Replacing the centrifuge to increase T-cell recovery and generate consistent data: The Laminar Wash™ approach.

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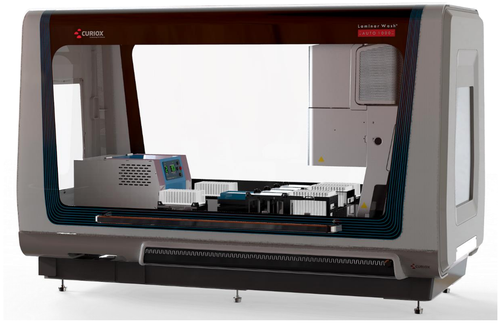
CURIOX'S LAMINAR WASH TECHNOLOGY

Fig 1 Curiox's centrifuge-less cell preparation platform is enabled by our **wall-less plate** and **laminar flow washer**.
(a) MINI1000 is a personal bench-top instrument that processes 8-wells simultaneously, on a strip plate consisting 16-wells.
(b) HT2000 washes 96 wells in a fully automated process, with touch-screen display and optional automated buffer exchanger.
(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 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

LAMINAR WASH™ retained endogenous protein expression

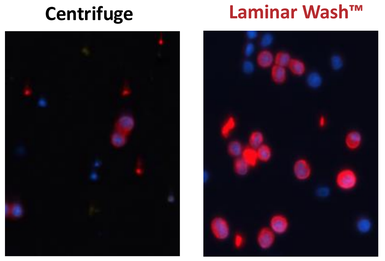
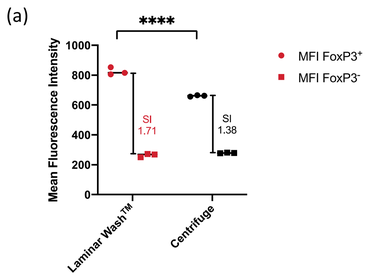
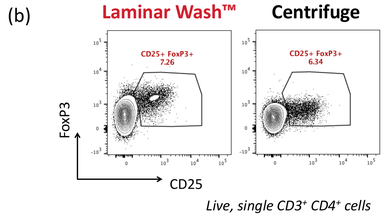


Fig 2 In processing of low cell numbers (50-100 cells), LW retained higher cell count with more viable cellular morphology and brighter endogenous fluorescent expression. The dim protein expression is evidence of mechanic stresses caused by centrifugation.

Data from a biotech company in San Diego

LAMINAR WASH™ improved stain indices of fluorescent antibodies while increased identification of rare Treg population





	Laminar Wash™	Centrifuge
Mean %Treg	7.18	6.38
CV% (n=3)	1.90	3.22

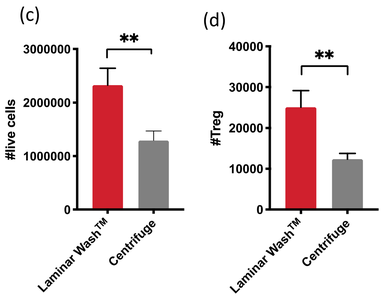


Fig 3 (a) LW improved positive signal of FoxP3 intranuclear marker in mouse splenocytes, while maintaining low background, increasing the overall stain index of FoxP3. (b) Higher MFI of FoxP3 in flow cytometry dot plot improved resolution of rare Treg population, identifying higher percentage of Treg while keeping %CV low. Greater total live cell retention (c) and higher frequency of Treg resulted in nearly double the absolute number of identified regulatory T cells (d).

LAMINAR WASH™ improved retention with low starting cell numbers, while maintaining cellular integrity of isolated T cells

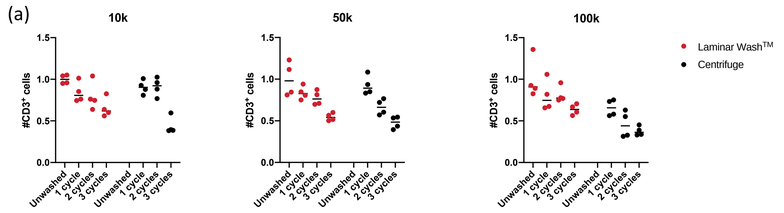


Fig 4 (a) LW retained more than 50% of initial naïve T cell count after multiple incubation and wash cycles, at starting cell numbers as low as 10k per well. Isolated naïve T were aliquoted to 10 000, 50 000 or 100 000 cells per well, and incubated and wash by either LW or centrifuge. Each set of incubation and wash accounted for 1 'cycle', simulating multiple rounds of antibody incubation.

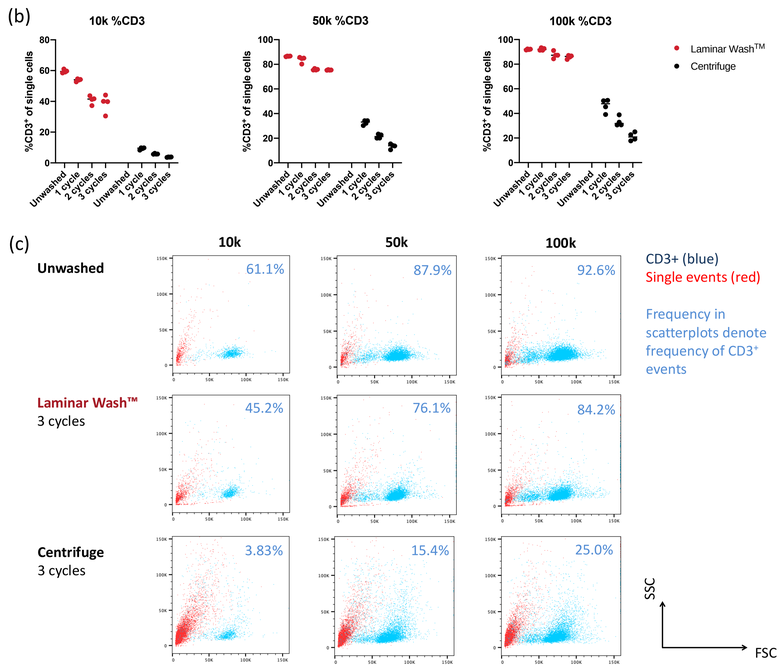


Fig 4 (b) Progressive incubation and wash of naïve T cells by centrifuge resulted in drastic loss of T cell frequency, while LW maintained near-native %CD3+ events. (c) Scatter plots revealed increase in debris events in centrifuged samples, indicating cell destruction from centrifugation.

LAMINAR WASH™ enabled accurate identification of Tumor Infiltrating Lymphocyte (TIL) population

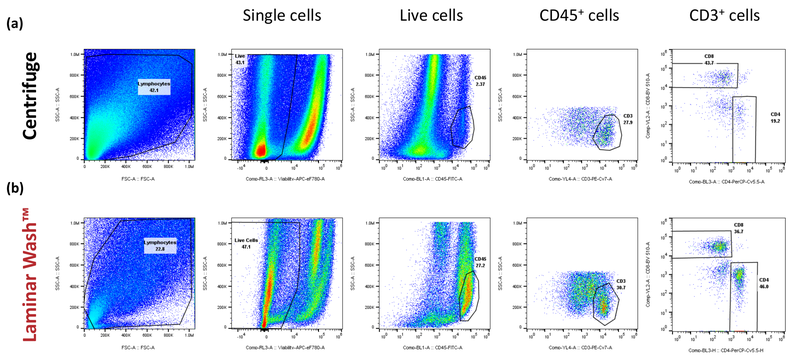


Fig 5 (a) **Centrifuge** wash method – cell loss and mechanical stress through sequential pelleting and resuspension. (b) **Laminar Wash™** method –TILs data shows less tumor debris, higher viability, higher retention of TILs and better resolution of populations.

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We would like to give special thanks to Dr. Christoph Eberle at Charles River Laboratories in Worcester, MA (Fig 5) for sharing their valuable insights and data.

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Headline session: "Biology at its best for all modalities" Friday 16th at 11:35am. Be ready with a notepad for a transformative 30 mins that will change the way you work forever. Global companies detail why they 'Go Centrifuge-Free' across different modalities. **Data workshop**: Real data from real customers. Thursday 15th 14:35 in CRUK track. We discuss data and simple steps to 'Go Centrifuge-Free' for cell and bead-based assays. Email: milesr@curiox.com

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