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# An Analysis of Several Slide Preparation Techniques for Human Peripheral Blood Cultures

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SUMMARY

Several slide preparation procedures were studied in order to render a semiquantitative indication of an optimum technique or techniques, concerning the over-all cytological quality of the chromosome spreads effected by each technique. A method of scoring for karyotypically analyzable spreads is given. It is concluded that one of the techniques (technique D) be utilized for routine karyotypic studies while a combination of two of the techniques (techniques D and E) should be employed for more extensive studies.

One of the methods used in human cytogenetics is to spread the chromosomes through fixative ignition on clean cold slides which have been coated with siliclad. The purpose of this communication is to analyze various technical modifications. An attempt is made to determine the over-all cytological quality of the preparations in a semiquantitative way.

## Materials and Methods

Venous blood from a normal, healthy subject was cultured according to a micromethod proposed by Hungerford (1965) with several modifications: 4 ml aliquots of medium containing Difco TC Medium 199 were dispensed in 14 × 74 mm screw cap test tubes; 0.15 ug of colchicine (Nutritional Biochemicals) per ml of medium were added during the last two to three hours of incubation. The incubation temperature was 37-38°C.

SLIDE PREPARATION TECHNIQUES. The slide preparation techniques themselves are referred to as techniques A through F and are the following.

A. Clean silicladed (Clay-Adams) slides were placed in cold 70% ethanol and placed in the refrigerator until the cellular suspension was in fixative.[1] One drop was then placed on several slides and the entire slide was ignited by means of an alcohol burner. The slides were stained in Unna's polychrome methylene blue solution prepared according to Turpin and Lejeune (1965). The slides were placed in a slide bank (Eberbach) for several hours to one day and subsequently mounted in Damar (Hartman-Leddon Co., Philadelphia).

[1] The fixative is a mixture of glacial acetic acid and methanol, 1 : 3.

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Acta Genet. Med. Gemellol. (1971), 20: 85-95

B. This technique is similar to A except that no siliclad coating of the slides was employed. This technique is a modification of the technique for slide preparations stated in Merchant et al (1964).

C. The clean slides were immersed in crushed ice and ethanol. One or two drops of suspension were placed on the slides and quickly rotated. They were immediately passed through the flame for fixative ignition, shaken well after the fixative had burned off, air-dried and processed similar to technique A. Technique C is employed routinely at the Idaho State School and Hospital at Nampa, Idaho.

D. One or two drops of suspension were placed on clean silicladed slides previously immersed in cold distilled water stored in the refrigerator. Subsequently the fixative was ignited and burned off; the slides were shaken well to facilitate drying and were allowed to completely air-dry. They were then processed similar to technique A. Technique D is a modification of the techniques given by Schera (1962), Merchant et al (1964), and Difco (1968).

E. Small drops of suspension were placed on dry clean cover slips and spread evenly by blowing on them. The cover slips were subsequently processed similar to that of technique A. Technique E was derived from the technique stated in Gibco (1967).

F. This technique is similar to E except the cover slips were immersed in cold distilled water, previously stored in the refrigerator. All other variables are described in the articles from which the techniques were derived.

SCORING. A bright-field American Optical Research Microscope was utilized for all of the observations. The term " Scan " refers to observing and scoring under low power ($\times$ 100). Several of the scan observations were further checked with high dry power ($\times$ 450). In scanning under low power the positions of various spreads were noted using a Carl Zeiss Objektfinder. After scoring, the spreads to be further studied were relocated using the Objektfinder and analyzed under oil immersion ($\times$ 1000).

DEFINITIONS AND ABBREVIATIONS. The following four parameters were utilized to select analyzable spreads for general karyotypic analysis:

1) All chromosomes must be clearly distinguishable morphologically.

2) All spreads must be intact or fragmented[2] to the extent that the individual chromosomes or fragments are not further from the main body of the spread than the length of the longest radius from the periphery of the main body of the spread. If the spread is greatly distorted, the radius used will be the mean between the two radii.

3) If the spread is aligned other than the above, but clearly isolated from other cells and the concerned chromosomes are obviously from the same cell, the spread will be considered analyzable; that is, if the chromosomes are similar in contraction and the chromosomes and/or spread fragments are within the above mentioned limits, the spread is considered analyzable.

4) All analyzable spreads must have at least 40 chromosomes.

Intact and fragmented analyzable spreads can be seen in Figs. 1 and 2, respectively. Fig. 1a is the actual print while Fig. 1b is a diagram showing the limits of the spread and

[2] Fragmented implies a fragmented spread, not a fragmented chromosome. It applies to a group of chromosomes or it may even apply to a single chromosome isolated from the main body of the spread. It does not imply chromosome fragments.

indicating intactness. Fig. 2a is again a print of the actual spread while Fig. 2b outlines the limits of the fragments. Below are abbreviations used throughout the paper.

L (Legal spread). A spread that under scan was apparently intact but several or many of the chromosomes were not morphologically distinguishable rendering the spread unanalyzable.

Ls (Legal spread - low power). A spread that was initially scored as acceptable under scan and should be further examined for numerical and morphological analysis under oil immersion.

$Ls_1$. The Ls amount observed during the first 100 (approximately) observations under scan.

$Ls_2$. The total Ls amount observed under scan.

$Ls_3$. The revised Ls amount observed under oil immersion.

I.S. (Illegal Spread). An unanalyzable spread because it does not fit one or more of the parameters mentioned for analyzable spreads in general karyotypic analysis. This is determined under scan.

I.A. (Illegal Area). A group of chromosomes scattered or fragmented in an area and having different contraction and morphology. The " spread fragments " or scattered chromosomes are presumed to have been derived from two or more cells. This is determined under scan analysis and also high dry.

L.I. (Legal Intact). An intact analyzable spread. That is, none of the spread is fragmented into groups of chromosomes or individual chromosomes. This is determined under oil immersion.

L.F.S. (Legal Fragmented, Scattered). A fragmented analyzable spread. The chromosomes or groups of chromosomes are scattered, that is, not intact, but still fit the criteria of an analyzable spread. This is determined under oil immersion.

N.C. (Not Countable). All observations whose chromosomes were not clearly distinguishable and hence not analyzable.

P. (Photographable). All analyzable spreads acceptable for karyotypic analysis.

C.O. (Count Only). Analyzable spreads which are acceptable for numerical analysis only as opposed to both numerical and morphological (karyotypic) analysis.

The term scan would infer the following: relative to the abbreviations $Ls_1$, L, I.S., and I.A., it would refer to the observations scored during the first hundred observations (approximately). That is, scanning under low power I.A., I.S., L, and $Ls_1$ were scored as they were observed in the order of the first hundred observations encountered. $Ls_2$, already defined, was obtained by continuing the scan after the first hundred observations were made, but by recording with the Objektfinder only Ls type spreads as opposed to recording I.A., I.S., L, and Ls. Here we were sampling for approximately 100 Ls cells referred to as $Ls_2$. The $Ls_2$ cells were relocated and analyzed under oil immersion. They were evaluated for the intactness of the spread, the chromosome number, and the general cytological quality of the spread as indicated in the Tables (II, III, and IV, respectively).

During this analysis revision of the $Ls_2$ number was expected because of the higher magnification involved. Any modifications in the $Ls_2$ number were manifested in the final revised Ls amount or $Ls_3$. All percentages in Tables II, III and IV were based on this revised number, the $Ls_3$ number. The percentage mentioned in Tab. II, L.I. and L.F.S., were determined by means of $Ls_3$ minus the amount of cells which were less than or equal to 39 chromosomes in number. The percentage in Tab. III was determined by dividing each of the actual values by the $Ls_3$ amount while Tab. IV percentage was determined

by dividing the quantity $Ls_3$ minus the number of cells which contained less than or equal to 39 chromosomes.

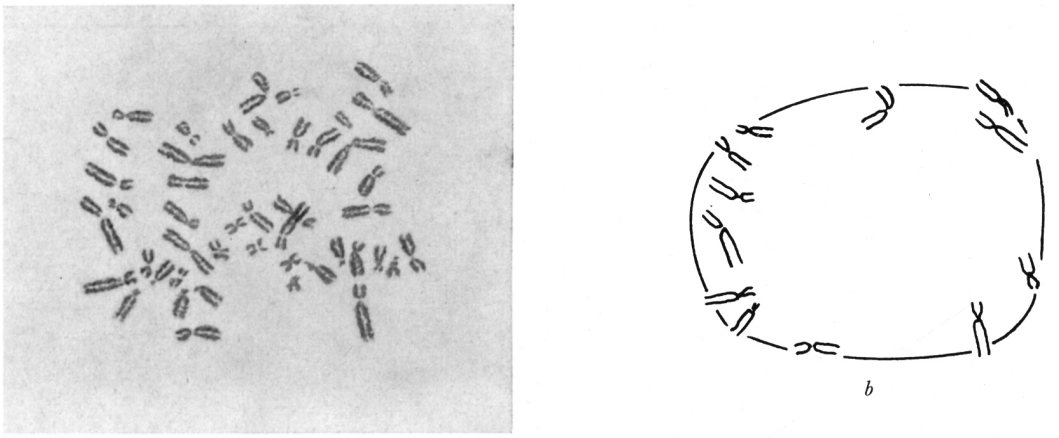
PHOTOMICROSCOPY. All photomicroscopy was carried out by means of a Zeiss II Photomicroscope using phase-contrast microscopy at a final magnification of × 1300. A Zeiss GG-14 filter was used for phase-contrast photomicroscopy.

## Observations and Results

Tab. I shows the actual (Act.) and percentage values of $Ls_1$, $Ls_2$, $Ls_3$, L., I.S., and I.A. It can be seen from Tab. I and Fig. 3 that technique C exhibited the highest percentage of acceptable spreads since 46.4% of the first 99 observations encountered were apparently analyzable while techniques A, D and F were quite close together with technique B showing the lowest percentage of acceptable spreads, while technique E was next in the order of magnitude.

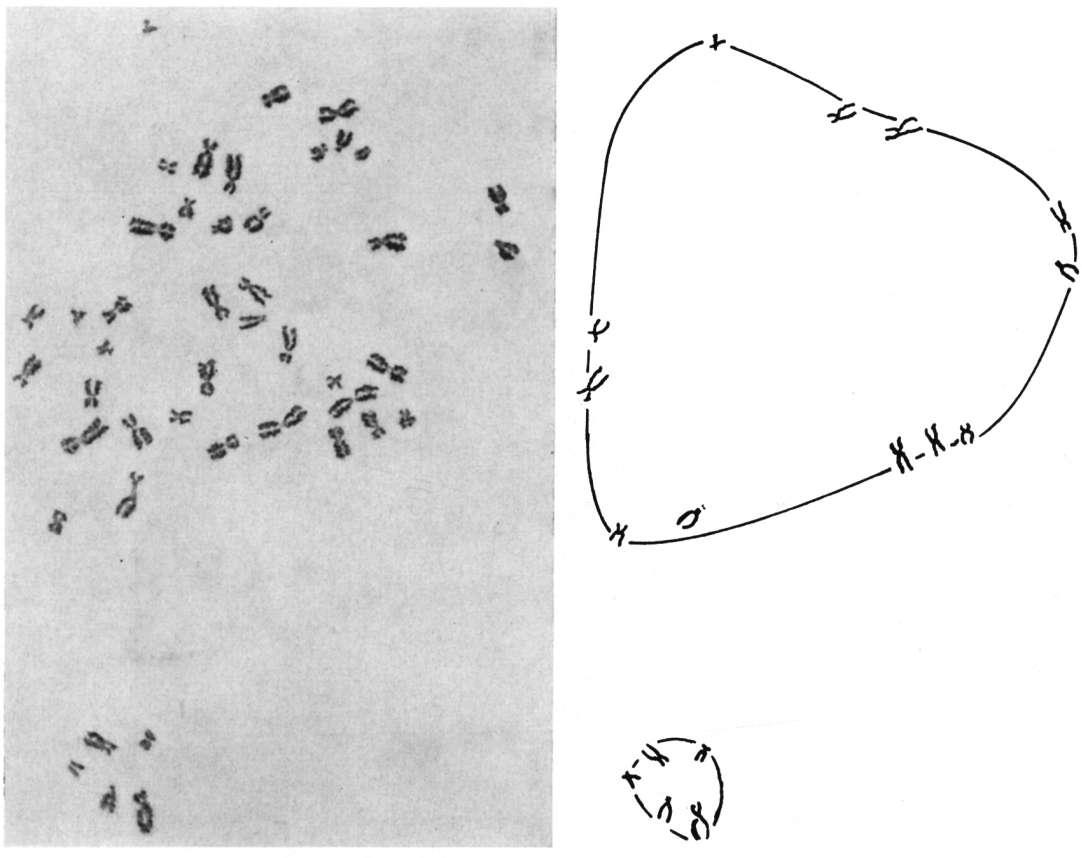
**Analyzable spread, chromosome number and cytological quality frequencies**

| | | A | | B | | C | | D | | E | | F | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Act. | % | Act. | % | Act. | % | Act. | % | Act. | % | Act. | % |
| **Tab. I** | $Ls_1$ | 46 | 42.6 | 24 | 24.5 | 46 | 46.4 | 42 | 41.6 | 33 | 34.0 | 43 | 43.0 |
| | $Ls_2$ | 74 | | 96 | | 95 | | 103 | | 94 | | 90 | |
| | $Ls_3$ | 73 | | 93 | | 94 | | 101 | | 91 | | 91 | |
| | L. | 6 | 5.6 | 10 | 10.2 | 7 | 7.1 | 2 | 2.0 | 23 | 23.7 | 33 | 33.0 |
| | I.S. | 40 | 37.0 | 49 | 50.0 | 40 | 40.4 | 55 | 54.5 | 40 | 41.2 | 21 | 21.0 |
| | I.A. | 16 | 14.8 | 15 | 15.3 | 6 | 6.0 | 2 | 2.0 | 1 | 1.0 | 3 | 3.0 |
| **Tab. II** | L.I. | 56 | 76.7 | 73 | 78.5 | 47 | 50.0 | 72 | 81.8 | 81 | 93.1 | 72 | 85.7 |
| | L.F.S. | 17 | 23.3 | 20 | 21.5 | 47 | 50.0 | 16 | 18.2 | 6 | 6.9 | 12 | 14.3 |
| **Tab. III** | $\leq 39$ | 1 | 1.4 | 4 | 4.1 | 6 | 6.4 | 13 | 12.9 | 4 | 4.4 | 7 | 7.7 |
| Chromosome | 40-47 | 54 | 73.0 | 84 | 86.6 | 76 | 80.9 | 69 | 68.3 | 41 | 45.1 | 59 | 64.8 |
| number of | 48-68 | 0 | 0.0 | 2 | 2.1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 2.2 |
| the $Ls_3$ cells | 69-92 | 1 | 1.4 | 0 | 0.0 | 2 | 2.1 | 0 | 0.0 | 1 | 1.1 | 3 | 3.3 |
| | N.C. | 18 | 24.3 | 7 | 7.2 | 10 | 10.6 | 19 | 18.8 | 45 | 49.5 | 20 | 22.0 |
| | 40 | 1 | 1.4 | 0 | 0.0 | 1 | 1.1 | 1 | 1.0 | 0 | 0.0 | 1 | 1.1 |
| | 41 | 3 | 4.1 | 5 | 5.2 | 3 | 3.2 | 1 | 1.0 | 0 | 0.0 | 0 | 0.0 |
| | 42 | 6 | 8.1 | 4 | 4.1 | 2 | 2.1 | 3 | 3.0 | 1 | 1.1 | 4 | 4.4 |
| | 43 | 2 | 2.7 | 10 | 10.3 | 7 | 7.4 | 8 | 7.9 | 0 | 0.0 | 10 | 11.0 |
| | 44 | 9 | 12.2 | 8 | 8.2 | 10 | 10.6 | 2 | 2.0 | 1 | 1.1 | 4 | 4.4 |
| | 45 | 11 | 14.9 | 15 | 15.5 | 14 | 14.9 | 6 | 6.0 | 2 | 2.2 | 6 | 6.6 |
| | 46 | 22 | 29.7 | 40 | 41.2 | 39 | 41.5 | 44 | 43.6 | 37 | 40.7 | 32 | 35.2 |
| | 47 | 0 | 0.0 | 2 | 2.1 | 0 | 0.0 | 4 | 4.0 | 0 | 0.0 | 2 | 2.2 |
| **Tab. IV** | P. | 35 | 47.9 | 35 | 37.6 | 39 | 41.5 | 61 | 69.3 | 34 | 39.1 | 37 | 44.0 |
| | C.O. | 20 | 27.4 | 51 | 54.8 | 45 | 47.9 | 8 | 9.1 | 8 | 9.2 | 27 | 32.1 |
| | N.C. | 18 | 24.7 | 7 | 7.5 | 10 | 10.6 | 19 | 21.6 | 45 | 51.7 | 20 | 23.8 |



*a* *b*

Fig. 1. An intact, analyzable spread. *a*: photomicrograph (× 1300); *b*: diagram outlining the limits of the spread and indicating intactness.



*a* *b*

Fig. 2. A fragmented, analyzable spread. *a*: photomicrograph (× 1300); *b*: diagram outlining the limits of the fragments.

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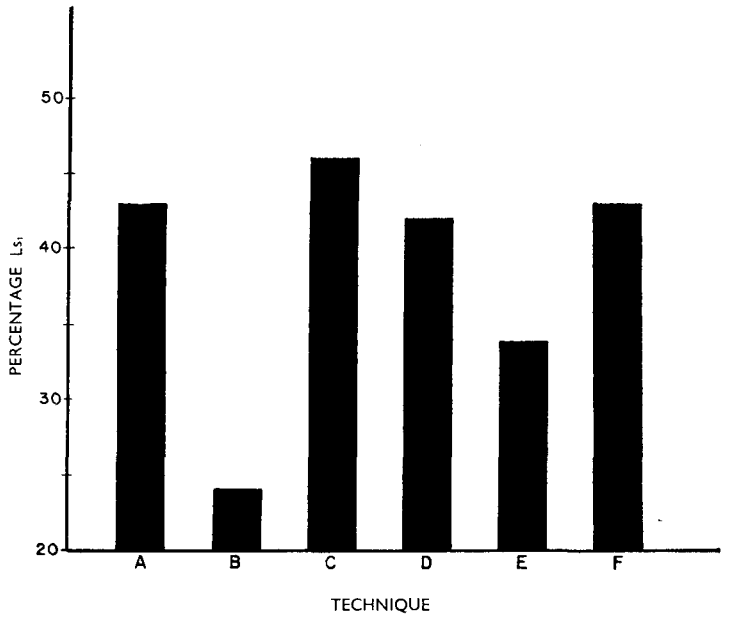


Fig. 3. The percentage of analyzable spreads ($Ls_1$) for techniques A-F

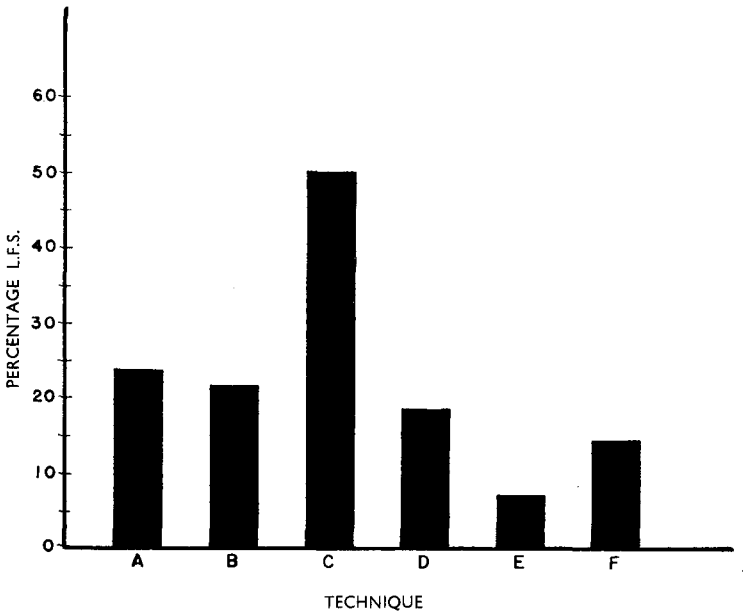


Fig. 4. The percentage of fragmented analyzable spreads (L.F.S.) for techniques A-F



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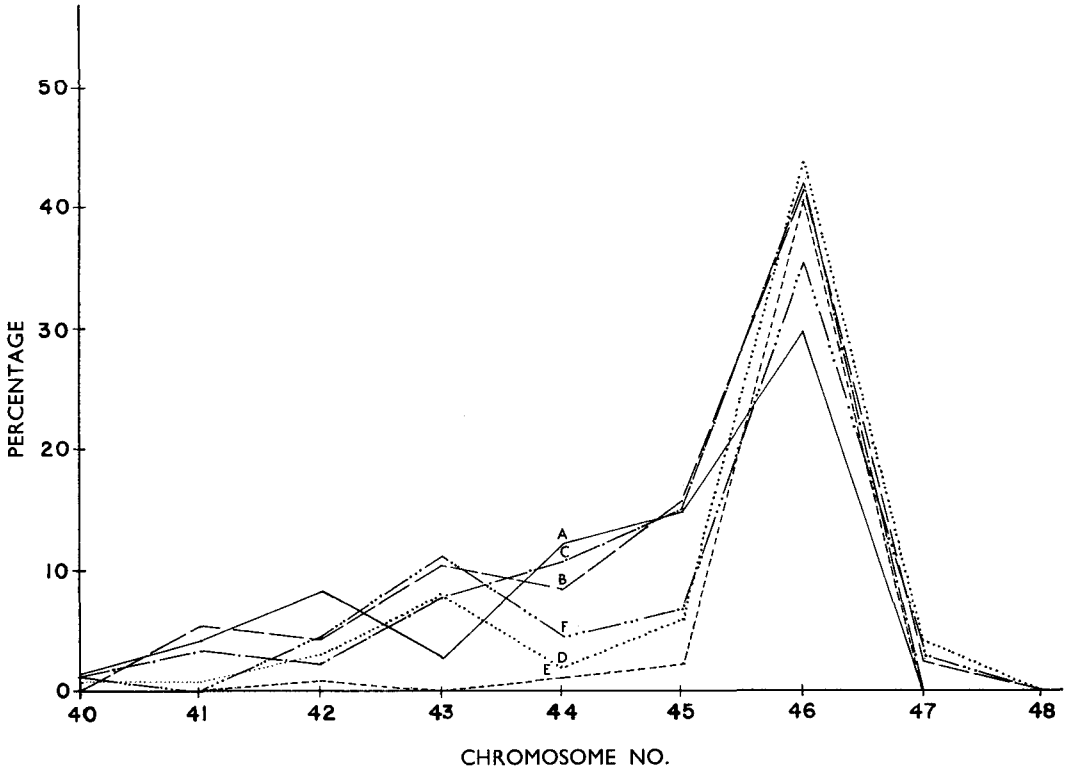


Fig. 5. The incidence of chromosome numbers 40-48 for techniques A-F

Tab. II shows the actual values and percentage values of intact and fragmented analyzable spreads. It can be seen from Fig. 4 that technique C manifested the largest percentage of fragmented spreads because 50% of 94 $Ls_3$ cells were fragmented. The technique with least fragmentation was E with technique F next in size while techniques A, B, and D were quite similar.

Tab. III shows the chromosome number (39-92) of $Ls_3$ spreads in actual and percentage values as well as indicating the countability (N. C.) of the $Ls_3$ cells. The values recorded in the row with the 39 chromosome number indicate the error on the part of the microscopist in being able to estimate under low power the number of chromosomes in a spread. It can be seen from the table and Fig. 5 that technique D had the largest percentage of spreads with the expected modality of 46, while techniques B, C and E were quite close to this value and technique A was the lowest value. Fig. 5 also shows that technique E deviates least from the expected modality of 46 chromosomes.

Tab. IV shows the actual and percentage values of analyzable cells for both numerical and morphological analysis (P). It also indicates the value which can

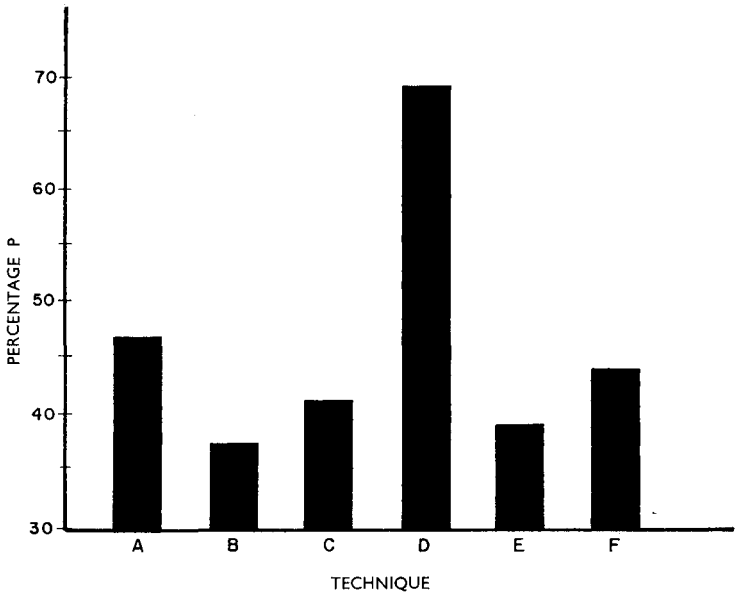


Fig. 6. The percentage of karyotypable spreads (P) for techniques A-F

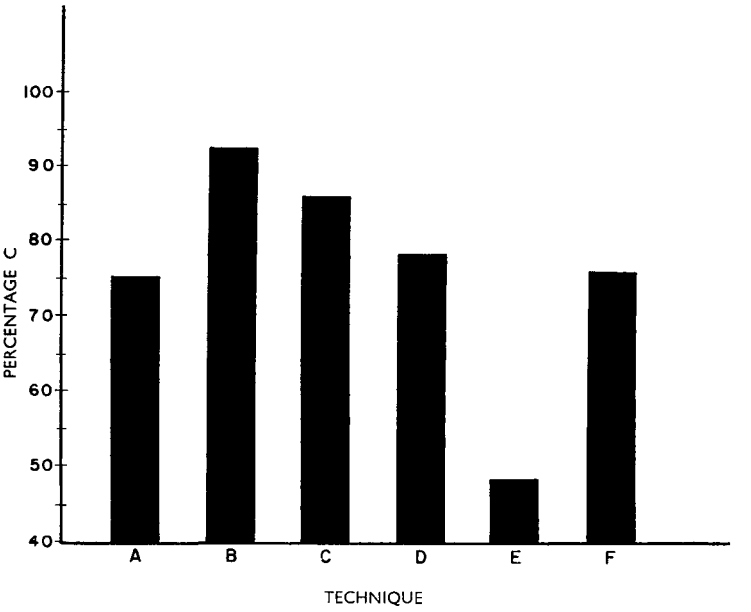


Fig. 7. The percentage of numerically analyzable spreads (C) — a combination of P and C.O.



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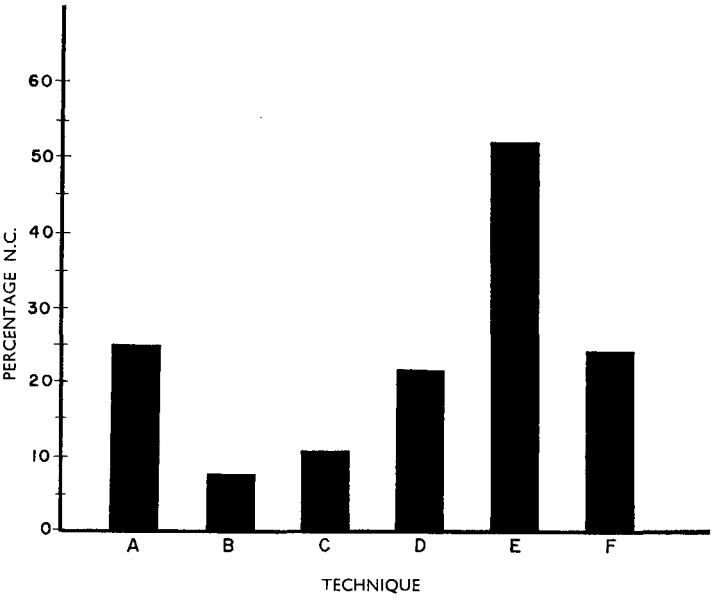


Fig. 8. The percentage of uncountable spreads (N.C.) for techniques A-F

be utilized for numerical analysis only (C.O.) and finally, shows the number of $Ls_3$ spreads which were not suitable for numerical or morphological analysis (N.C.). Tab. IV and Fig. 6 show that technique D had almost $1^1/_2$ times as many analyzable spreads as any other technique in this study. Techniques A and F were next in the order of magnitude, while techniques E and B were least.

Fig. 7 indicates the total number of numerically analyzable spreads (C) — a combination of P and C.O. It can be seen that technique B effected the highest number of countable cells, 92.4%, while techniques A, C, D, and F were all over 75% with technique E effecting the lowest value of 48.3%.

Fig. 8 contains the percentage of uncountable spreads (N.C.) for techniques A-F. Tab. IV and Fig. 8 indicate that technique E had at least twice as many uncountable spreads as any other technique studied. Techniques A, D, and F were quite similar, while techniques C and B effected the least number of uncountable spreads respectively.

## Discussion

It is realized that our definition of an analyzable spread may not be acceptable to all investigators but it is thought that this description is a practical one.

Bearing in mind that this was a semiquantitative analysis, it seems that techniques A, C, D and F (Fig. 3) are among those to be employed when one is interested in obtaining the highest possible percentage of acceptable spreads under scan

analysis. The technique exhibiting the highest number of acceptable spreads was C. On the other hand, if one wished to insure that the highest possible percentage of cells was unfragmented then one would certainly avoid technique C and employ technique E as evidenced in Tab. II and Fig. 4. Further, as shown in Tab. III and Fig. 5, if one wanted to insure that the spreads under examination deviated least from the expected modality, which was 46 in this case, one would choose technique E although in quantity techniques D, B, C and F were quite close to this value. If one wished to insure the maximum number of karyotypically analyzable cells, one would choose technique D, as shown by the values in Tab. IV and Fig. 6. If one wanted to insure the highest possible percentage of numerically analyzable spreads, one would confine one's choice of techniques to A through D and F. Technique B exhibited the highest number.

It should be realized that any of the techniques mentioned in the study are usable because they can be standardized in order to determine the amount of artifactual chromosome number deviation. As seen from Figs. 4 and 5, however, the lowest percentage of fragmented spreads and the least deviation from the expected modality have been effected by technique E. Thus, this technique would be the easiest one to standardize relative to artifactual numerical modulation. Further, it would also be desirable to standardize technique D because it has the highest amount of karyotypically analyzable cells and compares quite favorably with all of the other techniques in the graphic presentations.

Therefore, it is recommended that for routine karyotypic analyses, technique D be employed, while for more extensive research efforts, a combination of techniques E and D should be utilized.

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Riassunto

Sono state studiate varie tecniche preparative al fine di ricavarne un'indicazione semiquantitativa di una o più tecniche ottimali per la qualità citologica delle piastre cromosomiche. Viene anche proposto un metodo di valutazione quantitativa delle piastre analizzabili. Si conclude suggerendo di usare la tecnica D per gli studi cariotipici di routine, e, per studi di più ampio respiro, una combinazione delle tecniche D ed E.

Résumé

Différentes techniques de préparation ont été analysées dans le but d'obtenir une indication sémi-quantitative d'une ou plusieurs techniques optimales pour la qualité cytologique des plaques chromosomiques. Une méthode d'évaluation quantitative des plaques analysables est aussi proposée. En conclusion, l'on propose d'utiliser la technique D pour les études caryologiques de routine, et une combinaison des techniques D et E pour des études plus complexes.

Zusammenfassung

Untersuchung verschiedener Präparationstechniken zur Gewinnung einer semiquantitativen Indikation einer oder mehrerer optimaler Methoden zur Bestimmung der zytologischen Qualität der Chromosomenplatten. Unter anderem wird eine Methode zur quantitativen Bewertung der analysierbaren Chromosomenplatten vorgeschlagen. Abschliessend wird geraten, für karyotypische Routine-Untersuchungen die Technik D und für weitläufigere Untersuchungen eine Kombination der Techniken D und E anzuwenden.

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