

Table 1. Defining events in optimizing CD4 T cell enumeration by modern flow cytometry

year	(A) QA programmes	(B) Sample handling	(C) Generic antibodies	(D) Gating methods	(E) Volumetry	(F) Red-diode laser	(G) Multiplex assay
1980-85	—	Lyse-no-wash method [1]	Characterisation of CD45, CD4, CD8 Abs [2-4]	—	—	—	—
1986-89	—	Directly conjugated Mabs	Generic CD4 Mabs are deposited at NIBSC [5]	Primary CD45 gating for leucocyte differentials [6]	—	—	—
1990-92	NEQAS for lymphocyte subsets [7]	↓	↓	Primary CD3 gating [8]	—	—	—
1993-94	↓	↓	↓	↓	—	Red-diode for CD8 [9]	—
1995-96	Stabilized cells for multi-centre studies [10]	CD45 gating is more reliable than scatter used on its own [11]	↓	↓	Powerful volumetric flow cytometer with large biosampler [12]	Detailed concept of red-diode laser cytometry [13]	—
1997	CDC NCCLS guidelines [14]	↓	↓	↓	↓	↓	—
1998	QASI launched for Canada & the developing world [15]	TransFix blood stabiliser is launched [16]	↓	↓	↓	↓	Serology on multiplex beads (suspension array) [17]
1999	NEQAS and NIBSC launch the EuroStandard QA programme in 14 European countries using both long and short-term stabilized preparations [20]	↓	The first paper is published about the possibility of cost-effective CD4 enumeration by flow cytometry [18]	↓	↓	↓	HIV viral load tests using multiplex beads [19]
2000	TransFix is used for intercontinental sample transfer [21]	Generic Mabs are deposited in South Africa and labelled with different fluorochromes	Primary CD4 gating is performed on volumetric flow cytometer using a single CD4 antibody [22]	Cy-Flow (Partec) is launched on the Internet [23]	Industry largely ignores the invitation to produce simple dual purpose instruments [24]		
2001	QASI and NEQAS collaborate in providing complementary, non-profit QA [25]	Panleucogating works well in Transfixed blood [26]	The concept of generic Mabs is supported at the GMHC conference & by WHO [27]	Panleucogating (CD45/4) performs well on double platforms [26]	High capacity (400/d) affordable testing is documented [28]	All concepts of modern flow cytometry work well with red-diode [29]	The clinical need for multiplexing in resource poor setting is summarised [30]
(predictions for) 2002	QA is introduced into "industry-sponsored" HIV monitoring schemes [31]	Industry pays attention to provide test tubes with stabilisers for sample transport	Charity supports bulk production at cost and QC, and provides generic reagents for WHO & UNAIDS	Windows-compatible softwares are written for auto-gating, bar-coding and feedback for customers	Red-diode laser equipped and battery operated prototype instruments are constructed using Windows compatible systems in order to run both cellular flow cytometry and diagnostic tests on multiplex beads – to replace ELISA tests. The latter expand the differential diagnosis of infectious diseases. This is to modernise cytometry that is currently using old, expensive equipment designed >12 years ago [32].		

By the end of 2001 the areas 'A-E' have apparently merged into one major field of interest (cf. Refs.21 and 26)

In 2002 these areas are likely to remain the subject of active study

References: [1] Hoffman, PNAS 1980; 77:4914. [2] Beverley Nature 1980; 287; 332. [3] First Leucocyte Typing Conference, 1983. [4] Bofill CEI 1992; 88: 243. [5] National Institute for Biological Standards and Control, Catalogue, U.K. [6] Loken, Cytometry 1990; 11: 453. [7] Barnett & Reilly, review JCP 2001; 54: 508. [8] Mandy, JIM 1992; 156: 151. [9] Doornbos, Cytometry 1994; 15: 267. [10] Connelly, Cytometry 1995; 22: 200. [11] Nicholson, Cytometry 1996; 26: 16. For more recent confirmation see: Bergeron, submitted. [12] Mercolino, Cytometry 1995; 22: 48. [13] Shapiro: Practical Flow Cytometry, 3rd Ed. 1996. [14] Nicholson for CDC revised guidelines, 1997; NCCLS Document H42A, 1998. [15] Bergeron & Mandy, Cytometry 1998; 33: 146. [16] Barnett, Cytometry 1998; 38: 88. [17] reviewed in Mandy, Suspension Array Technology in: Clin.Lab.Med. 2001. [18] Sherman, JIM 1999; 222: 209. [19] McDade in www.Luminexcorp.com [20] EuroStandards – EU Commission supported international study (chair: Barnett) 1999-2003. [21] Jani, JIM 2001; 257: 145. [22] Janossy, BJH 2000; 111: 1198. [23] www.Partec.de [24] www.AffordCD4.com see ref.18 [25] *AffordCD4 Conference*, London, 21th March 2001; *NEQAS Conference*, Sheffield, 22nd March 2001. [26] Glencross, Cytometry (CCC) in press (Feb.2001) [27, 28] Gonsalves, Summing-up and Janossy, Poster at Monitoring and Diagnostic Tools for the Management of ART in Resource-Poor Settings, GMCH meeting, 11-13.Nov.2001, Washington [29] Janossy, Cytometry (CCC) in press [30] Jani, Lancet Inf.Dis. submitted [31] Multinational companies are currently formulating the plan to organise HIV monitoring for their employees in Africa with the latest QA schemes in place. [32] Mandy, CD4 T cell enumeration for GAP members, Health Canada document. 2001.

RECENT DEVELOPMENTS IN THE AREA OF AFFORDABLE CD4 T CELL ENUMERATION

The observations about the recent trends of flow cytometry in general, and that of CD4 T cell enumeration in particular, are based on the notion that the quality of these assays are of paramount importance. Consequently, ‘economies’ can be regarded as legitimate only when the inexpensive assays maintain optimal quality and efficiency. These considerations are also influenced by requirements that samples need to be processed optimally even when arrive to the laboratory after a relatively long travelling under harsher than normal conditions, i.e. when being dispatched in developing countries. It is also considered that the laboratory equipment used needs to be frugal with a low appetite for electricity and clean water, but useful in performing a diversity of laboratory assays in the differential diagnosis of infectious diseases.

When considered from such a perspective, optimised flow cytometry might be developed and improved as a co-ordinated action in seven related areas (Table I above) which are as follows:

- (i) The introduction of international **quality assurance** programs, such as the NEQAS and QASI, operating with stabilised cell preparations as standards (‘**A**’ in Table I; see ‘*QC and Blood Stabilisers*’ on the website www.AffordCD4.com);
- (ii) The development of simple protocols for **sample preparation** and the availability of short term fixatives (‘**B**’ in Table I; see ‘*QC and Blood Stabilisers*’ on the website);
- (iii) The provision of inexpensive **conjugated reagents** (‘**C**’ in Table I; referred to as the concept of ‘generic’ monoclonal antibodies);
- (iv) The Introduction of easy-to-learn, easy-to-operate automated Windows-based **gating technology** for efficient large scale operation (‘**D**’ in Table I; see ‘*Primary CD4 Gating*’ and ‘*CD4 on Double Platform*’ on the website);
- (v) Simple **volumetric** cytometer design with a single laser for 3-parameter analysis and equipped with a large auto-biosampler capacity (‘**E**’ in Table I; see ‘*CD4 on Single Platform*’ on the website);
- (vi) Reduction of costs by using **red-diode lasers** in battery operated flow cytometers (‘**F**’ in Table I; see ‘*Inexpensive Red Diode Lasers*’ on the website www.AffordCD4.com), and
- (vii) The development of suspension array technology, including **flow-based multiplexed immunoassay**, for the differential diagnosis of infectious diseases using the same or similar portable flow cytometers (see ‘**G**’ in Table I).

When the defining events are listed in a chronological order (Table I) it is apparent that important priming steps have been introduced as early as 1980-1985. The first pioneering paper about the explicit possibility of cost-effective but accurate CD4

enumeration has been published in 1999 (Sherman et al. 1999). During the last two years all the relevant areas have been regularly revisited.

The fast progress in all fields together with the need for affordable CD4 monitoring during inexpensive ART has now led to a new constellation. By the end of 2001 the international scientific efforts in the different fields have congregated into two major fields of interests (see poster *'Affordable CD4 enumeration'* presented at the ECCATH meeting in Athens and CISMA meeting in Ougadougou)

The first major field ('A' to 'E') now involves QA, improved sample handling, simple gating methods using 'generic' monoclonal antibodies (Jani et al., 2001; Glencross et al., 2002) with examples of high capacity affordable CD4 T cell enumeration (see poster *'CD4 T cell enumeration'* presented at the GMHC meeting in Washington).

The second major area ('E' to 'G') is the development of simpler, economical and modern flow cytometers for both affordable flow cytometry and flow-based multiplexed immunoassays. The need for this development is illustrated by three simple facts. First, amongst the flow cytometers currently available on the market, even the latest, most modern instruments were designed as long as 10-12 years ago - without an explicit need for economy. Since then digital processing, red diode laser technology, computer design and house-hold electronics have all changed beyond recognition. Second, informative scoring systems have been constructed to assess the relative merits of CD4 counting methods (ref Mandy: *'Report on CD4 T-cell enumeration for GAP members'*, 2001, for future release). While a new design is valued at a score of 1585, the current technologies can only 'earn' scores <1000 – leading to a question whether we could afford not to progress with such a new venture. Third, the availability of these instruments would inevitably lead to the establishment of a new concept in the clinically led differential diagnosis of infectious diseases (Jani et al., 2002).



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