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# Exclusion of Chromosomal Mosaicism: Tables of 90%, 95%, and 99% Confidence Limits and Comments on Use

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If one evaluates cytogenetically $n$ metaphases and finds identical karyotypes in all, what degree of mosaicism may be excluded with 95% or 99% confidence? Standard tables (e.g., [1, 2]) of what are described as 95% and 99% (or 0.95 and 0.99) confidence limits for proportions are often applied to this question. For 0 (or $n$) out of $n$ observations, however, applicable to the question of excluding mosaicism raised here, the limits given or derived from these tables (for $0/n$ or $n/n$ observations) are really 0.975 and 0.995 confidence limits. (See [1], p. 185 for an explanation.) The consequent differences are not trivial. For example, to exclude 10% mosaicism or greater with 0.95 confidence, evaluation of at least 29 cells is necessary, whereas the cited tables indicate the need to evaluate at least 35 cells (20% more) which is in fact the number required to exclude 10% mosaicism with 0.975 confidence. There would appear to be a use for tables specifically tailored to the problem of excluding chromosomal mosaicism at 95% and 99% confidence limits.

## METHODS

If one counts $n$ cells and finds no chromosomal mosaicism then the smallest level of mosaicism excluded with confidence level $1 - \alpha$ may be found by solution of the equation: $p^n + (1 - p)^n = \alpha$ where, if $1 - p$ is greater or equal to 0.50 (50%), then the level of mosaicism excluded is $p$ or greater, up to a maximum of 0.50 (50%). For example, for a confidence level of 95% (0.95), $\alpha = 0.05$, and if six cells are evaluated ($n = 6$), then $1 - p = 0.599$ and $p = 0.401$. Thus, the observations of six cells with the same cytogenetic pattern excludes, with 95% confidence, mosaicism of 41% (but not 40%) or more. (The method of calculation used was a determination of the lowest integral value of $n$ such that for a given $p$, the calculated $\alpha$ is less than or equal to (to five decimal places) the specified $\alpha$. Thus, for $p = 12\% = 0.12$ and $n = 36$ the calculated $\alpha$ is 0.01003, just above 0.01. To exclude mosaicism of 12% or greater with 0.99 (99%) confidence, an $n = 37$ is required, which in fact yields a calculated $\alpha$ of 0.00828, well below 0.01.) For results, see table 1.

## DISCUSSION

Several factors must be emphasized concerning application of table 1. Use of these values assumes that the probability of mosaicism is constant and independent in the cells examined. Cells which are evaluated provide data for fair inferences only concerning that population of which they are an unbiased sample.

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For these reasons, the table is most appropriate for evaluation of cells in the first metaphase division in vitro (e.g., PHA stimulated lymphocytes after 40–48 hr cultures or bone marrow analyzed using "direct" techniques). For cells examined after relatively long-term cultures (e.g., fibroblasts or amniotic fluid cells), there is the possibility of clonal outgrowth. If, for example, one single clone gives rise (in vitro) to all the metaphases analyzed from a culture of amniotic fluid, this would not provide data for inferences concerning the population of amniotic fluid cells from which the original cell was derived. Thus, for cytogenetic studies after relatively long-term cultures, the table is more appropriately applied to the number of independent colonies or clones rather than

TABLE 1

PERCENT MOSAICISM EXCLUDED WITH 0.90, 0.95, AND 0.99 CONFIDENCE IF SPECIFIED NUMBER OF CELLS ARE EVALUATED AND FOUND TO HAVE IDENTICAL KARYOTYPES

| No. Cells ($n$) | Confidence Levels | | | No. Cells ($n$) | Confidence Levels | | |
|---|---|---|---|---|---|---|---|
| | 0.90 | 0.95 | 0.99 | | 0.90 | 0.95 | 0.99 |
| ≤4 | ... | ... | ... | 36 | 7% | 8% | 13% |
| 5 | 38% | ... | ... | 37 | 7% | 8% | 12% |
| 6 | 32% | 41% | ... | 38 | 6% | 8% | 12% |
| 7 | 29% | 35% | ... | 39 | 6% | 8% | 12% |
| 8 | 26% | 32% | 46% | 40 | 6% | 8% | 11% |
| 9 | 23% | 29% | 41% | 41 | 6% | 8% | 11% |
| 10 | 21% | 26% | 37% | 42 | 6% | 7% | 11% |
| 11 | 19% | 24% | 35% | 43 | 6% | 7% | 11% |
| 12 | 18% | 23% | 32% | 44 | 6% | 7% | 10% |
| 13 | 17% | 21% | 30% | 45 | 5% | 7% | 10% |
| 14 | 16% | 20% | 29% | 46 | 5% | 7% | 10% |
| 15 | 15% | 19% | 27% | 47 | 5% | 7% | 10% |
| 16 | 14% | 18% | 26% | 48 | 5% | 7% | 10% |
| 17 | 13% | 17% | 24% | 49 | 5% | 6% | 9% |
| 18 | 13% | 16% | 23% | 50-55 | 5% | 6% | 9% |
| 19 | 12% | 15% | 22% | 56 | 5% | 6% | 8% |
| 20 | 11% | 14% | 21% | 57–58 | 4% | 6% | 8% |
| 21 | 11% | 14% | 20% | 59–63 | 4% | 5% | 8% |
| 22 | 10% | 13% | 19% | 64–73 | 4% | 5% | 7% |
| 23 | 10% | 13% | 19% | 74 | 4% | 4% | 7% |
| 24 | 10% | 12% | 18% | 75 | 4% | 4% | 6% |
| 25 | 9% | 12% | 17% | 76–89 | 3% | 4% | 6% |
| 26 | 9% | 11% | 17% | 90–98 | 3% | 4% | 5% |
| 27 | 9% | 11% | 16% | 99–112 | 3% | 3% | 5% |
| 28 | 8% | 11% | 16% | 113 | 3% | 3% | 4% |
| 29 | 8% | 10% | 15% | 114–148 | 2% | 3% | 4% |
| 30 | 8% | 10% | 15% | 149–151 | 2% | 2% | 4% |
| 31 | 8% | 10% | 14% | 152–227 | 2% | 2% | 3% |
| 32 | 7% | 9% | 14% | 228–229 | 2% | 2% | 2% |
| 33 | 7% | 9% | 14% | 230–298 | 1% | 2% | 2% |
| 34 | 7% | 9% | 13% | 299–458 | 1% | 1% | 2% |
| 35 | 7% | 9% | 13% | ≥459 | 1% | 1% | 1% |

NOTE.—If $n$ = no. cells counted, then the degree of mosaicism or greater that is excluded with given confidence limit (in the population of which the cells are an unbiased sample) appears in the appropriate column. For example, if 52 cells are evaluated without detection of mosaicism, then the lowest level of mosaicism excluded with 95% confidence is 6%. Alternatively, since 50% is the greatest magnitude of mosaicism possible, evaluation of 52 cells without detection of mosaicism excludes with at least 95% confidence mosaicism between 50% and 6% inclusive; it does *not* exclude levels of mosaicism of 5% or less with 95% confidence. To determine what number of cells to count to exclude a specific level of mosaicism for example, 10% mosaicism or greater, choose that lowest value $n$ for which 10% appears in the appropriate column. In this case, for 0.90 confidence, 22 cells; for 0.95 confidence, 29 cells; and for 0.99 confidence, 44 cells. (See Discussion for limitations on the inferences drawn from these tables.)

the total number of cells evaluated (unless of course only one metaphase from each colony was examined). If colonies are mixed and analysis done upon cells from the mixture, there is no ready way to determine the extent of mosaicism excluded by the finding of the same karyotype in any specified number of cells—unless the number of metaphases from each colony,contributing to the mixture were known.

An analogous but less serious problem arises with PHA stimulated blood after 72 hr in culture since this usually results in mixtures of cells arrested in the 1st, 2d, or 3d in vitro division [3]. "Sister cells" that have arisen from in vitro divisions may appear close to each other on a slide, even resulting in an "in situ" small clone [4], the members of which may all be scored. If each metaphase evaluated is chosen from a separate field, this would diminish the likelihood of the problem but a priori would not eliminate it. A cautious rule, based on the results of an analysis of the distribution of in vitro divisions of cells from adults and infants [3], is as follows: If $n$ metaphases are evaluated from a 72 hr culture of adult blood, apply the table as if $3n/4$ cells were evaluated; if the $n$ metaphases are from infant blood, apply the table as if $n/2$ cells were evaluated. This is a conservative approach, and if attempts are made to avoid adjacent metaphases and if the total number of metaphases analyzed is a relatively small proportion of the total available from the culture, then it is very likely $n$ itself could be safely used.

Lastly, the results here cannot help in determining if a deviation from normal is attributable to technical factors (e.g., loss (or gain) of a chromosome in preparation of the slide). Inconsistencies between cells in the identity of an absent chromosome may suggest random loss, and, of course, one may wish to count more than a prespecified number of cells before deciding whether observation of variant cell(s) is to be taken as evidence of random loss or "true" mosaicism.*

## SUMMARY

Tables specifically tailored to the exclusion of cytogenetic mosaicism at three confidence levels are presented. The consequences of the assumption of independence in application of the binomial theorem to this question are discussed. The tables are most applicable to the number of cells evaluated from cultures in which all mitoses are arrested in the first in vitro division. For long-term cultures the tables are conservatively applicable to the number of separate colonies evaluated. If $n$ cells have been evaluated from phytohemagglutinin stimulated peripheral blood after 72 hr in cultures, the tables are applicable to between $n/2$ and $n$ cells.

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* I thank a referee for calling my attention to calculations by Bochkov et al. [5] of the probabilities of detecting seven prespecified levels of mosaicism if between five and 30 cells are evaluated, a slightly different question. Unfortunately, the formulas given for calculating the probabilities in this reference $(1 - (1 - p)^n$ are inappropriate to the values actually provided in their table. In addition, strong exception may be taken to the classification in this reference of mosaicism of less than 25% as "artefact" and the illustrated applications of the probabilities derived.

difference between the values calculated and those derived from published tables of alleged 95% and 99% confidence intervals.

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