Four-color T and B cell ELISPOT for simultaneous detection of analytes

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ABSTRACT:

ELISPOT assays are a key research tool for enumerating antigen-specific T and B cells in PBMC. As both T and B cells occur in major classes, immune monitoring has to be concerned with identifying these as well. So far, ELISPOT assays have been primarily done single or double color. We report the development of four color T and B cell ELISPOT assays. Cells secreting any of the four analytes can therefore be identified unambiguously and without overlap in an automated fashion, without the need for compensation. Cells co-expressing analytes can be identified by superimposing the individual detection planes. Studying B cells and T cells experimentally has permitted us to verify the accuracy of co-expression measurements. Each B cell secretes only one type of Ig class/subclass. T cells, in contrast, frequently co-express cytokines. Serial dilution experiments showed that for T cells the numbers of co-expressers linearly decreased with the numbers of cells plated. For B cells, no co-expressers were found.

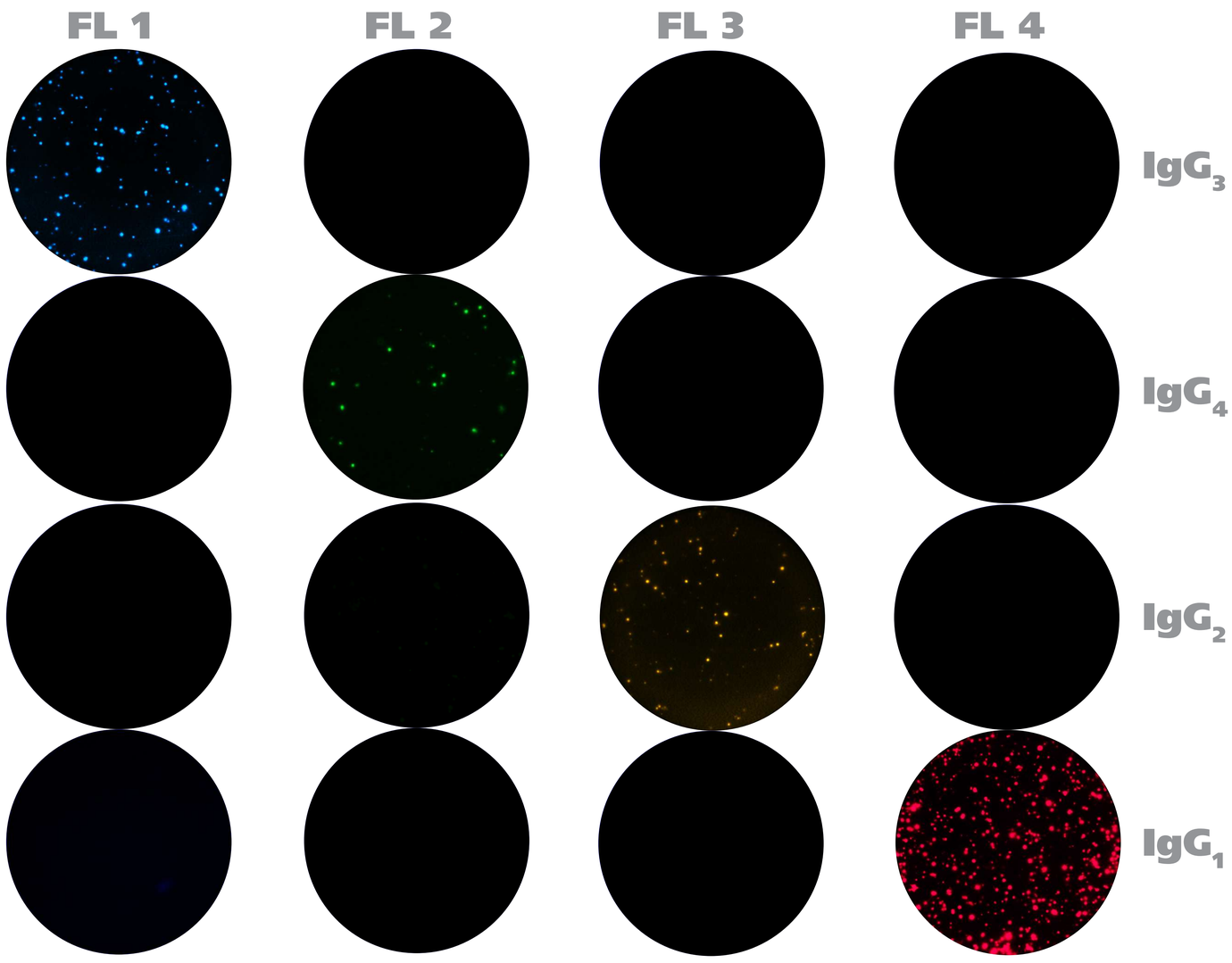

Figure 1: **Unambiguous detection of the four fluorescent tags identifying the individual IgG subclasses.** In each row a single-color B cell ELISPOT assay was performed using the respective IgG subclass-specific detection reagent, as specified. Each assay was analyzed with filter settings optimized for the individual colors. Note: there is no cross-bleeding of colors.

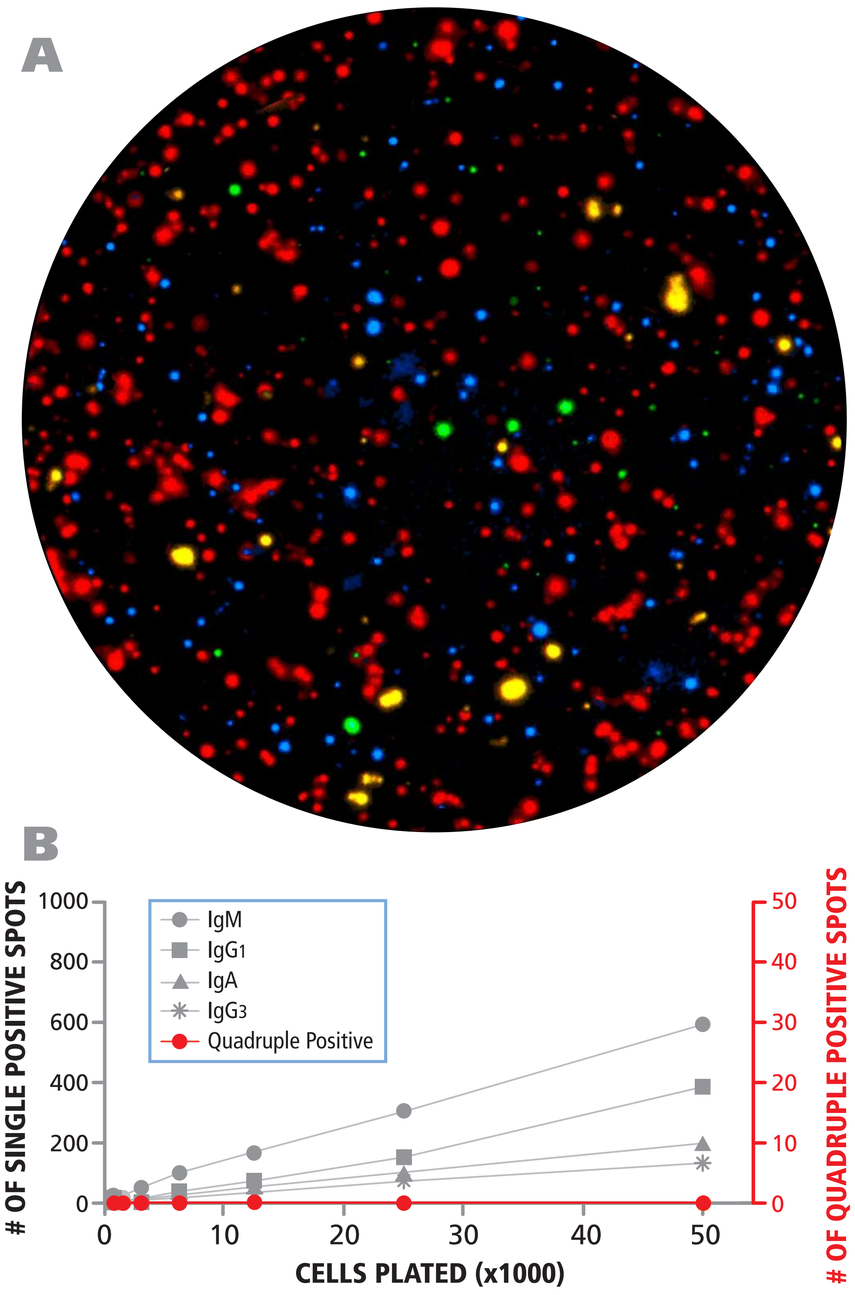

Figure 3: **Representative four-color B cell assay (A) and its analysis (B).** Pre-activated B cells were seeded in a four-color B cell assay in the specified cell numbers per well (X axis) detecting the number of cells secreting the specified individual antibody classes/subclasses in the four non-cross-bleeding detection planes (number of single color spots, Y axis on the left). A linear relationship is seen for all four individual analytes. No quadruple positive spots were seen at the cell numbers tested (shown in red). Spots apparently quadruple positive would have resulted from random overlays of single color spots because individual B cells are known to secrete only one antibody class/subclass.

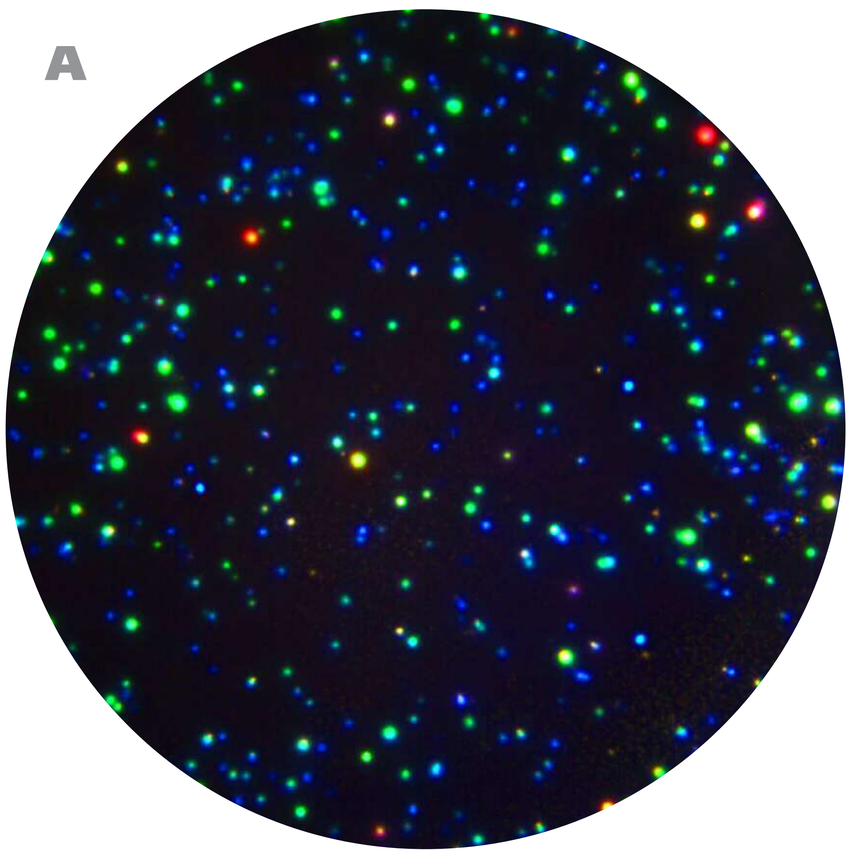

Figure 2: **Representative four-color T cell assay (A) and its analysis (B).** Pre-activated T cells were plated in the cell numbers specified per well on the X axis and a four-color T cell assay was performed detecting cells secreting Granzyme B, IFN-γ, TNF-a, and IL-2, as specified by symbols in the insert. Cells secreting all four analytes (quadruple positives) were determined for each cell dilution (shown in the red line) referring to the Y axis on the right, also in red. The linear relationship between cells plated and quadruple secretors suggests co-expression by individual cells rather than by random overlay of cells. (The linear relationship was also seen at the level of dual and triple expression for all of these cytokine combinations – data not shown). The number of quadruple positive cells relative to the number of cells plated is shown in percentage above each data point in parentheses, the average being 11.3%.