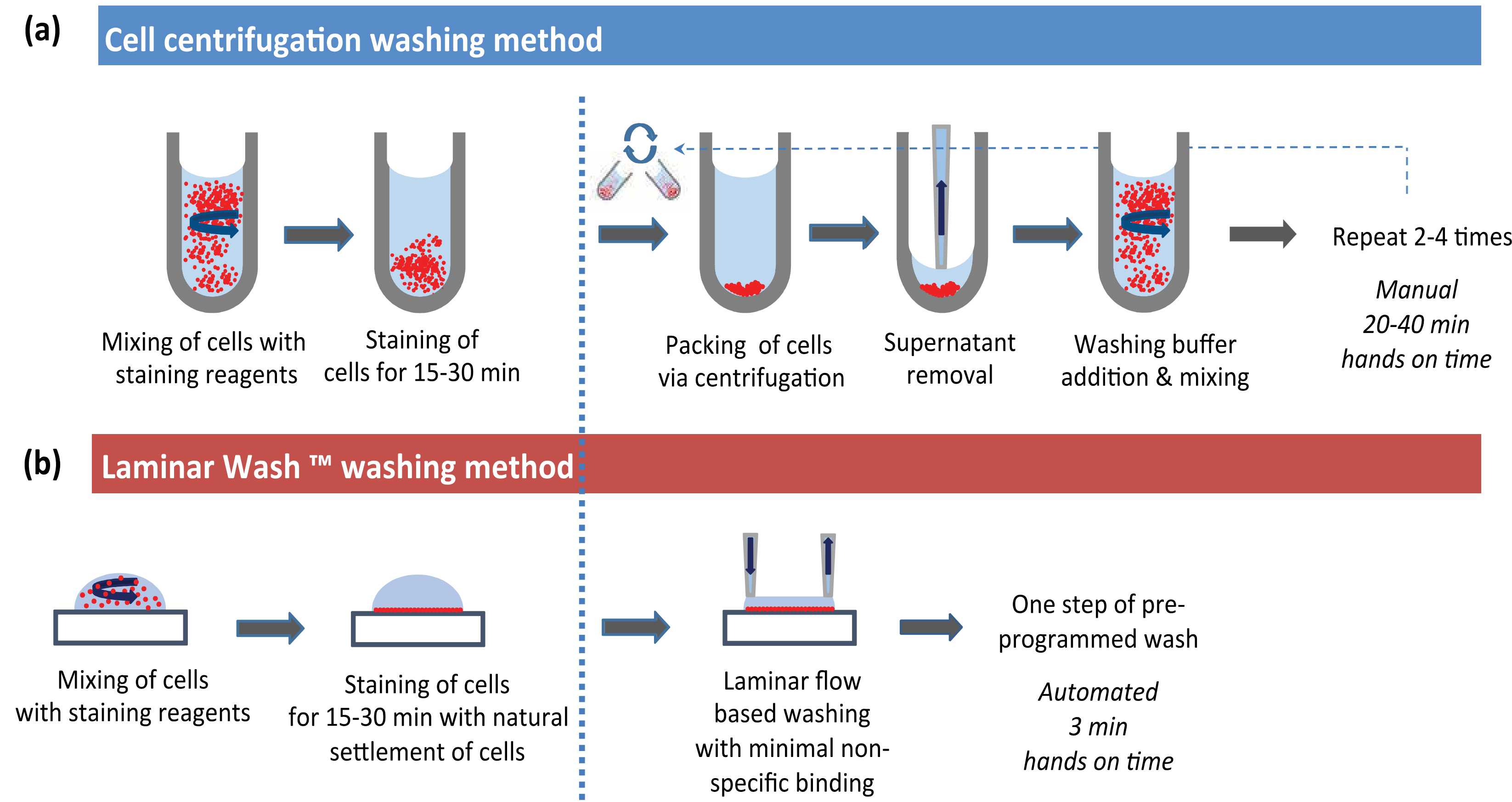


# Centrifuge-less Red Blood Cell Lysis and Immunostaining Whole Blood for Flow Cytometry using Laminar Wash™ Technology

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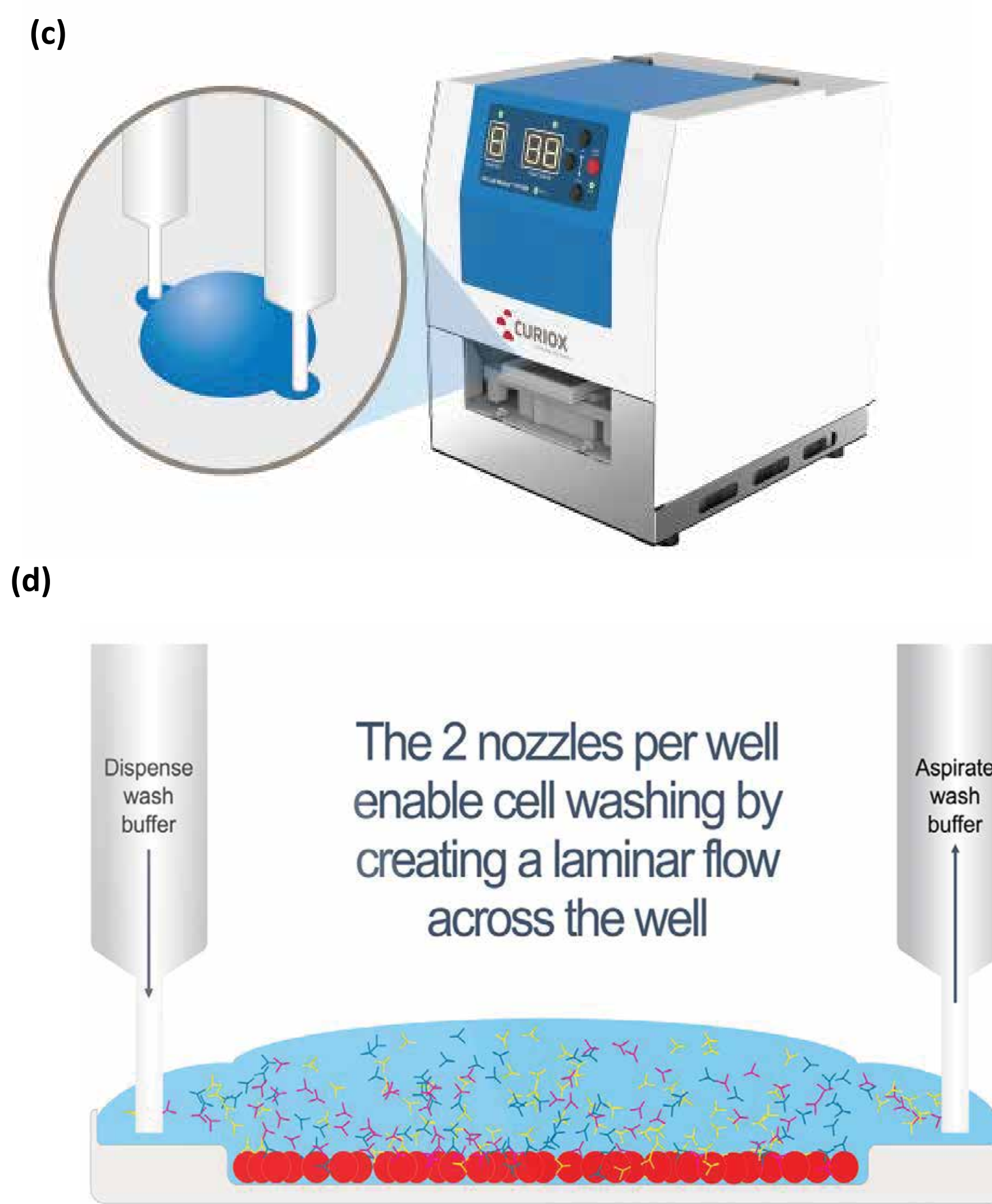
## CURIOX'S LAMINAR WASH TECHNOLOGY



**Fig 1** Schematic showing comparison of cell staining and washing by conventional centrifuge-based method (a) or Laminar Wash™ method (b).

**Fig 1(c)** Laminar Wash™ LW washer with dispensing and aspirating nozzle in each of 96-well illustrated.

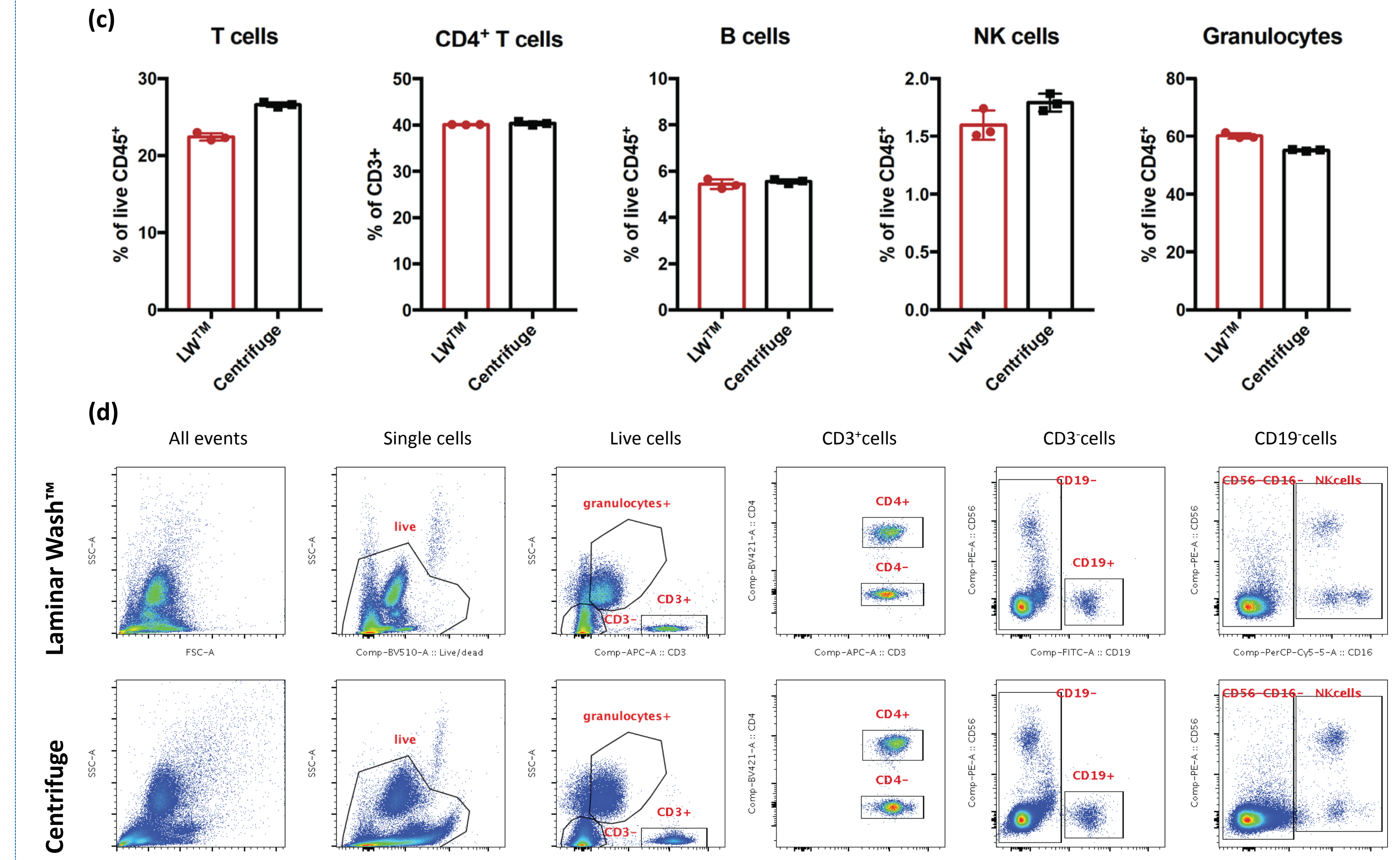
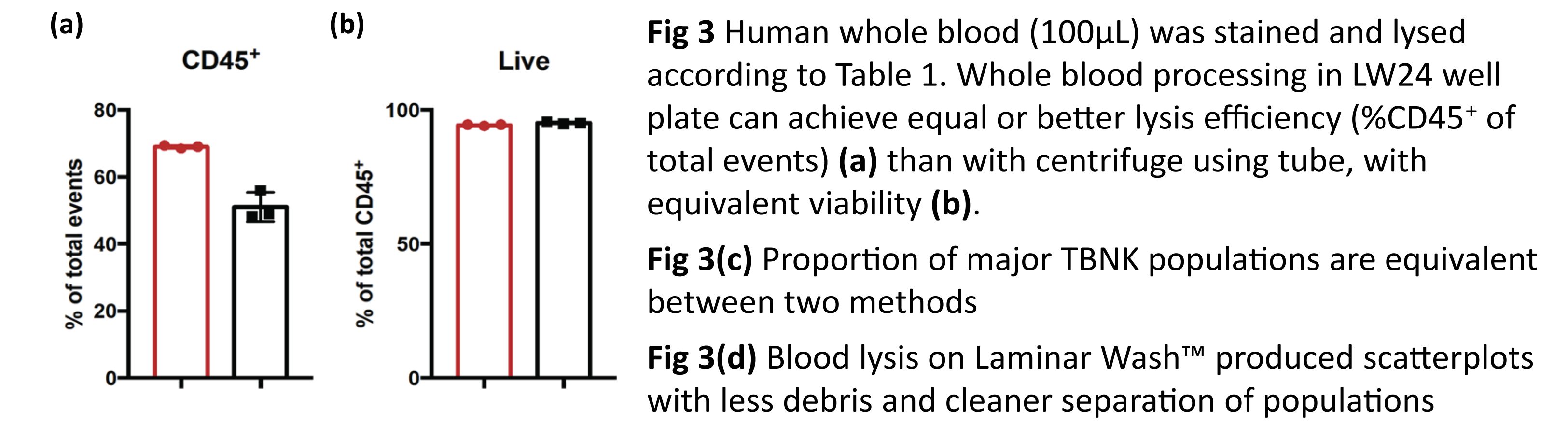
**Fig 1(d)** Schematic of efficient wash by laminar flow. Low flow rate ensures cells settled on plate are not resuspended and removed, while multiple exchange of buffer during wash ensures dilution and removal of reagents in less than 6 minutes.



Curiox's centrifuge-less cell preparation platform is enabled by our **wall-less plate** and **laminar flow washer**. The Laminar Wash™ (LW) 96-well and 24-well plate consists of an array of hydrophilic spots surrounded by hydrophobic surface, which functions as a virtual wall that separates each spot.

Each spot on 24-well plate (LW24) is capable of staining and lysing up to 100µL whole blood. During lysis, non-erythrocytes settle naturally while erythrocytes are lysed. Laminar wash removed lysed erythrocytes. The lymphocytes were then stained with tetramer and antibodies on LW plate, followed by laminar washes and acquisition on flow cytometer.

## IMMUNOPHENOTYPING of WHOLE BLOOD with LYSING SOLUTION



## WHY CENTRIFUGE-LESS WASH for WHOLE BLOOD PROCESSING?

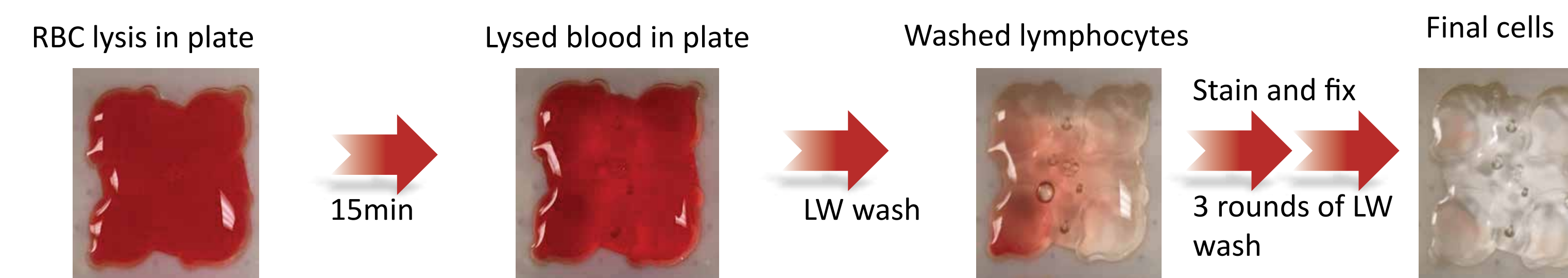
- Reduces aerosolization and exposure of blood products
- Less manual handling for improved consistency
- Potential for high throughput processing with automation
- Efficient dilution accomplished in 3 minutes for one centrifuge-equivalent of washing
- Gentle laminar wash improves staining and identification of rare or low-affinity binding populations

Wish to automate your whole blood processing? Come see the **Laminar Wash™ AUTO 1000** at Booth #207

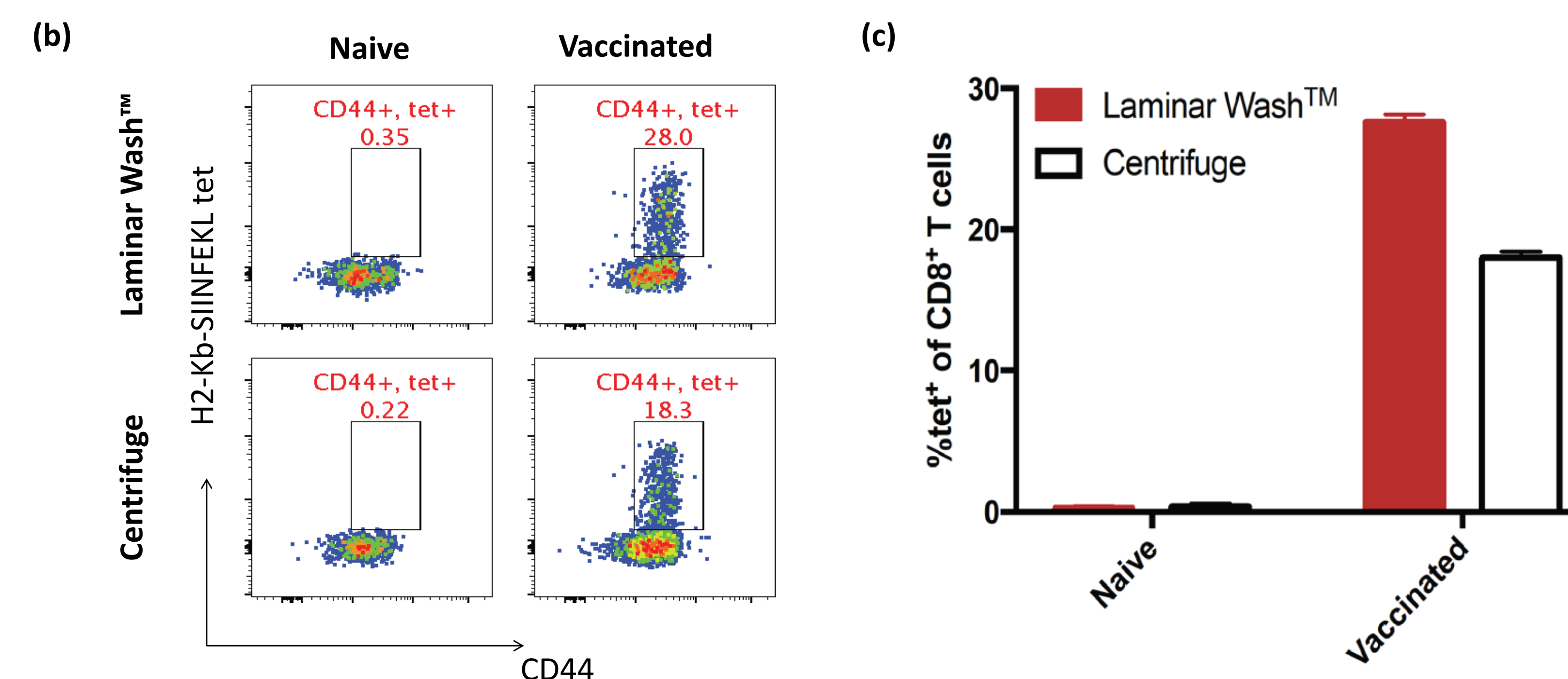
The **AUTO 1000** provides easy, turnkey automation and exceptional flexibility. In addition, **AUTO 1000** is much more compact, affordable and lower maintenance than automation systems built around centrifugation.



## TETRAMER STAINING in MURINE PERIPHERAL BLOOD



**Fig 2(a)** Ammonium chloride lysis of 30µL murine blood was carried out in LW 24-well plate. During 15min incubation, lymphocytes settle naturally while erythrocytes are lysed. Laminar wash removed lysed erythrocytes. The lymphocytes were then stained with tetramer and antibodies on LW plate, followed by laminar washes and acquisition on flow cytometer.



**Fig 2(b)** Scatterplot of single, live CD3<sup>+</sup>, CD8<sup>+</sup> cells showing CD44<sup>+</sup>, H2-K<sup>b</sup>-SIINFEKL tetramer<sup>+</sup> gate. **(c)** Laminar Wash™ was much less disruptive to tetramer-TCR binding, elucidating 10% more rare antigen-specific T cells compared to centrifuge wash.

## OVERVIEW of BLOOD PROCESSING WORKFLOW

Time (min)	Centrifuge	Laminar Wash™	Time (min)
35	Add antibody to 100µL whole blood in tube. Incubate for 30min.	Add antibody to 100µL whole blood in LW24 plate. Incubate for 30min.	35
32	Add 1mL Lysing Solution. Incubate 30min.	Gently add 400µL Lysing Solution. Remove 350µL volume and vortex plate. Add 450µL Lysing Solution, pipette to mix. Incubate at least 30min.	34
8	Add 1mL wash buffer, centrifuge 5min. Aspirate supernatant.	Wash plate 18X using Laminar Wash™ HT1000 washer.	10
16	Repeat wash twice more.	N.A.	0
1	Add 100µL of buffer.	Transfer cells from LW24 plate to conventional plate or tube with addition of 100µL buffer.	5
	Acquire or sort.	Acquire or sort	
95	Total Hands On Time		
	Laminar Wash™ significantly reduced manual handling and operator exposure		

**Table 1** Example of whole blood processing workflow using LW24 plate and HT1000 washer