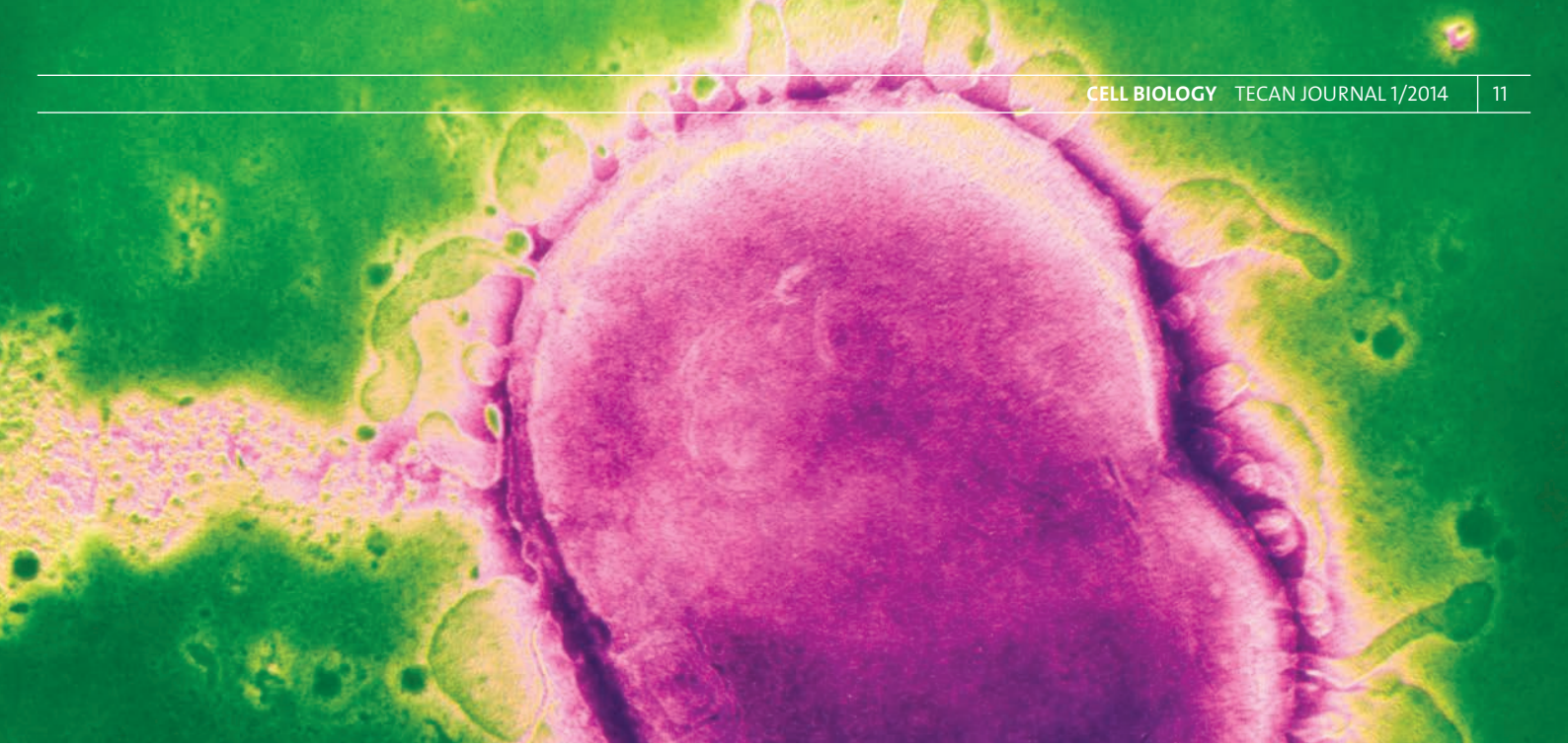
Automation paves the way for faster vaccine development

Charybdis Vaccines in Italy has automated phage display/deep sequencing techniques on a Freedom EVO® platform, resulting in faster identification of bacterial antigens and protective antibodies. This system is helping to drive the development of new vaccines against pathogens of major worldwide concern.

Charybdis Vaccines
A spin-off of the University of Messina

Charybdis Vaccines Srl provides vaccine discovery, testing and development services to pharmaceutical companies through its diverse team of microbiologists, immunologists and molecular biologists. Spun out from the University of Messina in 2011, the Company's primary goal was to extend ongoing collaborations between the University and scientists and managers working for pharmaceutical and biotechnology companies. Professor Giuseppe Teti, Research Director at Charybdis, explained: "We are focusing on bacterial pathogens, using phage display techniques and animal models of infection to identify novel antigens of gram-positive bacteria, particularly group B *Streptococcus* and *Streptococcus pneumoniae*, which persist as major health problems worldwide. In phage display, we 'chop' DNA extracted from these pathogenic bacteria into small pieces and insert them into phage genomes. These viruses then express fragments of bacterial proteins on their surfaces, allowing us to use the phages as bait to capture antibodies in patients' sera. We can use this technique to identify bacterial antigens capable of producing antibody responses, which often turn out to be excellent vaccine candidates. We have also used phage display methods to develop a breakthrough technology called PROFILER – Phage-based Representation OF ImmunoLigand Epitope Repertoire – that allows us to probe and categorize the antibody repertoire induced by infection or vaccination in humans or experimental animals."

Staff at Charybdis Vaccines are helping to drive the development of new vaccines against pathogens of major worldwide concern. Left to right: Salvatore Benfatto, Veronica Lanza Cariccio, Deborah D'Aliberti, Maria Domina



In 2012, Charybdis decided to automate its workload to improve throughput, choosing a Freedom EVO 200 platform configured with Liquid Handling and Robotic Manipulator Arms, a six-position incubator, an Infinite® 200 PRO multimode reader for DNA quantification, a HydroSpeed™ plate washer, Te-Shake™, Te-MagS™ and Te-VacS™ modules, and labware carriers for tubes and plates, all controlled by Freedom EVOware® software. "We chose Tecan automated systems because we liked the simplicity and versatility of the systems, as well as the ease of programming them for complex applications," Professor Teti continued. "Another factor was the excellent help and advice we have had from Tecan to automate our techniques. Although we use the Freedom EVO platform for several different applications, the most interesting has been automating the antibody-based selection of phage display libraries, and for this we have worked very closely with the Tecan specialist team."

Automation of the phage display technique begins with the addition of antibody-containing serum to protein G-coated magnetic beads in 96-well plates. Serum and beads are then mixed with phage libraries containing bacterial antigens displayed on phage surfaces, followed by magnetic bead separation of antibody-bound phages from those without antibodies attached. This library selection procedure is followed by pre-sequencing steps on the Freedom EVO platform, including amplification of phage inserts from entire selected or unselected libraries, addition of Illumina® adaptors and amplicon purification. Next generation sequencing on an Illumina MiSeq® platform generates millions of DNA sequences daily from phage display. Professor Teti explained: "We developed this technique in collaboration with Professor Franco Felici (University of Molise) and Professor Concetta Beninati (University of Messina), and it is already opening up exciting possibilities in our work, particularly for vaccinology. Until now, we have only been able to think about antibody response to vaccination or infection in terms of the quantity of antibodies induced. This new technique provides us with qualitative results, giving us a detailed picture of antibody specificities with information about the epitopes and/or protein fragments that are being recognized by the antibodies contained in individual serum samples."

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"We also use the Freedom EVO for automation of phage ELISAs," he added. "The ELISA we run most frequently is for library selection; in this assay, coating of plates with anti-phage antibodies is followed by addition of selected or unselected libraries or individual phage clones, followed by addition of the antibody or serum sample and appropriate secondary antibodies. After validation is complete, we plan to run up to 100 library selections daily, including same-day selection and sequencing. This huge workload would simply be impossible without the Freedom EVO platform, and automation has also reduced the volumes of sera we need to use; we often have only very small quantities of sera from laboratory animals, volunteers and patients, so this is very important. The high dispensing precision that we can now achieve is particularly important for our work, and factors such as human error and hands-on time – which are both major bottlenecks in any high throughput workflow – have been minimized. Sample security, which is always an issue with human sera, is another major advantage of Freedom EVOware, as is data tracking; our main customers in the pharmaceutical industry greatly appreciate total traceability in the services we provide."

Professor Teti concluded: "In the near future we plan to automate cloning and expression of the protein fragments identified by phage display. This will require ligation-independent cloning and automation of expression and purification of resultant proteins, but our experience has shown that the Freedom EVO is simple to use and to program – as well as very flexible – and we look forward to this next step in automating our workflow."

To find out more about Tecan's Freedom EVO platform, visit **www.tecan.com/freedomevo**

To find out more about Charybdis Vaccines, visit **www.charybdis.eu**