with biotin and FITC labeling. (2) Universal LAMP-LFA design

(3) LAMP assay amplicon detection with lateral flow assay. (4) LAMP-LFA results shown with positive and negative controls.

LAMLITE

Heater



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It is not as simple as it looks to coordinate with Tropical zoonosis control programs

Identify the methods

Standardize

Validate at OIE referral lab

Manufacture

With GMP guidelines

Synchronize with

Control program

Still ……..!

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GENOMIX
Molecular Diagnostics Pvt. Ltd.,

Genomix has a total of 20 Research Fellows (working for Ph.D.,) that are dedicated to work on Animal human Healthcare Diagnostics and Vaccines

ISO 2008-9001;

WHO approved Malaria; GMP; GLP; DSIR Recognized

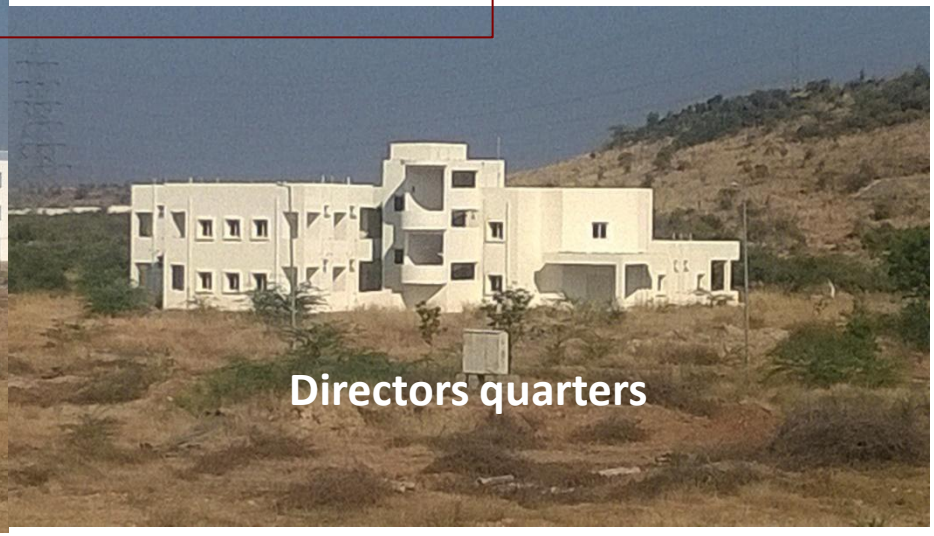
100 products Drug Control Approved

20 Research scholars /Ph.D scholars

GENOMIX CARL Pvt. Ltd

Facilities

GENOMIX CARL Facilities



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Thank You