SPECIAL REPORT

Convention on Nomenclature for DNA Cytometry

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The Committee on Nomenclature of the Society for Analytical Cytology presents guidelines for the analysis of DNA content by cytometry. These guidelines cover: staining of DNA; cytogenetic and cytometric terminol-

ogy; DNA index; resolution of measurements; and cytometric standards.

Key terms: DNA cytometry, DNA index, standards

Analysis of cellular DNA content by cytometry is important in clinical and biological research. Measurements are used widely to assess the relative DNA content of tumor stemlines and to assist in the detection and evaluation of malignant diseases. A review of the literature on DNA measurements in solid tumor and leukemias reveals a confusing variety of terms applied for the description of similar results. In order to facilitate the understanding of data and to standardize the terminology for DNA analysis, a questionnaire was distributed to more than 500 investigators. Subsequently, a workshop on terminology was held at the Combined Conference on Analytical Cytology and Cytometry IX and VIth International Symposium on Flow Cytometry. Schloss Elmau, West Germany, October 18–23, 1982. The workshop nominated a nine-member committee to develop guidelines for nomenclature to be used in reporting results from analyses by DNA cytometry. The committee was charged by the Council of the Society for Analytical Cytology to complete this task and to publish its recommendations in Cytometry and in Cancer Genetics and Cytogenetics. The following guidelines are based on the questionnaires returned and the discussion at the workshop; they represent the unanimous recommendations of the committee.

The five guidelines given below apply to measurements of relative DNA content of cells that have been stained appropriately and analyzed by cytometry.

STAINING OF DNA

DNA cytometry usually requires that the DNA be stained with an absorptive or fluorescent dye. Since proportionality between stain intensity and the cellular DNA content depends on the cell preparation and staining procedures, an adequate description of these procedures should be included in reports on DNA measurements.

CYTOMETRIC AND CYTOGENETIC TERMINOLOGY

Results of DNA cytometric analyses should be differentiated clearly from data obtained by cytogenetic techniques. Thus, use of the terms normal and abnormal DNA stemline is recommended instead of diploidy and aneuploidy; the latter are reserved for cytogenetic evaluation. In practice, it might be difficult to avoid completely use of the term an euploidy; but to emphasize the difference between DNA aneuploidy and "true" aneuploidy derived from karyotypic evaluation, the prefix DNA must be used. Thus, the term DNA aneuploidy may be used as a synonym for abnormal DNA stemline. It should be noted that the absence of an abnormal DNA stemline does not exclude the existence of an abnormal karyotype such as balanced translocations and that a negative result should be referred to as "no evidence of abnormal DNA stemline."

DNA INDEX

The degree of DNA content aberration should be expressed by the DNA index. DNA index (DI) is the ratio

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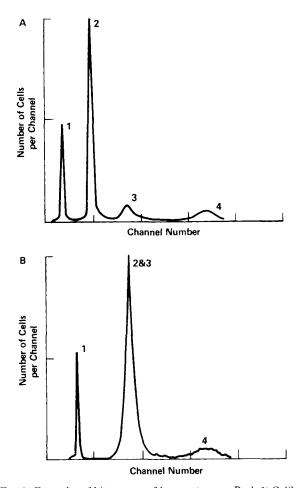


Fig. 1. Examples of histograms of human tumors. Peak 1) Calibration microspheres or avian erythrocytes. Peak 2) Normal diploid reference cells in tumor sample. Peak 3) Abnormal $G_{0/1}$ cells. Peak 4) Abnormal G_2 + M cells. In (A) the DI is (peak 3)/(peak 2), using the modes (or the means) of the two $G_{0/1}$ populations. In (B) the two $G_{0/1}$ modes obviously cannot be resolved, and the DI is not significantly different from 1.0. Note that the gain for B is almost twice that for A.

of the mode (or mean) of the relative DNA content of the $G_{0/1}$ cells of the sample divided by the mode (or mean) of the relative DNA measurement of the diploid $G_{0/1}$ reference cells. Cells with a normal diploid karyotype have, by definition, a DNA index of 1.0. The diagnosis of "abnormal DNA stemline" or "DNA aneuploidy" should be reported only when at least two separate $G_{0/1}$ peaks are demonstrated.

RESOLUTION OF MEASUREMENTS

Any report on DNA measurements should include the coefficient of variation of the $G_{0/1}$ peak of the cells analyzed. Scientific communications also should include the method used to calculate the coefficient of variation and, if necessary, a comment on the shape of the peak. In the case of multiple DNA stemlines, the coefficient of variation should be reported for each $G_{0/1}$ peak.

CYTOMETRIC STANDARDS

Reference cells should always be used when determining the DNA index of an unknown cell population. Cells from the reference should be mixed with the sample before staining. The ideal reference cells are diploid cells from the same tissue and the same individual ("individual and tissue specific reference"). Normal cells from another species, such as avian or fish erythrocytes or nonbiological standards such as fluorescent beads are useful standards for instrument calibration, but should not be used in calculating the DNA index.

The two exemplary DNA flow histograms of Figure 1 illustrate these guidelines.