WHITEPAPER
Increase the Physiological Relevance of HIV Models with RoboSep™

HIV research using human primary cells

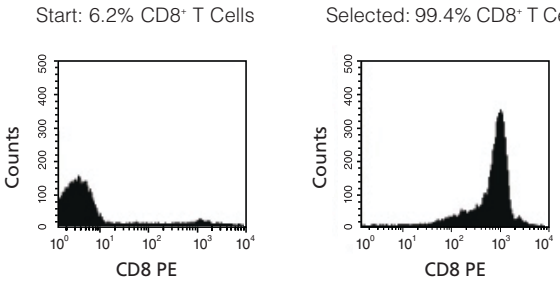
Despite intense research in the 30 years since the discovery of HIV/AIDS, the disease remains a global epidemic with no credible vaccine available. Many theoretical and empirical models in HIV research have proven to be of limited relevance to the in vivo dynamics of HIV infection, and this represents a major obstacle to vaccine development. As several recent reviews emphasize, research needs to turn towards more physiologically relevant models to gain a better understanding of the mechanisms of HIV pathogenesis.[1-4]

More physiologically relevant models are required for several reasons. For example, although the major HIV antigenic sites are well characterized, this information may not translate directly into the immunogenic structure of an effective vaccine.[5,6] Similarly, many in vitro approaches to studying HIV pathogenesis do not adequately approximate the in vivo dynamics of HIV infection since they assess cellular responses to HIV peptides rather than the intact virus. As Virgin & Walker (2010) point out in a recent paper, "studies using assays that more closely replicate the in vivo situation in which viral antigens are processed and presented by primary cells rather than being provided as synthetic peptides at supra-physiological doses are urgently needed."[3]

In order to mimic the natural physiological environment more closely, assays should begin with populations of viable, unstimulated human cells isolated from fresh blood.[3] Since it is difficult to obtain samples from enough subjects to allow for reasonable statistical power, it is extremely important to reduce error during sample preparation so that each sample yields usable results. Researchers therefore require reliable methods of isolating cells of interest rapidly and consistently, with high recovery and minimal sample handling.

In this paper we discuss automated cell separation with RoboSep™ as a key component of research into HIV vaccine development. We review recent ground-breaking research from the lab of Professor Françoise Barré-Sinoussi, which uses RoboSep™ to design and carry out ex vivo experiments into the underlying cellular mechanisms for HIV resistance in rare infected individuals known as HIV controllers (HIC).

FIGURE 1. FACS histogram results using EasySep™ Human CD8 Whole Blood Selection Kit.



Starting with fresh whole blood, the CD8+ T cell content of the selected fraction typically ranges from 97.1% - 99.9%.

Automated cell separation facilitates standardized, portable ex vivo HIV models

Using natural ex vivo cell populations to study the mechanistic basis for HIV infection and resistance is essential for developing effective vaccines. These cell populations must be isolated consistently and gently, and sample handling should be minimized to reduce exposure to the virus and the potential for human error. Automated cell isolation addresses these requirements, providing highly purified functional cells (Figure 1). Automation also allows standardized protocols to be carried out consistently between different labs. This fosters collaboration between research groups, and helps ensure that results can be replicated.

For these reasons, cell separation using RoboSep™ has become an essential tool for HIV labs. RoboSep™ is a fully automated instrument from STEMCELL Technologies that carries out cell separation protocols using EasySep™ immunomagnetic reagents. It is widely used in leading HIV research labs world-wide, such as the Laboratory of Immunoregulation at the National Institute of Allergy and Infectious Diseases (NIAID), the Ragon institute, and the Institut Pasteur in Paris. At the Institut Pasteur, RoboSep™ is an important tool in some of the most promising HIV research currently being conducted. In the laboratory of Françoise Barré-Sinoussi (incumbent president of the International AIDS Society and winner of the Nobel Prize in Medicine for the discovery of HIV), Asier Sáez-Cirión and colleagues investigate the cellular mechanisms for HIV resistance in a rare group known as HIV Controllers (HIC).

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Case study: The dynamics of HIV suppression in HIV Controllers

HIV Controllers (HIC) are a population of HIV-infected individuals capable of controlling HIV-1 infection to undetectable levels for more than 10 years.[7-9] They provide a clue for researchers working on HIV vaccine development: by understanding the basis for their resistance to HIV, it may be possible to develop a vaccine capable of recreating the HIC phenotype in typical HIV patients.

Researchers have learned that HIC are characterized by a robust $CD8^+$ T cell response to infection, and in vitro studies have shown that $CD8^+$ T cells in HIC have a number of unusual characteristics, including a high degree of functionality and the capacity to proliferate and generate a multifunctional response to HIV infection.[10-12] However, because these studies often used cells activated in vitro with HIV peptides rather than exposure to infected $CD4^+$ T cells, their relevance in the control of infection is uncertain.[13-14]

In order to investigate the basis for HIV suppression in HIC under more naturalistic conditions, Sáez-Cirión's research group at the Institut Pasteur has developed an ex vivo T cell-based suppression assay that allows HIV-specific $CD8^+$ T cells to be evaluated in terms of their response to infected $CD4^+$ T cells, rather than stimulation by HIV peptides[14] (Figure 2).

Ex vivo HIV suppression assay protocol

The protocol for Sáez-Cirión et al.'s assay protocol involves isolating $CD4^+$ T cells from fresh whole blood or peripheral blood mononuclear cells (PBMCs) by positive immunomagnetic selection. The negative fraction, which is depleted of $CD4^+$ T cells, is then used as the source for $CD8^+$ T cells. These cells are isolated by negative selection (i.e., by selectively removing all other cell types). These separation steps are carried out rapidly and consistently by RoboSep™, ensuring high purity and recovery. Sáez-Cirión et al. routinely obtain purities of >95% for $CD4^+$ T cells and $CD8^+$ T cells (see reference 14 for full protocol).

These purified cells are used in studies investigating HIV infection in pure $CD4^+$ T cell cultures versus infection of $CD4^+$ T cells cocultured with $CD8^+$ T cells. Sáez-Cirión's group provides protocols for assessing levels of infection by either of two proxies: HIV-1 p24 levels in culture supernatants as measured by ELISA, or the percentage of infected $CD4^+$ T cells as assessed by staining for intracellular Gag proteins. This allows the protective effects of $CD8^+$ T cells to be reliably quantified, and the dynamics of infection to be studied in a more physiologically relevant context (i.e., using whole cells rather than peptides). Furthermore, the protocol is readily adaptable to different HIV strains by simply substituting the strain of interest in the viral inoculate.

Do $CD8^+$ T cells from HIC suppress HIV infection?

Sáez-Cirión's research group predicts that ex vivo $CD8^+$ T cells from healthy donor controls should have no significant effect on levels of $CD4^+$ T cell infection, while $CD8^+$ T cells from HIC should be able to reduce viral infection by an order of magnitude or more.[14] The results of early experiments support this prediction (Figure 3). The door is now open for HIV labs to further test these hypotheses using the ex vivo T cell-based suppression assay, and to investigate the ultimate mechanistic basis for the protective effects of $CD8^+$ T cells from HIC.

This is just one example of how automated cell separation assists in creating simple, robust and portable experimental designs for HIV research. Sáez-Cirión et al. have also recently performed cell separation using RoboSep™ to study HIV resistance in $CD4^+$ T cells from HIC versus normal, non-infected individuals, and showed that $CD4^+$ T cells in HIC have a measure of intrinsic resistance to infection.[15] While this leading-edge research is ultimately driven by scientists and their ideas, cell separation technologies such as RoboSep™ help remove the barriers that once prevented HIV researchers from working with functional cells isolated directly from human blood.

FIGURE 3. HIV infection rates in $CD4^+$ T cell monocultures versus $CD4^+/CD8^+$ T cell cocultures as measured by HIV-1 p24 levels in culture supernatants. Adapted by permission from Macmillan Publishers Ltd: Nature Protocol, Sáez-Cirión et al. 2011, copyright 2011.

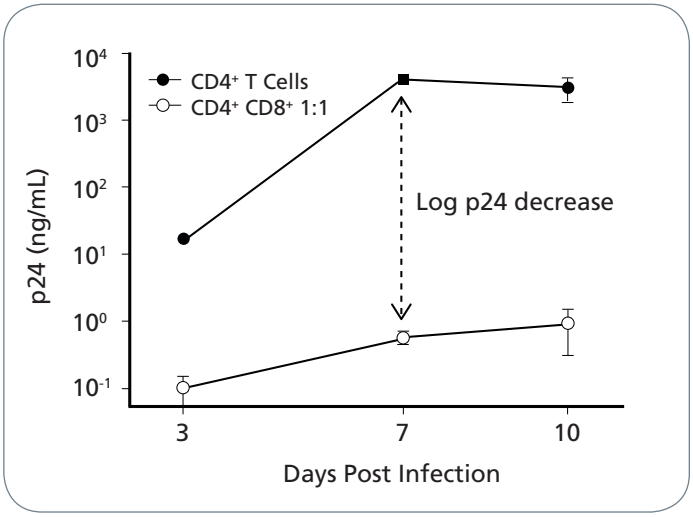
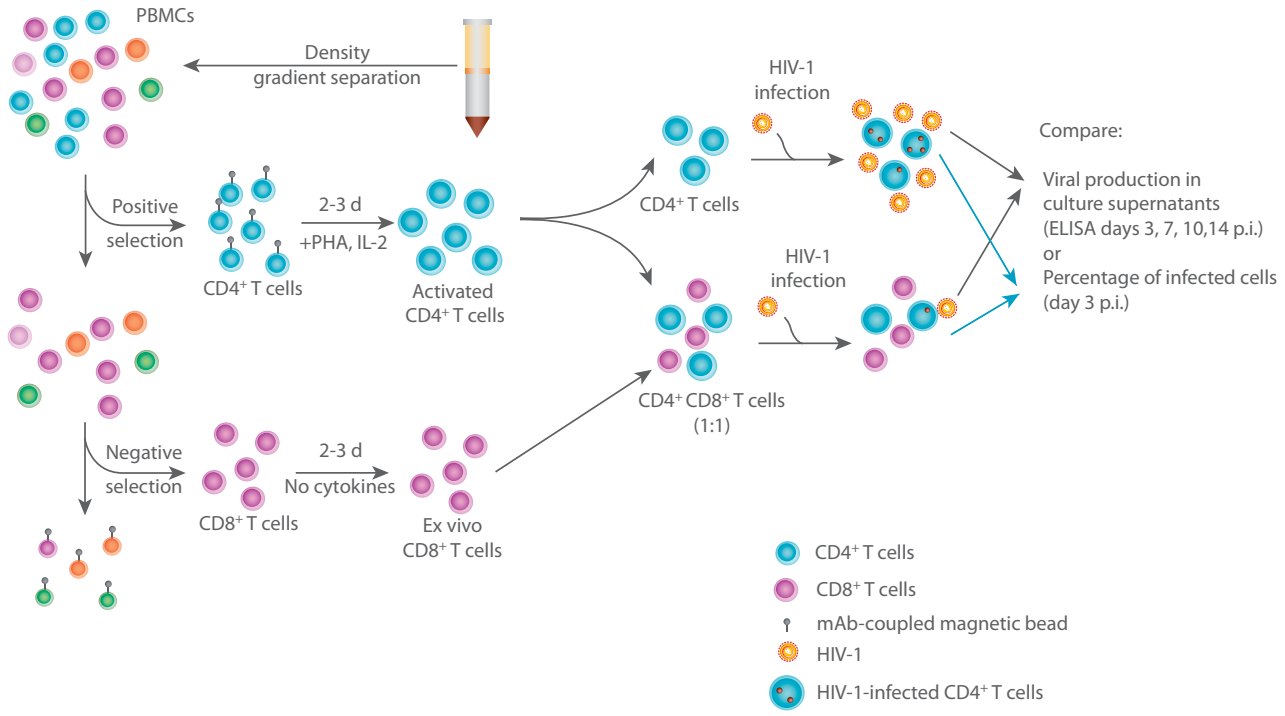


FIGURE 2. A diagram of the protocol for Sáez-Cirión's T cell-based suppression assay.[14] First, $CD4^+$ cells are separated from PBMCs using the RoboSep™ Human CD4 Positive Selection Kit. $CD8^+$ cells are then isolated from the remaining fraction using the RoboSep™ CD8 T Cell Enrichment Kit. (See Table 1 for catalog numbers). Isolated $CD4^+$ T cells can then be infected with HIV, and studied both in isolation and in coculture with $CD8^+$ T cells. Adapted by permission from Macmillan Publishers Ltd: Nature Protocols, Sáez-Cirión et al. 2011, copyright 2011.



Conclusion

The use of physiologically relevant laboratory models is crucial in HIV vaccine development. Automated cell separation facilitates these models by enabling viable cell populations to be isolated directly from human blood. Automation also allows standardized protocols to be carried out consistently and reliably, aiding collaboration between different research groups.

By developing and sharing protocols and assays such as those described above, scientists are stimulating research into the basic mechanisms of HIV pathogenesis and resistance, and exploring promising avenues towards vaccines capable of controlling HIV infection. The work being performed by Sáez-Cirión's group and other leading laboratories exemplifies the type of research that is now most urgently needed, and of which automated cell separation systems such as RoboSep™ form an integral part.

For more information about RoboSep™ kits for HIV research, please see the next page.

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RoboSep™: Automated Cell Separation for HIV Research

HIV research often requires cell isolation procedures that minimize sample handling in order to avoid cross-contamination of samples and exposure to virus. RoboSep™, from STEMCELL Technologies, is a powerful tool for rapidly isolating purified immune cells affected by HIV with minimum human manipulation. RoboSep™ fully automates cell isolation procedures by using a robotic pipettor to perform all cell labeling and magnetic separation steps in a column-free system. Only 5 minutes of technical "hands-on" time is required per run. RoboSep™ can process up to 4 different cell types at once from a variety of sources including peripheral blood mononuclear cells, whole blood, and buffy coat. The instrument can fit into a standard laminar flow hood for sterile operation, and uses disposable plastic-ware tips – instead of multi-use columns and tubing – to ensure safe handling of blood products and avoid sample cross-contamination.

Cells can be isolated using negative or positive selection either for direct use in downstream assays, or as a pre-enrichment step prior to flow sorting. It is also possible to carry out sequential isolations (for instance, isolating $CD4^+$ and $CD8^+$ T cells from the same sample). After completion of the cell separation cycle, the cells of interest are immediately available for further studies. Optimized reagents and protocols are available for isolation of highly purified $CD4^+$ T cells, $CD8^+$ T cells, Naïve or Memory $CD4^+$ and $CD8^+$ T cells, Regulatory T cells, Monocytes, Dendritic cells, NK cells, B cells, and more.

For more information about RoboSep™ or our other products, please visit **www.stemcell.com**.

Products for HIV Research

HUMAN CELL TYPE	SELECTION METHOD		% PURITY[1]	ROBOSEP™[2] REAGENT KIT[3] (CATALOG #)
T cells	Positive ($CD2^+$)	Whole Blood[4]	95.7 - 99.6	18687RF
	Positive ($CD2^+$)	PBMC[5]	86.3 - 98.0	18657RF
	Positive ($CD3^+$)	Whole Blood	98.2 - 99.8	18081RF
	Positive ($CD3^+$)	PBMC	95.0 - 99.8	18051RF
	Negative	PBMC	97.5 - 99.5	19051RF
$CD4^+$ T cells	Positive ($CD4^+$)	Whole Blood	97.1 - 99.9	18082RF
	Positive	PBMC	96.0 - 99.9	18052RF
	Negative	PBMC	92.5 - 98.6	19052RF
$CD8^+$ T cells	Positive ($CD8^+$)	Whole Blood	97.1 - 99.6	18083RF
	Positive ($CD8^+$)	PBMC	92.0 - 99.6	18053RF
	Negative	PBMC	82.7 - 98.2	19053RF
Naïve $CD8^+$ T cells	Negative	PBMC	85.0 - 92.0	19158RF
Memory CD^+ T cells	Negative	PBMC	72.0 - 92.0	19159RF
Naïve $CD4^+$ T cells	Negative	PBMC	81.5 - 97.0	19155RF
Memory $CD4^+$ T cells	Negative	PBMC	86.0 - 98.0	19157RF
Regulatory $CD4^+CD25^+$ T cells	Positive	Whole Blood	90.0 - 98.0	15862RF
B cells	Positive ($CD19^+$)	Whole Blood	94.3 - 99.6	18084RF
	Positive ($CD19^+$)	PBMC	95.0 - 99.4	18054RF
	Negative	PBMC	97.9 - 99.5	19054RF
Naïve B cells	Negative	PBMC	92.0 - 98.0	19254RF
Memory B cells	Negative	PBMC	85.0 - 95.0	18164RF
NK cells	Positive ($CD56^+$)	Whole Blood	89.7 – 99.8	18085RF
	Positive ($CD56^+$)	PBMC	85.0 – 98.0	18055RF
	Negative	PBMC	90.0 – 97.0	19055RF
Monocytes	Positive ($CD14^+$)	Buffy Coat[6]	96.5 - 99.6	18088RF
	Positive ($CD14^+$)	PBMC	91.0 - 99.6	18058RF
	Negative	PBMC	83.0 - 95.0	19059RF
	Negative (without CD16 Depletion)	PBMC	73.0 - 81.0	19058RF
Pan-Dendritic cells	Negative	PBMC	40.0 – 80.0	19251RF
Plasmacytoid DC	Negative	PBMC	87.0 - 97.0	19062RF
"Do-It-Yourself" Selection	Use your own mouse IgG_1 antibody			18099RF

NON-HUMAN PRIMATE			% PURITY[1]	ROBOSEP™[2] REAGENT KIT[3] (CATALOG #)
Custom Order (CD4, CD8, Treg, etc.)	Negative	PBMC	-	19809RF

1. EasySep™ and RoboSep™ purity data.
2. **Required equipment - RoboSep™ (Catalog #20000)**
3. RoboSep™ Reagent Kit contain a selection cocktail, magnetic particles, two boxes of RoboSep™ Tip Filter Racks (16 Racks in total), and a 250 mL bottle of RoboSep™ Buffer. Whole Blood kits contain an additional 10 mL bottle of EasySep™ RBC Lysis Buffer (10X concentrate). All reagents are also available for manual separation. Contact us for more information.
4. Each kit contains enough reagents to process 60 mL of whole blood/buffy coat, unless otherwise stated.
5. PBMC = peripheral blood mononuclear cells; each kit contains enough reagents to process 10^9 cells.
6. For 30 mL of buffy coat