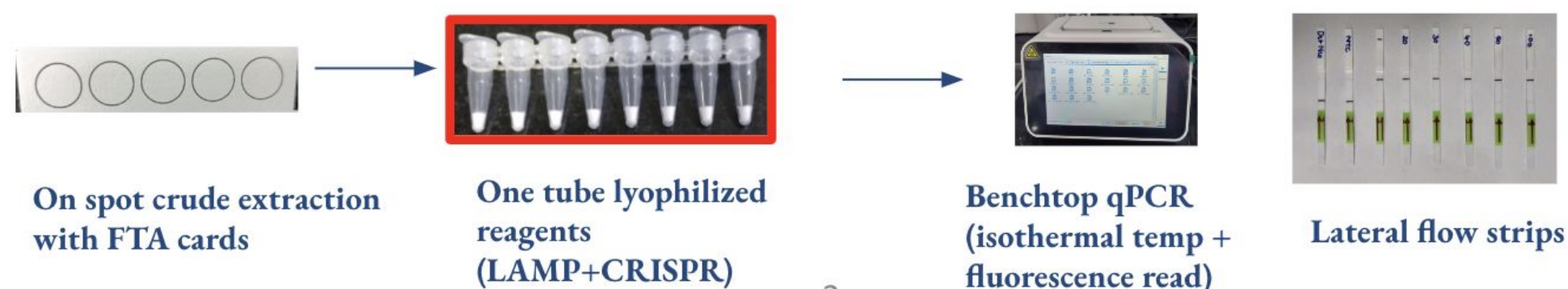




Technology Match Maker | Veterinary Diagnostics | Dec 2024

Title of the tech: PathCrisp-CRISPR based diagnostic platform at Point-of-need



Founder: Dr. Vijay Chandru

Organization: CrisprBits Private Limited

Email: [Info@crisprbits.com](mailto:Info@crisprbits.com)

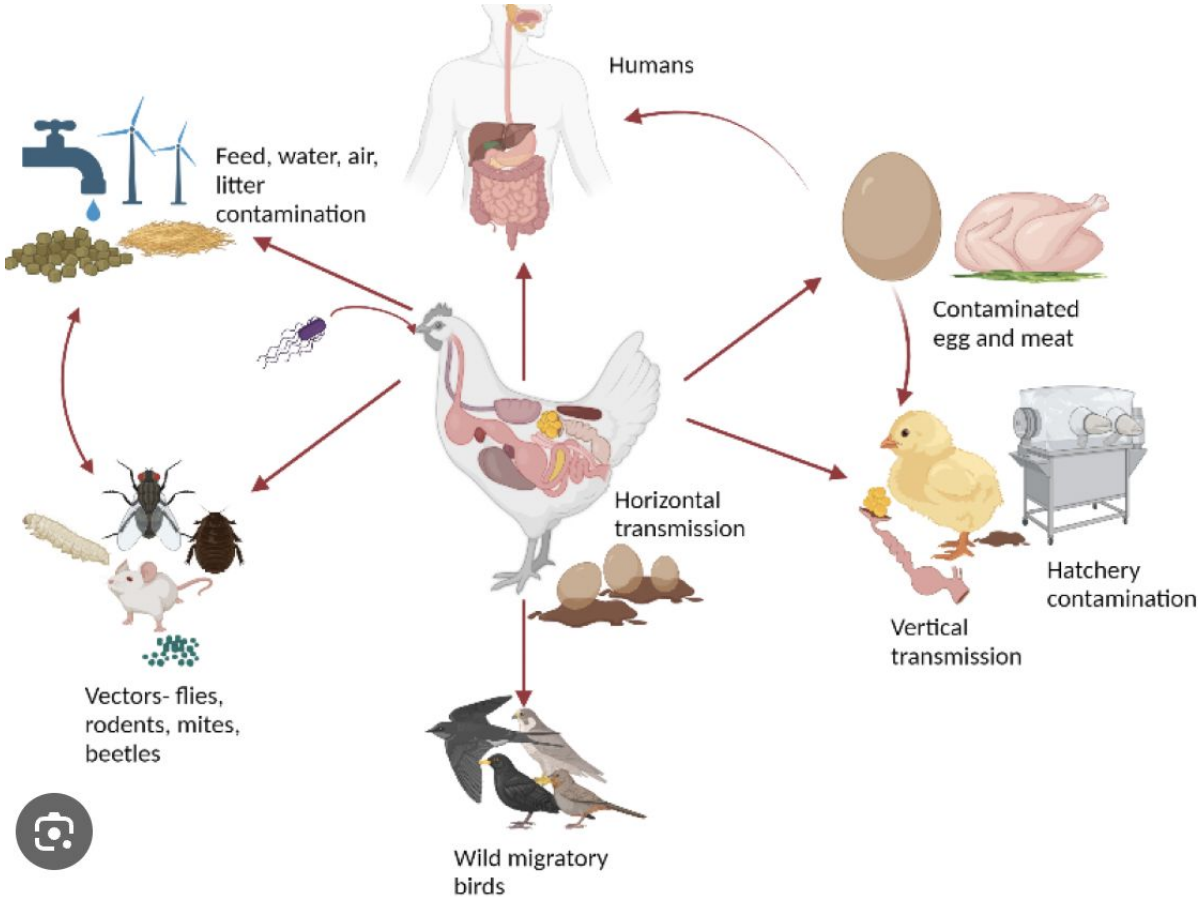
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supported by:



# Problem Addressed

- Lack of **accessible** and **rapid diagnostic tools** at the **point of need** in Veterinary sector is a critical challenge that threatens **global animal health, food security, and public health.**
- Livestock diseases alone result in annual economic losses exceeding **\$300 billion**, driven by **reduced productivity, high mortality rates, and trade restrictions.**
- Salmonella & FMD outbreaks can cause severe economic losses due to **decreased milk production, reduced livestock weight, death in young ones** and **trade restrictions.**

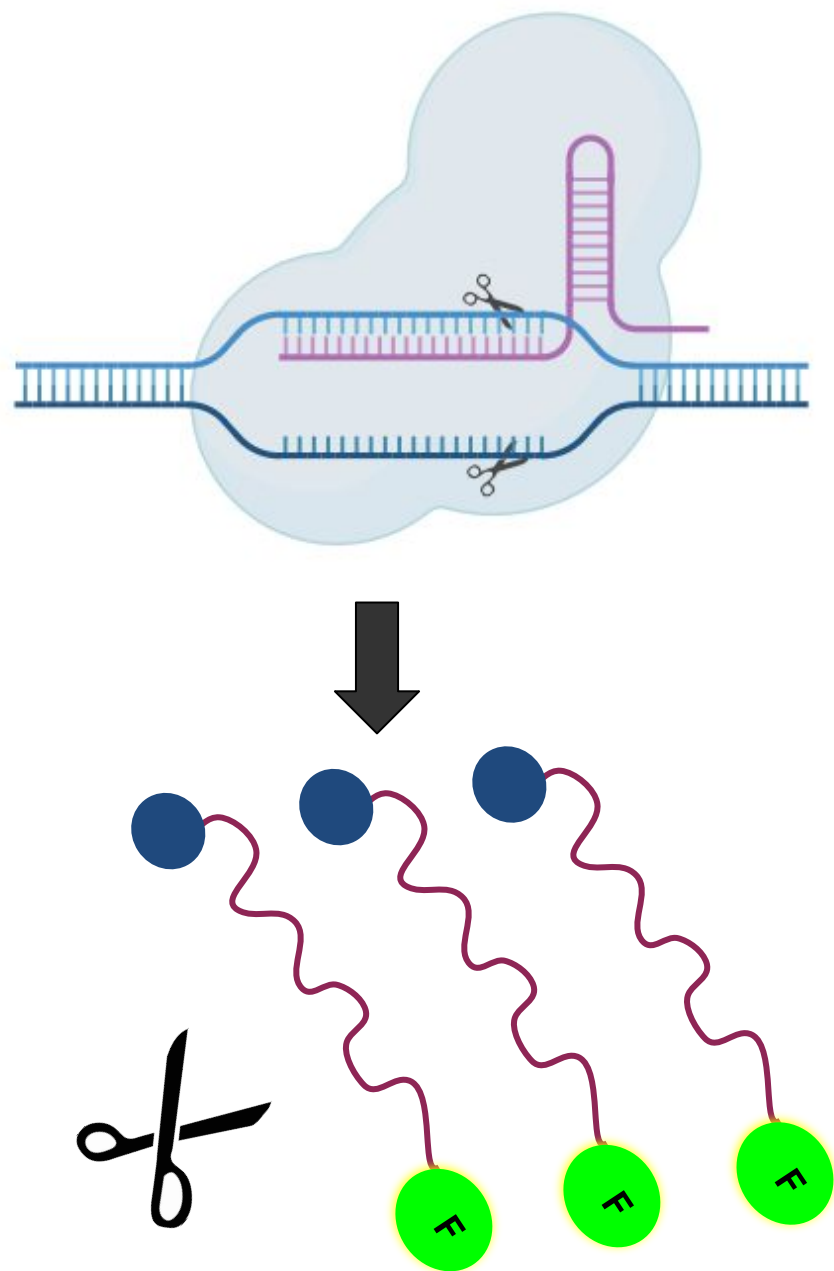


| Salmonella Outbreak in Meat & Animal Feed |                |                  |   |
|---|----------------|------------------|---|
| Outbreak                                  | Region         | Economic loss    | Remarks   |
| Foster Farms Chicken Outbreak (2015)      | USA            | ~50 Million USD  | Patients hospitalized, Foster farms temporarily halted production |
| Salmonella in Pork Products (2016)        | European Union | ~200 Million USD | Culling of animals, Product recalls                               |
| Salmonella Outbreak in Animal Feed (2016) | European Union | ~200 Million USD | Animal Egg recall   |
| Minced Beef Recall (2020)                 | Australia      | ~3.5 Million USD | Trade Restrictions, Temporary closure of implicated plants        |
| Poultry & Feed Industry Annually          | India          | ~60 Million USD  | Reduced productivity, Foodborne illness, Trade restrictions       |

| Foot & Mouth Disease |             |                    |  |
|----------------------|-------------|--------------------|--|
| Outbreak year        | Region      | Economic loss      | Remarks  |
| 2001                 | UK          | ~11 billion USD    | Livestock Culling, Trade restrictions  |
| 2010-2011            | South Korea | ~2.6 billion USD   | ~3.5 million animals culled  |
| 2013                 | India       | ~0.5-1 billion USD | Reduced milk production, death of high yielding cows and young livestock etc |
| 2005                 | China       | ~1.5 billion USD   | Large scale culling, vaccination drives etc                                  |
| 2005                 | Brazil      | ~1.5 billion USD   | Export restrictions (meat)   |

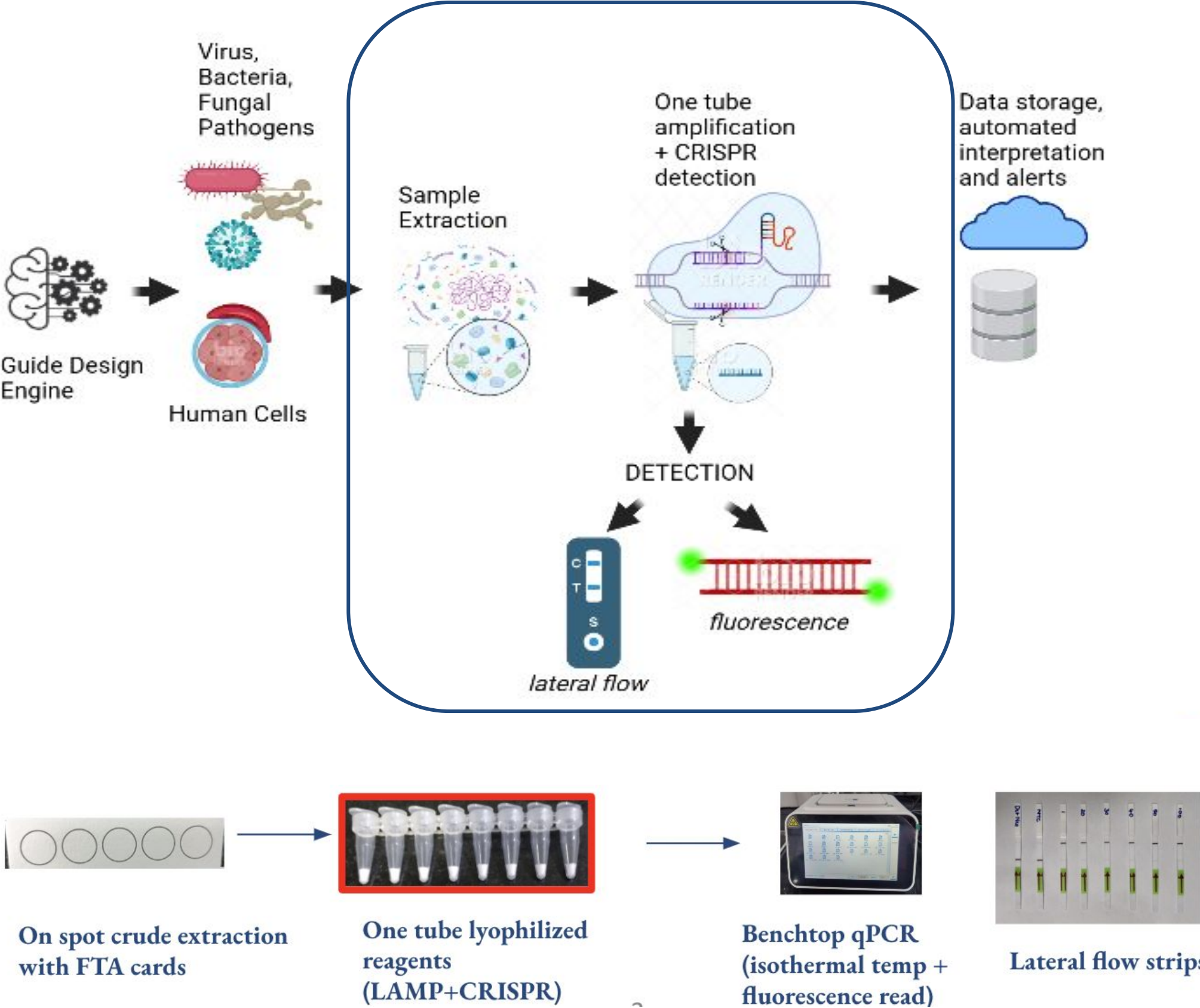


# Our Solution-PathCrisp-CRISPR-based diagnostic platform



## Principle behind CRISPR Diagnostics: Trans-Cleavage Activity of Cas12

Release of fluorescence via collateral-cleavage of nucleic acid reporter subject to cis-activation of Cas-RNP complex



# Value proposition: Molecular Diagnostics at the *Point of Need*



Point of  
Need

Single tube

Accuracy

RT  
shipment

Rapid TAT

Low  
resource

Versatile

Affordable

**Pervasive Diagnostics**

**Molecular testing at the point of need with the accuracy of qPCR/NGS and simplicity of Immunoassays (Rapid test)**

- **High specificity and Sensitivity:** Detects even single copy
- **Rapid TAT (60 minutes) (No thermal Cycling)**
- **Precisely target the desired DNA sequence** - minimize false-positive or false-negative results.
- **Versatility-** Can adapt to wide range of pathogens/SNPs
- **Multiplex-** detect multiple pathogens/markers, allowing for rapid and comprehensive screening.
- **Minimal Equipment-**accessible to healthcare providers in a variety of settings, including farms, field hospitals, PHC etc (low resource setting) with reagents shipped & stored at Room temperature (Lyophilised)
- **Multiple formats** e.g. portable devices, point-of-care tests, and lab-based assays.
- **Affordable:** (a)Eliminate expensive equipment (Low CapEx) (b)**No Skilled hands**, thus allowing more testing, more effective and timely diagnosis



# Use-Case 1: *PathCrisp*-Salmonella Test for Ensuring Safety in the Food Industry (Meat and Animal Feed)

- Salmonella infection in **meat** and **animal feed** can cause **illness in animals, foodborne diseases** in **humans, economic losses**, and contribute to **antimicrobial resistance**.
- Currently **Microbiological (culture based) testing** is employed which take about 72hrs (Pre-enrichment+Enrichment+Plating the media)
- For poultry, the standards specifically address ***Salmonella enterica* serovars Typhi, Typhimurium, and Enteritidis**
- FSSAI, European Union & FDA guidelines for testing salmonella in meat is **random sampling of meat cuts, ground meat, and other preparations**, with samples typically sized **25g**.
- The regulation requires testing of **animal feed materials**, particularly those of **animal origin** (e.g., meat meal), for *Salmonella* presence in **25g samples**.



Meat



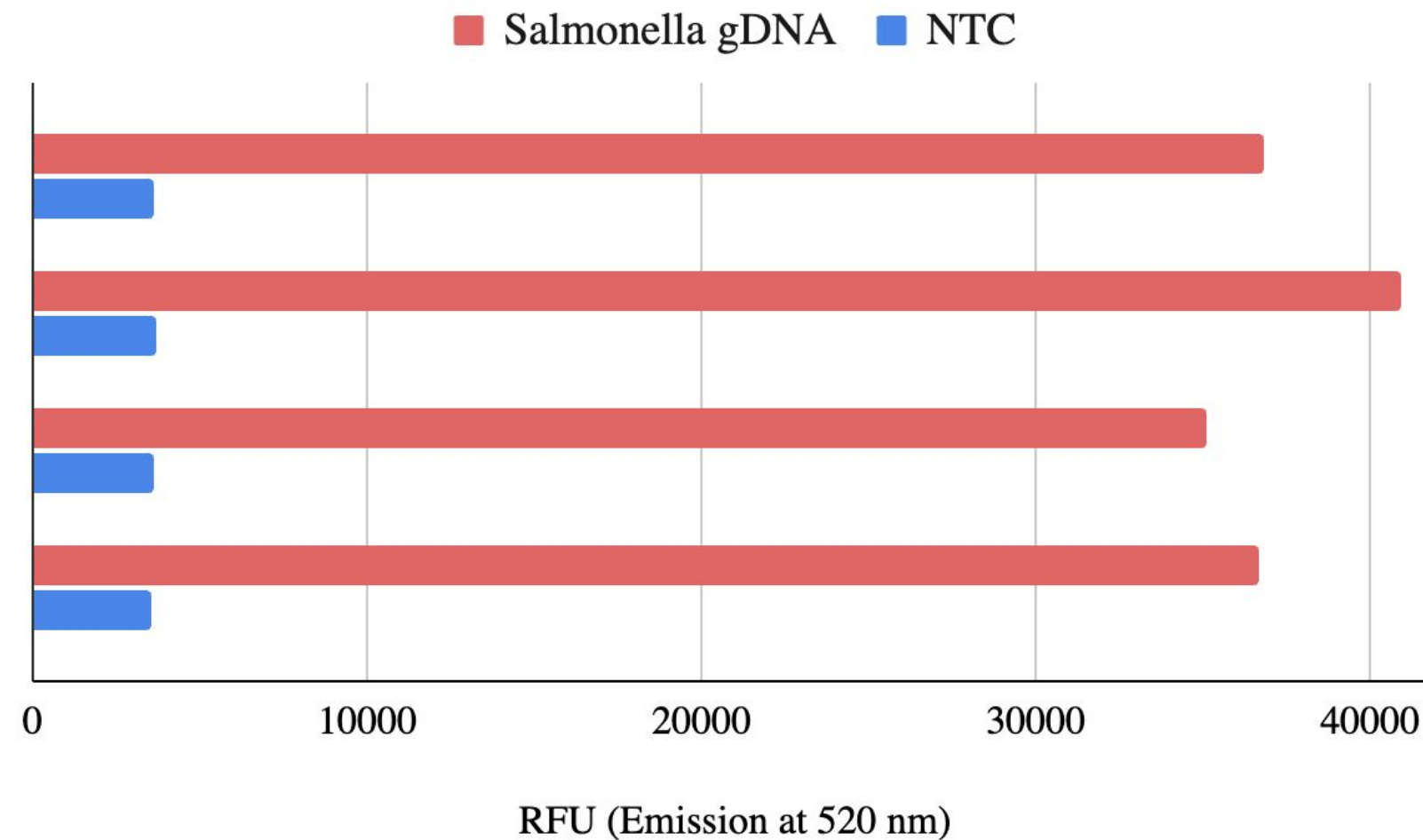
Animal Feed

## Value Proposition (Molecular testing)

- **High specificity and Sensitivity:** Detects even single copy
- **Rapid TAT (60 minutes):** No thermal cycling
- **Minimal Equipment:** Low CapEx
- **Simple to use:** not requiring skilled hands
- **Test every batch:** On-site

# PathCrisp-Salmonella test for Clinical and Food testing

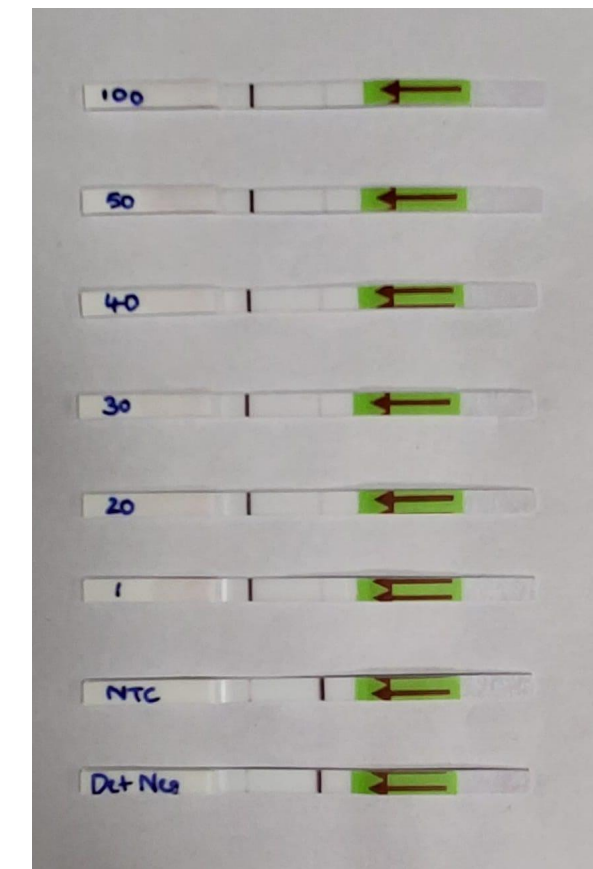
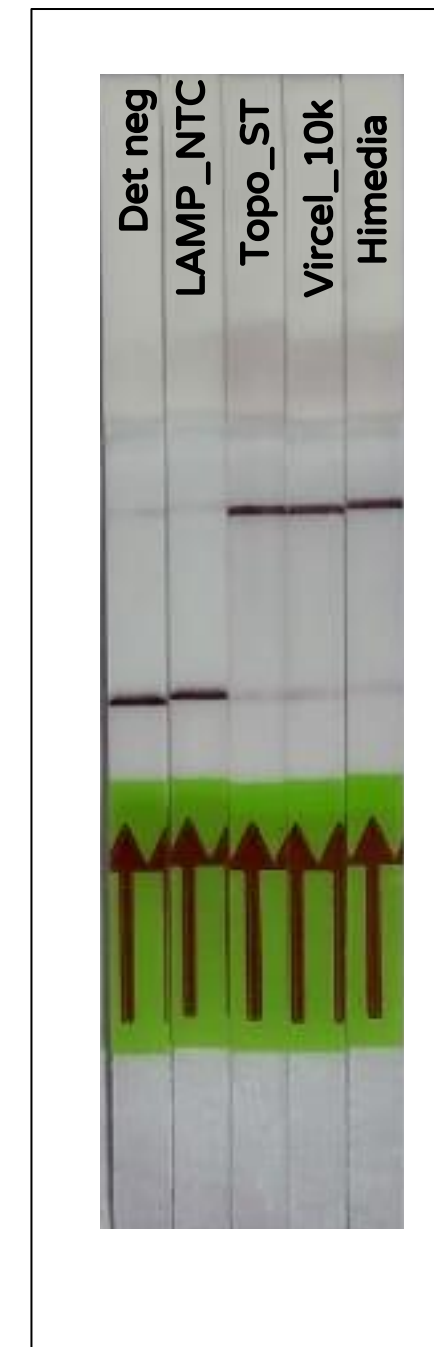
## Fluorescence end-point read out data



Single **lyophilized tube of reagents** (amplifies by **LAMP** and detects by **CRISPR**) Salmonella at 8-10 fold over background level. Manual step is to add the crude extract of DNA

*Awarded ICMR grant for assay dev of Salmonella and its Antibiotic resistance markers*

## Lateral Flow end-point

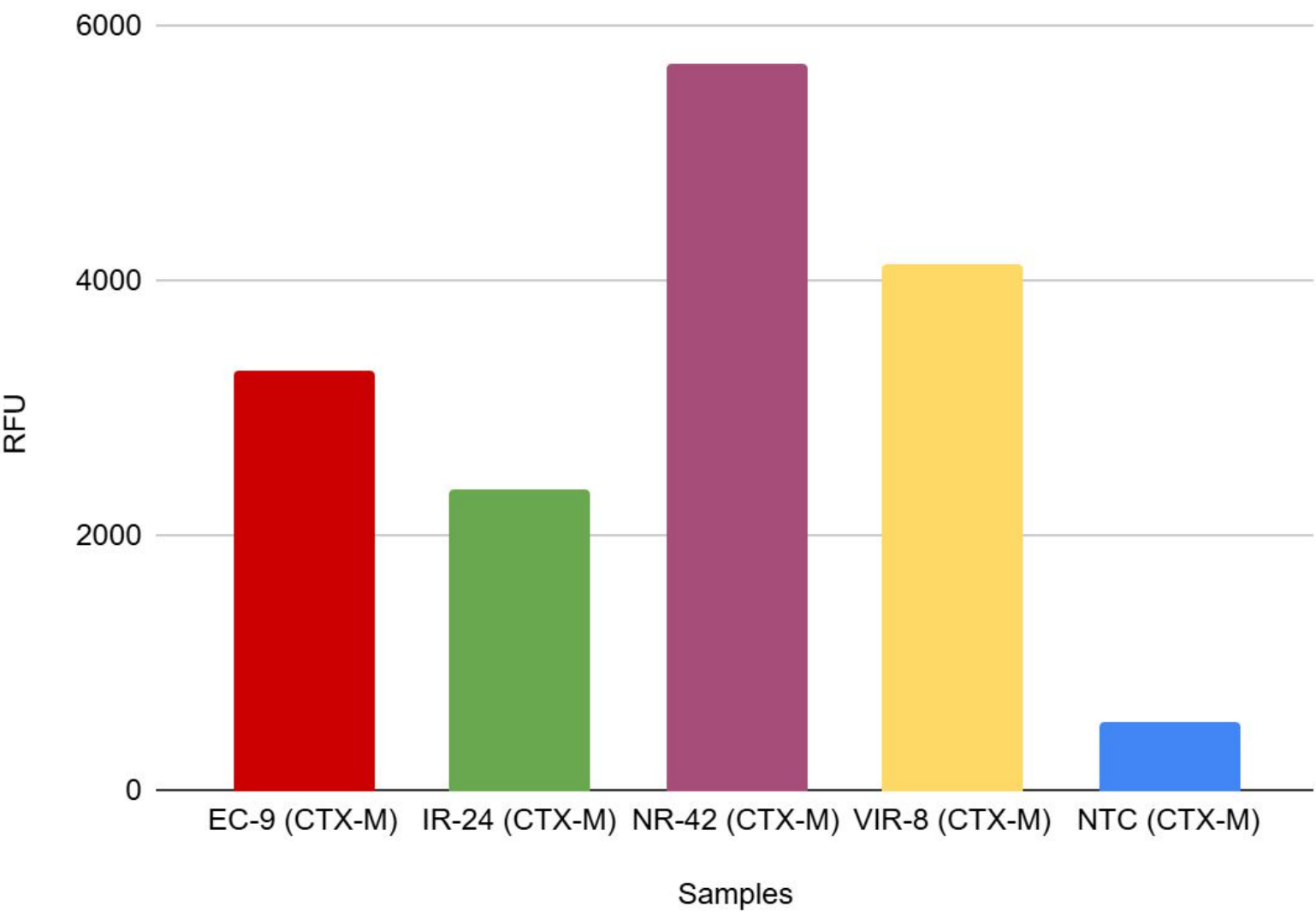


As low as 1 copy is detected via our test in spiked milk sample

1000 copies of Salmonella DNA (Vircell) detected using lateral flow strips.

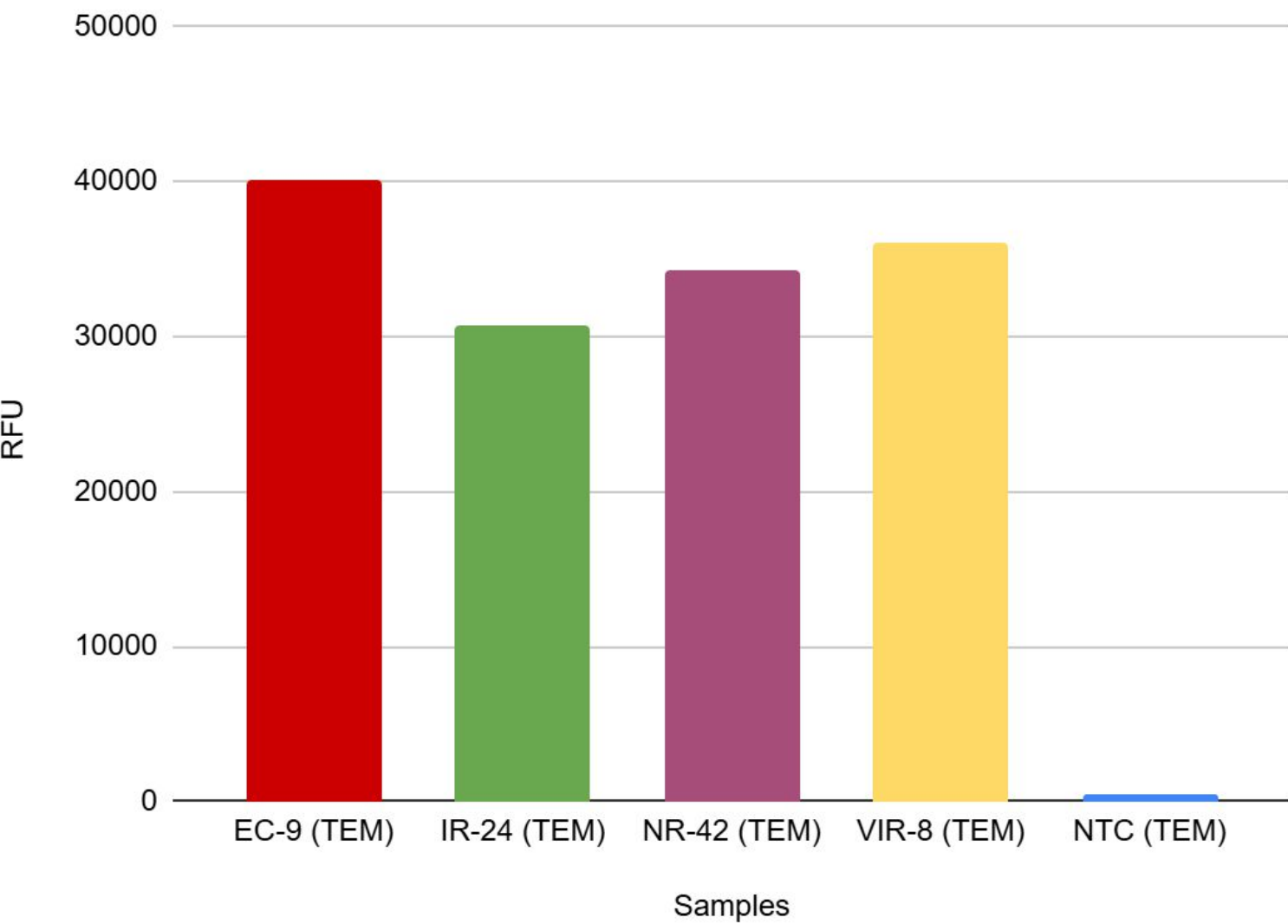
# PathCrisp-Salmonella AMR CTX-M and TEM testing

One Pot - Cas12b\_IDTgRNA\_CTX-M



| Samples       | RFU  | Fold Change |
|---------------|------|-------------|
| EC-9 (CTX-M)  | 3289 | 6.1         |
| IR-24 (CTX-M) | 2375 | 4.4         |
| NR-42 (CTX-M) | 5703 | 10.5        |
| VIR-8 (CTX-M) | 4130 | 7.6         |
| NTC (CTX-M)   | 541  |             |

One Pot - Cas12b\_IDTgRNA\_TEM



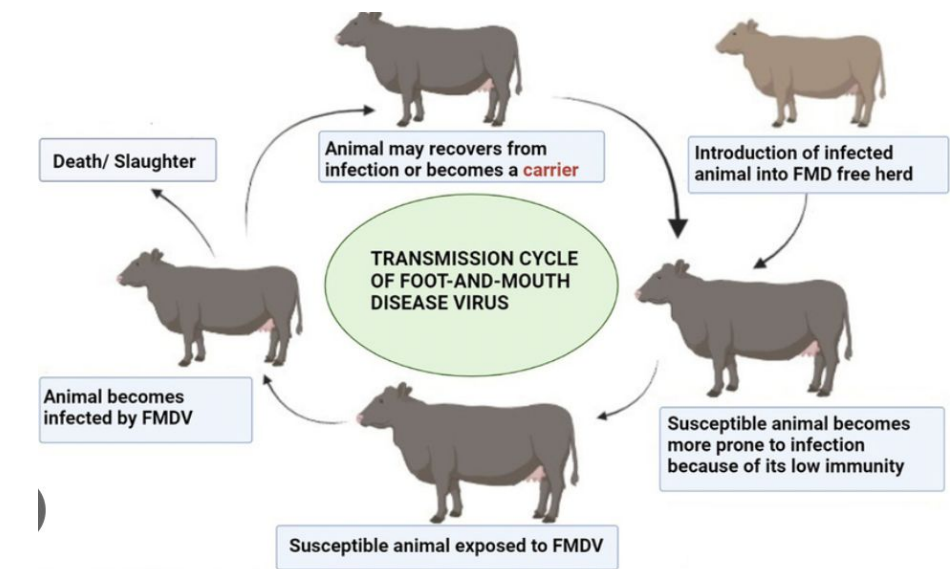
| Samples     | RFU   | Fold Change |
|-------------|-------|-------------|
| EC-9 (TEM)  | 40172 | 77.7        |
| IR-24 (TEM) | 30760 | 59.5        |
| NR-42 (TEM) | 34360 | 66.5        |
| VIR-8 (TEM) | 36108 | 69.8        |
| NTC (TEM)   | 517   |             |



# Use-Case 2: *PathCrisp*-Foot-and-mouth disease virus (FMDV)

## Detection Test for cattle health

- Foot-and-mouth disease virus (FMDV) is a **highly contagious** virus of the Picornaviridae family that affects **cloven-hoofed animals**, causing **fever, blister-like sores, lameness**, and **potential long-term infection risks**, which enable its spread through **direct contact (biological fluids)** or **contaminated environments**.
- FMDV outbreaks lead to significant **economic losses**, including **reduced milk production, decreased livestock weight, and trade restrictions**.
- **Current testing** methods include **Real-Time PCR, ELISA**, and **Lateral Flow Devices (LFD)**. While LFDs **lack sensitivity** and **fail** to detect early infections, qPCR and ELISA require **costly equipment, skilled operators, and central laboratory processing**.



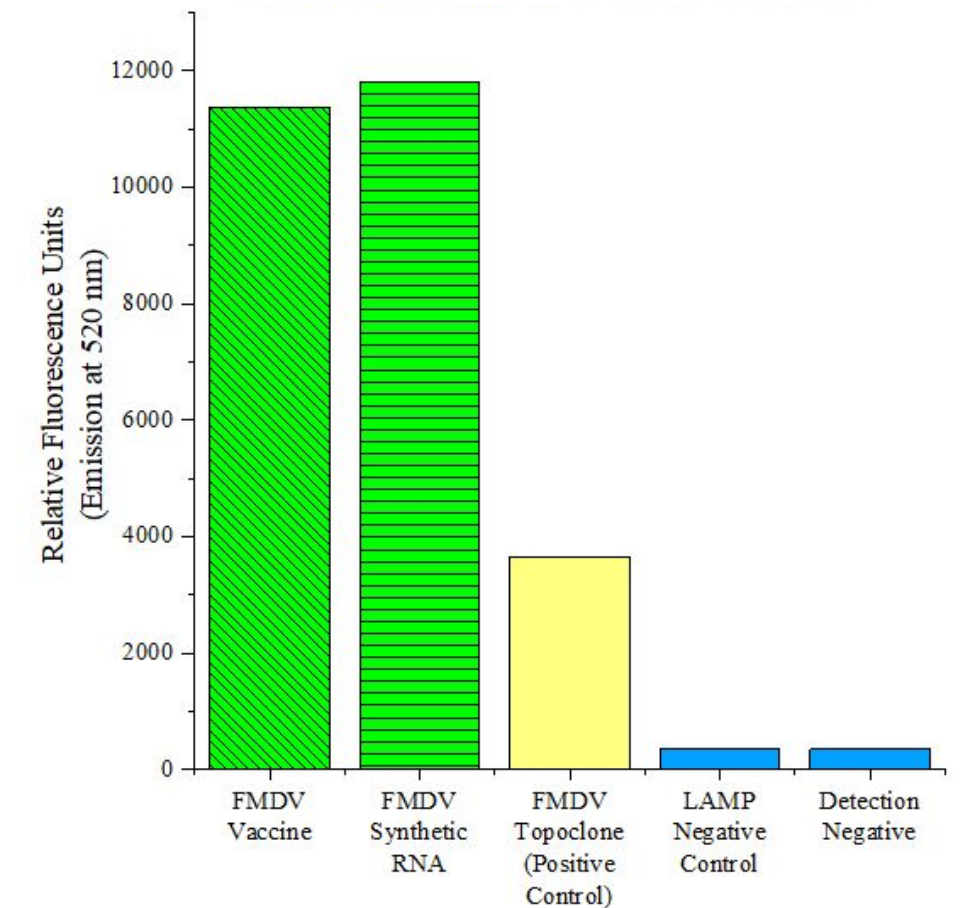
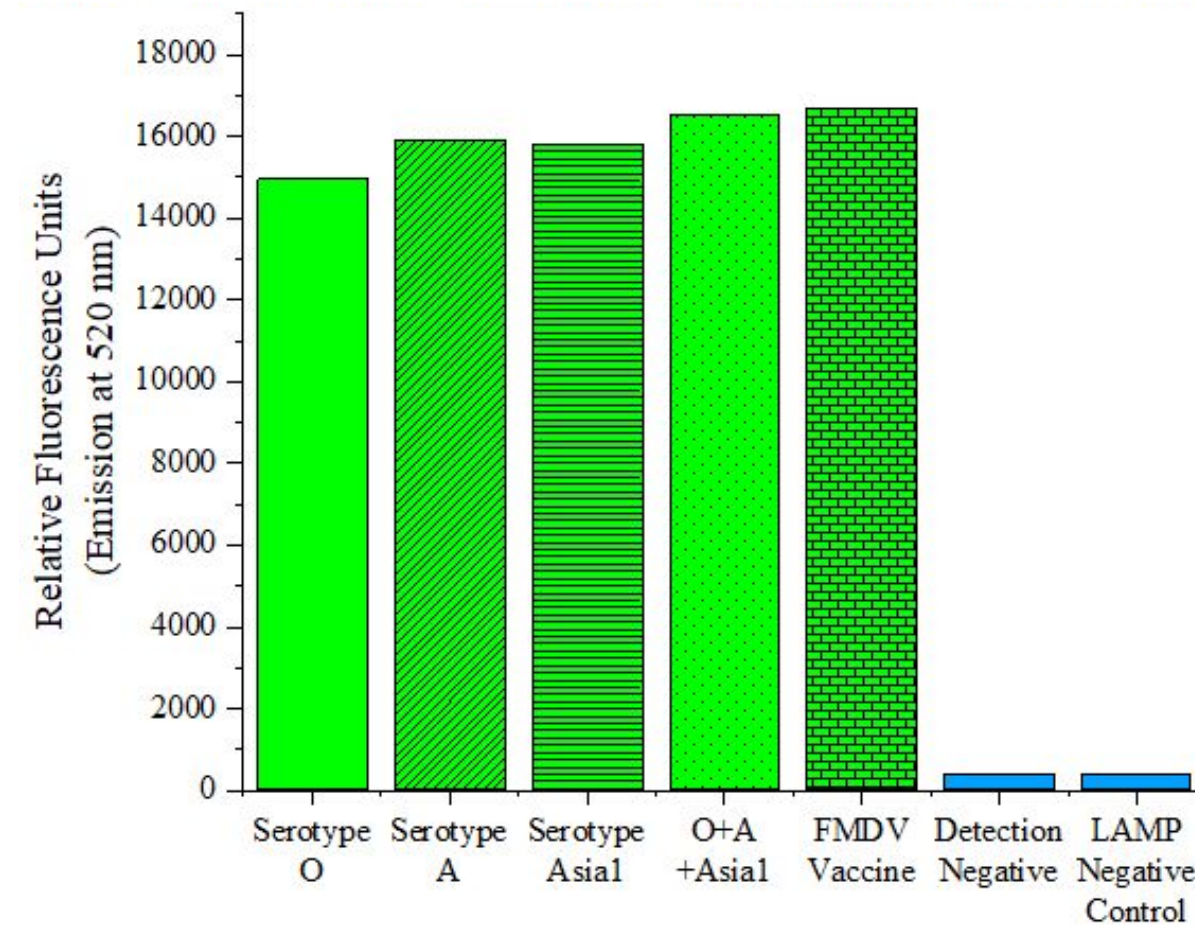
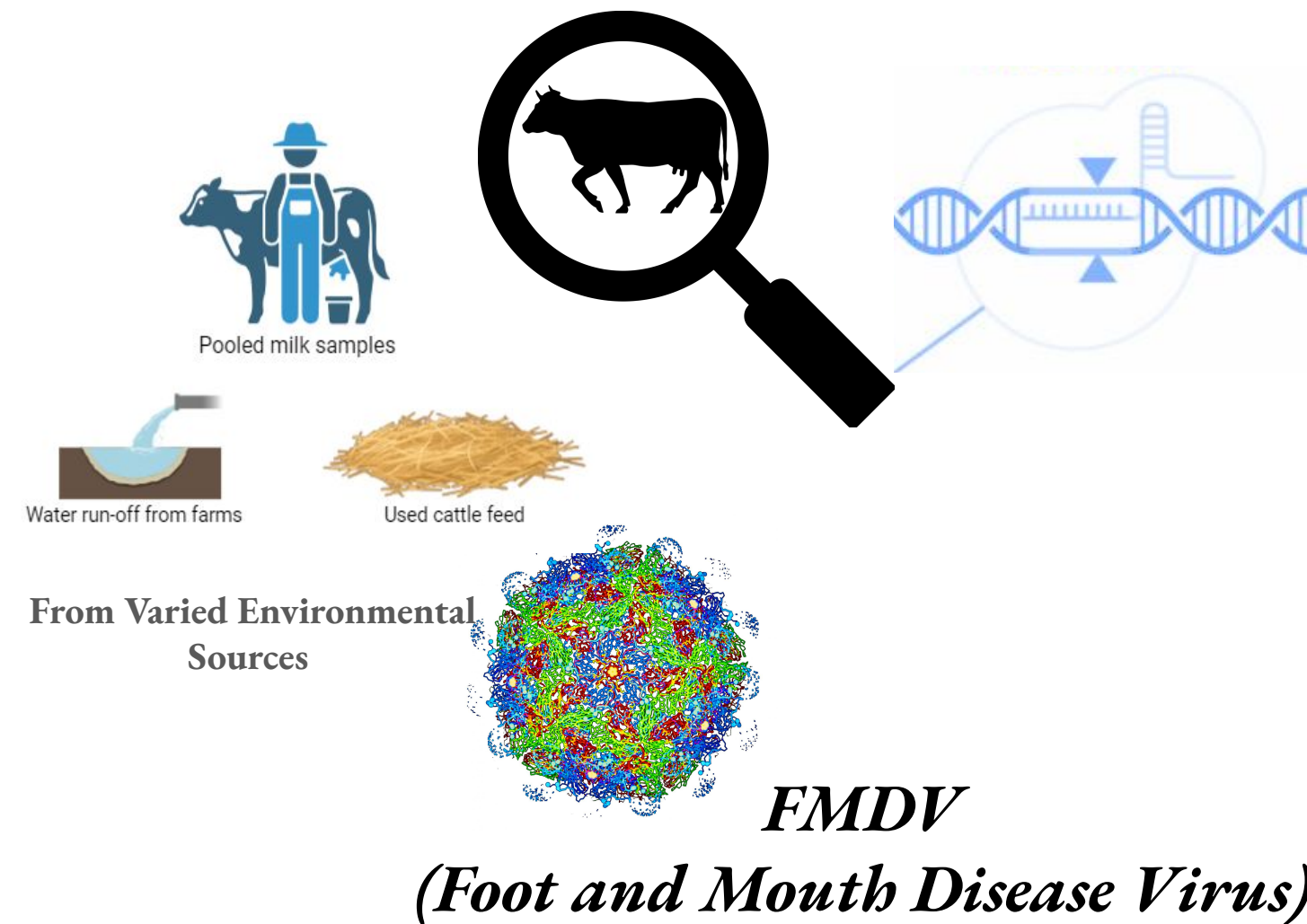
### Value Proposition

- **High specificity and Sensitivity:** Early detection for all positive animals
- **Rapid TAT (60 minutes):** Faster response time
- **Minimal Equipment:** Low CapEx
- **Simple to use:** not requiring skilled hands
- **Test on-Site:** Vet can perform the testing on site





# PathCrisp-FMDV Test

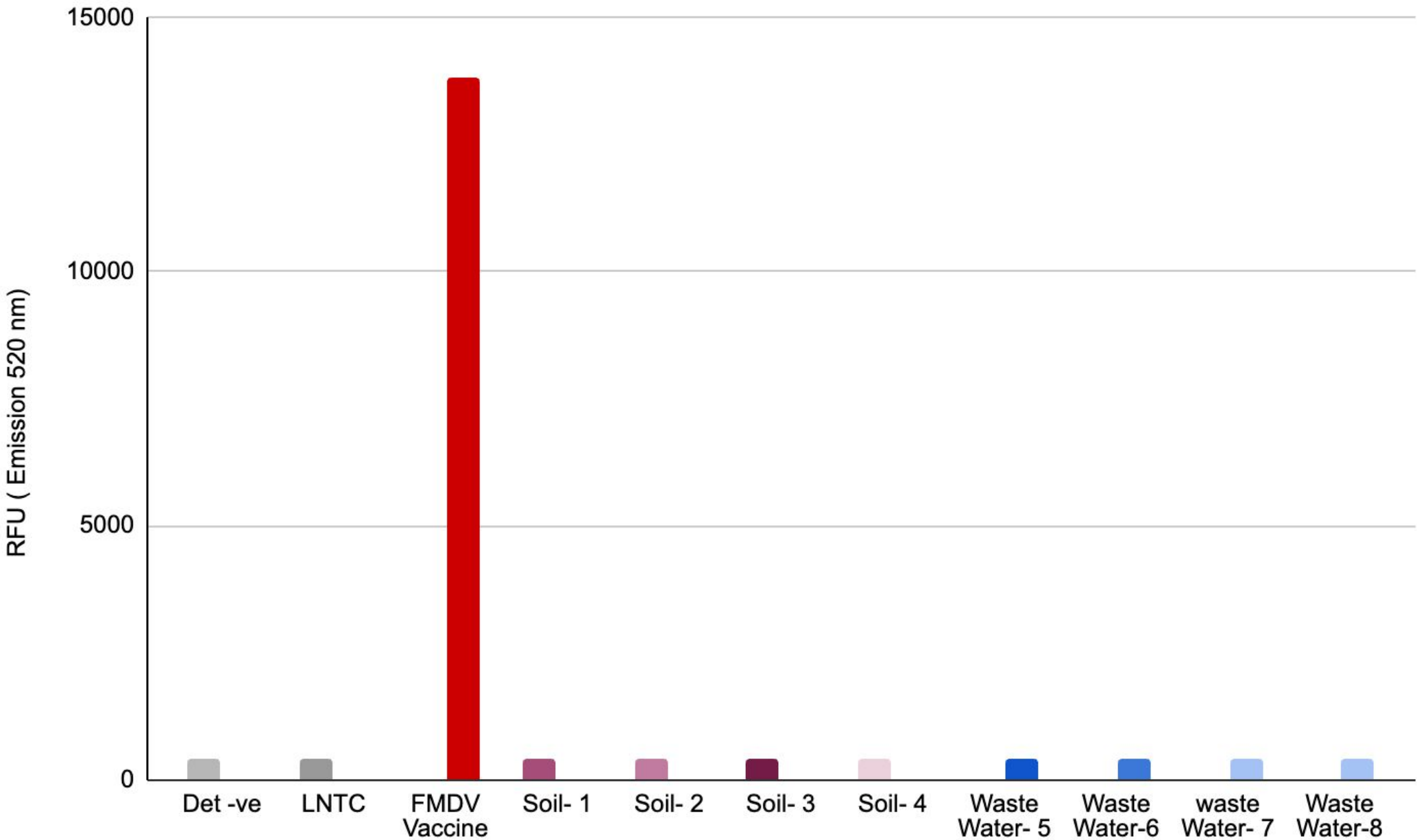


Our CRISPR Assay tested on 4 different **inactivated antigens** from all FMDV **serotypes** and from **Vaccine** sample. Synthetic RNA and TOPO cloned DNA was used as a positive control.

In collaboration with IISc-ARTPark for One-Health Surveillance Program

*Supported by a grant from PSO, Govt of India*

# PathCrisp-FMDV Test data



| Samples       | PathCrisp Fold Change |
|---------------|-----------------------|
| FMDV Vaccine  | 33.8                  |
| Soil- 1       | 1.0                   |
| Soil- 2       | 1.0                   |
| Soil- 3       | 1.0                   |
| Soil- 4       | 1.0                   |
| Wastewater- 5 | 1.0                   |
| Wastewater-6  | 1.1                   |
| Wastewater- 7 | 1.0                   |
| Wastewater-8  | 1.0                   |

← Positive control

FMDV-PathCrisp tested on 8 different environmental samples ( 4 from soil and 4 from wastewater ) collected from 2 different sites (healthy farms). FMDV Vaccine with inactivated antigens used as reference. Further we would be testing biological fluids (nasal swab, saliva etc) from cattle for checking carrier status.

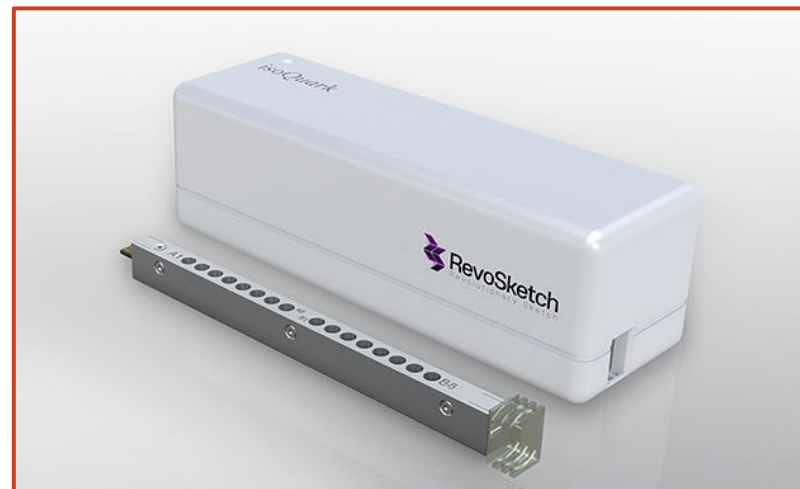


# Vision to develop Multiplexed tests

*automation, miniaturisation, and integration of sensors to rapidly detect on-site specific pathogens*

## Current Version

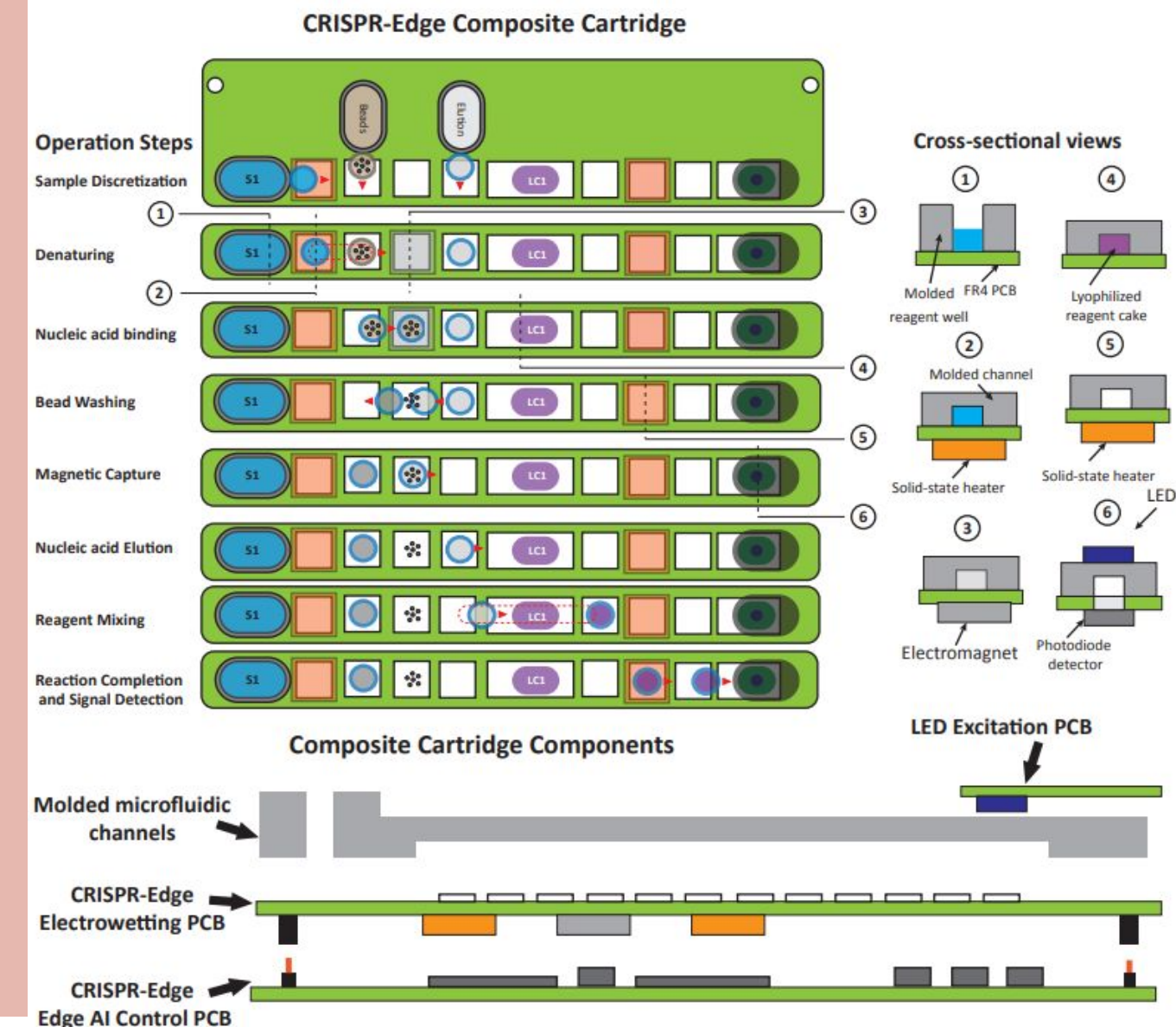
Lyophilized tubes for each marker



Portable Health block and Fluorescence Reader

## Future Vision

### Spatial Multiplexing using microfluidics



A top- and cross -sectional view showing the step-by-step time series operation (left to right) of the **Composite Cartridge**



# Market Analytics

**Global Veterinary Diagnostic Market Size:Size:**  
**\$ 3.1B CAGR: 0.5%**

## Target Customers/Market Opportunity



### Livestock Producers (QC on-site)

- Rapid disease detection
- Minimised production loss
- Compliance & trade benefits
- Reduced use of antibiotics
- Enhanced decision making



### Animal Feed producers (QC onsite)

- Improved brand reputation
- Enhanced Product safety
- Reduced risk of liability
- Streamlined quality assurance



### Veterinary Clinics & Hospitals

- Faster diagnosis
- Enhanced Biosecurity
- Cost efficiency
- Better disease management
- Regulatory compliance

Insurance companies (Farms with Insurance for cattle) benefit from lower claims costs as faster disease management reduces livestock losses and associated claims, helping them manage payout expenses effectively.



# IP Status

## Patents

- Sequences and Methods for the CRISPR based detection of **Omicron Variant** of SARS-CoV2 (Filed on (21/12/2022) (IN202211074360 entered **National phase** in India on Jun 28, 2024)
- Assay Device, Method Employing the same and features thereof (EP4460581, entered **National Phase in US, Europe and India**)-**SARI**
- Provisional patent: **CRISPR based system** and a method for detection assays, a point of need device with the isothermal and CRISPR assay on the **electrowetting technology** has been filed (202411001079).
- **Sickle cell Anemia** - Guide RNA, Kits comprised and Methods thereof ( Patent filed 202411084372)
- **PathCrisp platform** IP filing: *in preparation*.

## Publications

1. Sharma S., Manasa BP., Arora R., Siji A., Roy B., Sridhar V., Gupta N., Chandru V., Gupta V. *OmiCrisp: A CRISPR SARS-CoV-2 test with **Omicron detection***: Journal of Biotechnology and Biomedicine, Vol 7 (1), 2024
2. Patil S., Siji A., Mallur D., Gheewala N., Karve S., Kavathekar M., Tarai B., Naik M., Kruthika BS., Hegde V., Rangineni J., Gupta V., Chandru V., Pradeep BE., Arora R. *PathCrisp: An Innovative Molecular Diagnostic Tool for Early Detection of **NDM-Resistant Infections*** (medRxiv) - in review under Scientific Reports, 2024

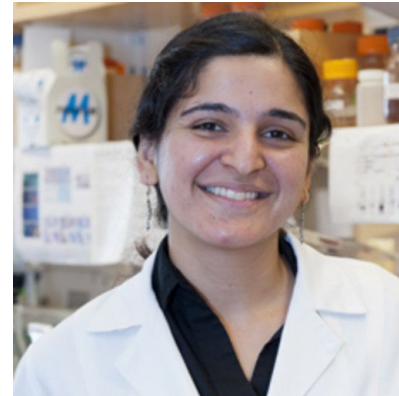
Trademarks for “*PathCrisp*”, “*OmiCrisp*” and “*EdiCrisp*” have been granted.

# CrisprBits Team

## Executive Team



**Dr. Vaijayanti Gupta**  
COO  
Healthcare & diagnostics  
Genomics & Molecular  
Genetics  
Translational research  
Product development



**Dr. Reety Arora**  
Principal Scientist  
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Gene editing  
Virology and Genetics  
Tumor Virology



**Vandana Hegde**  
VP-Business Development  
Biz Dev & Biz Ops  
Genomics  
Diagnostics  
Regulatory  
Market strategy



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Cancer, Cell Biology



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ex-TIGS



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Mouse Genetics  
Facility



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PhD.,  
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Entomology



Kanikah Mehndiratta  
M.Sc Medical Genetics  
and Genomics,  
ex-IGIB



Nancy Deep  
M. Sc of  
Science,  
Cellular and  
Molecular  
Biology,  
ex-Harvard,  
ex-Vertex

- ❖ Founding & Angel investment team: **5 distinguished Alumni of BITS Pilani**
- ❖ Scientific team (7 scientists, 1 QA, 2 Admin and 1 Informatics consultant)
  - Ph.Ds and Masters in **molecular biology** with Prestigious fellowships, **strong international academic credentials and work experience**
  - Experience in **launching clinical diagnostics** in the market
  - Prior **product development** with Pharma & biotech
  - Scientific board includes distinguished **CRISPR scientists** (Sam Sternberg), clinical experts in **infectious diseases** (Nancy Khardori) and pioneers in **gene therapy** (Roger Hajjar)



## *Founders / Board*

Founding & Angel investment team: *5 distinguished Alumni of BITS Pilani*



**Vijay Chandru**  
Entrepreneur,  
CSO, Healthcare,  
Computer  
Science  
(I.I.Sc, Strand,  
ArtPark)



**Rajeev Kohli**  
Professor  
Columbia  
University, USA  
(Marketing and  
Business)



**Sunil Arora**  
Entrepreneur,  
CEO  
(Automobile  
industry)



**Bharat  
Jobanputra**  
Retd. VP  
Operations



**Aditya Sarda**  
(Entrepreneur)



**Vijay Alreja**  
Founder & CEO,  
VJ Technologies,  
VJ Bio

## *Scientific Advisory Board*



**Sam Sternberg**  
(Faculty Columbia  
University, Ph.D  
Student of Jennifer  
Doudna)



**Dr. Nancy Khardori**  
(Medical Practitioner,  
Infectious Diseases  
VA, Norfolk hospital)



**Ajit Chande**  
(Faculty, IISER Bhopal,  
Virology and Infectious  
Diseases)



**Roger Hajjar, MD**  
Cardiology, Gene  
Therapy expert  
(Novoheart, Ring  
Therapeutics)



**Dhananjay  
Nawander** (Gene  
therapy/editing.  
Virology/ vector  
dev)

# Next Steps

- CrisprBits has developed the [platform technology](#), demonstrated [proof-of-concept](#) at lab-scale & currently in [validation stage](#).
- Next phase includes:
  - Signing manufacturing partner & beta testing
  - Regulatory approvals and commercialization

## Our Ask

- Industrial partners interested in sponsoring further [technology advancement](#) and scale up ([Engineering Multiplex format](#))
- Funded project focused on developing a QC method for food exporters/traders
- Funded project aimed at [developing a QC method](#) for incoming animal feed and feed additives.
- Industry partners interested in [paid pilot programs](#) leading to a purchase order



**For More Information Contact:**

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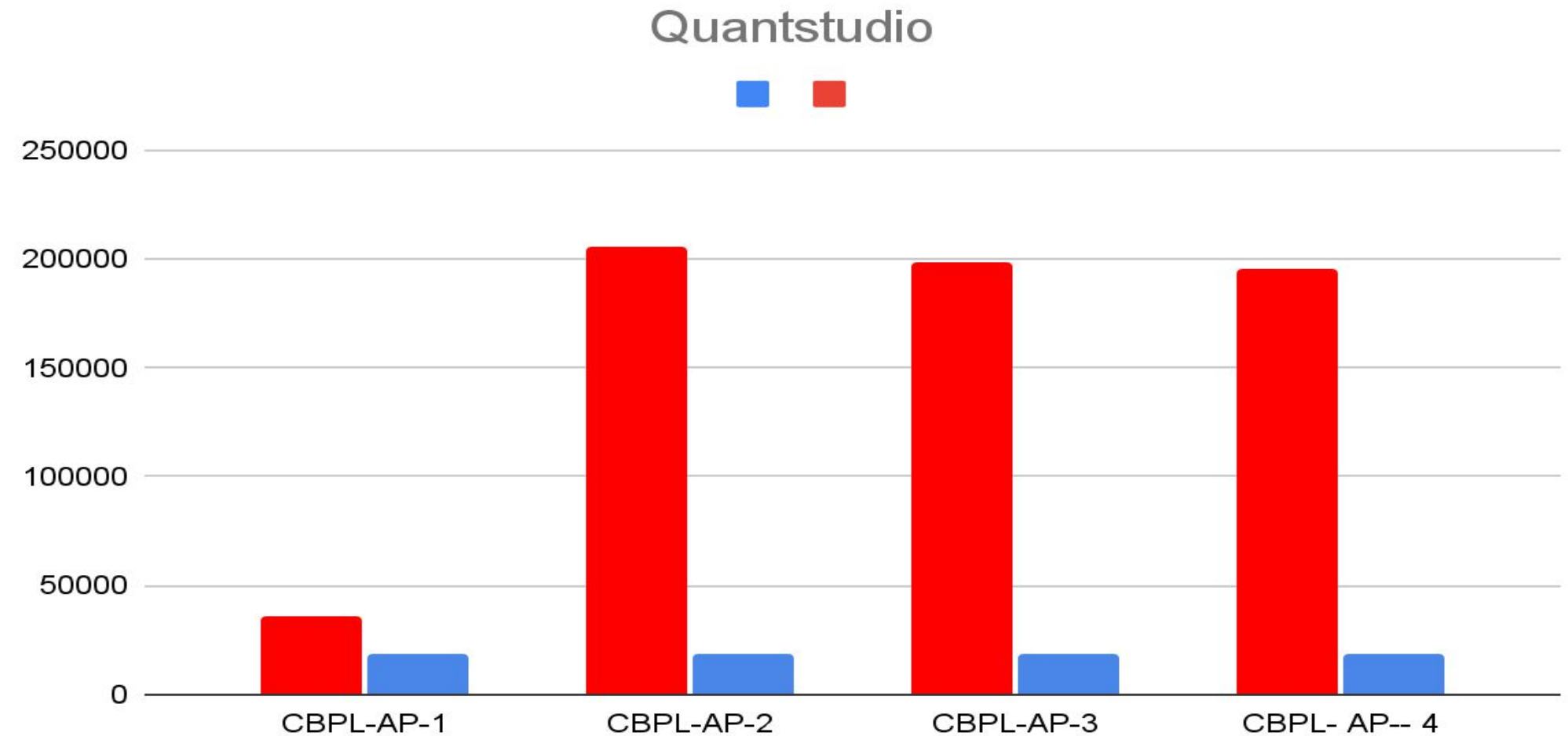
**Thank you**



# Backup Slides

# PathCrisp Assay for LSDV

| Sample Names | Source                    | Fold Change |
|--------------|---------------------------|-------------|
| CBPL-AP-1    | wound gDNA                | 1.94        |
| CBPL-AP-2    | wound gDNA                | 11.15       |
| CBPL-AP-3    | Amplicon from NGS library | 10.79       |
| CBPL-AP-4    | Amplicon from NGS library | 10.62       |

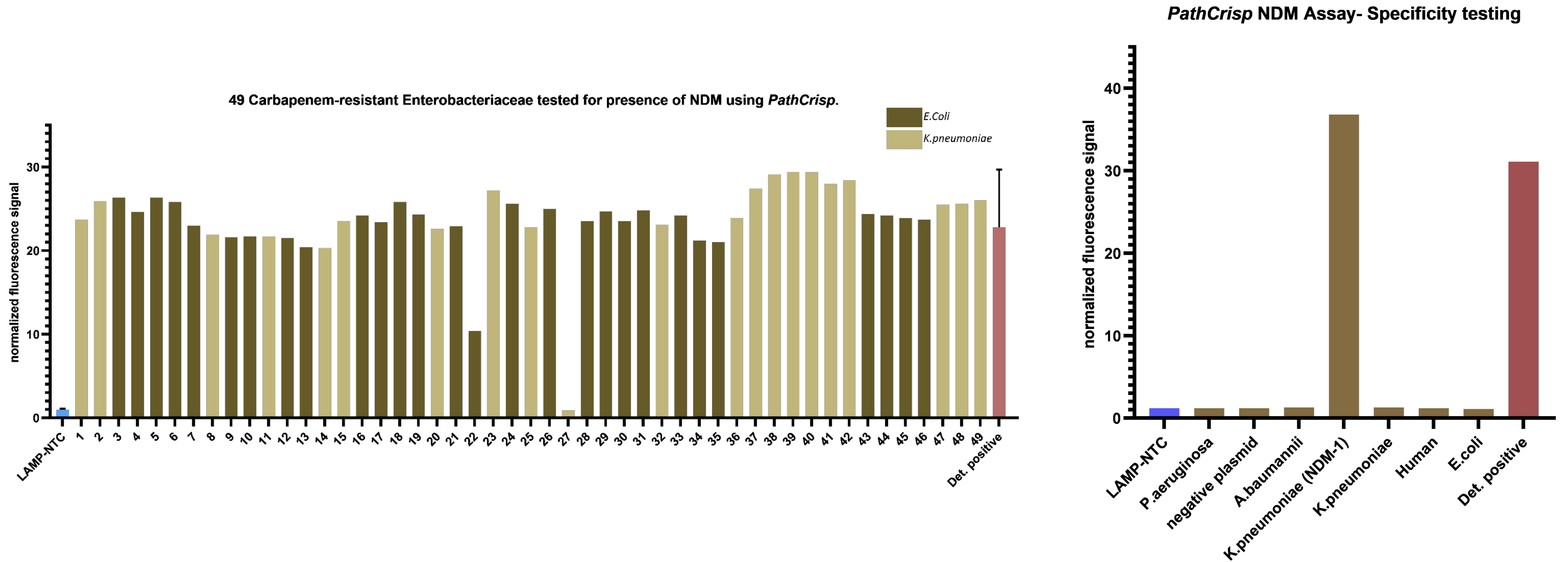


4 samples received from NCL Pune. One- pot CRISPR chemistry works on the LSDV samples (**genomic DNA** extracted from the diseased **cow wound** and **NGS amplicons**).

*Samples received from Dr. Dhanasekaran Shanmugam, NCL, Pune*



# *One pot - PathCrisp* for Carbapenem resistance (AMR) - NDM

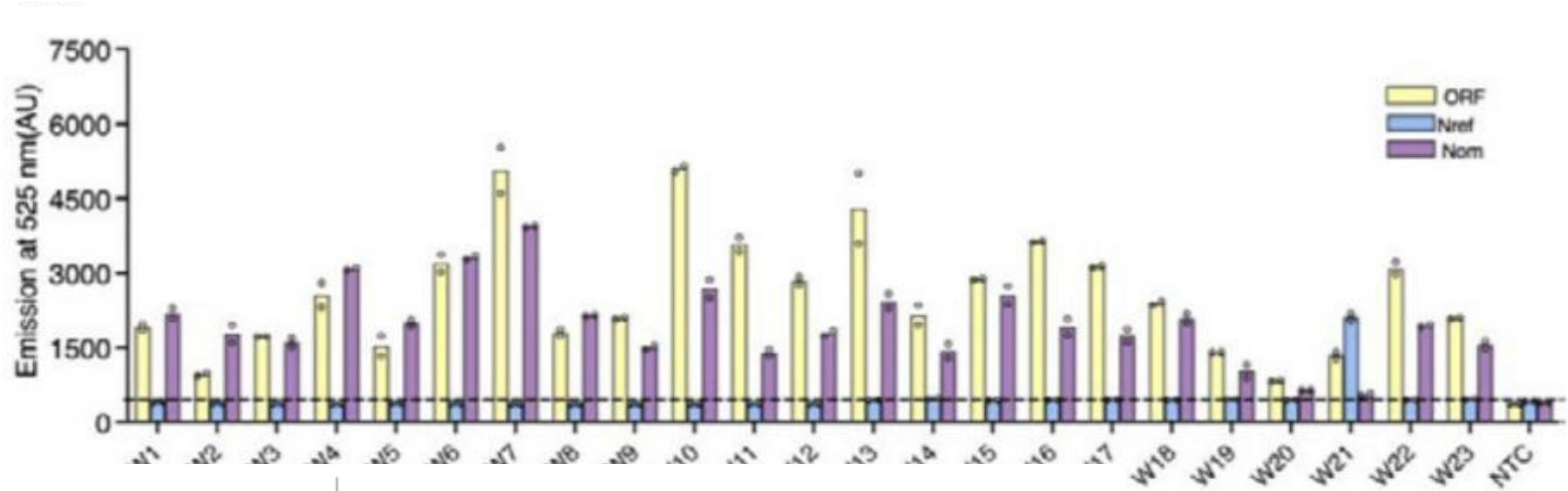


**Clinical Samples Batch 2 – 49** Carbapenem resistant Enterobacteriaceae samples ( from our collaborator at SSSIHL ) were tested using our assay and **NDM** was detected in 48 positive samples (confirmed through sequencing). Sample-27 was negative for NDM and we were able to detect as negative for NDM in our assay.

# Environmental Surveillance using our *PathCrisp* Assay for SARS CoV2 (Omicron)

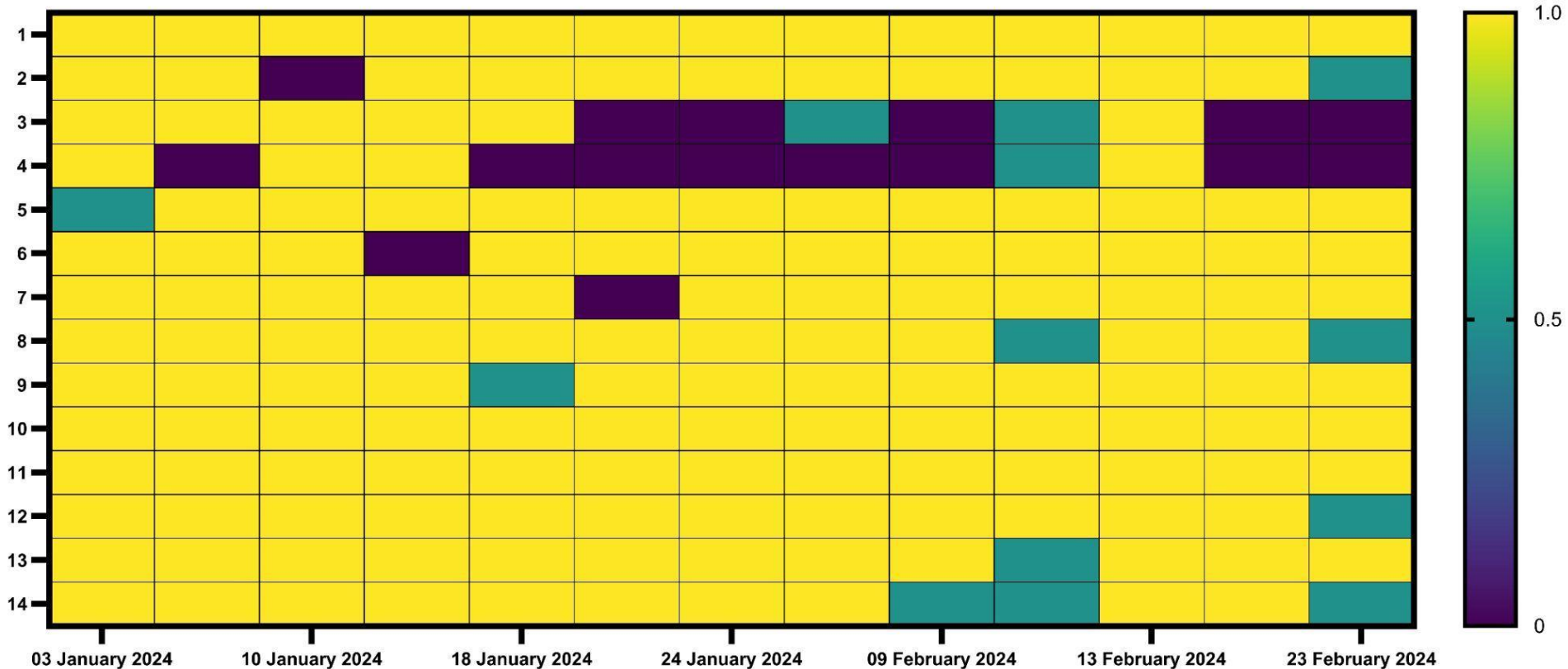
OmiCrisp is highly accurate in the detection of SARS-CoV2 in Sewage samples

| Samples     | RT_PCR kit 1 | OmiCrisp_v2 | RT-PCR kit 2 (Omicron +ve) |
|-------------|--------------|-------------|----------------------------|
| W1-W67      | Positive     | 66/67       | 61/67                      |
| W68-W96     | Negative     | 29/29       | 19/29                      |
| Sensitivity |              | 98.5        | 91                         |
| Specificity |              | 100         | 66                         |



Sharma S, et al., J Biotechnol Biomed 2024  
DOI:10.26502/jbb.2642-91280128

Our OmiCrisp Assay was used for waste water surveillance from Jan-March 2024



medRxiv THE PREPRINT SERVER FOR HEALTH SCIENCES

CSH Cold Spring Harbor Laboratory BMJ Yale

Advancing Sewage Sample Surveillance with OmiCrisp: A CRISPR-Based Platform for Variant-Specific SARS-CoV-2 Detection

BS Kruthika, Bidipta Roy, Varsha Shridhar, Vijayanti Gupta, Vijay Chandru, Reety Arora  
doi: <https://doi.org/10.1101/2024.05.28.24307854>



Supported by grant award from Cryptorelief



## *PathCrisp: Other tests in the pipeline*

|                          |                          |
|--------------------------|--------------------------|
| AMR-Ampicillin-Sulbactam | CMY                      |
| AMR-Colistin             | DHA                      |
|                          | MCR1                     |
| AMR-Fluoroquinolones     | GyrA                     |
|                          | TopoIV                   |
|                          | PMQR                     |
| Gram negative pathogens  | Klebsiella pneumonia     |
|                          | Pseudomona aeruginosa    |
|                          | Acinetobacter baumanii   |
|                          | E coli                   |
| VanA/B                   | Vancomycin               |
| Gram positive pathogens  | Enterococcus faecalis    |
|                          | Enterococcus faecum      |
|                          | Streptococcus pneumoniae |
|                          | Staphylococcus aureus    |
| Gastro Viruses           | Rotavirus                |
|                          | Norovirus                |
| Animal Feed pathogens    | Salmonella               |
|                          | Shigella                 |
|                          | Campylobacter            |
|                          | Listeria                 |
|                          | E coli                   |

- Several other tests including the **Animal Feed pathogens** are being built as tests in the laboratory.
- Our platform is ready and to build a new test taking about 2 weeks -1 month.