REPORT

Report on the participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. **Name and designation of ICMR-IF**: Dr G. K. Sivaraman, Principal Scientist.
2. **Address**: Microbiology, Fermentation & Biotechnology Division, ICAR- CIFT, Willingdon Island, Matsyapuri Post, COCHIN-29, Kerala.
3. **Frontline area of research in which training / research was carried out:** Micro Paper-Based Analytical System (µPAS) devices using AutoCAD for the rapid detection of Antimicrobial Use (AMU) and rapid diagnostic of Antimicrobial Resistance Bacteria (ARB) in aquaculture and marine fish samples.
4. **Name and address of Professor and host institute**: Dr (Prof). Xunli Zhang, Head & Professor of Bioengineering and Microsystems, Director of Chemical Engineering, University of Southampton, UK.
5. Duration of fellowship with exact date: 03 months (Three); 10th Feb to 10th May 2023
6. Highlights of work conducted

i). Technique/ expertise acquired:

1. Designing of paper-based sensors for AMR pathogen detection: In this training programme, designed multiplex paper-based devices for the simultaneous detection of AMR pathogen Methicillin Resistant *Staphylococcus aureus* MRSA (identification of Staphylococcus genus by Baird Parker Agar, *S. aureus* by Mannitol Salt Agar, MRSA by Oxacillin Resistance Screening agar) and with multiplexed antibiotic-resistance testing (methicillin-resistant by oxacillin @ 6µg/ml) as shown in the following figures 1a- 1e.

Materials required: The fluidic devices were fabricated within cellulose-based filter papers (CF1) from GE Healthcare, USA, cover tape to seal the device (Kenosha, Netherlands), transparency film (University of Southampton Office Depot, UK), photopolymer used for creating the boundary walls on transparency film and within cellulose-based filter paper (DeSolite® 3471-3-14 from DSM Desotech, Germany), which is an acrylate-based photopolymer with a viscosity of 10,000 mPa s at 25°C.

Fabrication of paper-based sensor device: A photopolymer was first locally deposited onto the substrate (cellulose filter) with a deposition nozzle at locations pre-defined by the device design. A laser beam that follows the deposition head subsequently illuminated the deposited polymer pattern inducing photo-polymerisation. These laser-cured patterns then define the solid walls of the fluidic structures that confine and transport liquid flows. The laser used for this LDW process was a 405 nm continuous wave (c.w.) diode laser (MLDTM 405 nm, Cobolt AB, Sweden) with a maximum output power of 110 mW). The dispenser platform used for the local deposition of the photopolymer onto the various substrates was a PICO® Pµlse™ dispensing system from Nordson EFD, UK. The bottom layer is built of a transparency film with an LDW patterned surface relief frame that contains a chromogenic agar that enables the permissive growth and therefore identification of the desired bacterial pathogen. A middle layer, based on a cellulose-based filter paper for the introduction of the sample. Once introduced via cellulose-based filter paper, the sample is uniformly distributed into the device via the capillary action. After the patterning, all components were sterilized by autoclaving at 121°C for 25 min. 1 mL of liquid agar with antibiotic(s) was pipetted into the circle/square of the bottom layer and this volume is just enough to cover the whole area. The bottom layer is then left at room temperature for 10 min for the agar to solidify. The sample was dropped onto a filter paper (20ul) by soaking into the sample with sterile forceps of place on the wells, layer the sample, and then cover with tape to seal the device from the top to stop evaporation and avoid contamination and then incubated at 35+2$_o$C.

More devices were produced on a sheet for the screening of samples and are sent to the MFB Lab of ICAR- CIFT, Cochin by post through Royal Mail for validation. Different capacities (size in diameter) and the number of cells were designed to simultaneously detect different types of AMR pathogens ranging from 2-10 and antibiotic resistance levels (2-10 with required antibiotic concentrations) viz., ESBL-producing Enterobacterales (*E. coli, K. pneumoniae, P. aeruginosa, A. baumannii*) and vancomycin resistant enterococci.

Figure 1a: Multiplex paper-based device for the detection of Methicillin Resistant *Staphylococcus aureus* MRSA. The 1,2,3 & 4 are the sample loading Whatman filter paper (left) and multichannel device for the detection of MRSA (Baird Parker Agar, Mannitol Salt Agar, Oxacillin Resistance Screening agar & 6ug/ml oxacillin on Mueller Hinton agar)

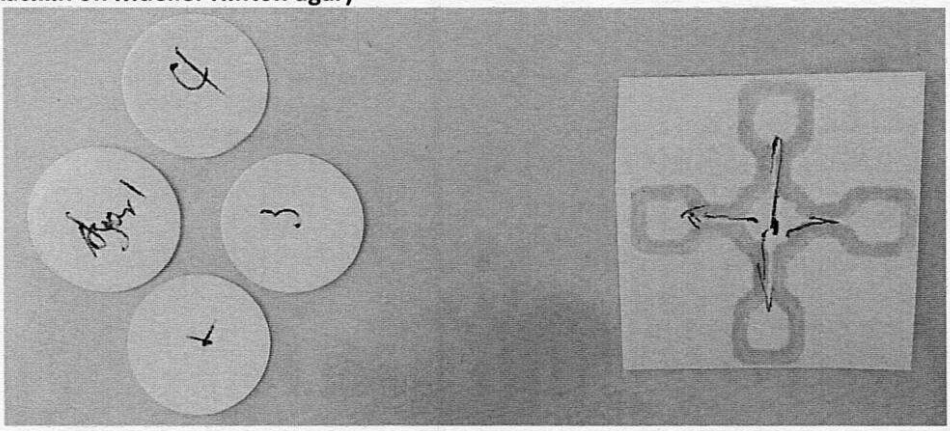


Figure 1b: Printed more devices on the transparency films (8 numbers) for the detection of more AMR pathogens and Antimicrobial Susceptibility Test (AST)

Figure 1a:

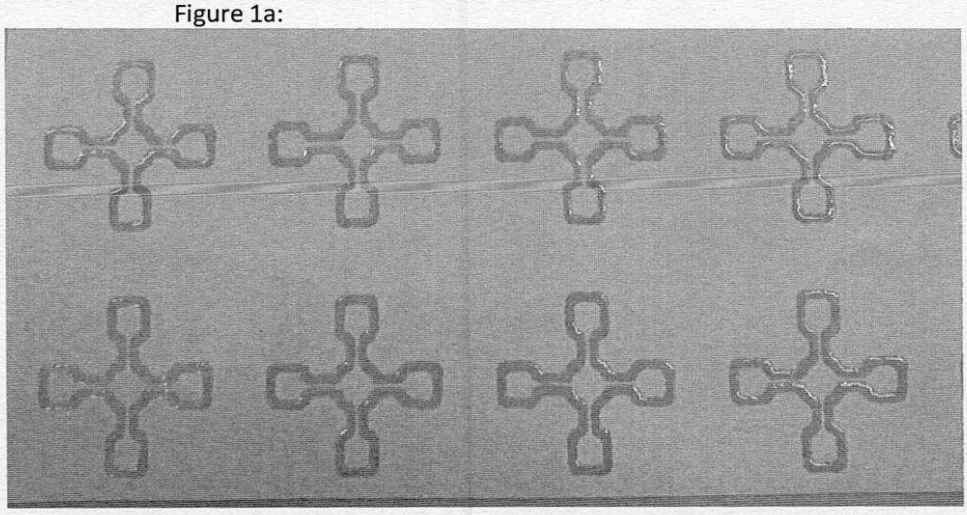
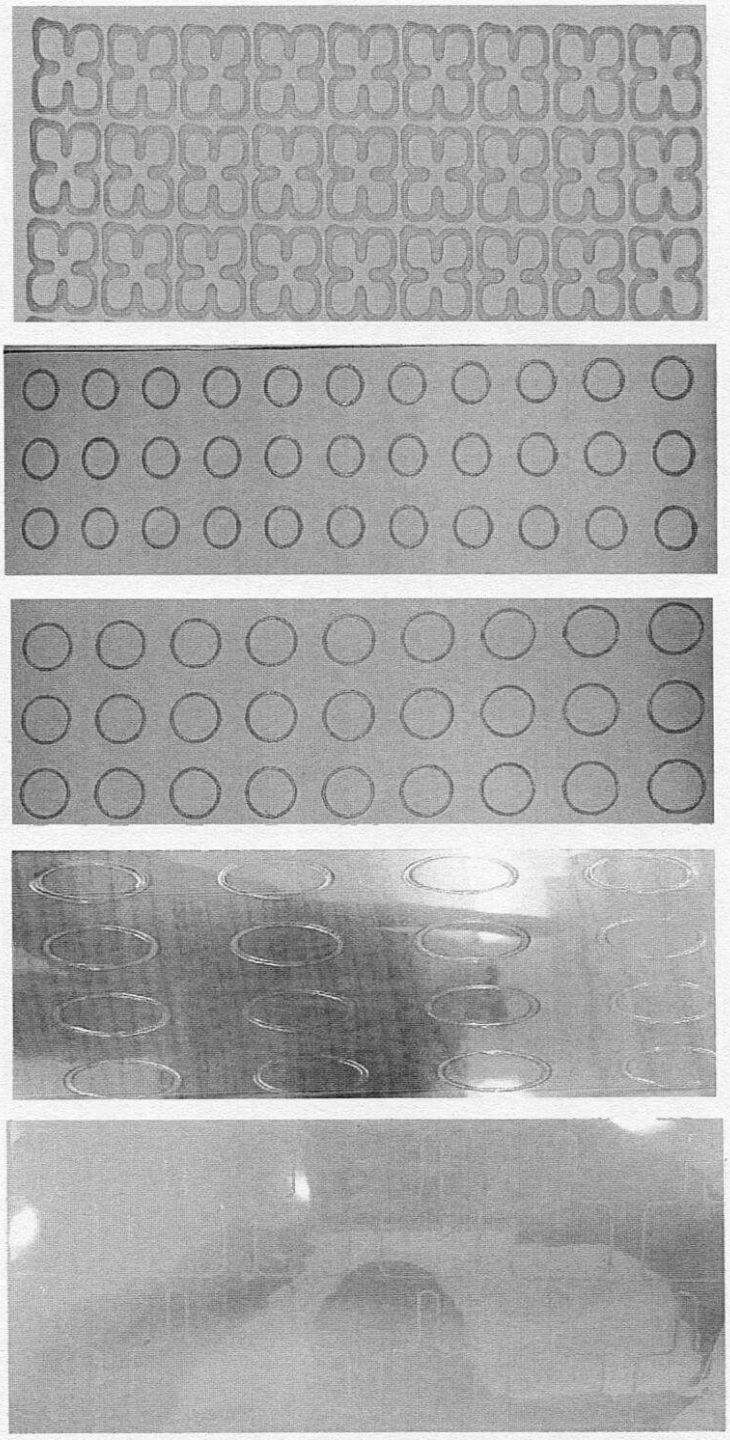
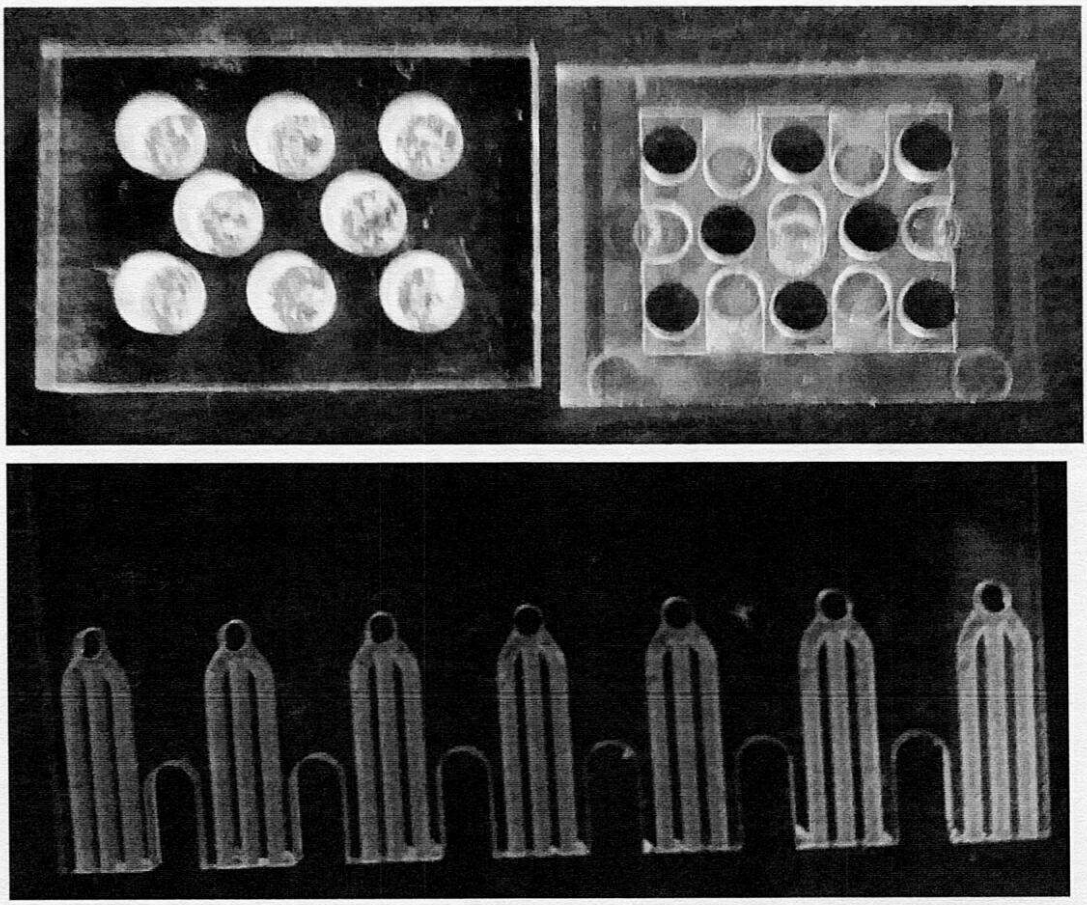


Figure 1 c: Different patterns and sizes of the paper-based sensor devices were printed for the detection of AMR pathogens and AST



2. Micro Paper-Based Analytical System (μ – PAS) device using AutoCAD: Currently, numerous screening assays (immunoassays, Liquid Chromatography with tandem mass spectrometry LC-MS-MS, PCR based

and Biochip) are commercially available for many drugs and generally take long reaction time. fewer numbers of drug detection, expensive instruments, and time-consuming. So, there is a growing need to introduce assays for the screening of many antibiotic residues at a time. We designed the Micro Paper-Based Analytical System (μ – PAS) device using AutoCAD and it consists of 2mm dia micro-milled pillars on a Poly methyl methacrylate (PMMA) surface and sample containers on a microchip. The Whatman Grade 3 filter paper disc (2mm dia) was selected and was fixed on the pillars. This device is suitable for the identification of AMR pathogens with selective & differential media and with chromogenic media (as shown in the following figures 2a & 2b.



Optimization of the developed μ – PAS device to detect antibiotic residues: We had developed a multiplexed μ – PAS device for the simultaneous detection of antibiotic residues. The multi-channel was designed for the most important antibiotic residues (3-4 channel) in a μ – PAS device viz., 1. tetracycline, oxytetracycline, and chlortetracycline; 2. sulphonamides, sulfamethoxazole, and trimethoprim, 3. chloramphenicol, thiamphenicol and florfenicol; 4. Beta lactam antibiotics (cefotaxime, ceftazidime,

cefpodoxime), and 5. Quinolones (ciprofloxacin, levofloxacin and ofloxacin), This LoC technology has several advantages such as less turnaround time (TAT), accuracy, high throughput analysis; minimal resource (sample volume, reagents, and electricity), low cost, user- friendly, field level deployable, and portable as compared to the conventional, molecular, automated systems and omics-based techniques. This device can be used for the detection of ß-lactam group of antibiotics residues such as penicillin's, cephalosporins (2, 3, 4 & 5^{th} generations), carbapenems, and monobactams by the induction of ß-lactamase in germinating spores in food/ fish/clinical samples in comparison with spiked samples:

3. Designing of Lateral Flow Assay for the detection of Antibiotic residues:

Lateral-flow devices (LFDs) are one of the simplest and most established formats of paper-based devices that allow the rapid detection of an analyte through the testing of complex samples (shrimp muscle extract, water, sediment, and fish feed). We optimized a local liquid-deposition-assisted laser direct-write process for structuring a range of porous materials such as different types of cellulose papers, glass fibre filters and nitrocellulose membranes. Then assembled an LFD by combining several specific patterned porous materials. The local deposition of bio-reagents (monoclonal antibodies and polyclonal against the chloramphenicol) onto the fabricated LFDs for the s detection of chloramphenicol antibiotic residues. We explored a range of different conductive photopolymers to find the optimal laser-writing parameters desired for producing conductive electrodes in a range of porous materials such as different types of papers and fabrics. The laser patterning of electrically conductive photopolymers followed by their electrical characterization. We optimized the simple electrochemical prototype biosensor that uses laser-patterned electrodes to detect the spiked antibiotic residues (shown in Figure 3). Different types of LFDs were designed to optimize the flow and binding of the analyte with antibodies and added gold nanoparticles with the conjugate. Designed 2 and 3 channels of an LFD for the simultaneous detection of 2 & no. of antibiotic residues and is shown in Figure 4. The LFD is stored in a bag with dry silica and also impregnated into the plastic mound for the ready-to-use. The designed Lateral Flow Immuno Assay (LFIA) for the simultaneous detection of 2-3 antibiotic residues in shrimp samples is based on the interaction of antigen and antibody and has many advantages simple to use, no specific expertise is to be required, highly sensitive information system, stable, specific, economical, and time-saving and scalable to high-volume production, stable-shelf-lives of 12–24 months often without refrigeration.

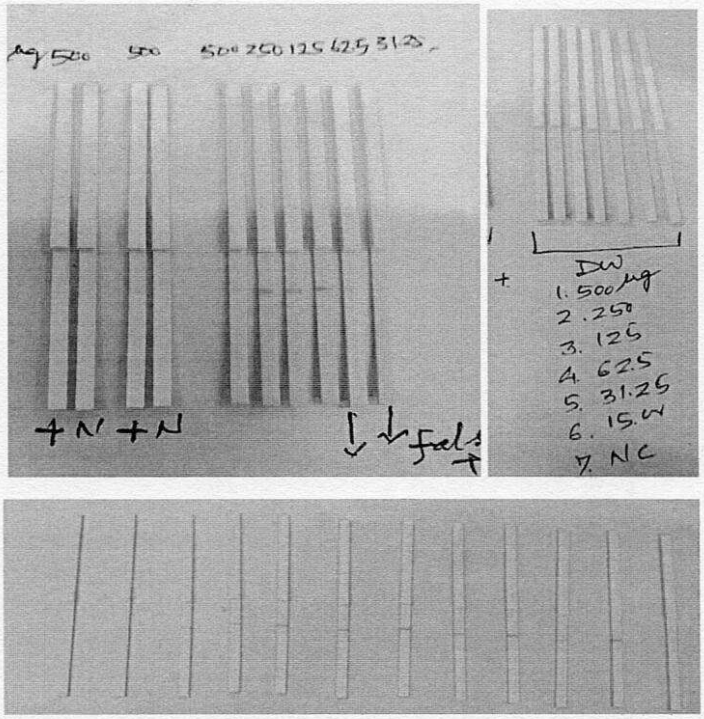


Figure 3 : Optimization of LFD for the detection of chloramphenicol antibiotic from 500 to 15 µg/ml of water

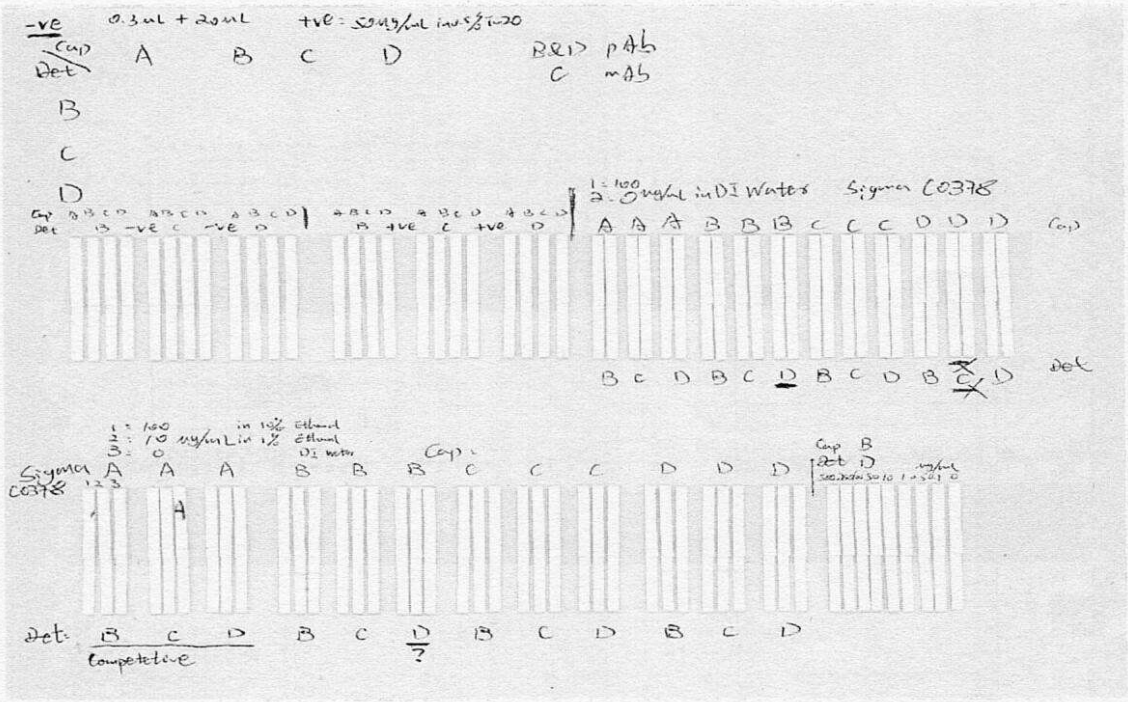
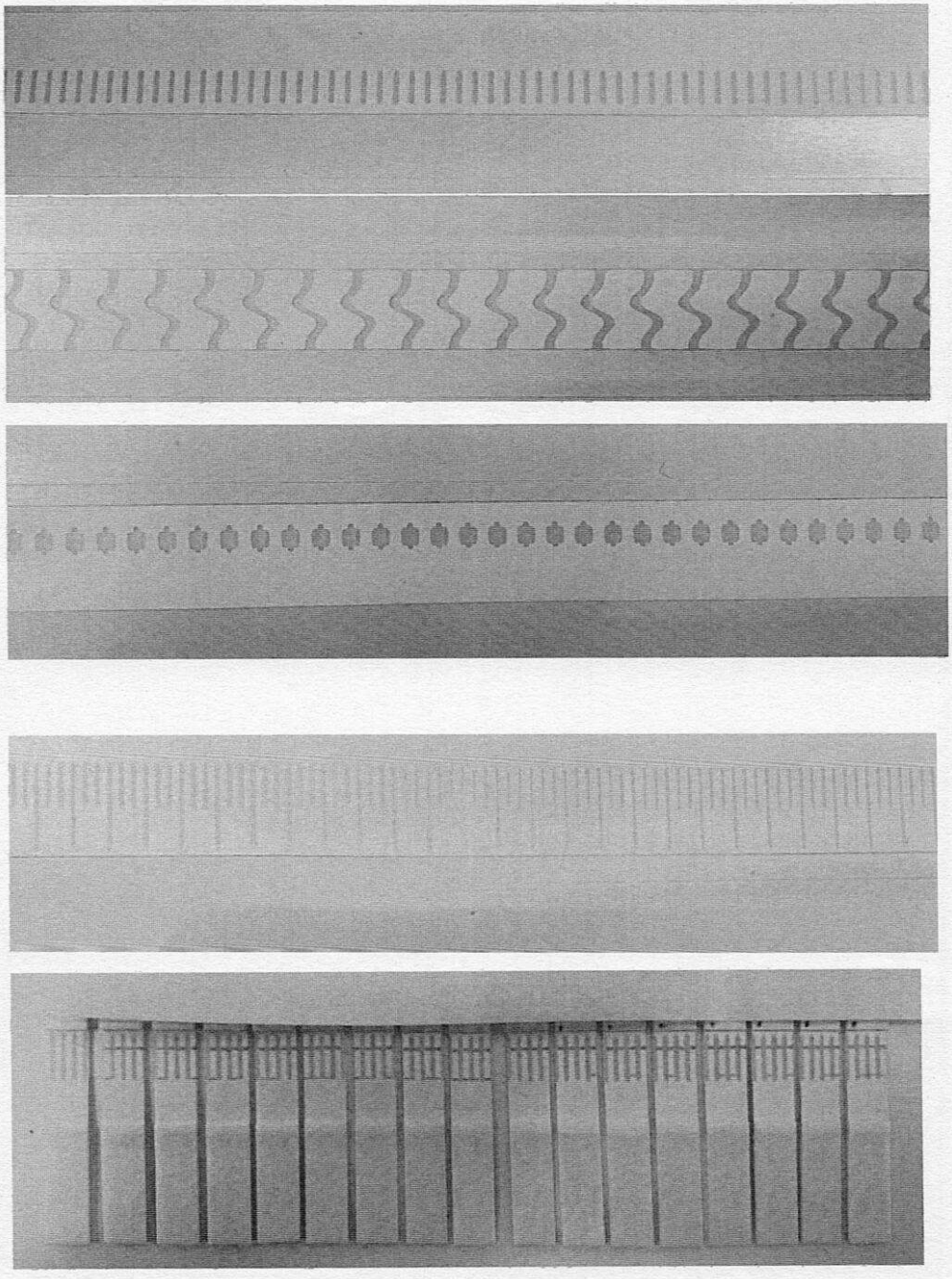
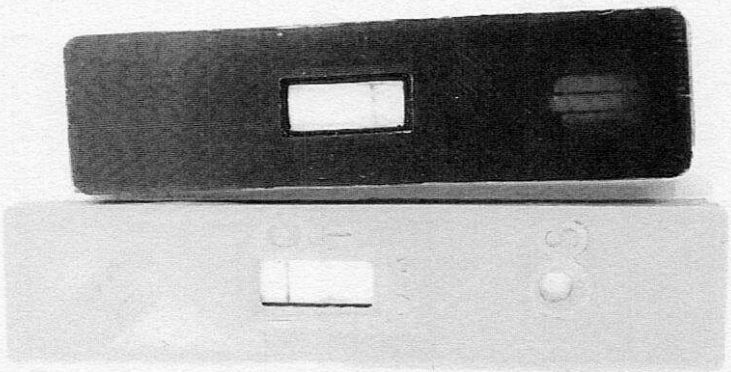


Figure 4: Designed different patterns and types (single channel, multichannel) of LFD for the detection of antibiotic residues.





ii). Research results, including any papers, prepared/ submitted for publication: The designed devices are prepared more in number for the field/ lab test evaluation in India.

iii). Proposed utilization of the experience in India: Based on this training, a project proposal under the UK Research and Innovation (UKRI) can be submitted in collaboration with the University of Southampton, UK. A project proposal submitted a proposal to the ICMR "Call for Investigator-Initiated Research Proposal for Small Extramural Grants (No.:BMI/ePMS/121273 dt: 01/03/2023) by collaborating with Govt. Medical College, Alappuzha and Wayanad Medical College, ICMR-NIV, Veterinary College, Kerala Fisheries College and ICAR-CPCRI in Kerala.

ICMR Sanction No. INDO/FRC/452/S-33/2022-23-IHD Dated 31st January 2023

10.5.2023

Signature of ICMR-IF

(G.K. SIVARAMAN)