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Evaluation of an Automated Image Analysis System for Counting Human Tumor Colonies

Sydney E. Salmon, Laurie Young, Joyce Lebowitz, Stephen Thomson, Janine Einsphar, Tony Tong, Thomas E. Moon

University of Arizona Cancer Center, Tucson, AZ, USA

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Abstract. The Omnicon FAS II image analysis system was applied to counting tumor colonies grown in a soft agar human tumor clonogenic assay with a detailed protocol designed to assess the instrument's sensitivity, specificity, precision, and accuracy. Comparisons of technician and instrument counts were done on a blinded basis. Sensitivity studies (which used metal microspheres) yielded a correlation coefficient (r) of 0.999 between technicians and the counter. A field-by-field analysis of the instrument's specificity for identifying individual objects correctly as tumor colonies rather than artifacts (as identified by the technician) was excellent ($r = 0.95$). In the precision studies (determined with repeated automated counting of the same samples for five days), the median coefficient of variation was $< 7\%$. Accuracy was evaluated on cultures of fresh biopsies from 30 human cancers obtained for drug sensitivity testing as well as on a series of tumor cell lines. The correlation between the mean number of colonies counted by the technicians and by the colony counter was greater than 0.91. Similar comparisons of mean percent survival of tumor colony-forming cells after drug exposure between technician and machine were also quite acceptable ($r = 0.85$). We conclude that the colony counter provided sufficient reliability to be applied to counting human tumor colonies grown in vitro. In addition, the colony counter performed the Petri dish counts ten times faster than experienced technicians and without associated operator fatigue.

Introduction

Evaluation of human tumor growth in vitro has been facilitated by the development of soft agar clonogenic assay systems [1, 2]. Tumor cells

Correspondence: Sydney E. Salmon, M.D., Department of Internal Medicine and the Cancer Center, University of Arizona College of Medicine, Tucson, AZ 85724 (USA)

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exhibiting colony-forming capacity in vitro are considered to be closely related to tumor stem cells, a key subpopulation of cells thought responsible for the metastatic process and for tumor recurrence after subcurative therapy in vivo [3]. The two-layer soft agar human tumor clonogenic assay (HTCA) system developed in our laboratory [1] has proven applicable to the study of fresh tumor biopsy specimens and permits quantitation of anticancer drug sensitivity [4]. Clinical trials from a number of centers have confirmed our results and shown that, in a variety of tumor types, in vitro sensitivity or resistance in HTCA correlates well with clinical response or resistance to a variety of anticancer drugs [5-12]. Other applications of HTCA have been to preclinical new drug screening [13-14], to in vitro phase II trials [15], and to various diagnostic applications [16, 17].

Shortly after development of HTCA, it became apparent that the development of an automated tumor colony counter was highly desirable, as the counting process proved to be very time-consuming and associated with error secondary to technician fatigue. Additionally, there was a considerable learning process required for new technicians to correctly identify tumor colonies. In recognition of its potential utility, an automated tumor colony counter using image analysis techniques was developed for HTCA [18]. In addition to colony counts, the automated counter was designed to provide quantitative information on the colony size range and utilize non-destructive testing methods so that substantial additional information could be obtained through serial studies. These additional parameters may prove to have a number of research applications in cancer biology as well as in clinical oncology. The purpose of the current investigation was to subject the automated tumor colony counter to a detailed assessment of its sensitivity, specificity, precision, and accuracy; and thereby evaluate its applicability to routine counting of tumor colonies grown in vitro in HTCA.

Materials and Methods

Microspheres

Metal microspheres (Bausch & Lomb, Rochester, NY) ranging in size from 60 μ to 160 μ in diameter were embedded in a clear plastic matrix in 35 mm Petri dishes (Nunc, Grand Island Biological Co., Santa Clara, CA). The concentrations per total scanning area in the Petri dishes were calculated to provide coverage of the range from 50 to 1600 objects. Microsphere circularity and size range were selected to fulfill the automated counter's criteria for "colonies."

Fresh Human Tumors

Surgical biopsies were handled aseptically by the clinical pathologist and a viable portion (at least 1-2 grams) of tumor tissue was transferred promptly to the HTCA laboratory at the University of Arizona Cancer Center. In the current study, tumor types studied were primarily epithelial type ovarian carcinoma, metastatic melanoma, and adenocarcinoma of unknown primary origin. Techniques for tumor disaggregation (mechanical and enzymatic), drug incubation, and culture in HTCA have been detailed elsewhere [19] and recently reviewed [20]. Tumor cells were plated at a concentration of 500,000 nucleated cells in the upper layer of 0.3% agar in each Petri dish. Preculture viability of each tumor cell suspension was determined with trypan blue dye, and all drug-treated cells and control cells were processed simultaneously. After gelation, the plates were examined to assure that they were comprised of single cell suspensions. Cultures were examined serially using inverted microscopy and were generally ready for colony counting on day 10-14 after plating. Colonies were defined as spherical aggregates of cells with a minimum diameter of 60 μ. (Sixty micron colonies generally contain 20-30 cells.) Under the standard conditions of culture in HTCA, fresh tumors which grow usually give rise to at least 30 colonies in HTCA (minimum number acceptable for all chemosensitivity testing results) and only infrequently give rise to more than 200 colonies per plate. When the cultures had grown, the culture plates were stored by refrigeration at 4° C in well-humidified plastic boxes. Slides for morphology were prepared with all starting tumor cell suspensions and from the colony containing cultures using a direct slide technique followed by Papanicolaou staining [21].

Tumor Cell Lines

Three human and one murine cell lines also were used in the evaluation of the automated counter and were of particular value for the higher colony count range because of their higher intrinsic cloning efficiencies than those of fresh tumors. The human lines were used in the reproducibility, precision, and accuracy studies along with fresh tumors, while the murine line was used along with the fresh tumors in the specificity study. Cell lines used are summarized in Table I.

Technician Counting

A microscope stage mask was prepared so that the total counting area viewed by inverted microscopy had similar field margins and area as that of the total field area of the automated colony counter. This was done to eliminate potential problems which might result from uneven microsphere or colony distribution in the dishes and the need for calculation of correction factors for relative field areas. Microsphere and tumor colony counts were performed directly by inverted microscopy on all plates by each of the two technicians, who recorded their scores independently for the coded plates. A total of nine technicians were involved in the phase I manual counting of colonies from fresh tumors. Cultures were counted by each of two technicians as well as by the colony counter. These included 11 ovarian, 9 melanoma, and 10 unknown primary cancers. Samples with > 2000 colonies/plate were excluded from this study as they were extremely difficult to count

Table I. Tumor cell lines used

Cell line	Plating concentration (cells/plate)	Counting day	% P.E.[a]	Supplier
Hep-2 Epidermoid Ca of larynx, human	$2.5\text{-}10 \times 10^3$	7	12.3%	ATTC[b]
WiDr Colon Adenocar. human	$3\text{-}25 \times 10^3$	7-14	= 21.2%	ATTC
147-D Breast Ca., human	$4\text{-}25 \times 10^3$	14	: 9.2%	O. Alibaster, M.D.[c]
Chinchman S91 Melanoma, murine	5×10^3	7-10	· 8.8%	ATTC

[a] Plating efficiency (%) in soft agar.
[b] American Type Tissue Collection, Rockville, MD.
[c] George Washington University, Washington, DC.

either by eye or by the automated counter. Counting the entire experiment was dependent upon a colony count of at least 15 in the control plate area. Two technicians alternated responsibility for this initial assessment (LY and JL). They were highly experienced in HTCA and counted most studies. There were 24 fresh tumor studies wherein they both counted the study. In only 3 of the fresh tumor studies did neither LY nor JL count the study.

Automated Colony Counting

The Omnicon model FAS II (Bausch and Lomb) was utilized as the automated colony counter (colony counter) in these experiments. Background concerning underlying theory and application to colony counting have been published previously [18]. The instrument equipped for this study incorporated a keyboard interface and printer, 36-position automated mechanical stage designed to hold 35 mm Petri dishes, an inverted microscope with a binocular port for technician observation, a television camera and video monitor, and a computer programmed for image analysis (Fig. 1). The standard program performs serial step evaluation of adjacent square fields within each circular Petri dish, with the total field area comprising approximately 51% of the area of the dish. Specific parame-

Fig. 1. Technologist utilizing Omnicon image analyzer for tumor colony counting. Agar-containing Petri dishes are located on the 36 position automated stage of the inverted videomicroscope. An individual field containing colonies is displayed on the monitor of the Omnicon

ical constants were used to define the circularity and density of objects classified as colonies, while instrument controls were used to "threshold" the light intensity delivered through the microscope to the analysis field. The computer's algorithms exclude from enumeration any colonies with significant bisection by two adjacent field boundaries. Operating in an automatic mode, a series of up to 36 Petri dishes can be loaded on the mechanical stage, with each experimental dish specified by keyboard entry as to dish position on the stage, after which the instrument functions automatically until all dishes have been counted and the results have printed out in a series of up to six selected colony size diameters. The counting procedure for 36 dishes takes from 35–40 min and can be accomplished unattended. As carried out in this study, colonies were counted without any overstaining. An additional computer program for semiautomatic counting permitted a single step advance between fields in each Petri dish. This latter program permitted direct microscopic and video monitor comparison in the Omnicon of all objects classified and counted by the technician and the colony counter.

Experimental Protocol and Statistical Analysis

The experimental protocol for evaluation of the colony counter included four separate studies: (1) sensitivity, (2) specificity, (3) precision, and (4) accuracy.

For the sensitivity studies, microspheres prepared in plates as described above were counted once by each of three technicians and twice by the colony counter. The comparison of technician and machine counts was analyzed as described below for the accuracy studies with regression and residual analyses performed on the scatter plot data.

For the specificity studies, agar plates containing approximately 200 colonies grown from 10 fresh tumors and one cell line were utilized. A field-by-field comparison was made using the standard field areas of the colony counter. First the technician identified tumor colonies as observed on the monitor of this colony counter. Then the colony counter analyzed the field and electronically tagged and counted the objects it determined to be colonies. Objects that were identified as tumor colonies by the technician and colony counter were compared. Scatter plots were prepared which related technician count versus colony counter count and the corresponding regression and residual analyses were calculated to provide an accurate and balanced assessment for the specificity studies.

For the precision studies, 10 plates containing WiDr colon cancer colonies were prepared with a colony size range of 50-160 μ and approximately 200-300 colonies were present in the scanning area of the plates. Using a constant dish orientation of each plate in the colony counter's mechanical stage, each plate was counted twice daily (a.m. and p.m.) over five days, and the coefficient of variation on the counts was assessed overall as well as for specific plates and counting times. Data were analyzed by logarithmic transformation, with statistics based upon the inverse of the transformed data.

For the accuracy study, 30 fresh tumors and 3 cell lines grown in HTCA were utilized. The cell lines were plated at required concentrations to assure that the higher colony count range (200-1000 colonies) would be adequately evaluated by the counter. In addition to the comparisons of mean colony counts by the technicians and the colony counter, analogous comparisons were also made of the mean percent survival of tumor colony-forming units (TCFU) as calculated from the technicians' and the Omnicon's colony counts:

$$\text{percent survival} = \frac{\text{no. colonies surviving drug exposure}}{\text{no. of control colonies}} \times 100$$

Five drugs were selected for testing the individual patient's tumor (in two concentrations) governed by the pharmacology of the specific agent [22]. For the fresh tumors, drugs were selected and individualized in relation to tumor type and clinical priorities. For the tumor cell line experiments, the five drugs selected were doxorubicin (DOX), cis-platinum, vinblastine, actinomycin, and 5-fluorouracil. All were tested at 1 μg/ml and 10 μg/ml, using standard incubation procedures. All control plates were counted in sextuplicate and drug-treated plates were counted in triplicate by the two technicians and the colony counter. Statistical methods used to carry out the analysis included scatter plots and linear regression analysis of: (1) technician 2 versus technician 1 (tech 2 vs. tech 1), (2) arithmetic mean of technician 1 and technician 2 versus the colony counter (techs vs. counter). This approach is used widely in quality control for biomedical instruments as it has the advantage of a simple presentation of the data which gives all data the same degree of importance.

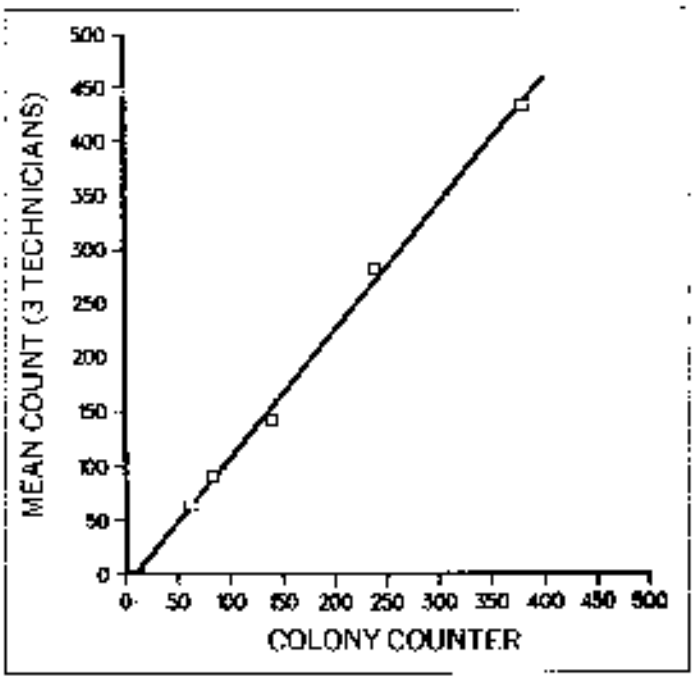


Fig. 2. Scatter plot of the mean of two technician counts versus the colony counter for varying size metal microspheres ($r = 0.999$).

Results

Sensitivity Studies

In these studies, metal microspheres in the size range from 60 μ to 160 μ were embedded in a clear matrix in the Petri dishes and counted by three technicians and the colony counter. There was good agreement between tech 1 and tech 2 as well as in the comparison of the mean of techs 1 and 2 versus tech 3. Figure 2 depicts a scatter plot of the mean technician count versus colony counter count of microspheres in each of five concentrations. The correlation coefficient was 0.999, and the regression and residual analysis showed a good linear relationship through the entire range of microspheres. However, the number of microspheres counted by the technicians was somewhat higher than that counted by the counter (means 202.6 and 181.4 respectively). This was also reflected by the fact that the 95% confidence interval for the slope of the regression line did not pass

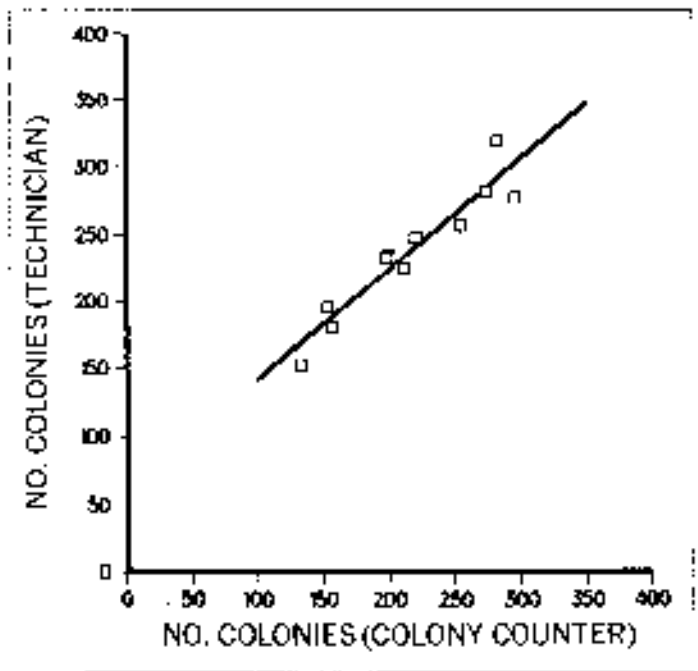


Fig. 3. Field-by-field comparisons of specific colonies counted for ten fresh human tumors and one melanoma cell line technician versus colony counter) obtained in studies of specificity. These results indicate an excellent linear association throughout the entire range of colony sizes.

through 1.0. This difference in the count rate is attributable to microspheres bisected by adjacent fields, and therefore is excluded from enumeration by the colony counter's algorithms.

Specificity Studies

In these studies, the field-by-field comparisons of technician and machine classification and counts were made for 10 fresh human tumors and 1 melanoma cell line. Figure 3 depicts the very close agreement which was observed between the number of colonies counted by the technician and the number counted by the colony counter. The calculated regression line is drawn through the area of the data points (correlation coefficient = 0.95). The results of regression and residual analysis yield an estimated slope of 0.82 (95% confidence interval: 0.60, 1.03) and an intercept for the

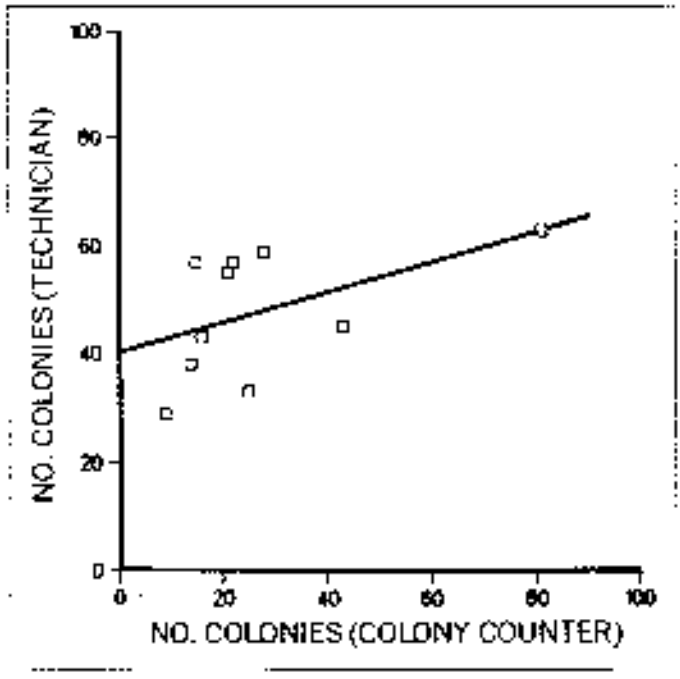


Fig. 4. Field-by-field comparisons of mismatches obtained in specificity studies depicted in Figure 3. The median mismatch count for technicians was approximately twice that of the colony counter ($r = 0.5$).

regression line of 6.0 (95% confidence interval: −0.15, 20.2). There was an indication, as seen in median or mean total colony counts, that the technician counted a slightly larger number of colonies as compared with the colony counter. As can be seen in Figure 4, there was not a good association between the mismatch colony count for the technician as contrasted to the colony counter (correlation coefficient = 0.50). This was further exemplified by the regression and residual analysis, as the slope for the regression line was not consistent with 1.0. The median mismatch count for the technician was approximately twice that obtained with the colony counter, thus indicating that the technician counted more colonies than the colony counter.

Precision Analysis

Precision of the colony counter was evaluated with colonies from a colon cancer cell line and counted 20 times on one day or twice daily for five

Table II. Precision study: repetitive counts twice a day for 5 days, first determination of each time

Day	Time	No. colonies (> 60 μ in diameter/plate)				
		Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
1	AM	1077	389	214	91	53
1	PM	1085	427	209	88	52
2	AM	1083	360	209	87	48
2	PM	1079	374	196	83	46
3	AM	1069	370	203	84	45
3	PM	1063	366	198	79	45
4	AM	1072	378	194	80	47
4	PM	1067	351	200	83	49
5	AM	1080	398	200	78	54
5	PM	1055	372	200	80	52
Mean		1070	378	199.7	85.3	48.0
SD		18.87	21.09	12.83	4.27	3.74
Range		(1053–1085)	(351–427)	(168–214)	(78–91)	(44–54)
Coefficient of variation (%)		1.76	5.73	6.43	5.13	7.80

days. The comparative colony counts between the 10 runs over five days were quite reproducible using either the mean of 4 counts or the first count of each plate per run. As an example of the precision results, the first counts of each plate are summarized in Table II. The coefficient of variation was below 10% in all of the precision studies.

Accuracy Studies

A total of 30 fresh tumors (11 ovarian, 9 melanoma, and 10 unknown primary carcinomas) were included in this study with two drug concentrations tested for each of five drugs plus untreated controls. Each was counted independently by two technicians and the colony counter. Thus, there were a total of 300 data points for the fresh tumor comparison between technician counts or the mean technician count versus the colony counter. In the cell line studies, a total of three different tumor cell lines

were each tested with two drug concentrations plus controls. There were a total of 90 data points available for analogous comparisons of counts on cell lines.

Technician Count Comparisons

The total number of colonies per plate in the studies of fresh tumors ranged from 0 to almost 900 colonies; however, relatively few plates had more than 300 colonies/plate. The comparison of technician counts (tech 1 vs. tech 2) of fresh tumor colonies yielded a regression line with a correlation coefficient of 0.93 (slope 1.11 with 95% confidence interval = 1.06, 1.16; intercept = 9.07 with 95% confidence interval = 21.5, 3.37). Based upon plots of residuals, the association was very good and linear throughout the entire size range of colonies. However, on the average, tech 1 tended to obtain slightly greater colony counts than tech 2. Separate analyses were done of the drug exposure experiments which were divided into high concentration and low concentration exposure for all five drugs. For 150 data points with high drug concentration exposure, the correlation coefficient between technician counts was 0.92, and for 150 data points at low drug concentration, 0.93. An additional subanalysis was performed to compare technician performance by tumor type. Based on both the regression analysis and corresponding residual analysis for ovarian cancer and melanoma, there was a linear association between the two technicians' counts (0.96 for each tumor type). However, there was less association for unknown primary tumor colony counts by the two technicians (correlation coefficient 0.69). This lower association and larger scatter of data points were largely due to data from two tumors. The lower association between the two technicians' counts for colonies from these patients with unknown primary cancers was observed at both low and high drug concentration studies.

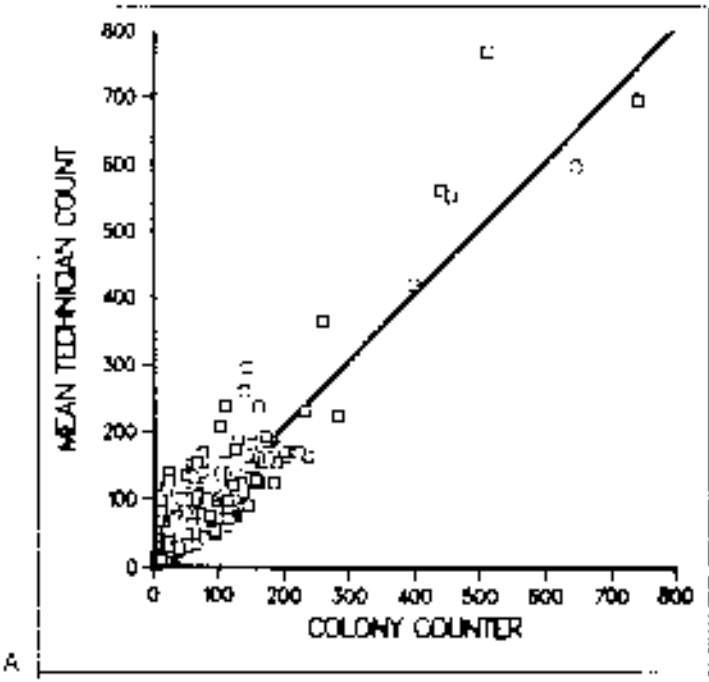
Analogous observations on comparative technician counts were made with the cell line studies. The total number of colonies per plate in these studies ranged from 0 to over 1100, with a significant number of plates containing more than 300 colonies/plate. The regression line (tech 1 vs. tech 2) yielded a correlation coefficient of 0.95 (slope 0.71; 95% confidence limits = 0.67, 0.76; intercept = 12.9, 95% confidence interval = −46.2, 72.0). By subdividing the studies to assess just high or low drug concentration exposure, the correlation coefficient for technician counts was 0.95 for each subset. In the cell-line studies, tech 1 counted on the average a smaller number of colonies than tech 2: tech 1 = 175.3 (SEM = 29.2), tech 2 = 226.6 (SEM = 38.9).

Technician Versus Colony Counter Comparisons

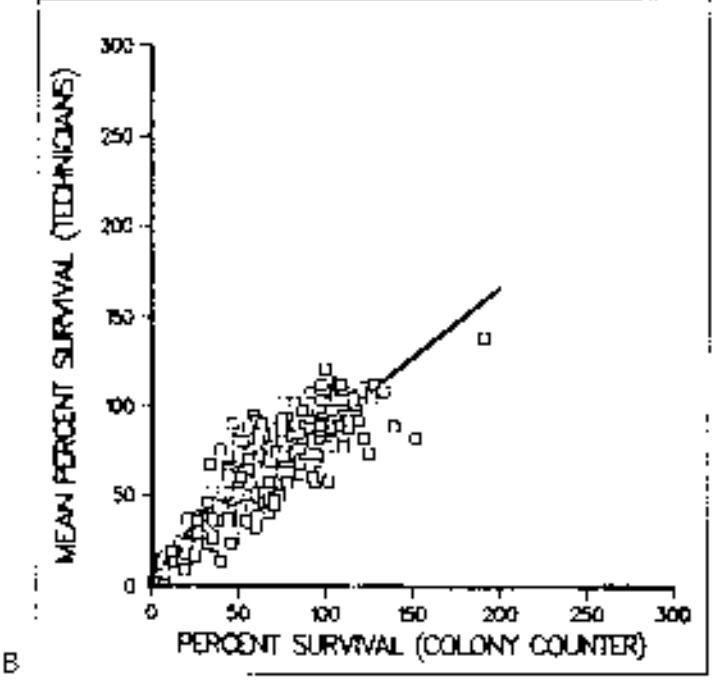
The comparison of mean technician counts (tech 1 + tech 2)/2 versus the colony counter for the 30 fresh tumors studied is depicted in Figure 5A. The results from the regression analysis as well as the plots of residuals indicated a linear association between mean technician count and the colony counter result. The mean value for the combined technician count (87.55 ± 5.4) was slightly higher than that of the colony counter (79.4 ± 4.9). Analysis yielded a regression line with a correlation coefficient of 0.91 (slope = 0.99 with 95% confidence interval = 0.94, 1.05; intercept = 8.39 with 95% confidence interval = −2.89, 19.67), permitting the conclusion that there was not a consistent difference between the enumeration of colonies by technician as compared to the colony counter. The comparison of percent survival of TCFU as calculated from the mean technician counts versus the colony counter for the 30 fresh tumors analyzed, is shown in Figure 5B. The regression line has a correlation coefficient of 0.85.

The colony count data were also analyzed in relation to high or low drug concentration exposure. At the high drug concentration exposure, the correlation coefficient between mean technician and colony counter results was 0.90, whereas at low concentration it was 0.93. As reflected in Figure 5, there was a linear association observed between mean technician and machine count through the entire colony size range. An additional subanalysis was performed to compare mean technician and colony counter results by tumor type. There was a very good linear association throughout the entire range of colony counts for both ovarian cancer and melanoma (correlation coefficients 0.93 and 0.95 respectively). In contrast,

Fig. 5. (A) Accuracy study: Comparison of tumor colony counts from 30 cancer specimens obtained by each of two technicians (mean value) and the colony counter. Data are plotted as absolute counts and include 150 data points from both drug treated and control cultures. A number of data points are superimposed, but the regression line is calculated for all points. (B) Accuracy study: Comparison of technicians and Omnicon data expressed as percent survival of tumor colonies after drug exposure. Points on this plot are calculated from the colony counts shown in (A). This analysis normalizes the data from low and high colony count tumors with most data points falling between [illegible] and 100% of control.


MEAN TECHNICIAN COUNT
COLONY COUNTER
0
100
200
300
400
500
600
700
800

A


MEAN PERCENT SURVIVAL (TECHNICIANS)
PERCENT SURVIVAL (COLONY COUNTER)
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50
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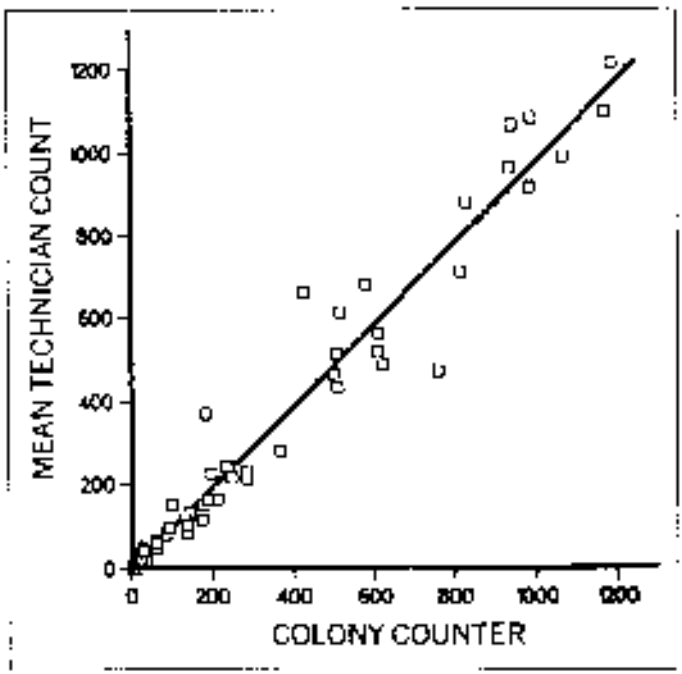

Fig. 6. Accuracy study on ten tumor cell lines with comparison of the mean of two technicians' counts versus the colony counter.

for patients with unknown primary carcinomas, there was a lower association between mean technician and machine counts (correlation coefficient 0.65). This lower association was primarily due to two patients for whom there were marked differences between the technician and the Omnicon counts. In these two patients, technicians consistently counted larger numbers of colonies by a factor of about 3 as contrasted to the colony counter. Separating the data on patients with unknown primary cancers by their low versus high drug concentration test did not yield additional information on this lower association in the unknown primary cancer group.

Accuracy data on the tumor cell lines were also compared for the mean technician counts versus the colony counter. Figure 6 depicts the scatter plot for the mean number of colonies counted by the technicians and the colony counter. An excellent correlation was obtained using tumor cell lines. Regression and residual analysis yielded a correlation

coefficient = 0.98 (slope = 0.98 and 95% confidence interval; 0.93, 1.01; intercept = −2.96 and 95% confidence interval −70.4, 64.5). The mean number of colonies counted by the technicians (301.2 ± 33.7) was quite similar to that counted by the colony counter (209.0 ± 33.9). Under the various conditions and lines tested, there was very close linear association between the mean technician count and Omnicon counts of cell lines throughout the entire range of colony sizes studied. Subanalysis, based on high and low drug concentration exposure, also yielded very good correlations (0.96 and 0.95 respectively) between mean technician and colony counter results.

Discussion

These systematic studies provide excellent evidence that the Omnicon image analysis system is a sensitive, specific, precise, and accurate means to count tumor colonies in soft agar. Based on these data, we believe it is a highly desirable alternative to manual counting by technicians. Our results markedly extend the initial report by *Kressner* et al. [18] and the recent paper by *Herman* et al. [23] who conducted an in-use evaluation of this colony counter. We found the colony counter to be as accurate as technician counts when either gross colony counts or percent survival after drug exposure was compared. It has been well established that marked heterogeneity is observed when clonogenic tumor cells from different patients (even with tumors of the same histopathology) are exposed to the same anticancer drugs [4–7]. For this reason, the availability of automated counting techniques could prove to be a great asset, as it provides sufficient speed and accuracy to permit relatively large drug assays to be set up with clonogenic assay methodology. In addition, the colony counter proved to be extremely accurate in its application to the counting of colonies grown from established tumor cell lines. Such cell lines provide excellent single cell suspensions as starting materials for clonogenic assay, and therefore there are fewer intrinsic artifacts present than is the case with cultures established from disaggregated fresh human tumors, which may contain "clumps" that could be confused with colonies. Studies of tumor cell lines with the colony counter could prove to have a wide variety of applications in research on cancer biology and growth factors. The precision studies clearly indicated that the colony counter can obtain remarkably similar counts when plates are counted repeatedly on the same day or sev-

eral times daily for a number of days. In our experience, most technicians have substantial difficulty doing colony counts for more than 3–4 h per day, principally because of operator fatigue. The machine's ability to perform colony counts automatically for as long a period of time as desired, as well as its intrinsically greater speed (averaging 1 min/plate as compared to 10 min/plate by the technician), represent two of its highly desirable characteristics. In the current studies, the instrument proved accurate in the assessment of surviving numbers of colonies after exposure to high or low concentrations of a variety of anticancer drugs. It was of interest to observe that when the data on colony counts were broken down by tumor type, significantly greater discrepancies between technician counts as well as technician versus machine counts were observed in the tumor category of "carcinomas of unknown primary." This group of tumors is a recognized but heterogeneous entity which often exhibits clinical and in vitro drug resistance to a wide variety of anticancer drugs. Such tumors arise from a number of distinct anatomical sites, with the major commonality being that their site of origin cannot be established. The discrepancies we observed with unknown primaries were attributable to two specific tumor specimens which exhibited substantial variability, as assessed by each of two technicians, and in the comparison of the technicians and the colony counter. The studies of accuracy clearly established that within the count range of up to 1000 colonies/plate, colonies from both fresh tumors and tumor cell lines could be counted reliably with automated methods. When significantly larger numbers of colonies are present in the Petri dishes (e.g., more than 2000/plate), the ability of the automated counter falls off substantially, and for that reason such high count samples were not included within the specimens considered for evaluation in this analysis. In our experience, plates with more than 2000 colonies/plate that are counted with the colony counter can yield an undercount of more than 25%. Such losses in counts are due to the presence of too many objects per field for analysis by the instrument, as well as significant overlap of colony margins which lead their borders to appear irregular in two dimensions and therefore are not subjected to automated counting. In the analysis of mismatches carried out as a component of the specificity studies, it became apparent that the technician counted more colonies than the colony counter. We believe that this discrepancy can be explained by at least two factors. First, the technician is not limited to counting a colony that fulfills the criterion of having a specified degree of circularity or uniformity in optical density. Examination by inverted microscopy does permit identi-

fication by the technician of occasional tumor colonies which have irregular margins, cellular extensions, or differences in internal density (e.g., due to cystic areas). Second, the colony counter has an algorithm for dealing with field adjacencies wherein those colony-shaped objects which are bisected at the field interface are excluded from the count. This is essentially because such a colony would be identified as more or less of a half-circle in each of the two fields. In this respect, the process of counting by the technician differs from that of the machine, as field adjacencies were not marked in any way in the dishes and not accounted for by the technician, whereas such field delineation was essential in relation to the mechanical stage transit and analytic features for microscopic measurements by the colony counter.

In the sensitivity studies wherein very circular microspheres of uniform density were counted, the agreement between the curves of technician and machine counts was excellent, and the undercount by the colony counter, which averaged 10.5%, likely reflected only the phenomenon of the "bisected colony." Inasmuch as the microspheres were distributed rather uniformly in the plates, this result thus defines the magnitude of intrinsic undercounting by the machine that results from the field adjacency bisection of colonies.

Based on these studies, we believe that the automated colony counter provides a useful and practical alternative to manual counting of tumors by technicians. It will have a wide variety of preclinical and clinical applications in oncology.

Acknowledgments

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