

Automated Spotting of Multiplex Lateral Flow Assays for Blood Group Genotyping Using the BIOSPOT® DX Platform

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Introduction

Lateral Flow Assays (LFAs) enable rapid, visual diagnostics at the point of care. For blood group typing, they provide a faster and simpler alternative to traditional serological and molecular methods, which are often time-consuming and require specialized equipment.

This Application Note presents the development of a dry-reagent, allele-specific multiplex LFA designed for visual genotyping of seven blood group alleles from the Kidd, Duffy, and MNS systems.

The assay combines Linear-After-The-Exponential (LATE)-PCR amplification with hybridization on a nitrocellulose membrane, producing clear red-dot results without requiring DNA extraction or expert interpretation. To ensure precision and reproducibility across 50 miniaturized 2×5 test matrices, capture and control probes were spotted at 50 nL volumes using the HAMILTON BIOSPOT® DX platform.

This automated, non-contact process reduces manual variability and enables walk-away manufacturing of up to 50 test strips per run. Validation on five blood donor samples (sample collection no. DC-2016-2689, approved by the French Research Ethics Committee SOOM1 CPP1-19-097SI6030) showed 100% concordance with existing genotype data.

Overall, BIOSPOT DX provides a scalable and precise solution for developing multiplexed, low-cost genotyping assays suitable for personalized diagnostics.



Figure 1: BIOSPOT DX in standard configuration with a flat deck for flexible substrate placement. The open, modular design supports customizable holders for a wide range of assay formats.

Key Highlights

- Reproducible spotting of 50 nL droplets on nitrocellulose membranes
- Multi-channel precision dispensing of oligonucleotides and proteins
- Compatible with multiplex genotyping workflows using LATE-PCR
- 100% concordance with expected blood group SNP results

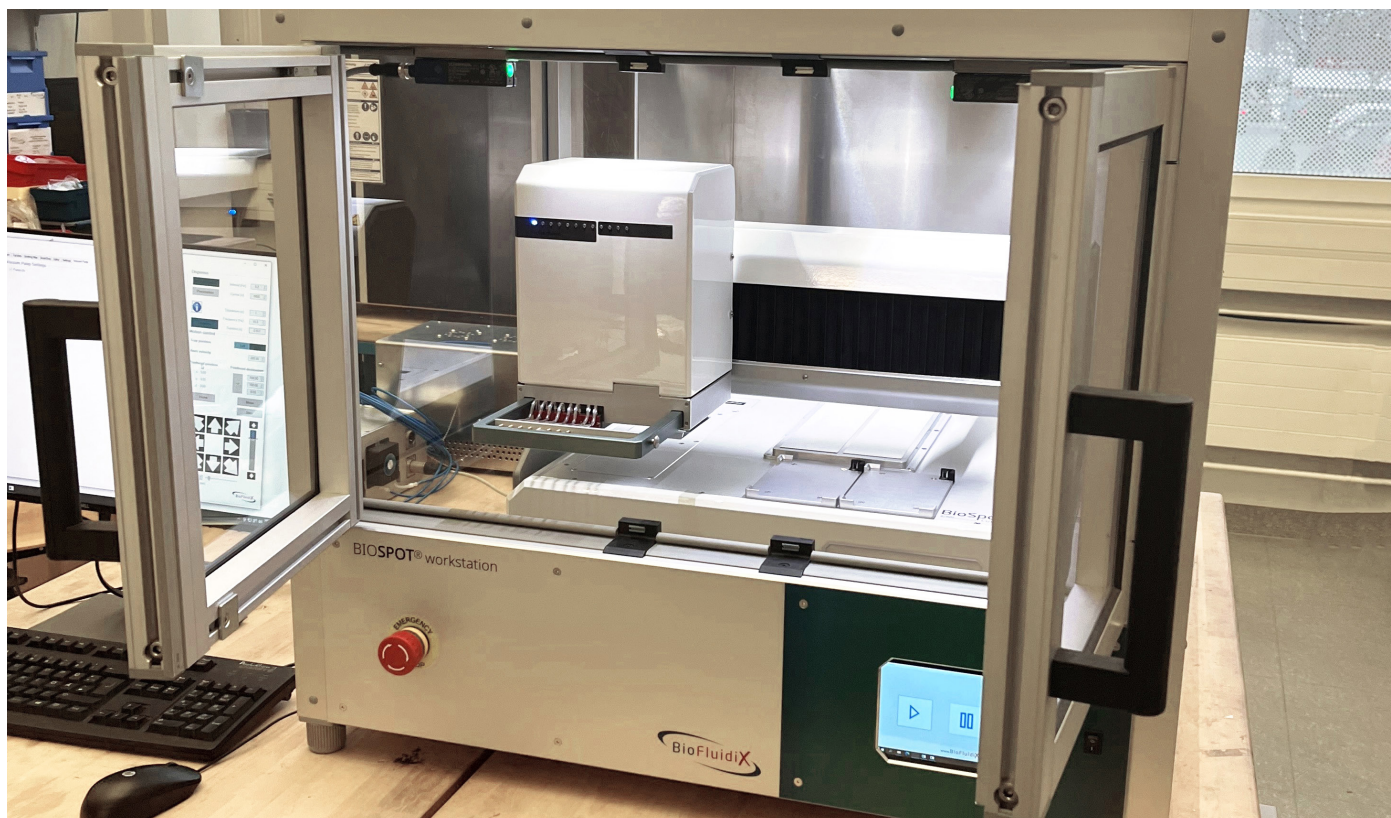


Figure 2: BIOSPOT DX configured for LFA membrane production. The system features a vacuum plate to securely hold nitrocellulose membranes during dispensing, ensuring consistent spot placement across multiple arrays. The enclosed housing provides operator safety and environmental protection, supporting clean and reproducible spotting of nucleic acid and protein reagents in walk-away mode.

Materials & Methods

Reagent and Components

- **Oligonucleotide probes:** Eurogentec, Belgium
- **Biotinylated Bovine Serum Albumin (BSA):** Thermo Fisher Scientific, USA
- **Spotting buffer:** 6X saline-sodium citrate (SSC) buffer (Thermo Fisher Scientific, USA), 2% methanol, 2% sucrose (Sigma-Aldrich)
- **Running buffer:** 1X SSC, 10% Tween-20 (Sigma-Aldrich), 0.5% sodium dodecyl sulfate (SDS), and 1M urea (Sigma-Aldrich)
- **Anti-biotin antibodies conjugated gold nanoparticles:** (BBI solutions, UK)
- **Dry reagent dipsticks:** Comprised of a wicking pad, glass-fiber conjugate pad, nitrocellulose membrane (Hi-Flow™ Plus HF135), absorbent pad, and adhesive backing card (Merck Millipore, Germany)
- **PIPEJET disposable pipes:** Dispensing pipe 500-L coated(part number 10187071)
- After probe deposition, membranes were dried in an incubator to stabilize spotted reagents.

Dispensing Conditions

- **Substrate:** 6.5 × 25 cm nitrocellulose membrane, held flat on BIOSPOT DX using a vacuum plate)
- **Spotting pattern:** 2×5 array per strip. 8x PIPEJET in parallel.
- **Volume:** 50 nL per droplet (auto-calibrated with SMARTDROP®) vs. 100 nL using manual pipette
- **Drying:** Membranes incubated post-spotting
- **Pipe washing:** Performed 3× between probe and BSA control depositions

PCR & Hybridization Protocol

- **Template:** PCR performed directly on whole blood samples
- **Amplification:** LATE-PCR generating biotinylated single-stranded DNA
- **Hybridization:** Conducted with 200 µL running buffer at 57 °C
- **Detection:** Red-dot visualization via anti-biotin gold nanoparticles

¹See reference at the end of manuscript.

Automated Dispensing Workflow

Using BIOSPOT DX, fifty LFA matrices were generated with high spatial and volumetric precision. Each matrix contained eight capture probes and one control probe dispensed using multi-channel, non-contact technology.

This approach minimized assay variability, improved reproducibility, and reduced manual labor. The integrated vacuum plate ensured consistent membrane positioning, while enclosed operation supported clean and contamination-free spotting.

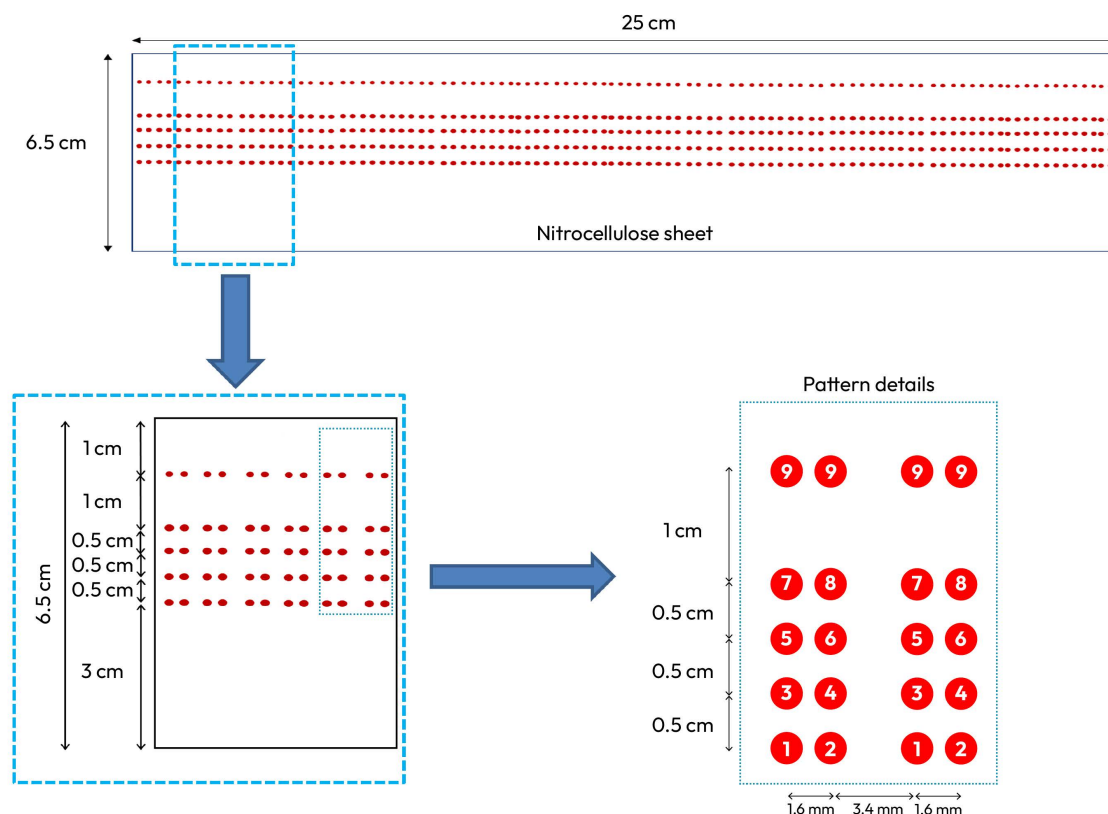
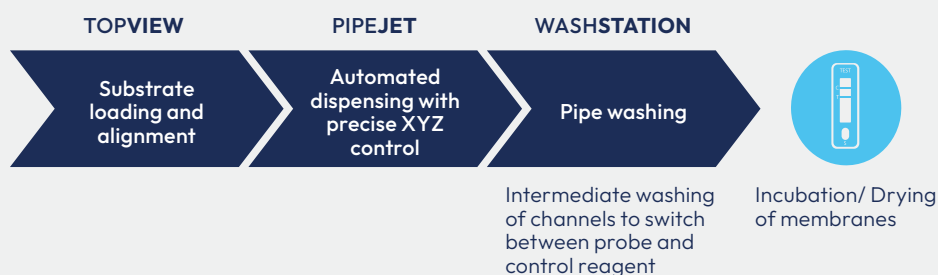


Figure 3: Spotting pattern for LFA test strips. Each 2x5 array comprises 10 droplets (8 oligos, [1-8] and 2 of the BSA control [9]), dispensed at 50 nL using BIOSPOT DX.

Visual Workflow



Results & Discussion

Five donor samples were analyzed using the multiplexed LFA strips. SNPs corresponding to seven blood group alleles (*FY*01*, *FY*02*, *FY*02N.01*, *GYPB*03*, *GYPB*04*, *JK*01*, *JK*02*) were successfully genotyped.

Hybridization between LATE-PCR amplicons and capture probes produced clear red-dot signals across all samples, confirming correct allele identification. The resulting test strips demonstrated strong signal intensity, high repeatability, and complete concordance with reference genotyping data.

Figures 4 and 5 illustrate the assay workflow and representative test strip results, showing robust and specific hybridization patterns across multiple genotypes.

These findings confirm that BIOSPOT DX enables reproducible and scalable production of multiplexed lateral flow membranes with nanoliter precision, a key factor for consistent SNP detection in genotyping assays.

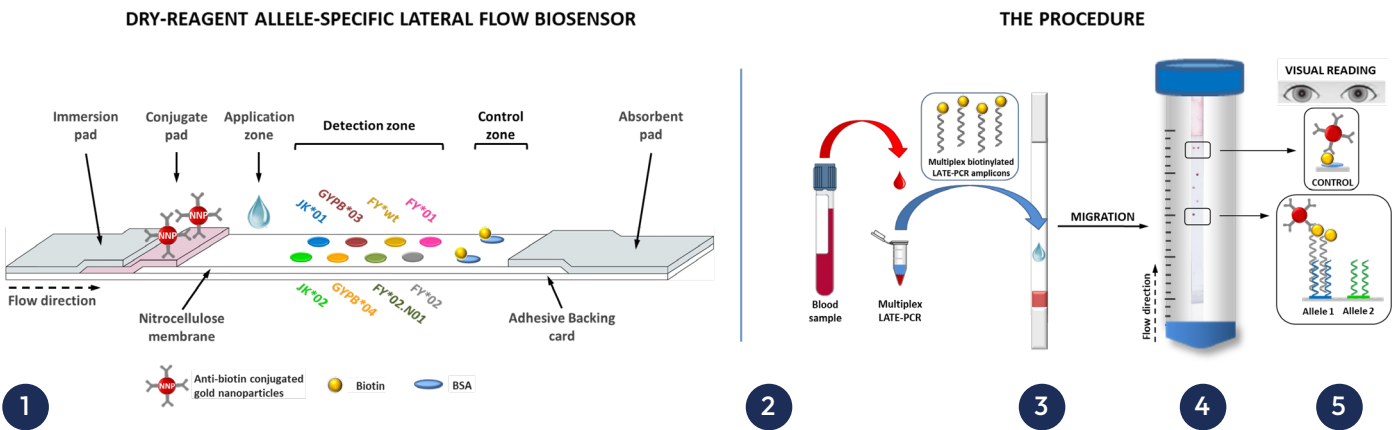


Figure 4: Workflow schematic for LFA genotyping assay: (1) Schematic view of the lateral flow strip, (2) DNA amplification by LATE-PCR, (3) Application on dipstick, (4) Hybridization with capture probes, and (5) Red-dot visual detection.

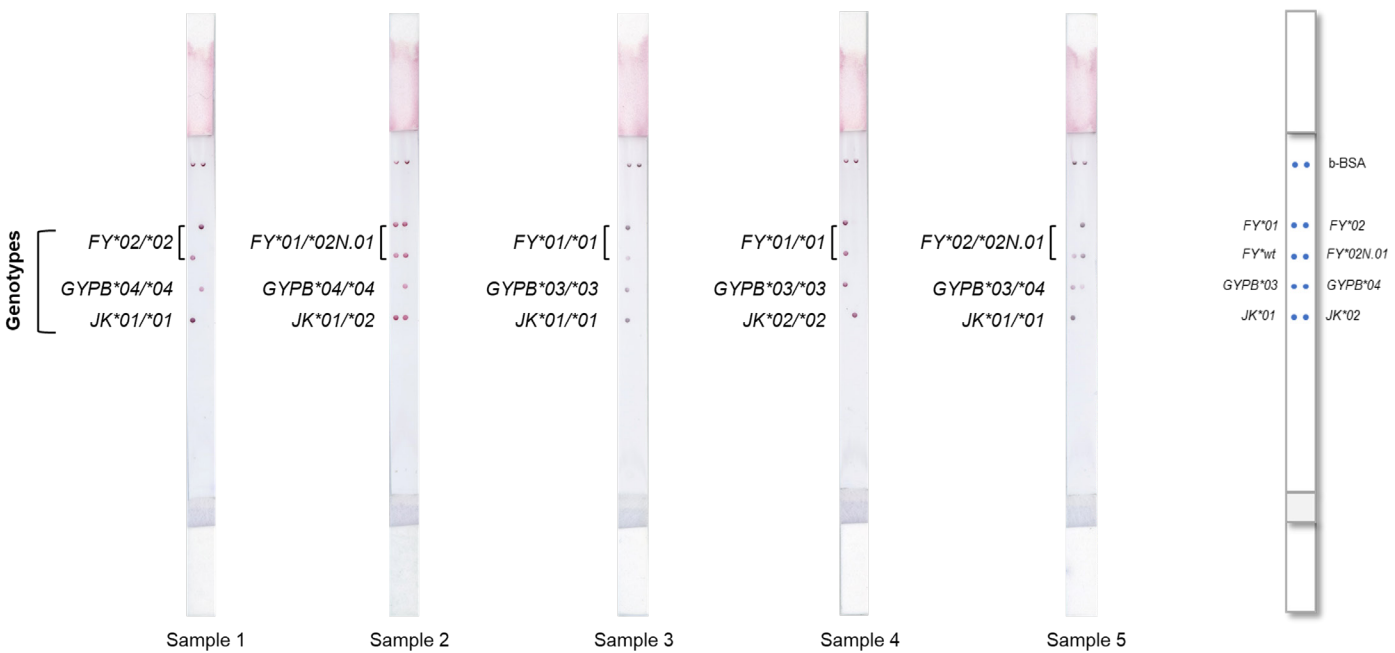


Figure 5: Example test strips showing specific hybridization signals across multiple genotypes. All 5 samples matched expected profiles with 100% concordance.

Conclusion

- **BIOSPOT® DX enables consistent multiplex spotting** of nucleic acid probes on nitrocellulose for LFA applications.
- **The multi-channel PIPEJET® system reduces** hands-on time and improves throughput during LFA strip production.
- Low-volume, non-contact dispensing minimizes reagent waste and prevents contamination.
- The platform is ideally suited for prototyping and scaling point-of-care genotyping assays, such as blood group SNP detection.
- Compatible with both nucleic acid and protein reagents, BIOSPOT® DX offers a versatile, reliable foundation for advanced LFA development.

Reference

Gomez-Martinez J, M. Silvy, J. Chiaroni, C. Fournier-Wirth, F. Roubinet, P. Bailly, J.C. Brès., Multiplex lateral flow assay for rapid visual blood group genotyping. Analytical Chemistry, 2018 May, 90(12):7502-7509.

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