

4-color B cell FluoroSpot for simultaneous detection of all IgG subclasses and Ig classes

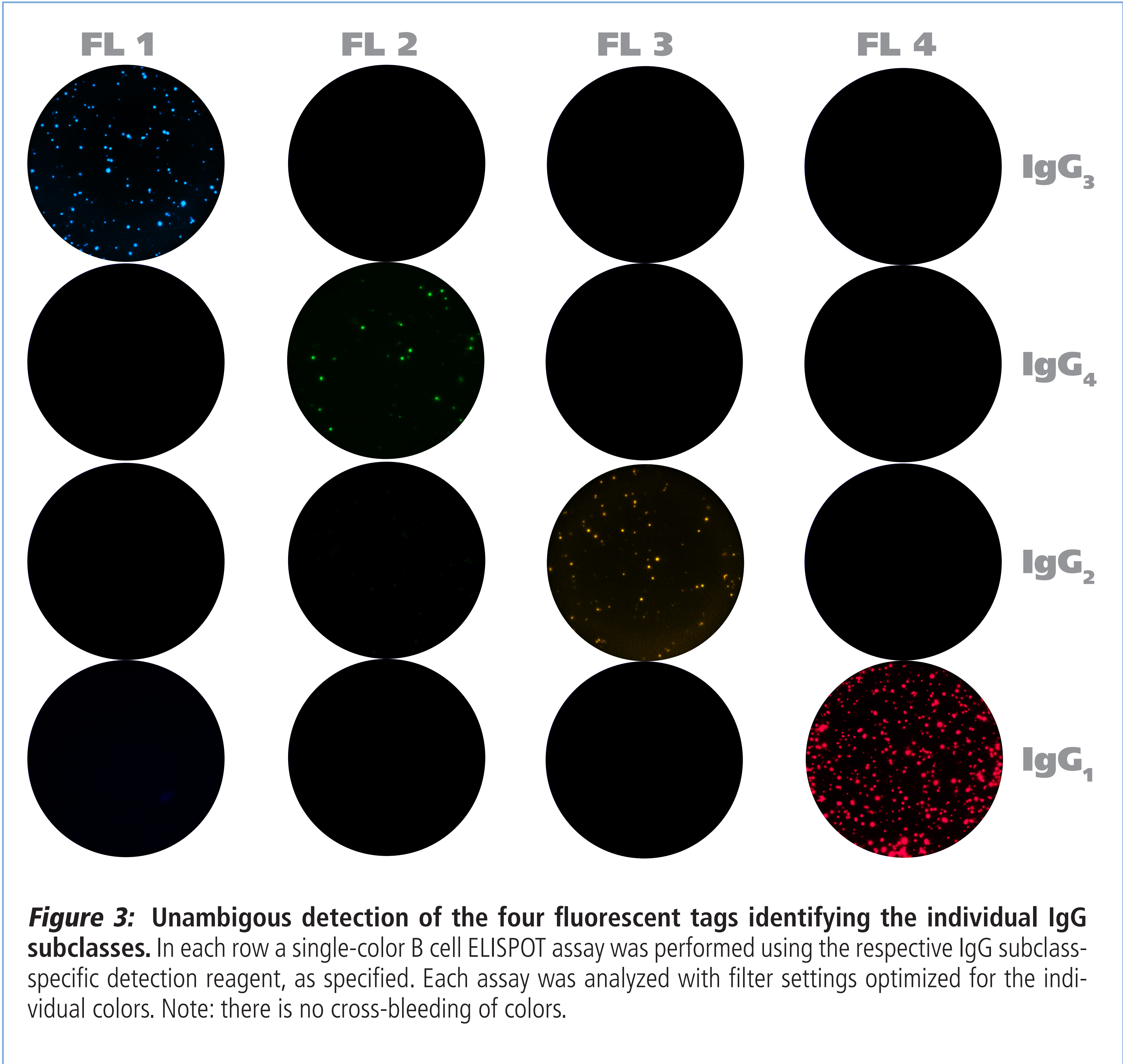
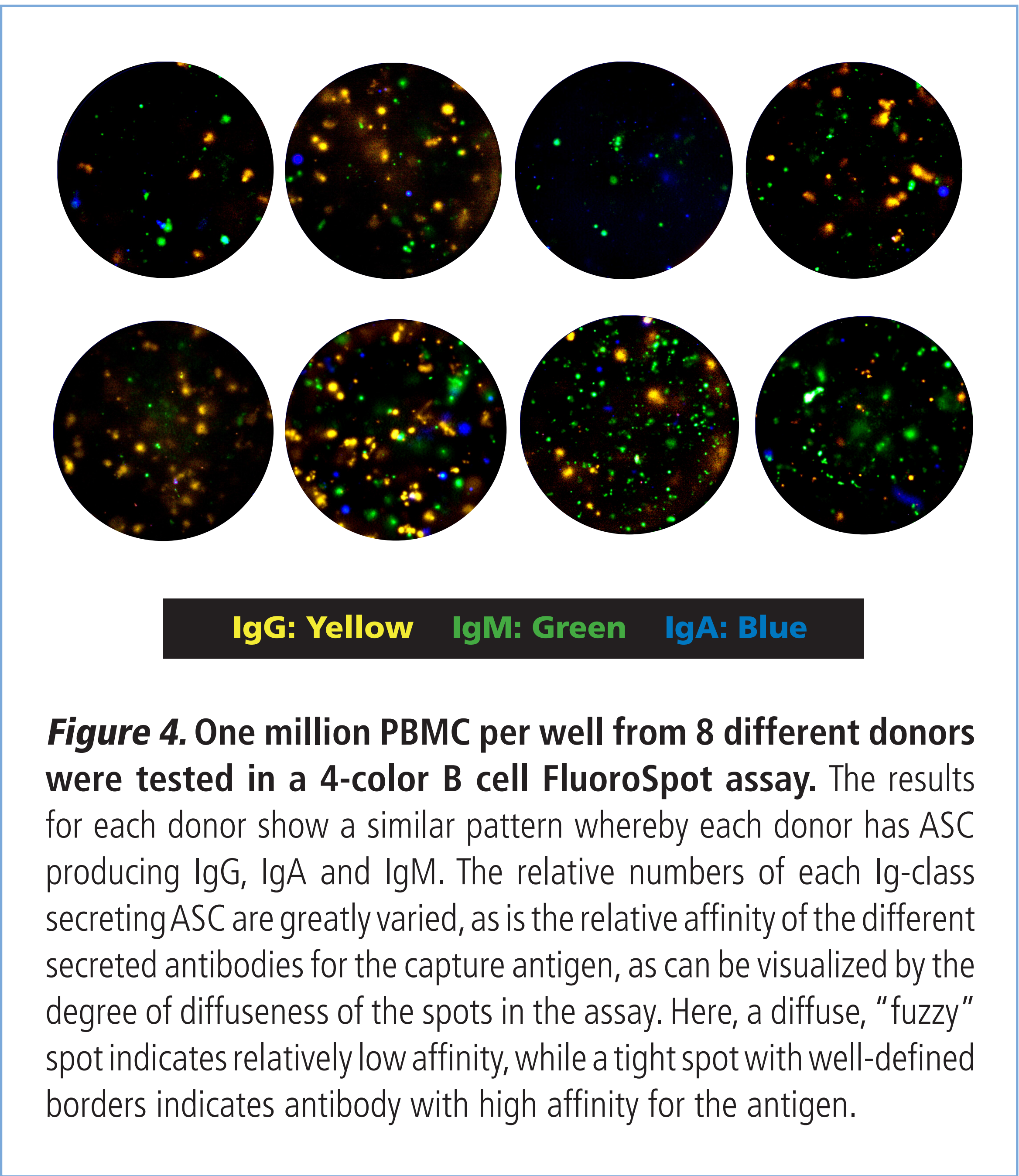
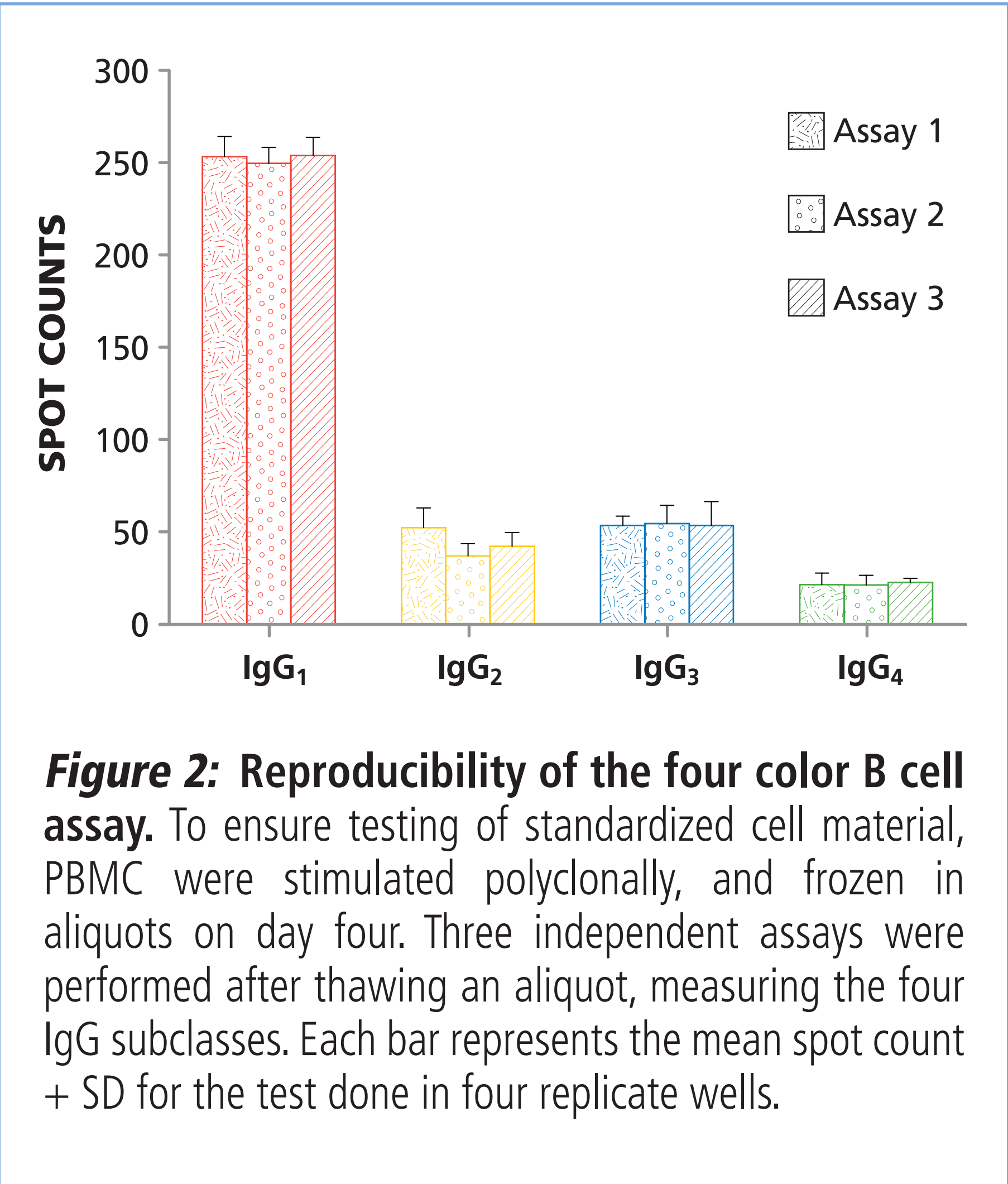
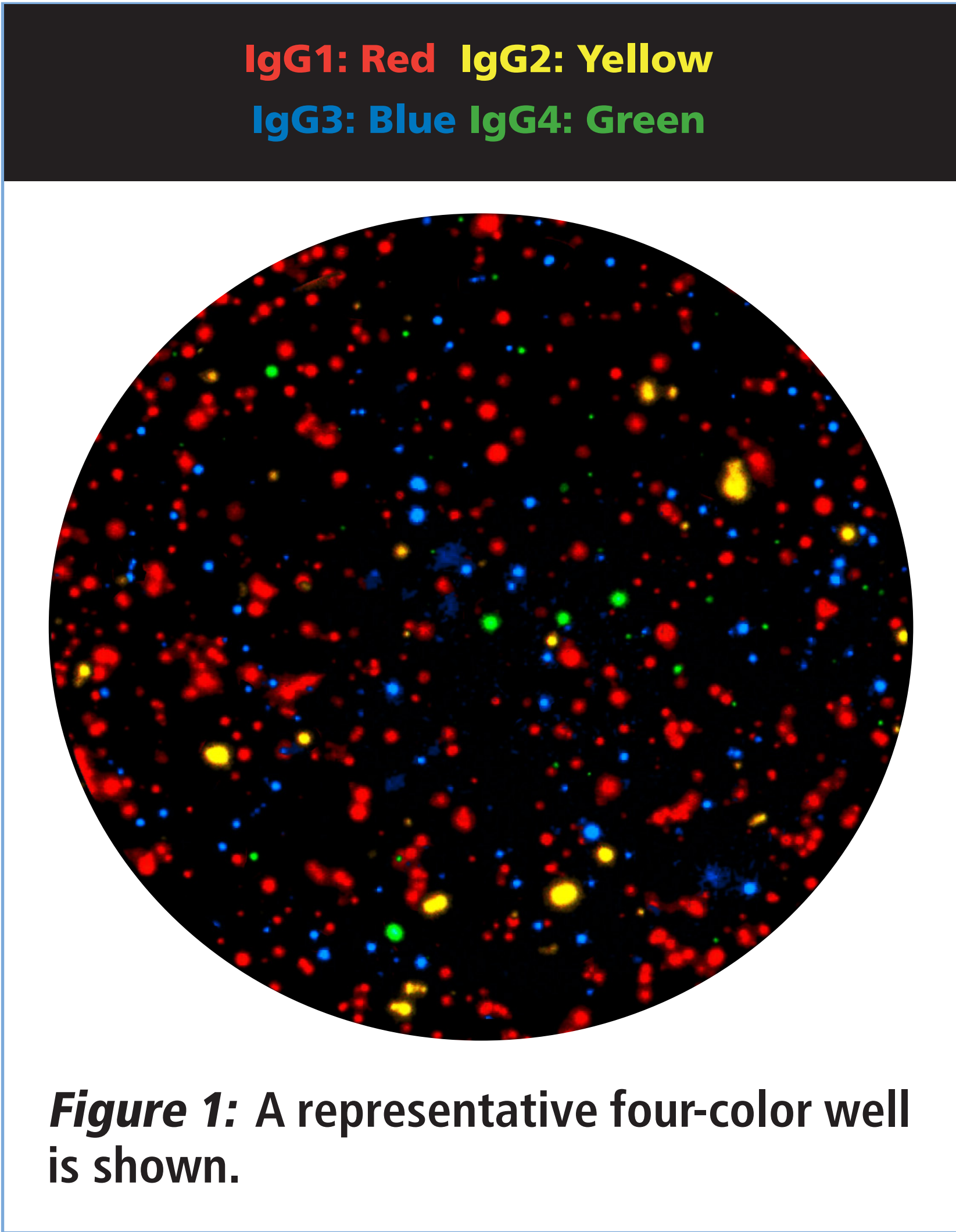
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INTRODUCTION: ELISPOT is a key research tool for enumerating antigen-specific B cells. Resting memory B cells can be detected in blood samples after polyclonal stimulation. In contrast, the spontaneous production of immunoglobulins (Ig) by B cells in freshly-isolated Peripheral Blood Mononuclear Cells (PBMC) signifies recent and ongoing antigen stimulation in vivo. ELISPOT has thus far been restricted to single-color analysis of each Ig class or IgG subclass for detection of Antibody-Secreting Cells (ASC). Consequently, a comprehensive study of all 4 Ig classes or subclasses requires fourfold amounts of PBMC, antigen and labor. We report here the development of a 4-color B cell FluoroSpot in which all IgG subclasses and Ig classes are detected simultaneously.

METHODS: Four-color sub-class detection: PBMC were cultured for 4 days with polyclonal B cell activator. On day 4, cells were washed, counted and plated at 50,000 cells/well in a low autofluorescence PVDF plate pre-coated with anti-human Ig capture antibody (Ab). During a 4h culture, the Ig secreted by the individual B cells was captured on the membrane. These plate-bound human Ig “spots” were visualized using IgG subclass-specific detection reagents, whereby each detection reagent is distinguished from the other 3 reagents through its unique fluorescence pattern. For detection of individual Ig-classes, (Figure 4.) PBMC were cultured on plates coated with human Cytomegalus virus antigen for X days, and the resultant spots representing antigen-specific ASC were detected using Ig-class specific fluorochrome-labeled detection reagents as above. The 4-color assays were analyzed using an ImmunoSpot® S6 Analyzer. All reagents and instruments used are available through CTL.

RESULTS/CONCLUSIONS: Our 4-color FluoroSpot has identical sensitivity for detecting individual B cells secreting IgG Ab subclasses as the respective single-color assays (Table 1), and the 4 fluorochromes can be discerned unambiguously, without spectral overlap (Figure 2). 4-color B cell FluoroSpot is equally suitable for monitoring B cell-mediated immunity as 4 single-color assays.



	IgG1	IgG2	IgG3	IgG4
Single-Color Enzymatic	241 +/- 13	60 +/- 8	67 +/- 8	29 +/- 7
Single-Color Fluorescent	248 +/- 16	59 +/- 3	43 +/- 5	25 +/- 3
Four-Color Fluorescent	250 +/- 9	37 +/- 7	55 +/- 10	21 +/- 5

Table 1: Single-color (SC) enzymatic and SC fluorescent B cell ELISOT assays performed to detect individual IgG subclasses provide the same ASC frequencies as the four-color (4C) fluorescent assay. Polyclonally stimulated B cells were seeded into SC enzymatic B cell ELISPOT assays that detect the four IgG subclasses. In parallel, SC fluorescent assays were performed, and a 4C fluorescent assay was done. The three different assays were each performed in four replicate wells. The spot numbers for each IgG subclass were counted, with the mean and SD of the replicates shown.