

Figure S1. Workflow diagram comparing commercially available bead based isolation techniques to the VerIFAST technique. Sample Prep includes whole blood applications, and refers to preparation via density separation.

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	Veridex	CTC Chip	Filter	VerIFAST
Assay time <2hr	✗	✗	✓	✓
Cell viability	✗	✓	✓	✓
Cell accessibility	✗	✗	✗	✓
Ease of use	✗	✗	✓	✓
Multiple analytes	✓	✓	✗	✓
Easy to image	✓	✗	✗	✓
Clinically proven	✓	✓	?	?
Negative selection	✗	✗	✓	✗

✓ = Yes ✗ = No ? = To be determined

Figure 2S. Comparison between the VerIFAST and other methods of cellular isolation - including commercially available methods. The items of the table for comparison and their definitions are: Assay time – the time from sample acquisition to imaging answer, cell viability – the viability of target cells following isolation, cell accessibility – the ability to remove target cells from the device following the assay, ease of use – the device is easy to actuate in terms of equipment, parts needed, multiple analytes – the device can capture a variety of input analytes (e.g. cells, protein, nucleic acid, etc.), easy to image – the automation, speed and simplicity of the imaging, clinically proven – the number and extent of clinical trials run with the device, negative selection – the independence of the method on the reliance of positive selection markers for isolation.

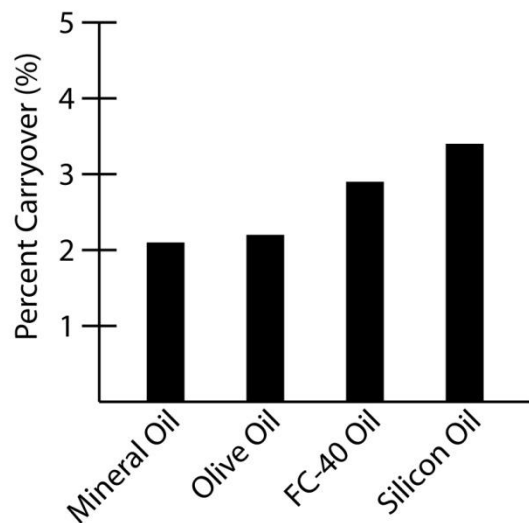


Figure 3S. Comparison of oils used in the VerIFAST. The percent carryover was characterized by loading a fluorescent die into the input, and measuring the level of fluorescence carried through the oil and into the output well.

Step	Reagent	Time for step	Temperature
Fixation	4% PFA in PBS	10 minutes	22.5C
Wash (3x)	0.1% BSA in PBS	1 minute	22.5C
Permeabilization	1% Tween-20 in PBS	30 minutes	22.5C
Primary Antibody	1:50 primary antibody in PBS with 0.1% BSA	24 hours	4C
Wash (3x)	0.1% BSA in PBS	1 minute	22.5C
Secondary Antibody	1:500 secondary antibody in PBS with 0.1% BSA	2 hours	22.5C
Wash (3x)	0.1% BSA in PBS	1 minute	22.5C

Table 1S. List of the steps required for the intracellular stained cells (shown in the main text). This entire process can be performed in the VerIFAST, without the risk of significant cell loss through the membrane. Furthermore, the VerIFAST enable beads to be removed from the cells prior to this staining process.