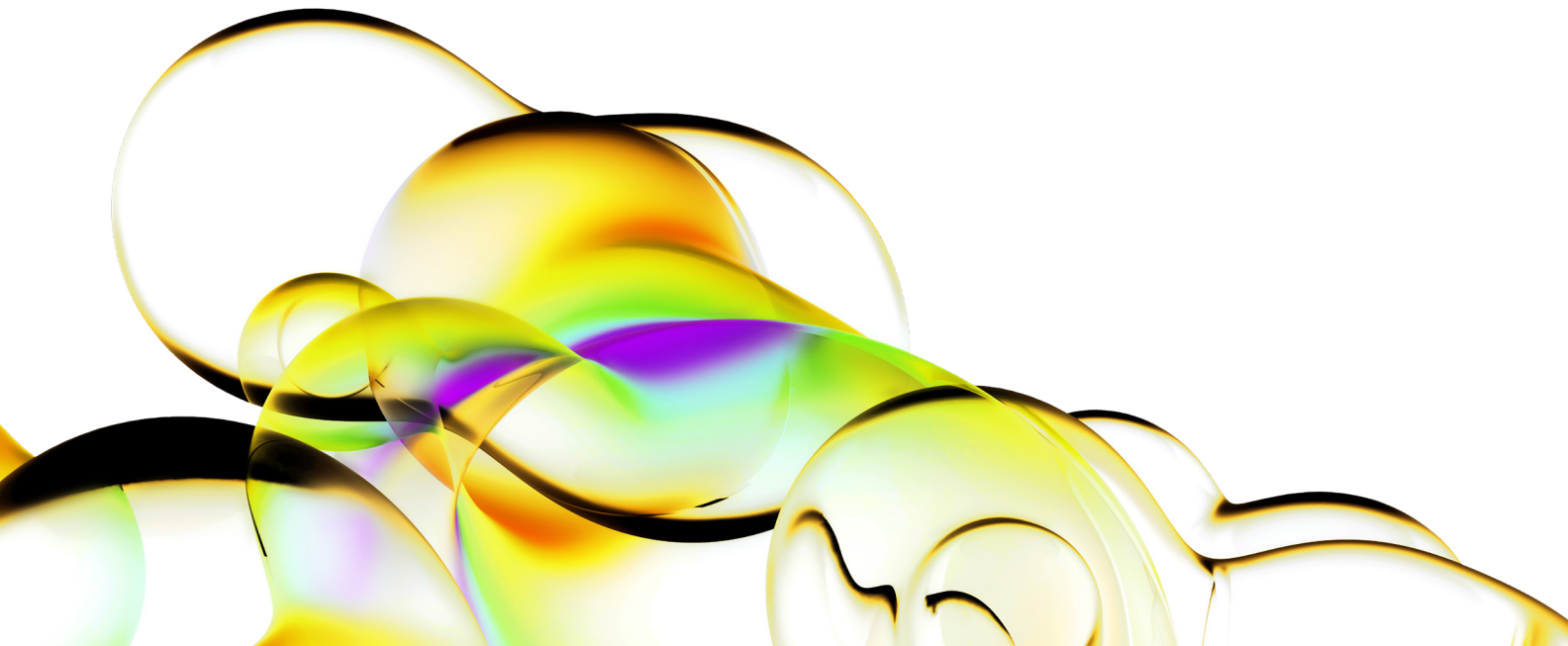
Overcoming challenges in primary sample processing with animal infectious disease models.

In the mid-nineteenth century, microorganisms were identified as the causative agent of infectious disease outbreaks occurring throughout history. These serious public health challenges continue to arise, correlating to an increased effort in understanding microbiology, infectious diseases, and immunology. Researchers are steadfast in developing novel therapeutics, antibiotics, drugs, and vaccines for the betterment of humankind. As these studies are necessary to acquire the knowledge required for the development of such therapeutics and they cannot ethically be conducted in humans, the use of animal models is vital for these efforts.

Therapeutic development through mimicry

A researcher working on infectious diseases typically uses mice, monkeys, and human samples from naturally occurring infections. Other animal models are also available for the study of some pathogens, including swine, the nematode C.elegans, and zebrafish (1,2). With animal studies, the animals are typically infected with the pathogen of interest, and the immune response is then evaluated in terms of cytokine production and immune cell subsets. Mice have certain advantages for immunology with the availability of knockouts, immuno-deficient mice, and humanized or human-tissue xenografted mice, as well as the lower cost of the experiments compared to other models (3).



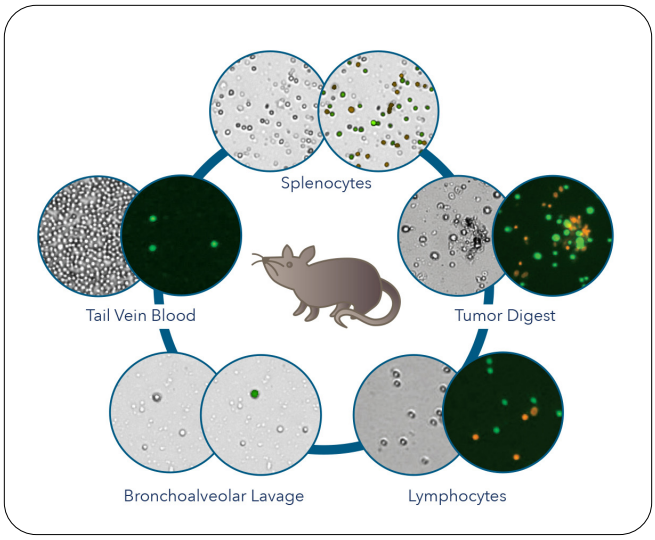


Figure 1: Types of primary samples that can be generated.

Mouse models lead to alternative subjects in comprehensive studies

The mouse model is the most commonly used when tackling an infectious disease but it is not without limitations including anatomical divergences from humans. Rhesus macaques can closely replicate the pathogenicity of human disease, making primates a great preclinical model to obtain more reliable information about what may happen in humans (4). Comprehensive infectious disease studies first study the fundamental immunology in the mouse model, then test the hypotheses generated in the monkeys, followed by determining if the human samples have similar patterns.

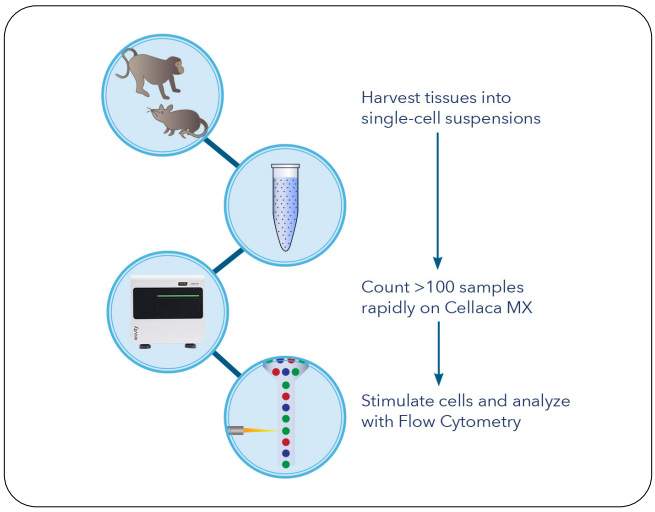


Figure 2: Primary sample workflow.

Accurate cell counts are needed for these samples as the right amount of stimulant or reagent needs to be added to properly evaluate them. For example, to evaluate a cytokine response, the number of viable cells in the samples needs to be determined to know how much stimulant to add. An accurate cell count must be determined per sample (Figure 2). Inaccurate cell counts will lead to non-optimal concentrations of reagents or stimulants in the downstream assays, skewing any data generated from these samples.

Obtain accuracy without sacrificing throughput

Animal studies evaluating the immune response to pathogens present cell counting challenges, as many different tissue samples from multiple animals may need to be counted. From a mouse, isolated samples may include lymph nodes, spleen, bronchoalveolar lavage (BAL), and whole or isolated blood cell samples (3). Some animal models may require an extraordinary number of samples. For example, a researcher investigating the pathogenesis of Mycobacterium tuberculosis may count 20-50 granuloma samples from individual rhesus macaques, in addition to the lymph nodes, spleen, peripheral blood mononuclear cells, and bronchoalveolar lavage samples. Accurate cell counts must be obtained from all of these samples (5), representing a serious bottleneck of time and potential error if not conducted properly.

Addressing the bottleneck

The Cellaca™ MX high-throughput cell counter saves researchers substantial amounts of time, samples, consumables, and energy with easy preparation and results with only 25 µL of cell sample needed. Increase efficiency by mixing the samples with the appropriate assay directly in the mixing wells.

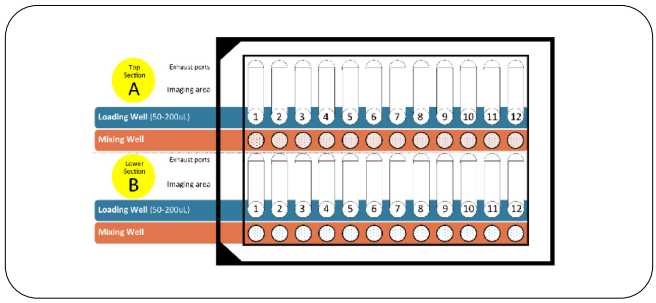


Figure 3: Cellaca MX plate loading template.

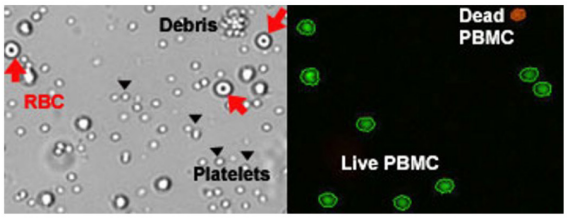
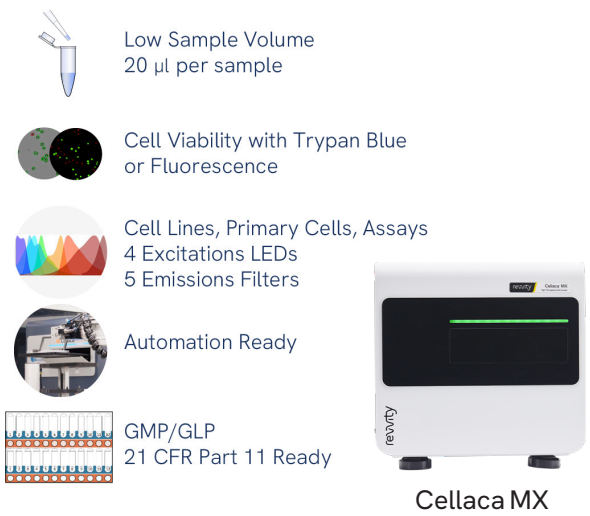


Figure 4: Primary cell counting with acridine orange/propidium iodide.

Simply load the sample cells along with Acridine Orange/ Propidium Iodide and the Cellaca will rapidly count 24 samples in less than three minutes to efficiently label all live cells green and dead cells red while excluding any debris, leading to extremely precise counts of only the cells of interest (Figure 4).

The Cellaca MX high-throughput cell counter is a versatile instrument, capable of performing brightfield and fluorescent concentration and viability measurements with ease. Studies show Cellaca is skilled at increasing both accuracy and throughput in biological workflows. It provides an efficient method of counting and analysis of multiple samples where one previously did not exist and proves to be a significant value to the cell line development and bioprocessing communities.



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