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# MONOGRAPH LABORATORY MEDICINE: CYTOMETRY

My responsibilities were:

- ☞ Worked with agency to identify content
- ☞ Researched topic extensively
- ☞ Wrote 40-page monograph, 25 references
- ☞ Completed glossary, DNA milestones, appendices

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## DNA Measurement by Flow Cytometry Improved by Automated Sample Preparation Techniques

### I. INTRODUCTION

#### II. DNA CONTENT ANALYSIS BY FLOW CYTOMETRY

Cellular DNA content can be measured by flow cytometric procedures. Neoplastic cells often present with DNA aneuploidy and disorderly cell division. In tumor analysis, identification of an aneuploid cell population is usually indicative of malignancy. DNA aneuploidy can indicate a high risk for tumor recurrence and shortened disease-free survival in several tumor types including bladder, breast, colon and ovarian. However, the presence of a normal DNA content does not always indicate a benign tumor.

Typically, pathologists use a number of factors to determine cell malignancy when examining histological preparations. These include morphologic criteria such as nuclear size and chromatin structure as well as DNA stainability, histologic grade and stage. While necessary, these methods are sometimes equivocal and can be laborious and time consuming. More recently, flow cytometric analysis of DNA content is being used to provide additional objective, quantitative results with ease and efficiency. In this context, DNA content measurement by flow cytometry complements morphological and other criteria, creating a more comprehensive picture of the patient's condition, particularly if morphology is inconclusive.

## Technical Monograph CYTOMETRY

effective and can be analysis.

The main indices use the Proliferative Index aneuploidy. The Pro phase of the cell cyc

ability of certain fluorescent cells or extracted nuclei ent dye such as A. In addition to PI, other When measured by flow proportional to the amount of DNA bound by the dye. By comparing the intensity of each cell to the intensity of cells containing normal diploid amounts of DNA, the relative quantity of DNA in the cells of interest can be determined.

The stained cells or nuclei are passed through an excitation light source (the laser of the flow cytometer) and emit fluorescence in proportion to the DNA content. Propidium Iodide excites at 300-380nm and 440-580nm, and emits at 560-680nm. The red fluorescence emitted by the bound dye is detected and displayed graphically as a histogram (Fig. 1). This fluorescence intensity is directly proportional to the amount of DNA present in the cell or nuclear preparation under study. With this technique, thousands of measurements can be made in seconds, thus improving the accuracy and precision of the measurement. Cell populations expressing DNA aneuploidy can be distinguished from those having no detectable DNA abnormality (8).

A common procedure for DNA content analysis by flow cytometry is single parameter analysis. By comparing the fluorescence intensity of the aneuploid population to the fluorescence intensity of cells containing diploid amounts of DNA (the G<sub>0</sub>G<sub>1</sub> peaks on the histogram, Fig. 2), the relative