

# High throughput hybridoma generation

Tecan's Freedom EVO® liquid handling platform has enabled Nurex to enhance its production of monoclonal antibodies, improving efficiency and increasing the number of clones obtained from each fusion.



From left to right: Dr Franca Mannu, Dr Marcella Orrù and Dr Franco Carta

Nurex is a biotechnology company based in Sassari and Turin, Italy, that specializes in the production of advanced diagnostic tools for biomedical applications in genomics, proteomics and clinical diagnostics. Dr Franco Carta, a researcher at Nurex, explained: "Nurex was established in 1995, developing and producing diagnostic kits for molecular biology applications such as the diagnosis of viral infections and some genetic mutations. Ten years on, we expanded the range to include products and services for proteomics, before introducing the antibody production service in 2007. We mainly supply these antibodies to private research groups or university researchers, and initially used classical manual techniques to produce monoclonal antibodies. As the demand for antibodies grew, we needed to increase production and turned to automation to achieve this."

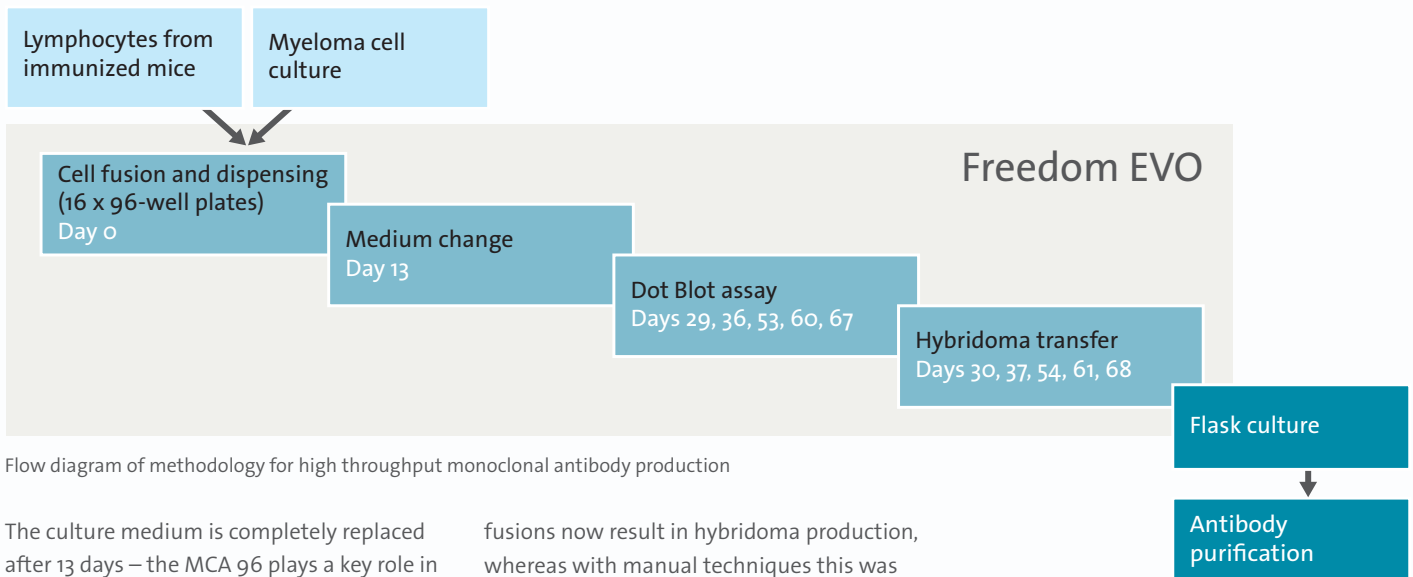
During the evaluation phase, Nurex visited several other laboratories that use liquid handling workstations from various vendors to manage cell culturing and hybridoma generation. "We were impressed by the high flexibility of the Tecan platforms that would allow us to implement a wide range of protocols, and the high throughput they achieve in order to reduce manual operation. We now have a Freedom EVO 200 platform equipped with a four-channel Liquid Handling (LiHa) Arm, a Robotic Manipulator (RoMa) Arm

and a MultiChannel Arm™ (MCA 96)." The configuration also includes a microplate hotel, an incubator and two heated Te-Shake™ modules for 50 ml tubes, to allow Nurex to meet the demand for increasing numbers of monoclonal antibodies. "The similarity of the language we already use for basic programming to that in Tecan's Freedom EVOware® software made the transfer from manual processing to automation much easier," Franco added.

Using the Freedom EVO liquid handling platform, Nurex has developed an innovative, high throughput methodology for monoclonal antibody production that allows hybridoma generation and cell culture maintenance to be performed in a sterile environment, with minimal user intervention to reduce the risk of errors and contamination. Automation ensures that a constant temperature is maintained throughout processing, which improves the quality of the results, and the LiHa guarantees rapid dispensing of consistent reagent volumes of media and buffers. The cellular fusion between a myeloma cell line and spleen cells from an immunized mouse is initiated by adding the required reagents while shaking and heating. The cells are then washed manually and the MCA 96 is used to transfer them to multi-well plates for incubation at 37 °C.

Monday	Tuesday	Wednesday	Thursday	Friday
Medium change	Fusion	Dot Blot	Positive hybridoma transfer	Positive hybridoma transfer
8 x 16 plates 13 day old fusions	8 fusions	8 x 16 plates 29 day old fusions	30 day old fusions	37, 54, 61, 68 day old fusions
	Dispensing		Dot Blot	
	8 x 16 plates		36, 53, 60, 67 day old fusions	

Schedule for high throughput monoclonal antibody production



Flow diagram of methodology for high throughput monoclonal antibody production

The culture medium is completely replaced after 13 days – the MCA 96 plays a key role in this – and a semi-automated Dot Blot assay is used to screen positive clones after 28 days. For this assay, Nurex uses the MCA 96 and a third party pin tool (V&P Scientific) to transfer 100 nl of the culture supernatant from up to 16 fusion plates onto a Hybond™ ECL™ nitrocellulose membrane saturated with antigen, generating a total of 1,536 dots for analysis. The membrane is then incubated with a dye-conjugated secondary antibody, and washed before identification of positive colonies using an Odyssey® infrared scanner (LI-COR Biosciences).

Based on the results from this assay, the Freedom EVO workstation is programmed to transfer the positive cultures from 96- to 48-well plates. The assay and the analysis is repeated on a weekly basis for four weeks and, on each occasion, the positive hybridomas are transferred to another plate format, from 48- to 24-well, 24- to 12-well, and so on. This methodology is at least 100 times more sensitive than ELISA, offering higher accuracy and selectivity, enabling generation of four or five monoclonal antibodies per fusion. In 2010, for example, Nurex performed over 300 fusions. This would be an impossible task if working manually, as only two fusions per day could be handled by manual processes. Automation has enabled Nurex to increase the throughput four-fold, and has eliminated the variable fusion times related to the poor temperature control associated with manual techniques.

“The Freedom EVO workstation has proved robust and easy to use for cell culture, allowing us to automate all our protocols, defining and standardizing all the parameters and generating more reproducible results. All

fusions now result in hybridoma production, whereas with manual techniques this was not always the case, and our quality control procedures – the routine assessment of affinity and specificity – are more accurate,” concluded Franco.

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

To learn more about Nurex, visit [www.nurexbiotech.com](http://www.nurexbiotech.com)

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