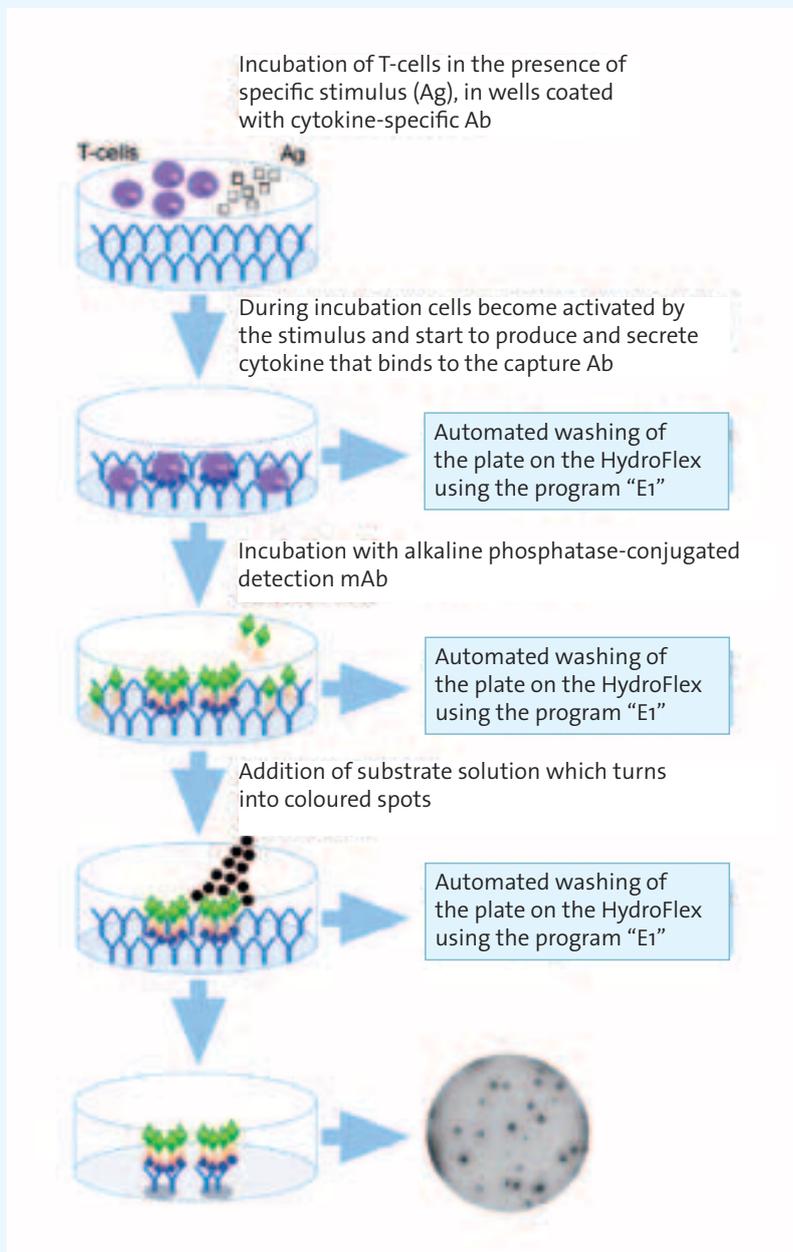


# Improved plate washing for ELISpot assays

Researchers at the University of Modena and Reggio Emilia, Italy, have introduced automated washing for Enzyme-Linked ImmunoSpot (ELISpot) assays, using the HydroFlex™ microplate washer.



Standing, from left: Giovanni Riva, Patrizia Barozzi, Leonardo Potenza, Eleonora Zanetti  
Sitting: Daniela Vallerini



Schematic assay procedure of ELISpot including the wash steps performed with Tecan's HydroFlex microplate washer

Hematologists at the University of Modena and Reggio Emilia's Department of Oncology and Hematology use ELISpot assays to perform detailed studies on the immune response of patients to viral, fungal and tumor antigens. Historically the group, led by Professors Mario Luppi and Giuseppe Torelli, has focused its studies on human herpes virus-8 (HHV8) and over the last 10 years has turned its attention to monitoring the T-cell immune response in post-transplant and leukemic patients. The aim of this immunological monitoring is to try to correlate the presence of an immune response with the development of a particular disease. Generally, when a person shows an immune response they will not have the disease, as this is the body's defense mechanism against a virus or tumor. Conversely, the lack of an immune response can result in recurring and life-threatening infections.

Dr Giovanni Riva, a postdoctoral scientist at the laboratory, said: "We are essentially performing clinical research studies, and what makes our approach a little bit different is that we start with the patient and then move the research into the laboratory. We have very close links to a local medical center and, by working with their clinicians, we are able to obtain samples directly from patients, either by venipuncture or bone marrow aspiration. This allows us to study the immune response in both peripheral blood and bone marrow samples, which is quite a new field of study. We have also expanded our field of research to include the study of polymorphisms in an attempt to correlate genetic differences



HydroFlex microplate washer

and disease, and are investigating some new applications involving siRNA (small interfering RNA), performing *in vitro* studies with siRNA-carrying immunoliposomes to try and identify possible treatments for some lymphomas.”

The department uses the Enzyme-Linked ImmunoSpot (ELISpot) assay, a very sensitive immunoassay, for the detection of secreted cytokines at the single cell level. The ELISpot technique can be applied to any system requiring single cell level investigation of protein secretion, although it is primarily used in T-cell analysis. Because of its high sensitivity, it has proved a valuable tool for the study of small populations of active cells, and is commonly used for investigation of specific immune responses in disease. ELISpot is a standard tool for the development and monitoring of new vaccines and vaccine candidates, and is also well suited to field studies.

The washing stages of the ELISpot assay are critical and can be performed either

manually with a multichannel pipette, or using an automated plate washer. Giovanni explained: “We used to carry out the washing stages of the assay manually, but this was very time consuming and labor intensive. Incubation times tended to be inconsistent, and the assay was not reproducible if performed in this way. As we are looking for different cytokines we can have quite a number of plates to wash – perhaps 10 plates a day, two or three days a week – often at different times. We wanted to improve the methodology, and decided to change to automated washing using the HydroFlex plate washer. We now have a standardized, reproducible, automatic plate washing method, which frees staff time, allowing them to perform other tasks.”

Giovanni concluded: “We have been able to use the standard HydroFlex protocols, just making small changes to adapt them for our specific needs, and the instrument has proved very reliable and simple to use. We just define the protocol for washing, insert a plate and then everything is done

automatically. The HydroFlex is a great improvement to the ELISpot assay and our laboratory.”

To find out more on Tecan’s HydroFlex, visit [www.tecan.com/hydroflex](http://www.tecan.com/hydroflex)

To find out more about the University of Modena, Department of Oncology and Hematology, visit [www.unimore.it/en/](http://www.unimore.it/en/)