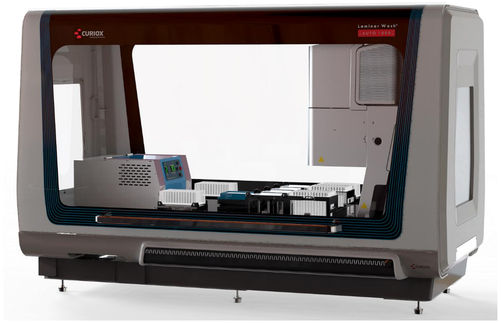
Walk-Away Automation of Cell Immunostaining Assays by Centrifuge-Less Laminar Wash™

Ira Kim[1], Melvin Lye[1], Nadiezda Fernandez Oropeza[1], Blanca Ponce-Ngo[2], Chyan Ying Ke[1], Yoke Keng Ngeow[1], Sigeeta Rajaram[1], Royce Pek[1], Kong Leong Cheng[1], Ih Chin Kon[1], Adriana Colovai[2], Namyong Kim[1]
Curiox Biosystems Inc., San Carlos, CA[1] Montefiore Medical Center, NY, USA[2]

CURIOX'S LAMINAR WASH TECHNOLOGY

Fig 1 Curiox's centrifuge-less cell preparation platform is enabled by our **wall-less plate** and **laminar flow washer**.
(a) MINI 1000 is a personal bench-top instrument that processes 8-wells simultaneously, on a strip plate consisting 16-wells.
(b) HT1000 washes 96 wells in a fully automated process.
(c) The Laminar Wash™ (LW) plate (96 well) or strip (16 well) consists of an array of hydrophilic spots surrounded by hydrophobic surface, which functions as a virtual wall that separates each spot. Each spot can process from a single cell to as many as 10 million cells without the mechanical stress and cell losses associated with centrifugation.

FULL AUTOMATION for CELLULAR STAINING and WHOLE BLOOD PROCESSING for FLOW CYTOMETRY ASSAYS

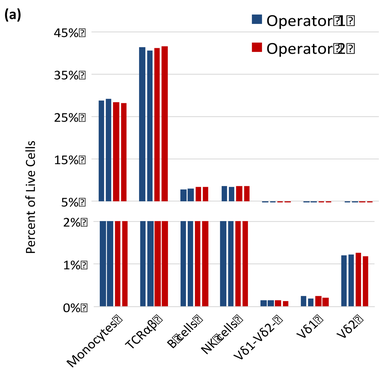


The **Laminar Wash™ AUTO 1000** software interface allows modifications to sample volume, buffer volume, antibody volume, number of washes, incubation time and temperature. In addition, it comes with pre-programmed protocols to ensure user consistency.

User-friendly Interface: The software prompts the user to enter the parameters of existing SOPs or protocols with no coding or programming of the liquid handling system.

- **Automated –** reduces manual pipetting errors and errors associated with multiple personnel changes
- **Turnkey surface and intracellular staining** – automation improves lab efficiency and reduces waste by reducing repeat experiments
- **Enables compliance –** every step of the protocol is recorded in the software
- **Rapid time to results –** AUTO 1000 processes 2 plates in under 2 hours using pre-programmed intracellular staining protocols
- **Consistent results –** across users and locations
- **Cleaner data –** Laminar Wash™ reduces the amount of background and debris, especially with tissue samples

AUTOMATED LAMINAR WASH™ PRODUCED CONSISTENT DATA with LOW COEFFICIENT of VARIANCE



(b)

Population	% CV
Monocytes	1.5
TCRαβ	1.0
B cells	3.4
NK cells	1.2
Vδ1	11.1
Vδ1-Vδ2-	10.1
Vδ2	2.4

Conventional method ~20% CV

Fig 2 By using Laminar Wash™ in a single wash step during immunophenotyping of human PBMCs, the user achieved **(a)** consistency and **(b)** lower coefficient of variance even between different operators. *Webinar available from www.curiox.com*

LAMINAR WASH™ ENABLED ACCURATE IDENTIFICATION of TUMOR INFILTRATING LYMPHOCYTE (TIL) POPULATION

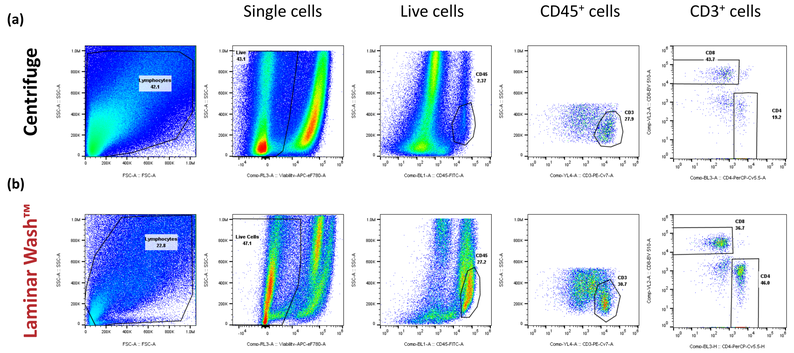


Fig 3(a) Centrifuge wash method – cell loss and mechanical stress through sequential pelleting and resuspension **Fig 4(b) Laminar Wash™** method – less tumor debris, but higher retention of TILs and better resolution of populations

LAMINAR WASH™ IMPROVED RETENTION and RETAINED ENDOGENOUS PROTEIN EXPRESSION

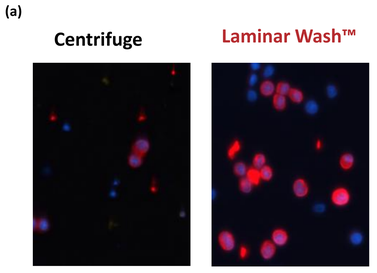


Fig 4(a) In processing of low cell numbers (50-100), LW retained higher cell count with better cellular morphology and brighter endogenous fluorescent expression. The dim protein expression is evidence of mechanic stresses caused by centrifugation.
Data from a biotech company in San Diego

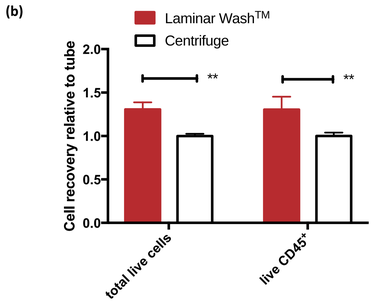


Fig 4(b) In subcutaneous murine melanoma tumor samples, LW retained 40% more live cells than centrifuge, with less tumor debris and better antibody staining.

LAMINAR WASH™ DECREASED BACKGROUND in B CELL CROSSMATCH without PRONASE TREATMENT

(a)

		Pronase	T Neg	B Neg	ΔB = B-T (Background)	T Pos	B Pos	Δ = B-T (Background)	T	B
Laminar Wash™	1	NO	261	264	3	599	672	73	338	408
	2	NO	259	259	0					
	3	YES	136	176	40	588	668	80	452	492
	4	YES	151	182	31					
Centrifuge	5	NO	258	374	116	589	708	119	331	394
	6	NO	265	371	106					
	7	YES	263	261	-2	685	779	94	422	518
	8	YES	269	268	-1					

(b)

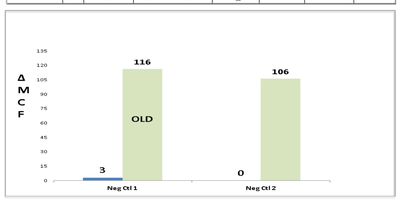


Fig 5(a) Decreased background in pronase-independent B cell crossmatching of PBL fresh cells with Laminar Wash™ **5(b)** ΔMCF was eliminated with Laminar Wash™ in pronase-untreated B cells bound to the wells of negative control serum. *Webinar available from www.curiox.com*

LAMINAR WASH™ LED to IMPROVED CELL VIABILITY of TILs and LESS CYTOMETER CLOGGING

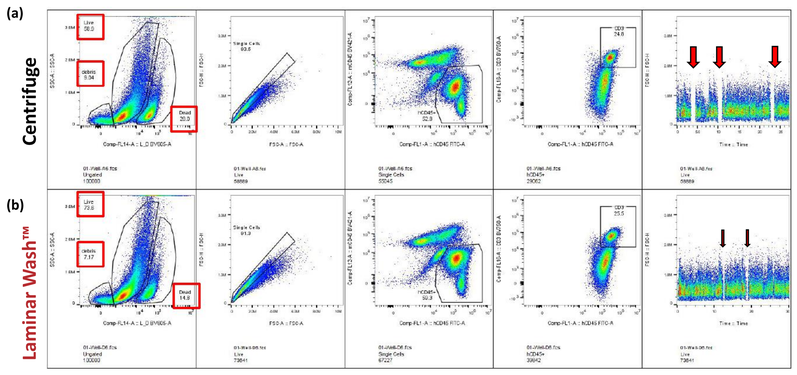


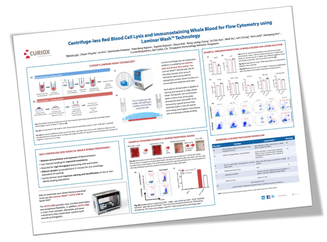
Fig 6 FACS plots by **(a)** centrifuge vs. **(b) Laminar Wash™** methods. The dissociated tumor samples from Raji-inoculated Balb/c mice showed higher viability and increased debris removal with Laminar Wash™, leading to less clogging and more consistent reading.

ACKNOWLEDGEMENTS

We would like to give special thanks to the following scientists: Dr. Arnaud Colantonio at Adicet Bio (Fig 2), Dr. Christoph Eberle at Charles River Laboratories in Worcester, MA (Fig 3), and Dr. Thomas Tan, Jonathan Hsu, and Benjamin Keller at Elstar Therapeutics, Cambridge, MA (Fig 6) for sharing their valuable insights and data.

Come see **Laminar Wash™ AUTO 1000** and your personal-sized **MINI1000** in action at Booth #207!

Learn more about how **Laminar Wash™** can benefit your **whole blood processing** assays at Poster #113.



Curiox Biosystems