The Use of Flow Cytometry for CD4+ T Cell Counts in HIV Testing

CD4+T CELLS: Subsets and the Th1/Th2 hypothesis as a logical explanation for acquired immune deficiency

by Nancy Banks MD

In the late spring of 1981, the National Cancer Institute (NCI) and the National Institutes of Health (NIH) were under increasing Congressional pressure to clean up the waste and corruption that characterized their failed ten-year War on Cancer. Threatened by potential funding cuts and layoffs, researchers were desperate to find a "new disease" to protect their careers.

These events coincided with the beginning of the AIDS era, which began with the reported rise of seemingly unrelated diseases in promiscuous homosexual men, junkies and drug addicts.

When politics prevented observers from linking GRID (*Gay-Related-Immune Deficiency*) to deadly behavioral decisions, scientists changed the name to the politically-correct AIDS (*Acquired Immune Deficiency Syndrome*). Under threat of being labeled as bigots and *homophobes*, politicians authorized billions of research dollars to blame something other than the self-destructive behavior of those afflicted.

By 1984, researchers claimed that a unique retrovirus they called HIV (*Human Immunodeficiency Virus*) attacks a subset of lymphocytes called CD4+T cells.[1] [2] [3] [4] They later asserted that *AID* was related to the decrease of lymphocytes that leads to the development of Kaposi's sarcoma, Pneumocystis carinii pneumonia, severe fungal infections and certain other "indicator" diseases, which constituted the *Syndrome* (the S).

This aggregate CD4+T cell hypothesis was based on a laboratory finding using a relatively new technology called **flow cytometry.**[5]

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[1] *Science* (1984) Vol. 224 pp. 497-501
[2] *Science* (1984) Vol. 224 pp. 501-503
[3] *Science* (1984) Vol. 224 pp. 503-506
[4] *Science* (1984) Vol. 224 pp. 506-508
[5] Flow cytometry is a technique for counting and examining microscopic particles by suspending them in a stream of fluid and passing them by an electronic detection apparatus.

Flow cytometry, is a technical procedure used to count microscopic particles. It was initially restricted to research labs including those that were doing blood studies on the original cohort of AIDS patients. To date, it's major disadvantage in its use to assess diseases in humans is that there have been no large-scale population studies to determine the upper and lower limits of *normal* cell counts and no method for standardization of the procedure.

The *Bioinformatics Standards for Flow Cytometry* states:

> "... **there are no standards to report flow cytometry experiments and thus the experiments are irreproducible and unverifiable by independent researchers. Moreover, the lack of standardization prevents a variety of collaborative opportunities to recreate experimental methods and results**."[6]

Despite this lack of standardization, the technology continues to be put to use in the identification and management of human disease – specifically AIDS, without knowing population *normals*. These unreliable and unverifiable test results are often used to treat otherwise healthy asymptomatic patients with deadly drugs. These results are used to send factually-innocent people to prison and to take children from responsible parents.

Low CD4+ T cell counts with level CD8[7] counts were found in many of the early patients who went on to develop the clinical syndrome. The CD4/CD8 ratio continues to be used to assess the progression from HIV positive to AIDS. It is recklessly assumed that flow cytometry results of low CD4+T cell counts is the *sine quo non* of the Syndrome; caused by a "killer retrovirus" identified as "HIV." These assumptions are not only unique to this syndrome but also have a predictive value in determining the trajectory towards death.

Since the definition of AIDS was expanded in 1993, the CD4+T cell count has been used as one of the AIDS predictors. Based on the results of this unsubstantiated and unstandardized test, clinicians prescribe mutagenic and genotoxic drugs to their patients.

The hypothesis that CD4+ T cell lymphocytopenia was a unique finding in AIDS was a theory that was never population-tested by identifying the range of expected values in people who had other illnesses and normal healthy controls of different ages, sexes, or racial ancestry.[8] [9] Since 1988,

[6] "Bioinformatics Standards for Flow Cytometry," http://flowcyt.sourceforge.net/
[7] http://www.tcells.org/scientific/CD8/
[8] Macy, Adelman "Abnormal T-Cell Subsets in Normal Persons," *NEJM* (1988) Vol. 319, pp. 1608-1609

researchers have determined that different sub-populations of CD4+T cells respond to different signaling molecules called cytokines. After 30 years and billions of dollars in wasted research funding, there is still no evidence that the retrovirus attacks cells (cytotoxicity).

Despite an unprecedented propaganda campaign (now called *marketing*) that permeates universities, public education and the media, the "AIDS establishment" has never proved that HIV kills CD4+T cells. But rather than re-evaluate the tenets of the original "killer virus" theory based on known functional parameters and the dual strategy of the immune system, the AIDS theory has evolved into a new claim that HIV works not by direct killing of CD4+T cells by taking over their DNA apparatus to replicate and proliferate, but by getting infected cells to secrete proteins that cause apoptosis (cell death) of "bystander" cells.[10] Like the original "killer virus" hypothesis, this bystander hypothesis is as implausible: "Viral and host mechanisms that lead to bystander apoptosis are not well understood."[11]

The evidence for this is not only thin, but it ignores some basic evolutionary biology and basic cell biology of T cell subpopulations.

IMPORTANCE OF THE TH1/TH2 BALANCE IN IMMUNE FUNCTION

Human blood consists of Red Blood Cells, White Cells, Platelets and Plasma. White cells consist of neutrophils, eosinophils, basophils, monocytes and lymphocytes. There are several subsets of lymphocytes of which the CD4+T cell is one. It is now known that the CD4+T cell matures from a single progenitor type (Th0) into one of four subsets: Th1, Th2, Th17 and iTreg.[12] [13]

AIDS is characterized by a fixed balance shift to the Th2 or humoral arm of the immune system.[14] This new understanding of the balance between Th1 or cell mediated immunity and Th2 humoral mediated immunity has profound implications for the scientific validity of the HIV/AIDS hypothesis.

[9] Jiang et al, "Normal Values fo…"*Clinical & Diagnostic Laboratory Immunology* (2004) Vol. 11 № 4 pp. 811–813
[10] Holm et al., "Apoptosis of Bystander T Cells Induced …" *Journal of Virology* (2004) Vol. 78 № 9 pp. 4541-4551
[11] Ibid
[12] Jimeno et al, "Effect of VIP on the balance between cyt…" *Immunology and Cell Biology* (2011) Vol. 1, № 9
[13] Zhou et al, "Plasticity of CD4+ T Cell Lineage Differentiation" *Immunity* (2009) Vol. 30
[14] Kremer "The Silent Revolution of Cancer and AIDS Therapy" *Raum & Zeit* (2003) Vol. 121, pp. 50-64

TH1 & TH2 BALANCE, REGULATION AND INVOLVEMENT IN DISEASE

By 1988, Mosmann and Coffman[15] presented a major study that first indicated that CD4+ T lymphocytes in fact contained two different types of T-helper subsets. They initially identified these subpopulations as Th1 and Th2[16] and demonstrated that naive Th cells (Th0) in the thymus and bone marrow are differentiated into two types of functional Th cell subpopulations (more have been subsequently identified). Depending on the cytokine signals received, the Th0 cells then mature into either the Th1 or the Th2 helper cells.

The Th1 cells are primarily involved in the regulation of cellular immunity. Cellular immunity is involved in the direct killing of intracellular pathogens such as fungi, viruses and mycobacteria. These cells have the ability to produce a noxious gas NO (nitric oxide) that is capable of destroying intracellular pathogens and is the main operation mode of this arm of the immune system.[17] [18] The majority of Th1 cells reside in the peripheral blood, and it is their depletion that is observed in the progression to AIDS.

Cell-mediated immunity is also involved in the rejection of foreign grafts and the elimination of tumor and virus infected cells. Clinically, cell-mediated immunity is involved in Delayed Type Hypersensitivity[19], contact sensitivity – such as skin rashes that people develop following contact with chemicals e.g. poison ivy, graft rejection in transplant patients, and pertinent to the AIDS issue, the killing of intracellular parasites with NO gas.

The Th2 cells cannot produce NO gas as a means of intracellular killing and are associated with what is called humoral immunity. Humoral immunity involves such cells as CD4+T lymphocytes acting as helper cells by traveling to the bone marrow where they interact with B cells which transform into plasma cells that produce large Y shaped proteins called antibodies. As the NO gas in the Th1 cells kills **intracellular** invaders, the production of antibodies is used to neutralize larger **extracellular** invaders. The Th2 cells reside mainly in the bone marrow and, to a lesser extent, in the lymph nodes. Th2 cells do not appear to become depleted in the progression to AIDS. In fact, their numbers have been observed to increase.[20] [21]

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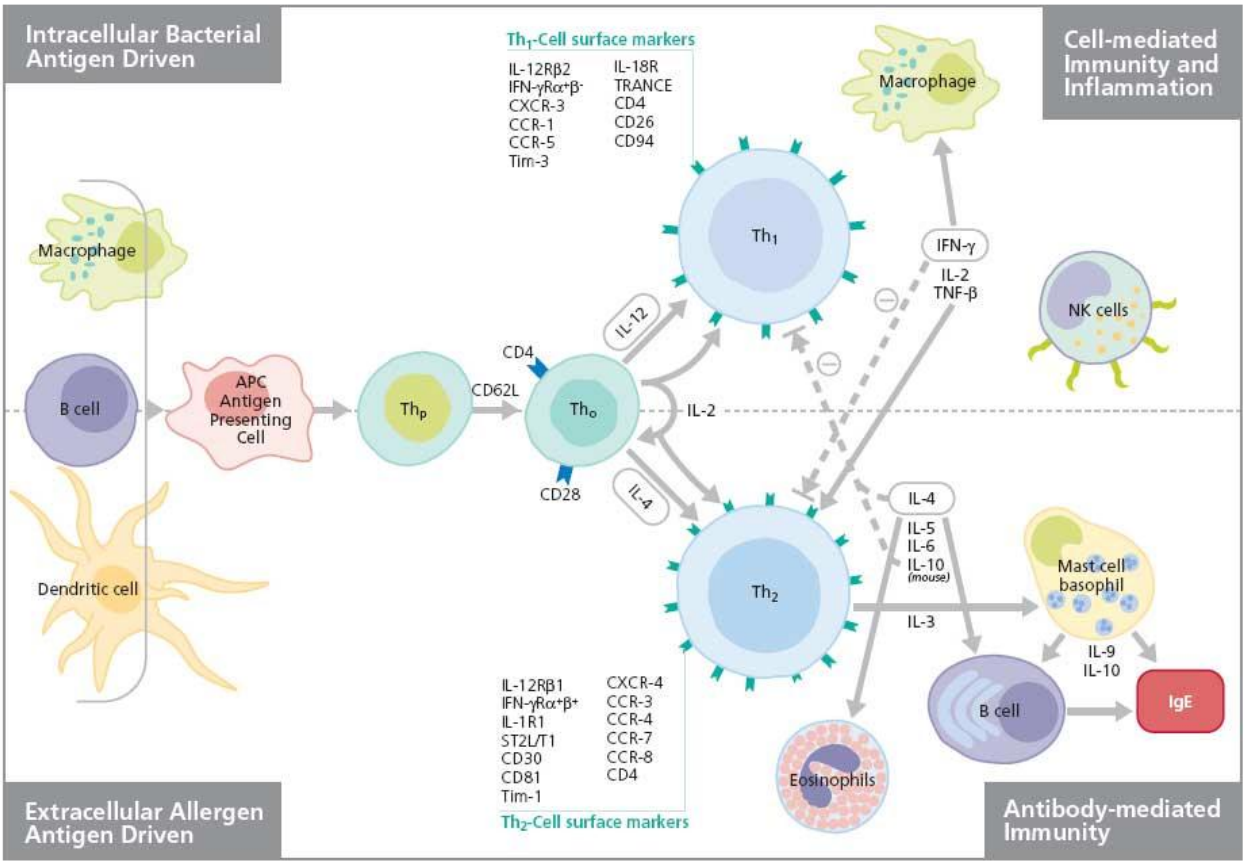
[15] Mosmann, et al., "Two types of murine helper T cell clone..." *J. Immunol*, (1986) Vol. 136 № 7 pp. 2348-57
[16] Mosmann & Coffman, "TH1 AND TH2 CELLS: Different Pat…" *Ann. Rev. Immunol.* (1989) Vol. 7 pp. 145-173
[17] van der Veen "Nitric oxide and T helper cell i…" *Int'l Immunopharmacology* (2001) Vol. 1 № 8 pp. 1491–1500
[18] Singh et al, "Role of Nitric Oxide in …" *Wayamba Journal of Animal Science* ISSN: 2012-578X; P97-P102 2011
[19] Abramson et al, http://emedicine.medscape.com/article/136118-overview#showall
[20] List – AIDS Risk Factors
[21] Mosmann, "Cytokine Patterns During the Progres…" *Science*, New Series (1994) Vol. 265, № 5169, pp. 193-194



Humoral immunity is involved in transfusion reactions, allergies and autoimmune diseases such as Rheumatoid arthritis, systemic lupus erythematosis, Grave's disease and Type I diabetes. It is also the humoral (increased antibody) response that is elicited after infections like measles or mumps which confers immunity in the future or is given passively as the result of vaccination. The vaccine industry claims that vaccines work because of this heightened antibody response. There is a functional humoral immunity in people who are allegedly infected with HIV.

The discovery of the Th1/Th2 dual strategy of the immune system illuminates the mechanisms of AIDS-related immune dysfunction. A gradual shift from Th1 to Th2 dominance is observed. The number of circulating T-cells in the periphery decreases and the number of T cells in the bone marrow and lymph nodes increases.[22]

[22] Ibid

We now know that every corresponding stimulation of the inflammatory Th1 CD4+T cells is answered with a corresponding counter-regulation: a balance shift to the increased stimulation of the antibody synthesis (Th2). This shift typically occurs after about 7 days, after which it is balanced again. This is the case after trauma or an operation. If the balance is not re-established after seven days, the danger of developing microbial sepsis may occur.

Paradoxically, AIDS patients develop no extra-cellular bacterial infections which could be blocked by antibodies. Instead, their elevated antibodies became the basis for the HIV antibody test. The logical consequence then was that the AIDS patients suffered from a lack of Th1 cells whose specialized job it was to send out signals after specific stimulation to other immune cells whose function is to destroy cells which are colonized by **intracellular** opportunistic agents (i.e., fungi, protozoa, mycobacteria and some real existing viruses) using NO. AIDS was the clinical appearance of opportunistic infections as a consequence of a transformation in function and a fixed balance shift in favor of one of the two subgroups of CD4+T cells--the increase in the Th2 cells at the expense of the Th1 cell.[23]

The Th1 to Th2 balance shift explains some of the major conundrums of the AIDS clinical syndrome: Specifically, AIDS is unlike classical immune deficiencies in that patients experience predominantly fungal and mycobacterial infections but very few or no typical bacterial infections. Further, elevated levels of antibodies, including autoantibodies, are characteristic of all AIDS patients – a finding consistent with a decrease in the Th1 subset with a concomitant increase in the Th2 subset.

This is why measuring the aggregate CD4+T cell count and assuming it has some predictive value is disingenuous at best. In many ways, it presents hallmarks of intentional fraud.

According to NIAID Director Anthony Fauci and Nobelist Rolf Zinkernagel, there is no evidence that HIV is cytopathic in vivo, and the focusing on peripheral blood lymphocytes, especially CD4+ T cells, is wrong.[24] [25] [26]

[23] Klein et al, "Demonstration of the Th1 to Th2 cytokine shift during …" *AIDS* (1997) Vol. 11 № 9 pp. 1111–1118
[24] Panteleo & Fauci, "Immunopathogenesis of HIV Infection" *Ann Rev Microbiol* (1996) Vol. 50, pp. 825-854
[25] Zinkernagel, "Are HIV-specific CTL responses salutary or pa…" *Curr Opin Immunol* (1995) Vol. 7, pp. 462-470
[26] Fauci, "CD4+ T-Lymphocytopenia without HIV Infection – No Lig…" NEJM (1993) Vol. 328, № 5 pp. 327-335

Fauci explained:

> "Although most studies necessarily focus on HIV infection of peripheral-blood mononuclear cells, the lymphocytes that are in the peripheral blood at any given time represent only about ***2 percent* of the total lymphocyte pool**, most of which is in the lymphoid organs. Hence, in certain pathologic processes involving lymphoic cells, the peripheral blood may not accurately reflect the status of disease. Specific immune responses are generated predominantly in lymphoid organs rather than in the peripheral blood."[27] (emphasis added).

By analogy, the absence of policemen at a city park does not necessarily mean that the park is unsafe. The park may be safer than a disturbance where policemen are found in larger numbers. While the overall numbers of policemen won't change in a city, their deployment depends upon when and where they are needed.

Despite claims that HIV kills these lymphocytes, Fauci declared:

> "...the primary mechanisms of CD4+T cell depletion in vivo remain unclear; there is no direct evidence that HIV is cytopathic in vivo, despite the fact that cytopathicity can be readily demonstrated in the artificial milieu of culture."[28]

Although no definitive population studies have been conducted to justify the use of CD4+T cell technology in the diagnosis and prognosis of AIDS, it has been found that many conditions affect the composition of lymphocyte subsets in the blood.[29] [30] [31] Therefore, finding a decrease in peripheral blood CD4+T cells does not relieve the clinician of assessing the clinical implications of this finding, even in the presence of a positive HIV test.

Perversely, a decrease in CD4+T cells in the absence of a positive HIV test is now called "idiopathic CD4+T cell lymphocytopenia or ICL". This is a non-specific condition associated with AIDS-defining diseases, non-AIDS defining diseases, and in 5-18% of healthy individuals, some of whom are denied insurance based on baseless flow cytometry tests.[32] Researchers at the University of California at Los Angeles School of Medicine found that 5% of

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[27] Ibid
[28] Panteleo & Fauci, "Immunopathogenesis of HIV Infection" *Ann Rev Microbiol* (1996) Vol. 50, pp. 825-854
[29] Ibid.
[30] Hässig et al., "Reappraisal of the depletion of circulating CD4+ lymph…" *Continuum* Vol.3 № 5 Jan./Feb. 1996
[31] Zonios et al, "Idiopathic CD4+ lymphocytopenia: natural history and…" *Blood* (2008) Vol. 112, № 2 pp. 287-294
[32] *NEJM* (1993) Vol. 328 № 6, pp. 373-379; Ibid. 380-385; ibid. 386-392; ibid. 393-398; ibid. 429-431

healthy persons seeking life insurance had abnormal T4 cell counts and that "in a subgroup of patients, the low T-cell numbers or ratios appear to be stable findings." They concluded:

> "In the absence of a history of a specific infection or illness or major abnormalities on physical examination, it is not worthwhile to attempt to find a specific cause for the abnormality of T-cell subsets... **A uniform approach to this problem throughout the medical community will help alleviate patients' anxiety and reduce the concern of the insurance industry about this relatively common problem."**[33] (emphasis added)

Brian Williams of the World Health Organization (WHO) and his colleagues studied HIV-positive and HIV-negative populations in eight African countries including Ethiopia, South Africa, Uganda and Zambia. They found that between 3 and 5 per cent of HIV-negative people had CD4 counts below 350 and that, when people with low pre-infection cell counts did contract HIV and received anti-retroviral drugs (ARV), they survived for about nine years - the same as people with high counts.[34] These findings call into question just how much is not understood about CD4 cells and their interaction with HIV.

Wrote Williams: "Generally, if you have high CD4 counts you can be considered to be doing pretty well and if you have very low counts, you're in trouble."

But CD4 counts vary naturally. Williams observed that by following the WHO (or CDC) guidelines to the letter, some people started on ARVs *would not even be infected with HIV*. William's assertion is borne out from the results of a literature review of the various disease entities that are characterized by low CD4+T cell counts.

A broad range of pathologies corroborate this laboratory finding, none of which has anything to do with being HIV antibody positive:

- patients of intensive care units
- people with various infectious processes
- patients exposed to injected foreign proteins (vaccines)
- people who have sustained traumatic injuries and burns
- pregnant women
- people who over-exercise or are malnourished

[33] Rett et al., "Abnormal T-cell subsets in normal persons" *NEJM* (1988) Vol. 319, № 24 pp. 1608-1609
[34] Williams et al., "HIV Infection, Antiretroviral …" *Journal of Infectious Diseases*, (2006) Vol. 194, pp. 1450-1458

- there are also daily and dirunal variations of CD4+ T cell counts
- psychological and social stress – especially social isolation – which often occurs with an HIV diagnosis
- people who are prescribed HIV medications (HAART)
- unexplained or "idiopathic" low counts.[35]
- elevated levels of cortisol[36]

The story is more complicated when we consider which T-cell subsets manifest features generally considered to be indicative of HIV, such as reverse transcription and various HIV-associated proteins. Paradoxically, HIV is expressed primarily in Th0 and Th2 cells, and is rarely found in the Th1 subset that is the subset that is actually diminished.[37] [38] [39]

These facts further challenge the prevailing HIV=AIDS theory, which fails to account for this selective decline in the Th1 sub-population. What the "AIDS Industry" calls HIV is not found in these cells, but in the Th0 progenitor cells and Th2 cells that actually increase. Unfortunately, the "medical standard of care" ignores these facts and continues to use flow cytometry to establish aggregate CD4+T cell counts.

VIRAL LOAD (VL) TESTING

Finally, there has been shown to be no relationship between the Viral Load as measured by the HIV-RNA level and changes in the CD4+T cell count. A study from *The Journal of the American Medical Association* found that changes in viral load were only able to explain 4% of changes in the CD4+ count in the patients observed.[40] Mathematically, this means that viral load is not able to explain 96% of the variation in CD4+T levels and therefore other mechanisms, as discussed above must be responsible for the observed decline. The implications of this are profound: if viral load is correlated neither with infectious virus nor with CD4+ T cell levels, this means the measurement (by flow cytometry) constitutes insufficient evidence for making clinical decisions.

[35] Irwin, "Low CD4+ T Lymphocyte Counts" VirusMyth, Feb 2001
[36] Hässig et al., "Reappraisal of the depletion of circulating CD4+ lymph…" *Continuum* Vol.3 № 5 Jan./Feb. 1996
[37] Abbas et al, "Functional diversity of helper T lymphocytes" *Nature* (1996) Vol. 383 № 31: 787-793
[38] Maggi et al. "Ability of HIV to Promote a TH1 to THO Shift and to Repl…". *Science* (1994) Vol. 265 pp.244-248
[39] Culshaw, "Mathematical Modeling of AIDS Progression: Limitations…" *JAPS* (2006) Vol. 11, № 4, pp. 101-105
[40] Rodriguez et al, "Predictive Value of Plasma HIV RNA Level o…" *JAMA* (2006) Vol. 296, № 12, pp. 1498-1506

For a more detailed discussion of the Th1/Th2, NO cell symbiosis theory please see Dr. Heinrich Kremer's: A Silent Revolution in Cancer and AIDS Medicine and The Concept of Cell Symbiosis Therapy.

With this basic understanding of the differentiation and function of the Th1 and Th2 subsets of CD4+T cells and knowing that only the aggregate CD4+T cell population is measured by flow cytometry, we can now evaluate the principles of flow cytometry and its very obvious failings as currently employed as a useful useful tool in the diagnosis and management of AIDS.

FLOW CYTOMETRY

The aggregate CD4+T cell count is measured by a process known as flow cytometry.[41] [42] Flow cytometry is the measurement of characteristics of single cells suspended in a flowing saline stream moving through a beam of light. Many scientific procedures involve obtaining measurements as average values for the whole population. Flow cytometry differs from flow cytometric analysis measurements, which are made on individual particles within the suspension.

Additionally, several parameters can be measured on tens of thousands of individual cells within a few minutes: relative size, relative granularity or internal complexity, and relative fluorescence intensity.[43] Any suspended particle or cell from 0.2-150 micrometers in size is suitable for analysis. The noted characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence.

However, flow cytometry has numerous intrinsically fatal shortcomings:

- While the observed cell stream is three dimensional, the scatter pattern that is generated on computer print-out of the cellular density stream is measured in two dimensions.

- The ability to identify live individual cells of a particular type from dead cells, clumps of cells and debris by a process known as *gating* is limited by the training and expertise of the observer-technician.

[41] Rahman, "Introduction to Flow Cytometry," ADB SeroTec (2006)
[42] "Introduction to Flow Cytometry," BD BioSciences (2000)
[43] http://en.wikipedia.org/wiki/Fluorescence Spectroscopy

- There are no established standards for the technology of operators. Procedures vary between each technician and lab.

- All FDA-approved flow cytometry devices are based upon "predicate devices" technologies that were marketed before May 28, 1976.

- Researchers claim that the distinguishing characteristics of live individual cells from dead cells and debris can be accurately preserved *following paraformaldehyde fixation* (**which kills all of the cells**).[44]

A **FLOW CYTOMETER** is comprised of three main systems: fluidics, optics and electronics.

1. The **FLUIDICS SYSTEM** transports particles in a stream to the laser beam for interrogation.

2. The **OPTICS SYSTEM** consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.

3. The **ELECTRONICS SYSTEM** converts the detected light signals into electronic signals that can be processed by a computer. For some instruments equipped with a sorting feature, the electronics system is also capable of initiating sorting decisions to charge and deflect particles.

Flow cytometry evolved from the development of several fields: microscopy; dye chemistry; electronics; and computers. It was the fusion and advances of these diverse technologies that allowed for the evolution of flow cytometry.

Modern flow cytometry started at the Los Alamos National Laboratories in New Mexico and entered the marketplace by the mid-1970s. After scientists alleged in 1984 that HIV was killing CD4+T cells, researchers developed an advancement they called **IMMUNOPHENOTYPING**.

Immunophenotyping is the analysis of heterogeneous populations of cells for the purpose of identifying the presence and proportions of the various populations of interest. Antibodies are used to identify cells by detecting specific antigens expressed by these cells, which are known as markers.

[44] Martz, Introduction to Flow Cytometry (2000)

These markers are usually functional membrane proteins involved in cell communication, adhesion, or metabolism. Immunophenotyping using flow cytometry has become the method of choice in identifying and sorting cells within complex populations and is used extensively in the diagnosis and management of AIDS. However, as noted above, the CD4+T cell population is not uniform and the nature of the Th1/Th2 shift has more to do with the development of AID than the aggregate decline of these cells.

FROM RESEARCH TO JUNK SCIENCE

In 1972, Congress established the OTA[45] (Office of Technology Assessment) to serve the legislative branch as an independent source of information and analysis about complex scientific and technical issues. OTA construed health technology broadly, including "all elements of medical practice that are knowledge-based, including hardware (equipment and facilities) and software (knowledge skills)... the set of techniques, drugs, equipment, and procedures used by health care professionals in delivering medical care to individuals and the systems within which such care is delivered."

By 1978, the OTA produced a shattering report on the state of scientific medicine, *Assessing the Efficacy and Safety of Medical Technologies*.[46] The report stated:

> "Evidence indicates that many technologies are not adequately assessed before they enjoy widespread use...Many technologies which have been used extensively have later been shown to be of limited usefulness"...and " ... **only 10 to 20 percent of all procedures currently used in medical practice have been shown to be efficacious by controlled trial."** (emphasis added)

The report implied that 80% to 90% of all routinely-performed procedures are unproven – a conclusion that implicates the technology of flow cytometry that uses immunophenotyping to identify antigen markers on various cell populations.

[45] http://www.fas.org/ota/
[46] Office of Technology Assessment, "Assessing the Efficacy and Safety of Medical Technologies" OTA, Sep 1978

The *U.S. News and World Report* issue of 23 November 1987 raised questions about HIV tests:

> "With public health officials and politicians thrashing out who should be tested for HIV, the accuracy of the test itself has been ignored. A study last month by the Congressional Office of Technology Assessment found that HIV tests can be very inaccurate indeed. For groups at very low risk – people who do not use IV drugs or have sex with gay or bisexual men – 9 in 10 positive findings are called false positives, indicating infection where none exists."

Clearly, OTA's warning continues to be ignored by the medical and scientific communities and the politicians and agencies that enable them.

In 1996, Congress disbanded the OTA, leading to the systematic deregulation of the various medical technology industries. The U.S. Supreme Court sealed the fate of future scientific transparency by ruling in favor or corporate interests in the Citizens United v. Federal Election Commission (2010), which provides personhood to corporations.

The OTA's demise closed a low-budget item that simply gave Americans too much information to make informed choices. It paved the way for the establishment of a medical and scientific knowledge monopoly that is now permeated by corruption and fraud (junk science).

As a result, the "AIDS industry" continues to use unproven technologies like flow cytometry to diagnose, alarm, and treat healthy people with toxic and expensive drugs that are designed to make them sick (see video).

Fundamental issues regarding the limitations of flow cytometry technology and the propriety of its use in the diagnosis of acquired immune deficiency have never been addressed:

- **PROOF** that an exogenous virus uniquely attacks CD4+T cells and is cytopathic (see above discussion). Further, the relationship of the Th1/Th2 balance shift in the CD4+T cell population has been ignored.

- **REPRODUCIBILITY –** samples drawn concurrently from one subject should deliver the same results in every machine and laboratory that receives those specimens.

Police officers have successfully used *gas chromatography* (GC) in the enforcement of drunk driving laws for many years. When properly operated, a functional and calibrated Breathalyser™ not only measures blood alcohol levels in breath samples accurately (sensitivity), but they can reliably differentiate between subjects that have – and have not – consumed alcohol (specificity).[47]

Police officers also use RADAR to enforce basic speed laws. But like GC, officers do not rely upon RADAR devices to enforce laws. Instead, officers rely upon their training and expertise to estimate intoxication and velocities. Once they develop articulable facts that indicate impairment or an unsafe speed, they use GC and RADAR *to confirm* what their training, expertise and observations initially tell them.

Unlike GC and RADAR, flow cytometry manufacturers invent proprietary algorithms to report spurious and unreliable cell counts. There is no reliable method for counting standardization for products and operators, and the substantial deviations in technical competency and quality control of test samples between labs render these tests wholly unreliable. Clinicians simply order blood draws and *presume* that lab results are accurate and meaningful. Clinicians who receive kickbacks from labs and drug companies have little incentive to question results of asymptomatic patients.

This protocol is akin to policemen who stop 35mph motorists because their RADAR device captured a 90mph reading. But while police agencies would train or terminate such derelict employees, this practice – when applied to biological testing and flow cytometry – is characterized as the "medical standard of care."

Flow cytometry employs two techniques to count cells:

- **DUAL PLATFORM SYSTEMS** – One component determines cell concentrations, while the second determines the relative number of CD4 and CD8 cells. Unless these two components count a common parameter, dual platform systems cannot accurately correlate the results.

- **SINGLE PLATFORM SYSTEMS –** These platforms are especially designed to count the absolute numbers of antibody-labeled cells. These devices are equipped with multiple sample loader, programming facility and computer support, which removes the need for using two

[47] www.intox.com/t-aboutalcoholtesting.aspx

different machine to determine the concentration of CD4 and CD8 cells.

The variability of results when comparisons are made between these two counting systems has been shown to be as high as 56%.[48]

- **STANDARDIZATION –** HHS, CDC, NIH and FDA have failed to produce meaningful guidelines for quality control, quality assurance or quality of test reagents.

Flow cytometric immunophenotyping is a relatively new technology that is highly complex, involves multiple components and procedures, and has numerous points for measurement vulnerability. As the technology moved from research laboratories to clinical laboratories, the need for standardization increased. In response to that need, guidelines addressing aspects of the CD4+T lymphocyte testing process – in particular, quality control, quality assurance, and consistency of reagents for immunophenotyping of lymphocytes were developed. (National Institute of Allergy and Infectious Diseases (NIAID)/AIDS Clinical Trial Group: Guidelines for hematologic and low cytometric analysis of ACTG specimens, 1992).

Hoping to assure the accuracy and reliability of CD4+T cell test results obtained within individual laboratories and to attempt to assure comparability of results between laboratories, the CDC established a list of standard methods for performing the test, as well as guidelines for quality control and quality assurance. The CDC's recommendations for flow cytometry apply to laboratory safety, specimen collection, specimen transport, maintenance of specimen integrity, specimen processing, flow cytometer quality control, sample analyses, data analysis, data storage, data reporting and quality assurance.[49]

As can be seen, there are multiple steps in this process, any of which that if violated can lead to a substantial alteration in the test results:

1. **Blood collection:** The type of vial, the time of day and the temperature at which the specimen is handled can all have an effect on test results. CD4+T cell counts are known to be higher in the afternoon than in the morning – a result of the response to the diurnal variation in steroid production from the adrenal gland.

[48] Whitby et al, "Quality Control of CD4 T-Lymphocyte Enumeration: R…" *Cytometry* (2002) Vol. 50, pp. 102-110
[49] CDC, "Guidelines for Performing Single-Platform Absolute CD4…" *MMWR*, January 31, 2003 / 52(RR02);1-13

2. **Specimen transport:** Was the specimen maintained at room temperature? If the specimen is too hot or too cold, cellular destruction might occur. This can be a major problem for transporting of the specimen outside of the collection facility.

3. **Specimen Integrity:** When the specimen was received, was it too hot or too cold? Was the blood hemolyzed or frozen? Are there visible clots? Has the specimen been received > 72 hours after collection? If so, the specimen must be rejected.

4. **Specimen processing:** The test should be run within 48 hours, but no later than 72 hours after drawing the blood. Procedures that must be followed:

    a. Gently rocking blood for 5 minutes to ensure uniform sample

    b. Pipetting accurate blood volumes; vortex sample tubes to mix the blood and reagents and break up cell aggregates

    c. Quality and type of reagent used

    d. Incubating tubes in dark during staining procedure

    e. A lyse/no wash method which requires following manufacture directions (each manufactures has a different set of directions and a different counting algorithm)

    f. An immediate capping and storing all stained samples in the dark under refrigeration until flow cytometric analysis is performed.

    g. It is advised that the specimens be stored no more than 24 hours unless the laboratory can demonstrate that scatter and fluorescence patterns do not change for specimens stored for longer periods.

5. **Machine calibration:** Variations in absolute lymphocyte counts obtained by different automated cell counters exposes another problem. A review of four widely used automated counters (Technicon H*1, TOA NE8000, Coulter STKS, and Abbott CD3000) indicate that analytic variability in the absolute lymphocyte counts due primarily to method variability, is significant and larger than the variability typically

observed on inter-laboratory trials of relative CD4+T cell counts. These method biases cannot easily be reduced by calibration, since **the cell classification algorithms are built in features** of the various counters.[50]

As can be seen, the process of flow cytometry using immunophenotyping requires not only a sophisticated level of technical skill, but a chain of delivery and processing events that is probably difficult to replicate from lab to lab, but also to substantiate. One study found errors of 18% and 35% of absolute CD4+T cell count,[51] while another study found the inter-laboratory variability so significant that it led to conflicting treatment recommendations.[52] Sax concluded that "when strict thresholds of CD4 cell counts are used as a basis for treatment recommendations or for diagnosis of AIDS, inter-laboratory variability may be sufficient to alter the decisions made."

PRODUCT RECALLS

Since 2004, the FDA has issued 66 recalls of flow cytometry products, devices, components and computer software.[53]

Examples:

Company: BD Biosciences
Product: FACSDiva Software
Reason: When data file containing one or no fluorescence (SIC) parameters is exported, the **software will automatically apply compensation** to this file and all subsequently exported files.
Units: 1074, US and worldwide distribution

[50] Simson et al, "Variability in Absolute Lymphocyte Counts Obtained by…" *Cytometry* (1995) Vol. 22 pp. 26-34
[51] Kunkl et al, "Grading of laboratories on CD4+T lymphocyte evaluations…" *Cytometry* (2002) Vol. 50:117–126
[52] Sax et al., "Potential Clinical Implications of Interlabo…" *Clinical Infectious Diseases* (1995) Vol. 21 pp.1121-5
[53] FDA, as of 29 Oct 2011

Company: Quantimetrix Synovialscopics
Product: Synovial Fluid Control
Reason: This recall was initiated due to **efficacy concerns with the stabilized erythrocytes, leukocytes and lymphocytes contained** in this product group
Units: 24,937 Nationwide distribution

Company: Cytosol Opthalmics
Product: ShellGell Sodium Hyaluronate 0.8mL syringe, 12 mg/ml.
Reason: Product **sterility may be compromised** due to incomplete heat seals in the cannula pouches that are included with the viscoelastic syringe.
Units: 3,720 California and North Carolina

Company: Beckman Coulter, Inc.
Product: Cyto-Stat/Coulter Cone B6-RD
Reason: Diminished expression on B-cell population when drawn in EDTA tubes, which **may lead to inaccurate interpretation of phenotype results**
Units: 1053 Nationwide and Canada

The FDA has issued numerous warning letters to **PointCare Technologies**, a leading developer and producer of flow cytometry products. In its latest warning letter dated 14 June 2011, the FDA cited PointCare's repeated and unresolved problems with their testing equipment, reagents and manufacturing facilities:

> *"...*two of these three lots **failed the specifications** for osmolality and optical density (OD)... **devices are adulterated**... their manufacture, packing, storage, or installation are **not in conformity** with the Current Good Manufacturing Practice (CGMP) requirements... **did not perform adequate stability studies** after changing the packaging of **CD4NOW** Gold Reagent to determine an accurate shelf-life for the product... functional performance of the gold pack was **not acceptable**... **failed to provide scientific justification** for performing gold functional testing on only one vial per lot... **Failure** to establish, maintain, and implement a corrective and preventative action procedure... due to an AC charging **cable/adapter bursting into flames** while service personnel charged the unit... **Failure to have quality audits** conducted by an individual that does not have

> direct responsibility over the matters being audited..." (emphasis added)

THE COOLFONT ESTIMATE

In May, 1986, a group of approximately 100 health experts from the CDC met in Coolfont, West Virginia to discuss the "AIDS Epidemic". Their agenda was, in part, to **estimate** the number of HIV infected persons in the U.S. They arrived at an **estimate** of between 1-1.5 million – a number based primarily on the 1948 *Kinsey Report,* which **estimated** the number of homosexuals, IV drug users, hemophiliacs and heterosexuals who occasionally took part in homosexual acts in 1948.[54]

Thirty years since the "AIDS Epidemic" began, the US population swelled from 228 million[55] to more than 312 million (one-third).[56] Despite the alleged threat and prevalence of HIV, the CDC's latest reports show that the initial estimates have remain unchanged at 1.2 million.[57] Even if these Kinsey-based estimates were reliable, they would represent a little more than ***one-third of one percent of the total US population***.

Throughout the history of HIV and AIDS, the CDC has struggled with profound statistical discrepancies not only in the United States,[58] but also in countries like Italy, [59] [60] Australia,[61] and South Africa [62] [63].

To adjust for these statistical shortcomings, the CDC:

> … revised its HIV testing recommendations to include implementation of routine, voluntary HIV testing in health-care settings for all persons aged 13-64 years. To implement these recommendations, the DCDOH (DC Department of Health) engaged multiple community-based and clinical providers throughout DC to perform rapid HIV screening, **launched extensive social marketing campaigns** to educate DC residents and providers about routine HIV testing, and trained

[54] Robert Sweet, "AIDS Statistics" White House Memorandum 18 Nov 1987
[55] http://www.census.gov/popest/archives/1990s/nat-total.txt
[56] http://www.census.gov/main/www/popclock.html
[57] "HIV in the United States," CDC (Nov 2011)
[58] Armstrong et al, "Trends in Infectious Disease Mortality in the United St…" *JAMA* (1999) Vol. 281, № 1 : 61-66
[59] Ruggiero et al, "Aids denialism at the ministry of health," *Medical Hypothesis* (2009)
[60] Ruggiero et al, "On the risk of contracting AIDS at t…" *IT.J. Anat. Embryol*, (2009) Vol. 114, № 2/3 pp. 97-108
[61] Annual Surveillance Report, Australia 2009
[62] South Africa Statistics - Surveillance Report, 1997-2003
[63] South Africa Statistics - Surveillance Report, 2007

> providers to facilitate immediate linkage to care among those testing HIV-positive… [64]

Although the FDA has NEVER approved HIV, PCR or VL tests for diagnostic purposes, the CDC promotes social marketing campaigns to increase testing and reporting based upon unreliable HIV, PCR and viral load tests, and CD4 counts (e.g. flow cytometry):

> "The proportion of cases that had a CD4 count within 3 months of a new HIV diagnosis was used as an indicator of entry to HIV care. Since the start of AIDS reporting, DCDOH has received laboratory reports of CD4+ cell counts, and in more recent years, HIV viral load tests, and has matched these reports to HIV case surveillance data. In accordance with national recommendations, DCDOH recommends that the first visit to a health-care provider be within 3 months of HIV diagnosis.[65]

Unsurprisingly, the CDC's social marketing campaign resulted in increases of alleged infections wholly unsupported by competent clinical diagnoses – based entirely upon tests that cannot be used for diagnostic purposes.

MARKETING

Of the 66 aforementioned recalls and warning letters, FDA complaints were issued for failures that typically result in low CD4 counts. For agencies that need low counts to claim high HIV infection rates e.g. revenues (and for clinicians who accept kickbacks and bribes from companies like Bristol-Myers Squibb [BMS] and Gilead Sciences), flow cytometry helps clinicians justify the unnecessary delivery of toxic HAART therapies to healthy patients.

Flow cytometry is especially helpful in places like Africa, where mining companies routinely dump toxins and heavy metals into waterways.

One company is Kilembe Mines Limited, which is trying to sell an operation that has polluted the environment and water supplies of southwestern Uganda since 1956.[66] Because foreign investors are reluctant to assume liability that comes with the purchase of toxic waste dumps, clinics and medical devices that blame pollution-caused ailments on HIV offer significant advantages to prospective investors.

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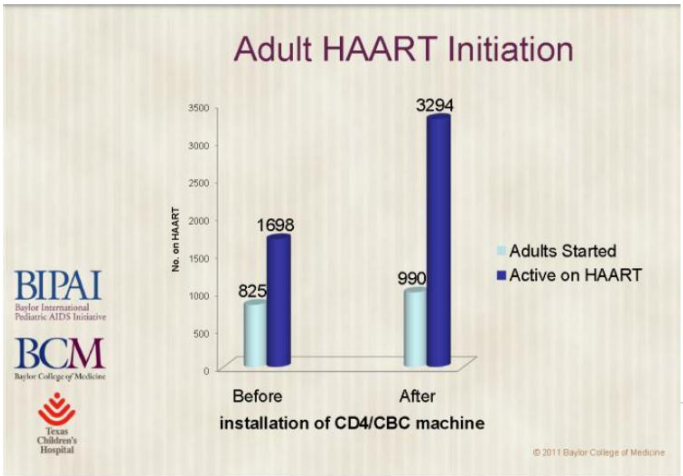
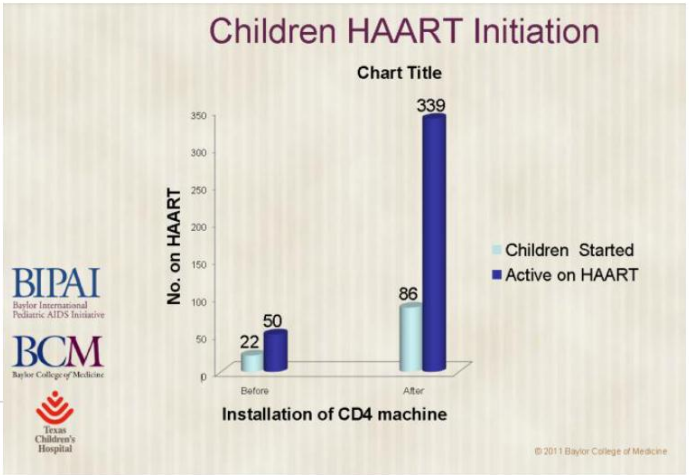
[64] CDC, "Expanded HIV Testing and Trends in Diagnoses of HIV Inf…" *MMWR*, June 25, 2010 / 59(24) ; 737-741
[65] Ibid. *Diagnosis* meaning "test results," not a clinical diagnoses as required by all HIV test package inserts.
[66] Hartwig et al, "Lake George as a Sink for Conta…" *Mine Water and the Environment* (2005) Vol. 24 pp. 114–124

According to this report, the Uganda Catholic Medical Bureau (UCMB) has distributed drugs in this area for 30 years.

In an effort to "scale up access to antiretroviral therapy…" UCMB advised that "treatment procedures and the monitoring of clients are **simplified so that lower cadres of health workers can be trained to carry out some of the simpler functions**… (of HIV testing, diagnosis and treatment)."

Gloria Kakuru of the Baylor University's International Pediatric AIDS Initiative (BIPAI) explains how PointCare Now helps these "clinicians" diagnose HIV in the area surrounding the Kilembe mines – claiming that the device is superior because it justifies earlier initiation of HAART medication in adults and children:





These inflated misdiagnoses are then used by agencies like UNAIDS, the World Health Organization (WHO) and drug companies like Bristol-Myers Squibb (BMS), GlaxoSmithKline, Merck (and others) to push deadly HIV drugs – usually at taxpayer expense.[67]

Although labs continue to use PointCare Now and its CD4 Gold reagents, the recent FDA warning letters do not appear on the PointCare website or in any of these marketing materials – nor is there any evidence that PointCare, clinical laboratories or advertisers made any attempt to contact patients who have been misdiagnosed by the faulty equipment and protocols, poisoned by HAART medications or criminally convicted for having sex.

[67] Most of these are heterosexual cases, which amfAR co-founder Joseph Sonnabend MD admits has been a "fraud and a hoax" since 1987, when it was designed to generate revenues.

RELIABILITY

Dr. Marion Joppe describes "The Research Process" this way:

> "The extent to which results are consistent over time and an accurate representation of the total population under study is referred to as *reliability*. In other words, if the results of a study can be reproduced under a similar methodology, then the research instrument is considered to be reliable."[68]

Theoretically, reliable tests should deliver the same results no matter how many times it is applied to random members of the same target groups. Because of the failure to conduct population studies that would have helped researchers understand CD4+ variability with age, sex, race, time of day or health status; along with the lack of standardization, the reliability of the HIV test results of the absolute CD4+ T cell value by flow cytometry is, at best, wholly unreliable.

While caution must be exercised in the interpretation of unreliable results, this has not been emphasized by the CDC. White blood cell counts can vary substantially from day to day and may account for shifts of as much as 50 to 150 in normal adults, although the degree of this change may be less in individuals with lower CD4 counts. Also substantial variability exists from laboratory to laboratory; those that do not perform cell count procedures frequently – or do not have quality assurance programs – can be expected to produce inaccurate test results. Compounding this problem, an extended delay of more than 48 hours between the time of sampling and actual specimen processing will result in inaccurate values. Therefore, if a laboratory does not perform the test on a daily basis, or if, for example, blood is drawn on Friday and processed on Monday, the test results may be inaccurate. Another common source of inaccuracy is refrigeration of the blood sample.[69]

Because caution conflicts with their ongoing social marketing campaigns, the CDC ignores the known science of CD4+T cell subsets, the Th1/Th2 dual strategy of the immune system, the function of nitric oxide in cell mediated immunity and the possibility for reversal of this immune imbalance with inexpensive nutracuticals, antioxidants and detoxification.

[68] M. Joppe, *The Research Process*, 2000
[69] *AIDS and the Law*, 3rd Ed. (1997) by David Webber

During the two years of its involvement in HIV-related criminal cases and its review of medical and laboratory records, OMSJ has found no evidence that US laboratory facilities exercise caution in the use of flow cytometry equipment or have met any of the CDC's recommended standards. Instead, laboratories shroud their operations in secrecy, which is only exposed when laboratories like Quest Diagnostics pay $241 million in fines for fraud and kickbacks or $302 million for illegally marketing misbranded diagnostic equipment.

Given these findings, there is no credible evidence that HIV is responsible for the decrease in CD4+T cells in acquired immune deficiency or that a decrease in CD4+T cells is a unique finding in people who are HIV+: Nor is there any credible evidence that the current usage of flow cytometry technology is justified as a diagnostic therapeutic tool in the current HIV/AIDS paradigm.