An Open Platform, High Speed CD4 T- Cell Monitoring Method Using Immunogold Conjugates: A Method Aimed at Reducing Loss to Follow-up in Resource-poor Settings

Peter Hansen[1], David Dombkowski[2], Michelle DeLelys[2], Amanda Restell[3], Dorothy Sylvia[3], Donald Barry[3], Artem Zeygerman[3], Petra Krauledat[3], Frederic Preffer[2]

1 Union Biometrica Tech, Inc., Canaan, NY, USA
2 Massachusetts General Hospital, Dept. of Pathology, Boston, MA, USA
3 PointCare Technologies, Inc., Marlborough, MA, USA

Immunofluorescent flow cytometry procedures have patients phlebotomized in the morning, samples stained and prepared for CD4 T-cell analysis in parallel, and then batch-analyzed in the afternoon. With this slow protocol, results are unavailable for patients and caregivers before the end of the day, by which time the patient is often geographically distant from the clinic. The need for the patient to return for test results and appropriate therapy can be difficult and contributes to patient “loss to followup”. Additionally, cold chain shipping and the need to maintain immunofluorescent monoclonal conjugates at 2-8C is unreliable and costly.

This study determined that an immunogold CD4 T-cell assay could be used on existing flow cytometers with greater speed and that the temperature stability of the conjugate was superior. A cationic surfactant was used to neutralize immunogold surface charge and enable rapid coverage of CD4 T-cells with gold nanoparticles. This coverage was achieved in 45 seconds and distinct CD4 T-cell clusters were readily detected with forward and side scatter using 488nm excitation. The time to prepare a sample for immunogold analysis was less than one minute, a thirty-fold improvement over immunofluorescent preparatory times. To elucidate the robustness of the immunogold, aliquots were stored continuously for one year at 42C and one week at 50C without diminished activity. In a CD4 T-cell methods’ comparison patient samples were analyzed using immunofluorescence and immunogold light scatter protocols on the same flow cytometer. Bland-Altman analysis showed satisfactory levels of agreement. These results indicate that fluorescence flow cytometers currently deployed for CD4 T-cell analysis can be used with immunogold monoclonal conjugates utilizing a different strategy where results are ready in minutes while the patient waits. The elimination of cold chain shipping and the extension of unrefrigerated shelf life reduce transportation and storage costs and ease procurement.