

Visitect CD4 T-cell test – from concept to the WHO essential diagnostics list

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Background: Measurement of CD4+ T-cell numbers (CD4 counts) is essential for monitoring of disease progression in HIV/AIDS. While CD4 counts are no longer used for treatment eligibility, detection of advanced disease (CD4 <200/mm³) is essential for appropriate management of tuberculosis and other OIs. Despite massive investment in flow cytometry, CD4 counts remain inaccessible in most resource-limited settings. We developed a simple, visual lateral-flow test to measure CD4 counts, based on the hypothesis that cell-associated (full-length) CD4 antigen could serve as a surrogate marker of CD4 T-cell counts.

Methods: We used monoclonal antibodies to the cytoplasmic and extracellular domains of CD4 to provide selectivity for only full-length CD4. HIV+ patients at The Alfred Hospital donated venous EDTA whole blood for analysis. Monocytes were depleted from whole blood using magnetic beads for ELISA, or customised RosetteSep reagents (StemCell Technologies) for lateral flow. CD4 reactivity in lateral flow (colloidal gold) was measured via visual comparison to an internal reference line representing the relevant cutoff (350, or 200 for “advanced disease”).

Results: After depletion of monocytes from whole blood, we showed close correlation of CD4 counts versus CD4 antigen reactivity in ELISA (n=28, 50-1,400 CD4/mm³, R²=0.92). Close correlation was also observed in lateral flow, with ≈90% sensitivity and specificity for visual scoring (either 350 or 200 cutoff) across various cohorts. Following GMP manufacture (Omega Diagnostics, UK), field validation studies in South Africa and other sub-Saharan countries demonstrated similar levels of diagnostic accuracy when used by different cadres of health-care workers. The Visitect™ CD4+ Advanced Disease test is now included on the WHO list of essential diagnostics.

Conclusion: Visitect™ CD4 T-cell tests are now being implemented in HIV programmes in many countries, providing access to accurate CD4 counts at all levels of healthcare facilities for the first time. The novel underlying principles of the test, including selective cell depletion and visual semi-quantitative readout, provide the basis for continuing product development.

Disclosure of Interest Statement: *DA, MG, SC, AL are inventors on the patent licensed from Burnet Institute to Omega Diagnostics Ltd (UK) for the commercialisation of the Visitect™ CD4 tests.*