Sequential sampling in clinical cytogenetics: a quality control viewpoint

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SUMMARY We have observed that there is some resemblance between the problems of sampling in industrial quality control and the process of diagnosis of mixed cell populations in cytogenetics. This resemblance enabled us to draw from the methodology of the former science to solve some diagnostic problems in the latter. We considered which of the several sampling procedures available for quality control would be more efficient and more suitable in clinical cytogenetics, concluding that 'sequential sampling' combines both features. We also studied the effect that some abnormalities due to technical factors had on sample size required to reach a diagnosis with a given level of confidence. We give a set of tables for sequential sampling in diagnostic laboratories, illustrating their use in discriminating between mosaicism and pseudomosaicism in prenatal diagnosis and in the diagnosis of the fragile X syndrome. Finally, we discuss the merits of sequential sampling as shown here, comparing it with the current method used in cytogenetics to exclude chromosomal mosaicism.

Verification of the existence of a second cell line in a clinical sample is a problem commonly found in the cytogenetic diagnosis of several pathological situations. It occurs in blood cultures and in prenatal diagnosis, if mosaicism is being considered. It has been suggested¹ that the cytogenetic diagnosis of fragile X presents a similar problem, since patients typically express the abnormal chromosome only in a proportion of their cells. In the diagnosis of leukaemia from bone marrow aspirates, the purpose is again to determine the presence of possible pathological clones identifiable by numerically or structurally abnormal karyotypes. These are 'pathological factors' that occur with a mixed cell population and obviously they pose a sampling problem, because if the size of the sample analysed is too small, the second line is likely to be missed.

The problem stated above is compounded by the fact that sometimes the growth medium, or poorly understood biological or technical factors, can provoke low frequencies of chromosomal abnormalities that mimic pathological cases. For instance, it would appear that culture conditions suitable for the expression of fragile X induce telomeric chromosomal changes on the X chromosome of normal subjects.^{2–4} There are random chromosome losses, the frequency and type of which can be predicted as

a function of size and other chromosomal parameters, in lymphocyte cultures from young adults.⁵ An age related factor is involved in the spontaneous loss of one X chromosome from cultured lymphocytes of normal women.⁶ 'Single abnormal cells' are randomly distributed in amniotic fluids, so that their frequency can be used to predict accurately the expected numbers of cases of pseudomosaicism and mosaicism when a particular method of harvesting is in operation.⁷ Lastly, abnormalities could occur through accidents in the process of making the slides. Here we will refer to all of these as 'technical factors', as opposed to the 'pathological factors' mentioned above. We see then that in cases where 'technical factors' are known to occur, taking a sample that is too large would increase the probability of including an abnormal cell produced by these, yielding a false positive result.

From the point of view of sampling, cytogeneticists are faced in these situations with a problem similar to the one confronted in industrial quality control. The object of this science is to sentence a production batch as acceptable or unacceptable by a potential customer, having established an upper limit (or acceptance number) to the number of defective items that are attributable to random variations of the production process and a lower limit (or rejection number) to the number of defectives that would indicate a fault in the production process. In cytogenetics, the problem is to diagnose a culture as

normal or pathological having established an upper limit to the frequency of abnormal metaphases that can be expected due to 'technical factors', and a lower limit to the frequency of abnormal metaphases that indicate 'genuine pathology'.

In both situations a sampling plan is required. Deciding on a sampling plan entails (1) selection of a sampling procedure, (2) determination of sample size, and (3) determination of acceptance and rejection numbers. Only one aspect of the second of these steps, that is, the sample size that allows exclusion of chromosomal mosaicism with a given level of confidence, has been dealt with in clinical cytogenetics,^{8,9} with some modifications for the case of amniotic fluids harvested by the flask method.¹⁰ In those references, consideration is not given to the effect of low frequencies of abnormal karyotypes due to the 'technical factors' mentioned above. The purpose of this paper is to draw from the methodology of industrial quality control to suggest the sampling procedure that would be most efficient and suitable in diagnostic cytogenetics, considering successively the three steps: procedure, sample size, and rules for acceptance and rejection.

Methods

We include the minimum amount of terminology, references, and formulae required to derive sampling plans or repeat calculations for conditions applying in any laboratory.

CONSTRAINTS

p_1 is the proportion of a second cell line occurring as an artefact (technical factors) even in normal samples. (α) is the probability of diagnosing as pathological a sample expressing $p_1\%$ abnormal cells only. The value of this probability may be at 5%. ($1-\alpha$) is then the probability of accepting such a sample as normal.

p_2 is the proportion of second cell lines of abnormal karyotype above which we will consider the sample as a pathological mixture. (β) is the probability of taking as normal a sample expressing $p_2\%$ abnormal cells. The value of this probability may be set at 5% or 10% and ($1-\beta$) is then the probability of diagnosing such a sample as pathological.

From the definitions above it can be gathered that to decide on a sampling plan from a particular situation and technique we require to know p_1 and p_2 and decide on α and β .

SAMPLING PROCEDURES

Single sampling

If we were to establish as a guideline for a laboratory

that from all blood samples of males queried as fragile X 100 metaphases should be scored, we would have settled for single sampling with $N_1=100$.

Double and multiple sampling

In the previous example, we could establish instead that two smaller samples of size $N_2 < N_1$ should be studied from each queried fragile X male. In this case we are doing double sampling. We could decide this for three reasons: firstly to prevent the tediousness of scanning a large sample, secondly because we may hope, at least in some cases, that time could be saved because a diagnosis could be reached with the first sample alone, and lastly, because of uncertainties attaching to the culture methods, we might wish to examine more than one culture combination. We could also decide on three samples of size $N_3 < N_2$, four samples of size $N_4 < N_3$, and so on. This would be multiple sampling.

Sequential sampling

Still with the example of the fragile X, we could decide not to have a fixed sample size but to analyse metaphase by metaphase, and decide on a diagnosis of the whole culture as soon as evidence is sufficiently strong one way or the other. This is known as sequential sampling and its mathematical development is due to Wald.¹¹

SAMPLE SIZE AND ACCEPTANCE AND REJECTION NUMBERS

Regrettably, there are no simple formulae to determine sample size or acceptance and rejection numbers for single or double sampling. However, having decided on the constraints involved in a particular problem, and on the sampling procedure, sample sizes and acceptance and rejection numbers can be determined by consulting the corresponding tables from references 12 and 13 for single and double sampling respectively, or reference 14 for all

TABLE 1 Master table for the selection of acceptable errors.

(Error type 1) fixed for all tables=0.05
(Error type 2)

=0.10		
$p_2=0.10$	p_1 variable	table 2
$p_2=0.05$	p_1 variable	table 3
=0.05		
$p_2=0.10$	p_1 variable	table 4
$p_2=0.05$	p_1 variable	table 5
Special cases		
$p_2=0.50$	$p_1=0.0002$	table 6
$p_1=0.007$	p_2 variable	table 7
$p_1=0.015$	p_2 variable	table 8

Error type 1=probability of diagnosing as pathological a sample expressing $p_1\%$ abnormal cells (same abnormality), which are due to technical factors.
Error type 2=probability of diagnosing as normal a sample expressing $p_2\%$ abnormal cells (same abnormality), which are due to pathological factors.

TABLE 2 Acceptance and rejection numbers in sequential sampling from mixed cell populations.

$\alpha=0.05, \beta=0.10, p_2=0.10$											
$p_1=0.05$ ASS=120			$p_1=0.04$ ASS=71			$p_1=0.03$ ASS=55			$p_1=0.02$ ASS=39		
A m	c	d	B m	c	d	C m	c	d	D m	c	d
16	—	6	16	—	5	12	—	4	6	—	3
29	—	7	31	—	6	30	0	5	25	0	4
42	0	7	35	0	6	47	1	6	44	1	5
43	0	8	46	0	7	64	2	7	63	2	6
56	1	8	50	1	7	81	3	8	82	3	7
58	1	9	61	1	8	98	4	9	101	4	8
69	2	9	65	2	8	116	5	10	120	5	9
71	2	10	76	2	9	132	6	11	139	6	10
83	3	10	80	3	9						
85	3	11	92	3	10						
97	4	12	96	4	10						
111	5	12	107	4	11						
113	5	13									
125	6	13									
127	6	14									
138	7	14									
$p_1=0.01$ ASS=28			$p_1=0.009$ ASS=27			$p_1=0.008$ ASS=26			$p_1=0.007$ ASS=25		
E m	c	d	F m	c	d	G m	c	d	H m	c	d
20	—	3	22	—	3	23	0	—	23	0	—
23	0	3	23	0	3	24	0	3	27	0	3
45	0	4	48	0	4	50	1	3	51	1	3
49	1	4	49	1	4	51	1	4	55	1	4
70	1	5	74	1	5	77	2	4	79	2	4
74	2	5	75	2	5	78	2	5	83	2	5
95	2	6	100	2	6	104	3	5	107	3	5
99	3	6	101	3	6	105	3	6			
120	3	7	125	3	7						
124	4	7									
145	4	8									
$p_1=0.006$ ASS=24			$p_1=0.005$ ASS=23			$p_1=0.004$ ASS=22			$p_1=0.003$ ASS=21		
I m	c	d	J m	c	d	K m	c	d	L m	c	d
23	0	2	22	—	2	4	—	2	7	—	2
30	0	3	33	0	3	22	0	2	22	0	2
52	1	3	53	1	3	37	0	3	42	0	3
59	1	4	64	1	4	55	1	3	57	1	3
81	2	4	84	2	4	70	1	4	78	1	4
88	2	5	95	2	5	88	2	4	93	2	4
111	3	5	115	3	5	103	2	5	113	2	5
118	3	6	126	3	6	121	3	5	128	3	5
134	4	6	146	4	6	135	3	6	148	3	6
147	4	71	156	4	7	153	4	6			
169	5	72	177	5	7						
176	5	82									
$p_1=0.002$ ASS=21			$p_1=0.001$ ASS=20			$p_1=0.0005$ ASS=19			$p_1=0.0002$ ASS=18		
M m	c	d	N m	c	d	O m	c	d	P m	c	d
11	—	2	17	—	2	21	0	—	21	0	—
22	0	2	22	0	2	23	0	2	33	0	2
50	0	3	62	0	3	73	1	2	82	1	2
61	1	3	67	1	3	76	1	3	93	1	3
88	1	4	108	1	4	125	2	3	141	2	3
100	2	4	112	2	4	127	2	4	153	2	4
128	2	5	153	2	5	176	3	4			
139	3	5	157	3	5						
167	3	6									
177	4	6									

(—) means that no decision can be made as to normal (c) or pathological (d). For interpretation of other symbols, see text.

procedures. By definition, in sequential sampling there is no fixed sample size (m), and instead a diagnosis is reached only on the basis of acceptance and rejection numbers, which in this case are determined as follows.

Acceptance number: $c = -h_1 + ms$. This is the maximum number of abnormal cells which can be accepted as being associated with 'technical factors'.

Rejection number: $d = h_2 + ms$. This is the minimum number of abnormal cells which would lead to

TABLE 3 Acceptance and rejection numbers in sequential sampling from mixed cell populations.

$\alpha=0.05$, $\beta=0.10$, $p_2=0.05$

$p_1=0.05$ ASS>1000			$p_1=0.04$ ASS>1000			$p_1=0.03$ ASS=408			$p_1=0.02$ ASS=164		
A m	c	d	B m	c	d	C m	c	d	D m	c	d
						14	—	7	29	—	5
						40	—	8	59	—	6
						65	—	9	72	0	6
						91	—	10	90	0	7
						116	—	11	103	1	7
						133	1	11	120	1	8
						142	1	12	133	2	8
						159	2	12	150	2	9
						167	2	13	164	3	9
						184	3	13	181	3	10
						210	4	14			
$p_1=0.01$ ASS=81			$p_1=0.009$ ASS=75			$p_1=0.008$ ASS=71			$p_1=0.007$ ASS=66		
E m	c	d	F m	c	d	G m	c	d	H m	c	d
10	—	3	15	—	3	20	—	3	26	—	3
50	—	4	53	0	3	52	0	3	51	0	3
55	0	4	56	0	4	63	0	4	71	0	4
90	0	5	95	1	4	95	1	4	96	1	4
95	1	5	98	1	5	106	1	5	116	1	5
130	1	6	137	2	5	139	2	5	142	2	5
135	2	6	140	2	6	149	2	6	162	2	6
170	2	7	178	3	6	182	3	6			
175	3	7	181	3	7						
210	3	8									
$p_1=0.006$ ASS=61			$p_1=0.005$ ASS=58			$p_1=0.004$ ASS=54			$p_1=0.003$ ASS=50		
I m	c	d	J m	c	d	K m	c	d	L m	c	d
32	—	3	39	—	3	47	—	3	47	0	—
50	0	3	49	0	3	48	0	3	59	0	3
79	0	4	90	0	4	102	1	4	106	1	3
97	1	4	99	1	4	156	1	5	118	1	4
127	1	5	141	1	5	157	2	5	166	2	4
145	2	5	150	2	5	211	3	6	178	2	5
175	2	6	191	2	6						
192	3	6	201	3	6						
222	3	7									
$p_1=0.002$ ASS=47			$p_1=0.001$ ASS=43			$p_1=0.0005$ ASS=41			$p_1=0.0002$ ASS=40		
M m	c	d	N m	c	d	O m	c	d	P m	c	d
8	—	2	21	—	2	35	—	2	44	0	—
46	0	2	45	0	2	44	0	2	53	0	2
74	0	3	101	0	3	126	0	3	154	1	2
112	1	3	124	1	3	136	1	3	163	1	3
140	1	4	180	1	4	218	1	3			
178	2	4	204	2	4						
206	2	5									

(—) means that no decision can be made as to normal (c) or pathological (d). The tables left blank give average sample sizes that are too large. For interpretation of other symbols, see text.

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rejection of the hypothesis that only 'technical factors' are involved. In the above equations,

$$h_1 = b/(g_1 + g_2)$$

$$h_2 = a/(g_1 + g_2)$$

$$s = g_2/(g_1 + g_2)$$

$$a = 1n[(1-\beta)/\alpha]$$

$$b = 1n[(1-\alpha)/\beta]$$

$$g_1 = 1n(p_2/p_1)$$

TABLE 4 Acceptance and rejection numbers in sequential sampling from mixed cell populations.

$\alpha=0.05, \beta=0.05, p_2=0.10$											
$p_1=0.05$ ASS=137			$p_1=0.04$ ASS=95			$p_1=0.03$ ASS=68			$p_1=0.02$ ASS=37		
A m	c	d	B m	c	d	C m	c	d	D m	c	d
10	—	6	11	—	5	9	—	4	3	—	3
22	0	7	25	—	6	25	—	5	22	—	4
35	0	8	40	—	7	39	0	5	35	0	4
48	0	9	54	0	8	41	0	6	41	0	5
61	0	10	60	1	8	55	1	6	53	1	5
67	1	10	68	1	9	57	1	7	60	1	6
74	1	11	74	2	9	73	1	8	72	2	6
80	2	11	82	2	10	74	2	8	79	2	7
86	2	12	88	3	10	88	3	8	91	3	7
93	3	12	96	3	11	89	3	9	98	3	8
99	3	13	102	4	11	104	4	9			
106	4	13	110	4	12						
117	5	14									
131	6	14									

$p_1=0.01$ ASS=37											
$p_1=0.009$ ASS=35			$p_1=0.008$ ASS=34			$p_1=0.007$ ASS=33					
E m	c	d	F m	c	d	G m	c	d	H m	c	d
17	—	3	19	—	3	22	—	3	24	—	3
31	0	3	31	0	3	30	0	3	30	0	3
42	0	4	44	0	4	47	0	4	51	0	4
55	1	4	56	1	4	56	1	4	57	1	4
66	1	5	69	1	5	73	1	5	78	1	5
90	2	5	81	2	5	82	2	5	84	2	5
103	3	7	94	2	6	99	2	6	105	2	6
114	3	7	106	3	6	108	3	6			
127	4	7									

$p_1=0.006$ ASS=32											
$p_1=0.005$ ASS=31			$p_1=0.004$ ASS=30			$p_1=0.003$ ASS=29					
I m	c	d	J m	c	d	K m	c	d	L m	c	d
27	—	3	29	0	—	3	—	2	5	—	2
30	0	3	30	0	3	29	0	2	29	0	2
55	0	4	59	1	3	34	0	3	40	0	3
58	1	4	60	1	4	61	1	3	63	1	3
84	1	5	89	2	4	66	1	4	74	1	4
86	2	5	90	2	5	93	2	4	97	2	4
112	2	6	119	3	5	98	2	5	108	2	5
115	3	6									

$p_1=0.002$ ASS=28											
$p_1=0.001$ ASS=27			$p_1=0.0005$ ASS=26			$p_1=0.0002$ ASS=25					
M m	c	d	N m	c	d	O m	c	d	P m	c	d
9	—	2	16	—	2	22	—	2	28	0	—
28	0	2	28	0	2	28	0	2	31	0	2
47	0	3	60	0	3	73	0	3	87	1	2
66	1	3	72	1	3	78	1	3	90	1	3
85	1	4	104	1	4	123	1	4	142	2	3
104	2	4									

(—) means that no decision can be made as to normal (c) or pathological (d). For interpretation of other symbols, see text.

$$g_2 = 1n[(1-p_1)/(1-p_2)]$$

and p_1 , p_2 , α , and β are as defined above.

To sketch the function expressing the average sample size in sequential sampling, Wald¹¹ gives the following five pairs of values:

- (1) for $p=0$, $m_{p0}=h_1/s$
- (2) for $p=p_1$, $m_{p1}=[(1-\alpha)h_1-h_2 \alpha]/(s-p_1)$
- (3) for $p=s$, $m_s=h_1 \cdot h_2/s(1-s)$
- (4) for $p=p_2$, $m_{p2}=[(1-\beta)h_2-h_1 \cdot \beta]/(p_2-s)$
- (5) for $p=1$, $m_1=h_2/(1-s)$.

TABLE 5 Acceptance and rejection numbers in sequential sampling from mixed cell populations.

$\alpha=0.05$, $\beta=0.05$, $p_2=0.05$

$p_1=0.05$ ASS>1000			$p_1=0.04$ ASS>1000			$p_1=0.03$ ASS=481			$p_1=0.02$ ASS=208		
A m	c	d	B m	c	d	C m	c	d	D m	c	d
						30	—	8	23	—	5
						55	—	9	52	—	6
						79	—	10	81	—	7
						142	0	11	94	0	7
						166	1	11	111	0	8
						190	2	12	124	1	8
						215	3	12	141	1	9
						240	4	13	153	2	9
						264	5	14	170	2	10
						289	6	14	182	3	10

$p_1=0.01$ ASS=105			$p_1=0.009$ ASS=99			$p_1=0.008$ ASS=93			$p_1=0.007$ ASS=87		
E m	c	d	F m	c	d	G m	c	d	H m	c	d
7	—	3	11	—	3	16	—	3	22	—	3
45	—	4	52	—	4	59	—	4	66	—	4
71	0	4	70	0	4	68	0	4	67	0	4
85	0	5	93	0	5	101	0	5	111	1	5
110	1	5	110	1	5	110	1	5	155	2	6
124	1	6	133	1	6	144	1	6			
150	2	6	151	2	6	153	2	6			
163	2	7									

$p_1=0.006$ ASS=81			$p_1=0.005$ ASS=76			$p_1=0.004$ ASS=71			$p_1=0.003$ ASS=66		
I m	c	d	J m	c	d	K m	c	d	L m	c	d
29	—	3	36	—	3	44	—	3	54	—	3
65	0	3	64	0	3	62	0	3	59	0	3
76	0	4	85	0	4	98	0	4	110	0	4
112	1	4	113	1	4	116	1	4	125	1	4
122	1	5	135	1	5	151	1	5			
159	2	5	163	2	5						
170	2	6									

$p_1=0.002$ ASS=62			$p_1=0.001$ ASS=57			$p_1=0.0005$ ASS=55			$p_1=0.0002$ ASS=53		
M m	c	d	N m	c	d	O m	c	d	P m	c	d
6	—	2	19	—	2	33	—	2	51	—	2
59	0	2	59	0	2	58	0	2	58	0	2
71	0	3	97	0	3	124	0	3	159	0	3
125	1	3	137	1	3	149	1	3	166	1	3
136	1	4	175	1	4	214	1	4	268	1	4
190	2	4	215	2	4						
201	2	5									

(—) means that no decision can be made as to normal (c) or pathological (d). The tables left blank give average sample sizes that are too large. For interpretation of other symbols, see text.

Results

TABLES FOR SEQUENTIAL SAMPLING

Using the previous methodology we have prepared tables 2 to 5 showing the maximum number (c) of abnormal metaphases that can be tolerated in a normal sample (acceptance number), and the minimum number (d, rejection number) that are necessary to diagnose the culture as pathological, when the total number of metaphases sampled by the sequential method is m. The choice of table should be made by reference to table 1. Tables 2 to 5 are themselves divided in subtables lettered from A to P according to different values of p_1 .

Here we will illustrate the use of the tables in discriminating efficiently between mosaicism and pseudomosaicism in amniotic fluid cultures harvested by the flask method. From the master table 1 we decide for example that we are prepared to let 10% of cases of mosaicism with less than 10% abnormal cells go undetected, that is, we settle for table 2. Let us suppose that after 10 cells sampled from the first flask we found two trisomic for chromosome 20, and that we know that in our laboratory the frequency of 'single abnormal (trisomic 20) cells' found with our culture conditions is

$p_1=0.0002$, as given by Moller and Ormerod.⁷ Therefore, we will follow table 2P. This table tells us that no diagnosis can be reached unless at least 21 cells have been analysed from a second flask. If after the 21st cell no further trisomy 20 has been found, the case can be reported as normal. If only one +20 cell has been found, the sample cannot be diagnosed as pathological as yet, and further sampling is required. If after a total of 33 cells scored no further +20 metaphases have been found, the sample can be reported as normal. If there were two or more +20 metaphases, the sample can then be reported as mosaic. The procedure would vary depending on the abnormality considered, since p_1 could vary for different chromosomes⁷ and also between laboratories. For instance, in the case of suspected mosaicism for an additional fragment ($p_1=0.003$ in reference 7) we should proceed according to subtable letter L. The tables can be used also to determine the number of colonies from which one metaphase should be analysed if the amniotic fluid was harvested by an in situ method. As a further example let us remember that, according to the experience of Djalali *et al*,¹⁵ mosaic trisomy 20 fetuses are at high risk of severe abnormalities only when we find $p_2 \geq 50\%$ fetal cells with that karyotype. We take again $p_1=0.0002$ as the frequency with which this karyotype occurs by accident in our culture conditions⁷ and we decide on $\alpha=0.05$ and $\beta=0.10$. Substituting these values in the formulae above for c and d, and giving successive integer values to m, we obtain table 6. This table tells us that the earliest decision could be made if having scored one metaphase we found it to be +20; the case could then be reported at least as a mosaic of the type defined above. If having scored four metaphases (or colonies) we found no +20, we could accept the case as normal (or mosaic with $p_2 \leq 50\%$, which according to the experience quoted¹⁵ would be clinically normal). If we found one or more +20

TABLE 6 Sequential sampling in the diagnosis of mosaic trisomy 20.

$\alpha=0.05, \beta=0.10, p_1=0.00002, p_2=0.50$		
m	c	d
1	—	1
4	0	1
9	0	2
16	1	2
21	1	3
28	2	3

ASS=1. See text for explanation of the choice of values of p_1 and p_2 in this case.

TABLE 7 Acceptance and rejection numbers in sequential sampling from mixed cell populations. (Example: diagnosis of fragile X in males.)

$\alpha=0.05, \beta=0.10, p_1=0.007$ (see ref 2)																	
$p_2=0.04$ ASS=64			$p_2=0.03$ ASS=114			$p_2=0.02$ ASS=294			$p_2=0.01$ ASS=4160			$p_2=0.001$ ASS=7433					
A m	c	d	B m	c	d	C m	c	d	D m	c	d	E m	c	d			
2	—	2	3	—	3	3	—	3	9	—	9	6	6	—			
20	—	3	66	—	4	23	—	4	115	—	10	60	7	—			
67	0	3	97	0	4	104	—	5									
73	0	4	130	0	5	171	0	5									
120	1	4	160	1	5	184	0	6									
125	1	5	193	1	6												
172	2	5															
178	2	6															

that with these plans the probability of diagnosing as pathological a sample expressing 4% fragile X will be 90%.

Using these constraints and the tables from Cameron,¹² we find that the required single sampling plan for males is 117 (2, 3) where 117 is the sample size, 2 is the acceptance number, and 3 the rejection number. This means that if after having scored 117 cells we found two or fewer metaphases with the fragile X, the case can be reported as normal. If we found three or more, the case could be reported as pathological. The equivalent plan for females is 313 (8, 9). Using the tables from reference 13 the corresponding double sampling plan for males requires two samples of size 96, the first one with $c=0$ and $d=3$, and if the number of positive cells is >0 but <3 , a second sample of the same size should be drawn with $c=2$ and $d=3$. For females, two samples of size 321, with $c=4$ and $d=10$ for the first and $c=9$ and $d=10$ for the second, are required. Making use of equation (4) above, we find that when we use sequential sampling with those same constraints, we only need to score 64 metaphases as

an average for males and 187 for females. The five pairs of points (1) to (5) are plotted for these conditions in fig 2 for males and in fig 3 for females. These figures also show the average sample size (ASS) required with single and double sampling plans for the purpose of comparison.

COMPARISON BETWEEN THE TABLES FOR THE EXCLUSION OF MOSAICISM⁹ AND SEQUENTIAL SAMPLING

The problem of sample size in the diagnosis of mixed cell populations has been approached before.^{8,9} The main difference between sequential sampling as proposed here and the reasoning behind the tables for the exclusion of mosaicism given by Hook⁹ is that we take into account more constraints. Hook's tables are the solutions for p of the first term of the binomial expansion

$$p^n + (1-p)^n = \alpha$$

where p is the proportion of second cell lines, n is the number of metaphases of identical karyotype observed, and α is the probability of missing the

FIG 2 The relative efficiencies of single, double, and sequential sampling in detecting different frequencies of abnormalities (fragile X, males). Average sample size (ASS) is lowest in most situations using sequential sampling.

FIG 3 Same as in fig 2, in the case of females with the fragile X.

minority cell line present in the proportion of $p\%$ when n cells have been scored and found identical. With this approach, it is not possible to assess the likelihood that the abnormal findings are attributable to technical factors. Here, we have used evidence available to the effect that in some cases like fragile X or trisomy 20, the abnormal cells could have two origins, that is, 'genuine pathology' (p_2) or 'technical factors' mimicking those pathological situations (p_1), and we have considered that it would be possible to discriminate between them if they occurred with different frequency; normally we would expect that $p_1 < p_2$. In the case of trisomy 20 in amniotic fluids, this is a reasonable expectation since it is based on the widely different figures given for the frequency of 'single abnormal (+20) cells' ($p_1 = 0.00027$) and the minimum percentage of trisomic cells in the amniotic fluid required for a clinically abnormal outcome ($p_2 \geq 0.5$).¹⁵ In the case of other aneuploidies, or the fragile X, there are some data for p_1 , quoted above in the introduction, but these may vary among laboratories and the minimum frequency required for pathological expression is less well defined. For this reason we have given tables 7 and 8 ordered according to different values of p_2 .

As a final example we will compare the use of Hook's tables and the tables presented here. The problem would be to determine sample size to rule out expression of fragile X in a male in more than 5% metaphases with 95% confidence ($\alpha = 0.05$). The use of Hook's table⁹ suggests that this could be done if we do not find any fragile X in 59 metaphases. A positive finding before that would justify making the hypothesis that the patient expresses in more than 5% cells, and the sample size would be adjusted accordingly. No further findings would suggest that the patient expresses in a lower proportion, and the sample size would be increased accordingly. For the use of our tables we will consider as before that $p_1 = 0.007$,² and we should choose table 3H which gives the following guidance: no diagnosis can be given before scoring 26 metaphases. If at this stage we have found three or more fragile X, the diagnosis is positive without further sampling. Similarly, sampling can be discontinued if at any stage between 26 and 71 cells we score three or more positives. The case could be reported as negative if at any stage between 51 and 96 cells scored no positive findings have been made. Only one sighting between 96 and 116 metaphases, or only two between 142 and 162, are not diagnostic of a positive case, but rather could be considered as technical artefacts in an otherwise negative sample. The difference in the advice obtained comes from the fact that, having introduced the concept of acceptance and rejection

numbers (or p_1 and p_2), more detailed interpretation of the findings can be given.

For the proper use of these tables, it is essential that individual laboratories, or manufacturers of culture media, investigate the values of p_1 for the different chromosomes and conditions, and that clinical cytogeneticists find out the minimum proportion of abnormal cells that produce noticeable clinical effects (p_2), as discussed above in the case of trisomy 20 and the fragile X.

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