Cytometry (Communications in Clinical Cytometry) 26:101–107 (1996)

Review Article

Quality Assurance Issues in DNA Image Cytometry

Alberto Marchevsky, Tanya Tolmachoff, and Stephen Lee
Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, California

DNA image cytometry is currently used in many clinical laboratories as a prognostic tool for the study of patients with malignant neoplasms. However, quality assurance (QA) procedures for image cytometry (IC) have not been standardized. National proficiency testing programs for DNA IC are yet to be developed. We describe our QA program for DNA IC. Indicators of quality; thresholds for evaluation; methods for collection, organization, and evaluation of data; and actions recommended to deal with problems are described for each of the phases of DNA IC. They include QA procedures for sample acceptability; staining technique; instrumentation, including linearity and calibration verification; and correlation with other cytometric methods. The difficult problem of standardizing the postanalytical phase of QA is discussed. The need for a consensus conference to adopt standardized QA procedures for DNA image analysis is emphasized.

Key terms: Quality assurance, image cytometry, DNA

DNA ploidy analysis is routinely used in many clinical laboratories as a prognostic tool for the study of patients with malignant neoplasms (9–12,29,37). Numerous studies have established that alterations in DNA ploidy and increases in the S-phase fraction (SPF) are associated with a poor prognosis in patients with a variety of neoplasms (2,6,8,16,17,20,27,28,31,33,35,36,40,41,44,45). DNA ploidy analysis can also be a helpful tool for the study of the progression of DNA changes in premalignant and malignant lesions, comparison of primary and metastatic tumors, and observation of differences between benign and malignant tumors (38). DNA ploidy analysis has also been applied to the study of fetal tissues and is a valuable tool in the differential diagnosis between complete hydatidiform mole, partial mole, and hydropic chorionic villi (7,14,22,23,30,34,39).

To our knowledge, there are no widely accepted technical standards or standardized quality assurance (QA) procedures for image cytometry (IC). In contrast, there are many published guidelines for flow cytometry QA, including a National Committee for Clinical Laboratory Standards (NCCLS) manual (13,19,24,25,42,43). We describe our QA program for DNA IC and emphasize the need for a consensus conference to develop national technical standards for QA in IC. Recently, the European Society for Analytical Cellular Pathology Task Force issued a consensus report for standardization of DNA (IC) (25).

It is currently mandated by legislation in the Clinical Laboratory Act Improvement Amendments of 1988 (CLIA 88) that clinical laboratories performing clinical tests develop QA programs designed to ensure the accuracy and reliability of test results (1). Detailed guidelines for the performance of DNA cytometry have been recently reported by Bocking and associates (11,12) and by Reith and Danielson (37). This following paper describes the QA procedures currently in place at our cytometry laboratory. DNA ploidy measurements are performed with a CAS 200 Image Analysis System (Becton Dickinson Cellular Imaging Systems, San Jose, CA).

To report reliable DNA ploidy results, laboratories must assess "quality" during all phases of testing: preanalytical, analytical, and postanalytical. Table 1 lists many of the technical factors that can influence DNA ploidy results (11,12,15,38). A written QA plan should be designed and updated annually to evaluate selected steps of the DNA ploidy measurement. We have structured our QA plan along a simplified outline based on the Joint Commission for Accreditation of Hospitals (JCHAO) multistep monitoring process, recommended for other procedures in Pathology and shown in Table 2 (3).

QUALITY ASSURANCE MONITORS FOR THE PREANALYTICAL PHASE OF DNA IMAGE CYTOMETRY

Sample Acceptability

A variety of sample types has been used for image DNA cytometry (Table 3). These include air-dried smears from touch preparations, fine needle aspirate biopsies, cytospin preparations, and paraffin-embedded tissue sections among others (12,32,37). Regardless of the sample

Received for publication March 13, 1996; accepted March 26, 1996.
Address reprint requests to Alberto Marchevsky, M.D., Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048.

Table 1
Technical Factors That Influence Image Cytometry DNA Ploidy Measurements

Specimen type:
Fine needle aspirate biopsy
Cytology preparations (touch preparations, cytospins from paraffin blocks)
Histologic sections
Specimen processing
Fresh cells
Fixed cells: formalin, Boehm's solution, others
Optical system
Illumination: glare, shading
Numerical aperture of objective
Characteristics of condenser
Focus
Video camera features
Signal to noise ratio
Gamma factor
Image analysis software
Nuclear segmentation procedure
Algorithms for glare correction
Algorithms for section thickness correction
Calibration
Internal controls
Lymphocytes
Epithelial cells
Others
External controls
Hepatocytes
Lymphocytes

Table 2
Quality Assurance Procedures for DNA IC

Assignment of responsibility
Selection of important aspects of DNA ploidy testing
Development of quality indicators
Identification of threshold for evaluation
Collection and organization of data
Threshold for problem identification/solving
Corrective actions
Assessment of the actions and document improvement

type chosen, the specimens must meet important criteria for acceptability. All specimens must be received in the cytometry laboratory accompanied by appropriate patient identification, information about sample type, hematoxylin and eosin-stained slide of the tumor, and surgical pathology report. Air-dried touch preparations and cytospins from fresh materials (body fluids, urine) should be prepared as soon as possible to prevent autolysis. They are then fixed in 10% neutral buffered formalin within 1–2 h for further processing. The presence of autolysis in these preparations is assessed prior to Feulgen staining, using a modified Wright stain. Specimens with prominent autolysis are not acceptable. Tissues fixed in 10% buffered formalin are acceptable for IC, whereas materials fixed in Bouin's solution or alcohol are generally not suitable for DNA IC in our laboratory. These latter fixatives induce changes in the nuclear chromatin that preclude optimal quantitation. In selected cases, when DNA ploidy determination is considered particularly important and no other materials are available, tissues fixed in these solutions can be postfixed in formalin or with Boehm's solution, with some improvement in results. Unacceptable specimens and corrective actions taken are recorded in a QA log.

Identification of an Adequate Number of Tumor Cells

Parallel sections or smears stained with hematoxylin and eosin or Papanicolaou stain must be reviewed microscopically by a pathologist to ensure that an adequate number of tumor cells is present. Appropriate tumor areas are identified by encircling them with a marking pen on the histologic slides, a technique that we refer to as "microdissection." The sample is rejected if only few preserved, nonoverlapping viable tumor cells are available. A list of technical problems identified and corrective actions implemented is documented in a daily correction action log. Technical procedures are reviewed if recurrent collection or sample-processing problems are noted.

Staining and Reagents

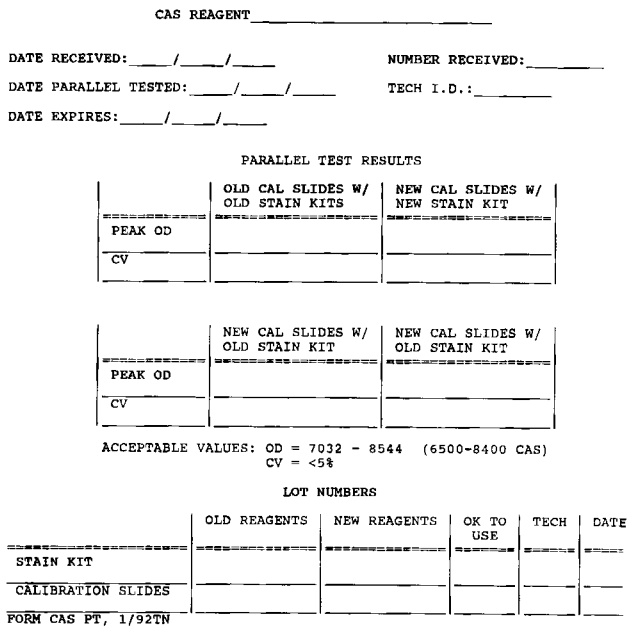
The Feulgen reaction provides a relatively simple, specific, and stoichiometric stain for DNA (11,12). It involves a strong hydrolysis step and a staining step in which the dye couples with the sugar aldehyde groups via the Schiff reaction. If tissues are not well fixed or have been subjected to lengthy hydrolysis times, the DNA is degraded, with suboptimal results. We use the Feulgen staining kit provided by the manufacturer (Becton Dickinson Cellular Imaging Systems). An unstained calibration slide and an unstained, in-house control (see analytical section) are included with each staining batch of patients' samples. These slides function as method controls; they are treated exactly the same as patient material. Deviation above or below the expected range indicates a possible staining problem. In addition, each new lot of staining reagents and calibration slides is parallel tested before using them to test patients' samples, comparing their DNA picogram values with those obtained from previously used calibration and control slide lots (Fig. 1). Parallel testing results are documented in a reagent log book, and procedures are reviewed when problems arise.

Instrument Preventive Maintenance and Function Checks

Multiple systems have been developed for image DNA cytometry (24,25,42,43). With rapid advances in computer hardware and image analysis software, it is now possible to assemble relatively inexpensive systems for DNA cytometry (18,21). These systems must be properly calibrated and maintained to ensure reproducibility of the DNA ploidy measurements. Instrument preventive maintenance and function checks must be performed on all components of the image analysis system. Our CAS 200 daily preventive maintenance includes cleaning and dusting of the system exterior, cleaning of the microscope lens and objectives, and checking of the printer ribbon and paper supply. The microscope lenses and objectives also should be inspected for scratches, blemishes, marks, separation of elements, and other problems. We

Table 3
QA Procedures for the Preanalytical Phase of DNA Ploidy Analysis

	Important aspect of DNA ploidy testing		
QA program	Sample selection	Reagents and staining	Function checks
Responsibility	Pathologist	Technologist	Technologist
Indicator of quality	Microscopic images	CAS calibration slides In-house controls	Set-light function Reproducibility Linearity Calibration verification System and method correlation
Collection of data	Daily QA log of sample acceptability	Controls processed with clinical samples	Record mV illumination Measure cells in different locations Divide CAS control cells into four classes Calculate pg DNA/cell Compare with flow cytometry
Threshold for problem identification	Fewer than 100 well-preserved tumor cells	Deviation from expected range	8.9–9.1 mV CV < 5% DIs of 1.0, 2.0, 3.0, and 4.0, respectively Expected range 11.88–14.52 pg DNA No threshold for minimal number of adequate cells
Actions	Request additional materials	Change reagents, review procedures	Check lighting system Correct microscope setup


CAS REAGENT_____

DATE RECEIVED:____/____/____ NUMBER RECEIVED:_____

DATE PARALLEL TESTED:____/____/____ TECH I.D.:_____

DATE EXPIRES:____/____/____

PARALLEL TEST RESULTS

	OLD CAL SLIDES W/ OLD STAIN KITS	NEW CAL SLIDES W/ NEW STAIN KIT
PEAK OD		
CV		

	NEW CAL SLIDES W/ OLD STAIN KIT	NEW CAL SLIDES W/ OLD STAIN KIT
PEAK OD		
CV		

ACCEPTABLE VALUES: OD = 7032 - 8544 (6500-8400 CAS)
CV = <5%

LOT NUMBERS

	OLD REAGENTS	NEW REAGENTS	OK TO USE	TECH	DATE
STAIN KIT					
CALIBRATION SLIDES					

FORM CAS PT, 1/92TN

FIG. 1. Example of quality assurance form for recording of parallel test DNA ploidy values.

document daily in a QA log the results of various function checks (Fig. 2). Function checks are necessary to ensure proper instrument performance and are performed by measuring calibration slides in conjunction with the measurement of each clinical sample. They include microscope setup and set-light functions. A modified Koehler illumination technique (microscope setup) is performed for every slide. This ensures correct placement of the microscope substage condenser and diaphragm. The value recorded by the CAS 200 instrument with the set-light function depends on correct microscope setup. It indicates the millivolts of light intensity required to obtain adequate illumination for DNA ploidy measurements. The acceptable voltage intensity range in our instrument is 8.9–9.1 mV. Deviations from this range indicate potential problems in the system's light source, such as light bulb deterioration, misalignment of the microscope, or improper Koehler illumination. In addition, the operator should be alert to the image resolution, contrast, and freedom from aberrations and other defects and should not acquire measurements from suboptimal images. Other function checks, such as for reproducibility, interobserver and intraobserver reproducibility, linearity, calibration verification, and system and method correlations, are performed periodically and at least biannually. Documentation is maintained for each of these monitoring checks.

Reproducibility is a function check designed to ensure that there will be no significant variation in DNA content measurements based on the location of a nucleus on the video screen. It is monitored by measuring the DNA content in picograms of the same cell or small group of cells in different locations. Coefficient of variations (CV) higher than 5% indicate a problem with the instrument. Problems with reproducibility of DNA content measurements can usually be solved by realigning the microscope and its light source. Interobserver and intraobserver variations are another source of lack of reproducibility that

MONTHLY INSPECTION FORM

INSTRUMENT: CAS 200
MANUFACTURER: BECTON DICKINSON
SERIAL NUMBER:

MONTH:_____ YEAR _____
LOCATION: ROOM 5617
PROPERTY NUMBER: _____

DATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
DAILY PM - PERFORM EACH USE																															
CLEAN AND DUST EXTERIOR OF SYSTEM																															
CLEAN LENS/OBJECTIVES OF MICROSCOPE																															
RECORD MV SET LIGHT																															
RECORD QDA CALIBRATION DATA																															
CHECK PRINTER RIBBON/ PAPER SUPPLY																															
OTHER PM - PERFORM AS NECESSARY																															
CHANGE LAMP BULB																															
CENTER LAMP ALIGNMENT																															
BIANNUAL FUNCTION CHECKS																															
REPRODUCIBILITY	Date Performed :																														
LINEARITY	Date Performed :																														
FCM CORRELATION	Date Performed :																														
CALIB. VERIFICATION	Date Performed :																														
TECH																															

CORRECTIVE ACTION: _____

5/24/91

INH,PROC [CAS INSPECT RES 5/94]

FIG. 2. Example of quality assurance documentation form for recording daily preventive maintenance and function checks data.

can be monitored by repeating the DNA ploidy measurements in approximately 10% of cases. Different technologists and each technologist at different times should obtain similar histograms and DNA indices that remain within 10%.

Linearity is a function check designed to ensure the correct placement of the DNA measurements of each nucleus on the histogram scale. It is established by using a morphological filter that classifies the measurements obtained from cells on the calibration slide into four categories: diploid, tetraploid, octoploid, and doubles. Approximately 100 cells are measured from each rat liver calibration slide to acquire a histogram. The mean DNA indices (DI) calculated for each class should be approximately 1.0, 2.0, 4.0, and 3.0, respectively. Aberrant linearity values usually indicate computer malfunction or improper use of the "filter" program.

Calibration verification is a function check designed to assess the picograms of DNA per nucleus used by the instrument in DNA ploidy calculations. It is calculated by measuring the DNA content from 100 cells present in calibration slides as a patient sample. The acceptable calibration verification range of picograms/cell measured with our instrument is 13.2 pg (±10%; 11.88–14.52).

System and method correlations are required by CLIA 88 legislation; if more than one instrument is available for testing, each system must be compared with the others and validated for concordance to ensure patient reproducibility (4,26,46). This comparison must be performed twice per year. It is difficult to design QA monitors to comply with this requirement; most laboratories do not have more than one expensive cytometer. In our laboratory we periodically compare the DNA ploidy results obtained with flow cytometry and IC from selected cases. We do not currently have thresholds for action; it is customary to retest each case when significant differences in the distribution of the ploidy histogram or DI values are detected and to try to analyze potential reasons for discrepancy, such as dilution of tumor cells by inflammatory or stromal cells, presence of necrosis, and other technical factors that influence flow cytometric measurements. Results are noted on the requisition slips for review by a pathologist. A more accurate assessment of different method concordance is to measure "known" samples, such as proficiency testing samples or cells from tissue culture. To our knowledge, national proficiency testing programs are not yet available for image DNA cytometry.

QUALITY ASSURANCE MONITORS FOR THE ANALYTICAL PHASE OF DNA IC

Quality assurance indicators to monitor the analytical phase of DNA cytometry are needed, including the ac-

Table 4
Quality Assurance Monitors for the Analytical Phase of DNA Image Cytometry

Review cell gallery to discard inadequate nuclear samples
Calibration monitors
Summed optical density
CV of the main peak
Calibration verification of DNA peak
Diploid controls
Internal
External
Method

quisition of histograms and the use of calibrators and controls (Table 4).

Histogram Acquisition

Histogram acquisition includes the selection of a sample size, sampling area, and acquisition mode. We routinely measure 100–200 intact, nonoverlapping nuclei per case. A pathologist selects the appropriate tumor cells for sampling. The selected tumor cells are stored in the computer in a "list mode," and the image sequence is reviewed by a pathologist in a gallery-type display of all nuclei selected for quantitation. Inadequate nuclei are deleted from the histogram. Criteria for nonacceptability of tumor cells include presence of nuclear fragments, multinucleated cells, out-of-focus images, and overlapping cells among others. We currently do not keep QA logs recording the results of this process.

Calibration

Calibration is performed daily before the patient samples or controls are measured, using commercially prepared slides containing unstained rat hepatocytes. These hepatocytes have a known amount of DNA content and thus are used to establish a one-point concentration curve for the CAS system. One calibration slide is included with each stain batch of patient samples. Two calibration criteria are recommended: summed optical density (SumOD) and CV of the diploid peak. CAS has an established acceptable calibration SumOD mean of 7,500 with a 95% (±2 SD) range of 6,500–8,400. Each laboratory must calculate its own acceptable range to account for individual instrument variability. Our SumOD mean is 7,788, with a 95% range of 7,032–8,544; this was determined on the basis of consecutive data collected over a 2 year period. The CV of the diploid peak is considered acceptable if it is smaller than <5%. In addition, daily calibration values are forwarded to Becton Dickinson Cellular Imaging Systems for participation in a QC program (Fig. 3). Becton Dickinson Cellular Imaging Systems compares our data with their established ranges for each calibration lot and also compares our data with

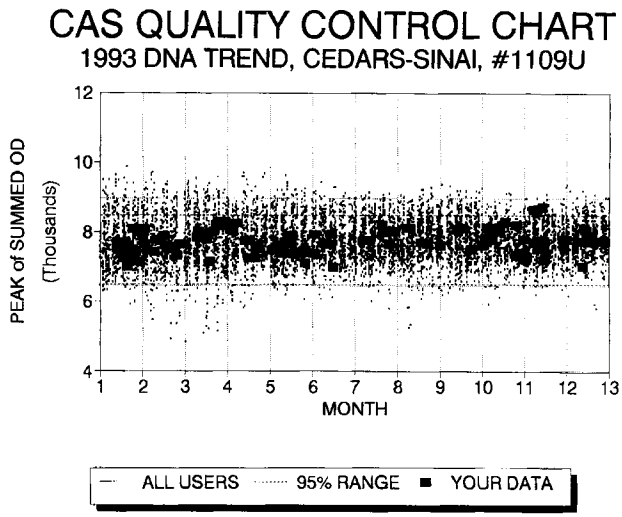
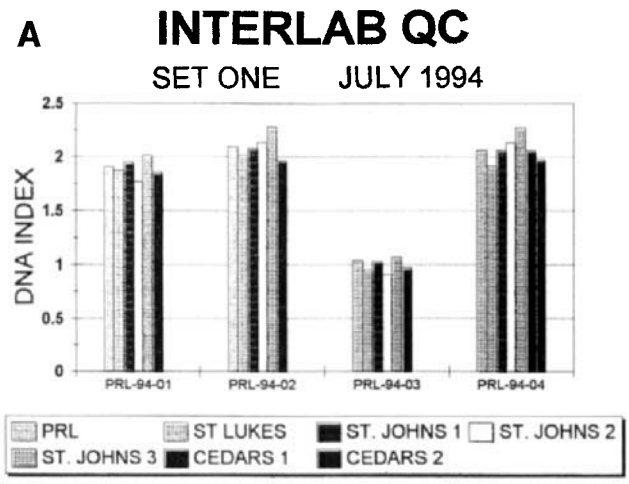


FIG. 3. Quality control data from the quality control program coordinated by the instrument manufacturer (CAS, San Jose, CA). Data from our laboratory are indicated by the dark squares. Most values reported by various laboratories are within the 95% peak of summed OD range.

other participant's data. We are able to monitor possible instrument shifts and trends with this QC program.

Diploid Controls

Diploid controls are classified as internal, external, and method controls. A population of "normal" cells is read as internal controls from each patient sample, to ensure a reference diploid peak. Internal controls can detect improper sample processing, such as inadequate fixation. We generally measure normal lymphocytes or benign epithelial or stromal cells for internal controls. These cells must exhibit a single narrow diploid peak, with a DI value ranging from 0.9 to 1.1, for the sample to be acceptable. External controls are materials with known, established DI ranges. They may be commercially purchased or prepared in house, but in both instances acceptable ranges must be established for each lot by repetitive testing. We use an in-house diploid control prepared from buffy coat smears of a chronic lymphocytic leukemic patients. We established 95% SD ranges for DNA content in picograms, DI, and peak CV by generating data on several slides over an extended period of time. Each newly prepared lot is parallel tested to validate acceptability of the established ranges. The ranges we have established for our in-house control are 6.4–8.0 pg DNA, DI 0.9–1.1, and peak CV <5%. Commercially available rat hepatocytes are used as method controls. Because the calibration slide is stained along with the patient samples, in exactly the same manner, and the CAS system is preprogrammed with the criteria for acceptable calibration material using the ranges discussed above, any unacceptable staining techniques will be detected. If the calibration material staining is too intense or too faint, instrument calibration will not meet acceptability criteria, and the stain batch must be rejected.



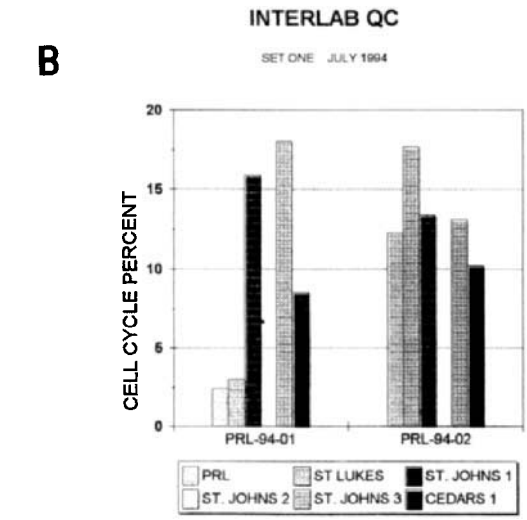


FIG. 4. **A,B:** Comparison of the results from our laboratory with those from other participants in a small proficiency testing program. Note that the DNA index values collected from measuring shared samples at all laboratories are within 10%. However, SPF values vary by as much as 30%; we do not routinely report S-phase values based on image cytometry. (Courtesy of K. Snyder and C. Vittori Physician's Reference Laboratory).

QUALITY ASSURANCE MONITORS FOR THE POSTANALYTICAL PHASE OF DNA IC

Interpretation of Histograms and Proficiency Testing

The interpretation of histograms is probably the most difficult area to standardize in DNA IC. A detailed review of this topic is beyond the scope of this outline. Various technical factors, such as sample size, scaling of histograms, range of measurements, and selection of histogram "peaks," can result in erroneous interpretations of DNA ploidy results measured via image analysis (5). To our knowledge, there is no widely used software that would analyze the DNA ploidy histograms based on consensus and well-accepted criteria. Further studies and guidelines reached by consensus are clearly needed in this area. All DNA ploidy histograms are performed at our laboratory by technologists and pathology residents and are reviewed by one of two pathologists. Discrepancies in selection of the main peaks or the histogram interpretation are documented upon review of consultation reports by a pathologist. Equivocal cases are submitted for flow cytometry, whenever possible, and additional reports are issued when needed. There is no national proficiency testing program for DNA IC. We are performing IC on cytospins prepared from survey samples sent to the laboratory by the College of American Pathology for flow cytometry. Results are compared to those obtained by flow cytometry. Results are self-evaluated, and discrepancies are noted. We have participated during the past year in a proficiency program organized by Physician's Reference Laboratory (Overland Park, Kansas) in which we share clinical samples that are retested at three other laboratories using similar instrumentation (Fig. 4). The survey demonstrates very good concordance for DI values (<10% variability among laboratories). However, SPF values vary by up to 30%, underscoring the unreliability of S-phase measurements by image DNA cytometry. We do not report SPF data generated by IC.

CONCLUSIONS

Much more work must be done in the area of quality assurance in DNA image cytometry to ensure that different laboratories reporting DNA ploidy results from clinical samples issue comparable findings. Interinstitutional guidelines, particularly for the interpretation of histograms, and proficiency testing programs must be developed. In our opinion, a consensus conference to discuss these topics is overdue.

LITERATURE CITED

1. Federal Register 57(40):7163–7169, 1992.
2. al-Abadi H, Nagel R: Deoxyribonucleic acid content and survival rates of patients with transitional cell carcinoma of the bladder. J Urol 151:37–42, 1994.
3. Association of Directors of Anatomic and Surgical Pathology: Recommendations on quality control and quality assurance in anatomic pathology. Am J Surg Pathol 15:1007–1009, 1991.
4. Bachner P, Howanitz PJ: Using Q-probes to improve the quality of laboratory medicine: A quality improvement program of the College of American Pathologists. Qual Assur Health Care 3:167–177, 1991.
5. Bacus JW, Bacus JV: A method of correcting DNA ploidy measurements in tissue sections. Mod Pathol 7:652–664, 1994.
6. Ballouk F, Ambros RA, Malfetano JH, Ross JS: Evaluation of prognostic indicators in squamous carcinoma of the vulva including nuclear DNA content. Mod Pathol 6:371–375, 1993.
7. Barclay, ID, Dabbagh L, Babiak J, Poppema S: DNA analysis (ploidy) of molar pregnancies with image analysis on paraffin tissue sections. Am J Clin Pathol 100:451–455, 1993.
8. Baretton G, Diebold J, DePascale T, Bussar-Maatz R, Weissbach L, Lohrs U: Deoxyribonucleic acid ploidy in seminomas with and without syncytiotrophoblastic cells. J Urol 151:67–71, 1994.
9. Berner A, Danielsen HE, Pettersen EO, Fossa SD, Reith A, Nesland JM: DNA distribution in the prostate. Normal gland, benign and premalignant lesions, and subsequent adenocarcinomas. Anal Quant Cytol Histol 15:247–252, 1993.
10. Biesterfeld S, Fuzesi L, Harle F, Bocking A: DNA-cytometric detection of euploid polyploidization in oral lichen Ruber planus. Anal Quant Cytol Histol 13:7–10, 1991.
11. Bocking A: DNA cytometry. In: Surgical Gynecologic Oncology. Spe-

cial Diagnostics, Burghardts E (ed). Georg Theime Verlag, Stuttgart, 1993, pp 250–252.
12. Böcking A, Striepecke E, Auer H, Fuzesi L: Static DNA cytometry. Biological background, technique and diagnostic interpretation. In: Compendium on the Computerized Cytology and Histology Laboratory, Wied GL, Bartels PH, Rosenthal DL, et al. (eds). Tutorials of Cytology, Chicago, 1994, pp 107–128.
13. Bringuier PP, Bouvier R, Berger N, Piaton E, Revillard JP, Perrin P, Devonec M: DNA ploidy status and DNA content instability within single tumors in renal cell carcinoma. Cytometry 14:559–564, 1993.
14. Cera G, Fruttero A, Rua S, Comino A, Abrate M: Flow cytometric studies in spontaneous abortions. Applications in the medico-legal practice. Forens Sci Int 54:167–175, 1992.
15. Chieco P, Jonker A, Melchiorri C, Vanni, G, Van Noorden CJF: A user's guide to avoiding errors in absorbance image cytometry: A review with original experimental observations. Histol J 26:1–19, 1993.
16. De Lena M, Romero A, Rabinovich M, Leone B, Vallejo C, Machiavelli M, Cuevas M, Rodriguez R, Lacava J, Perez J: Metastatic pattern and DNA ploidy in stage iv breast cancer at initial diagnosis. relation to response and survival. Am J Clin Oncol 16:245–249, 1993.
17. Deans GT, Williamson K, Hamilton P, Heatley M, Arthurs K, Patterson CC, Rowlands BJ, Parks TG, Spence RA: DNA densitometry of colorectal cancer. Gut 34:1566–1571, 1993.
18. Erler B, Chen K, Marchevsky AM: An image analysis workstation for the pathology laboratory. Mod Pathol 6:612–618, 1993.
19. Fossa SD, Berner A, Waehre H, Heiden T, Juul ME, van den Ouden D, Pettersen EO, Wang N, Tribukait B: DNA ploidy in cell nuclei from paraffin-embedded material—comparison of results from two laboratories. Cytometry 13:395–403, 1992.
20. Fossa SD, Berner AA, Jacobsen AB, Waehre H, Kvarstein B, Urnes T, Ogreid P, Johansen TE, Silde H, Nesland JM: Clinical significance of DNA ploidy and S-phase fraction and their relation to p53 protein, c-erbb-2 protein and hcg in operable muscle-invasive bladder cancer. Br J Cancer 68:572–578, 1993.
21. Fu YS, Cheng L, Huang I, Huang S, Wiesmeier E, Wettstein F, Weissman M: DNA ploidy analysis of cervical condyloma and intraepithelial neoplasia in specimens obtained by punch biopsy. Anal Quant Cytol Histol 13:187, 1989.
22. Fukunaga M, Ushigome S: Spontaneous abortions and DNA ploidy. An application of flow cytometric DNA analysis in detection of nondiploidy in early abortions. Mod Pathol 6:619–624, 1993.
23. Fukunaga M, Ushigome S, Sugishita M: Application of flow cytometry in diagnosis of hydatidiform moles. Mod Pathol 6:353–359, 1993.
24. Hedley DW, Shankey TV, Willis LL: DNA cytometry consensus conference. Cytometry 14:471–500, 1993.
25. Bocking A, Giroud F, Reith A: Consensus report of the ESACP task force on standardization of diagnostic DNA image cytometry. Anal Cell Pathol 8:67–74, 1995.
26. Howanitz PJ, Hoffman GG, Schifman RB, Zarbo RJ, Steindel SJ, Walker K: A nationwide quality assurance program can describe standards for the practice of pathology and laboratory medicine. Qual Assur Health Care 4:245–256, 1992.
27. Huddart SN, Muir KR, Parkes SE, Mann JR, Stevens MC, Raafat F, Smith K: Retrospective study of prognostic value of DNA ploidy and proliferative activity in neuroblastoma. J Clin Pathol 46:1101–1104, 1993.
28. Ikeda M, Watanabe Y, Nanjoh T, Noda K: Evaluation of DNA ploidy in endometrial cancer. Gynecol Oncol 50:25–29, 1993.
29. Kaern J, Wetteland J, Trope CG, Farrants GW, Juhng SW, Pettersen EO, Reith A, Danielsen HE: Comparison between flow cytometry and image cytometry in ploidy distribution assessments in gynecologic cancer. Cytometry 13:314–321, 1992.
30. Koenig C, Demopoulos RI, Vamvakas EC, Mittal KR, Feiner HD, Espiritu EC: Flow cytometric DNA ploidy and quantitative histopathology in partial moles. Int J Gynecol Pathol 12:235–240, 1993.
31. Koha M, Wikstrom B, Brismar B: Colorectal carcinoma. DNA ploidy pattern and prognosis with reference to tumor DNA heterogeneity. Anal Quant Cytol Histol 14:367–372, 1992.
32. Martelli G, Daidone MG, Mastore M, Gabrielli GM, Galante E, Pilotti S, Silvestrini R: Combined analysis of ploidy and cell kinetics on fine-needle aspirates from breast tumors. Cancer 71:2522–2527, 1993.
33. Morkve O, Halvorsen OJ, Skjaerven R, Stangeland L, Gulsvik A, Laerum OD: Prognostic significance of p53 protein expression and DNA ploidy in surgically treated nonsmall cell lung carcinomas. Anticancer Res 13:571–578, 1993.
34. Neuber M, Rehder H, Zuther C, Lettau R, Schwinger E: Polyploidies in abortion material decrease with maternal age. Hum Genet 91: 563–566, 1993.
35. Porschen R, Remy U, Bevers G, Schauseil S, Hengels KJ, Borchard F: Prognostic significance of DNA ploidy in adenocarcinoma of the pancreas. A flow cytometric study of paraffin-embedded specimens. Cancer 71:3846–3850, 1993.
36. Raviv G, Leibovich I, Mor Y, Nass D, Medalia O, Goldwasser B, Nativ O: Localized renal cell carcinoma treated by radical nephrectomy. Influence of pathologic data and the importance of DNA ploidy pattern on disease outcome. Cancer 72:2207–2212, 1993.
37. Reith A, Danielsen H: Assessment of DNA ploidy in tumor material. Preparation and measurement by image cytometry. In: Compendium on the Computerized Cytology and Histology Laboratory, Wied GL, Bartels PH, Rosenthal DL, et al. (eds). Chicago, Tutorials of Cytology, 1994, pp 185–193.
38. Russack V: Image cytometry: Current applications and future trends. Crit Rev Clin Lab Sci 31:1–34, 1994.
39. Sagot P, Nomballais MF, David A, Yvinec M, Beaujard MP, Barriere P, Boog G: Prenatal diagnosis of tetraploidy. Fetal Diagn Ther 8:182–186, 1993.
40. Schapers RF, Ploem-Zaaijer JJ, Pauwels RP, Smeets AW, van den Brandt PA, Tanke HJ, Bosman FT: Image cytometric DNA analysis in transitional cell carcinoma of the bladder. Cancer 72:182–189, 1993.
41. Schlichting E, Clausen OP, Hanssen AS, Buanes TA: Ploidy and survival in resectable pancreatic cancers. A retrospective study over 9 years. Eur J Surg 159:229–233, 1993.
42. Shankey T, Kallioniemi OP, Koslowski JM, Lieber ML, Mayall BH, Miller G, Smith G: Consensus review of the clinical utility of DNA content cytometry in prostate cancer. Cytometry 14:497–500, 1993.
43. Shankey T, Rabinovitch PS, Bagwell B, Bauer KD: Guidelines for implementation of clinical DNA cytometry. Cytometry 14:472–477, 1993.
44. Shyr YM, Su CH, Wu LH, Li AF, Chiu JH, Lui WY: DNA ploidy as a major prognostic factor in resectable ampulla of Vater cancers. J Surg Oncol 53:220–225, 1993.
45. Thornton JG, Ali S, O'Donovan P, Griffin N, Wells M, MacDonald RR: Flow cytometric studies of ploidy and proliferative indices in the yorkshire trial of adjuvant progestogen treatment of endometrial cancer. Br J Obstet Gynaecol 100:253–261, 1993.
46. Zarbo RJ, Hoffman GG, Howanitz PJ: Interinstitutional comparison of frozen-section consultation. A College of American Pathologists q-probe study of 79,647 consultations in 297 North American institutions. Arch Pathol Lab Med 115:1187–1194, 1991.