

Claims of diploid cancers analyzed.
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Summary: I have identified five reasons why solid-cancer flow cytometry is not widely used in clinical labs: 1) it's too technically demanding, which has led to a mass of conflicting results in the literature, 2) low sensitivity, 3) a conceptual error that considers significant only well-defined populations of aneuploid cells, 4) suboptimal sample preparation, 5) frequent use of cultured cells overgrown with normal diploid cells.

1) The primary reason that flow cytometry is not used to diagnose cancer is that it is too technically demanding for the routine measurement of DNA content in the clinical lab. This has led directly to the mass of conflicting results in the literature. The most recent evidence of this is a report that appeared recently in the journal *Cytometry* [6].

“Flow cytometry technique on fresh/frozen material requires compliance to a number of different technical steps. These include type of dissociation, type of DNA binding dye, instrument settings (e.g. assessment of instrument linearity, calibrations), conditions of data acquisition, external standard used, histogram analysis conditions thanks to different DNA analysis software, and the way the software is used.”

In 1993 the American Consensus (AC) Guidelines were published for the field of solid-tumor flow cytometry [5]. “Unfortunately, some of the AC guidelines were ambiguous, and numerous questions are still open to discussion” [3].

2) Another serious problem with flow cytometry is the low sensitivity for routine use. It requires the measurement of 10,000 to 40,000 cells for good statistics and the sample is lost at the end of the analysis. You can't go back and reevaluate it if needed or desired. By comparison, FISH needs only hundreds of cells for analysis and the slide is a permanent record that can be reexamined. A recent study sums it all up: “FISH is much more sensitive than flow cytometry for identifying aneuploidy...” [2].

3) The low sensitivity of flow cytometry is exacerbated by a conceptual error. The AC recommendations consider significant only those aneuploid cells that form a well defined population centered around a distinct DNA index that is clearly separated from other peaks. While the AC recommendations acknowledge that DNA content is a continuous variable, the insistence on discrete, identifiable peaks reduces the inherent quantitative power of the method to the qualitative categorization of tumors as either diploid, aneuploid, or non-diploid.

This practice of focusing on distinct peak populations can change the DNA ploidy type of the tumor [3]. For example, the asymmetrical and skewed peaks, especially those in the near diploid region, are lumped together with the major peak and classified as diploid cells. Such peaks often represent a heterogeneous mix of normal cells, lymphocytes, and tumor cells. However, image analysis has shown that these “skewed” cells and cells that don't form a distinct peak represent a “genuine DNA aneuploid population” [3].

Specialists in solid-cancer flow cytometry are trying to address, at least in part, the conceptual error of the AC guidelines by implementing software “fixes” that separate “overlapping peaks”. However, this problem has not yet been solved.

4) Yet another problem confounding the interpretation of flow cytometric results is the large number of reports that base their conclusions on the use of archived formalin-fixed, paraffin-embedded tumor specimens. It is now well-known that flow cytometric analysis using formalin-fixed, paraffin-embedded samples frequently fails to detect aneuploid cells that are readily detected in fresh or fresh-frozen suspensions of the same tumors [4,7]. The extensive use of formalin-fixed, paraffin-embedded tumor samples during the formative days of flow cytometry has contributed significantly to the confusion in the literature and has often led to claims that there are diploid cancers.

5) A sample size of hundreds of thousands of cells is needed in order to supply the 10,000-40,000 cells for flow cytometric analysis. As a result, tumor biopsies are often cultured to produce the large number of cells needed for flow cytometry. However, there is a problem with using cultured cells. In striking contrast to the consistent findings of aneuploidy in direct preparations of malignant tumors, there have been a number of studies in which culturing the same tumor cells for a week or longer resulted exclusively or predominantly in the growth of normal diploid cells (that are always present in the original sample) and the disappearance of the aneuploid cancer cells [1]. The use of cultured tumor cells overgrown with normal diploid cells has led a number of researchers to erroneously conclude that some primary cancers are diploid.

References

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