Clinical Genetics 1991: **40**: 441–451

Tests of performance of four semi-automatic metaphase-finding and karyotyping systems

GERT KORTHOF[1] AND ANDREW D. CAROTHERS[2]

[1]Department of Human Genetics, University of Leiden, Leiden, Netherlands and
[2]M.R.C. Human Genetics Unit, Edinburgh, Scotland, U.K.

Four commercially-available semi-automatic cytogenetic systems (Cytoscan, Ibas, Magiscan and Miamed) have been evaluated for both metaphase-finding and karyotyping performances, using a common set of test slides and uniform criteria. Comparisons have been made in respect of timings, number and nature of operator interactions, and false positive and negative rates. Amongst the general conclusions are the importance, for metaphase-finding performance, of a facility for ranking candidate metaphases according to their 'analysability', the need for some systems to reduce the time taken to relocate candidate metaphases, and the ability of all systems tested to detect analysable metaphases that were initially overlooked by a skilled cytogeneticist. In spite of automation, karyotyping remains a highly interactive process, strongly dependent on the skill and judgment of the operator, and therefore difficult to evaluate fully objectively.

Received 5 February, revised 2 May, accepted for publication 11 May 1991

Key words: automation; cytogenetics; karyotyping; metaphase-finding; performance.

Many automatic or semi-automatic cytogenetic systems have become available in recent years but, to quote a recent and very thorough review by Lundsteen & Martin (1989), performance data for such systems are 'woefully scant'. Where evaluations have been carried out, they have involved individual systems in isolation (Philip & Lundsteen 1985, Shippey et al. 1986), specialised applications (Zahed et al. 1989) or both (Finnon et al. 1986). Manufacturers' claims are difficult to assess since each system has been tested under different operating conditions and with different qualities of material. The present study was initiated by the European Concerted Action on Automation in Cytogenetics (CAACG) in order to fill this perceived gap in knowledge, at least in part. A full evaluation of each system, involving long-term assessments of its impact on the economics and working practices of a cytogenetic service laboratory, would have required considerable resources and has rarely been attempted (Lundsteen et al. 1987). It was therefore decided to commission a more limited study, in which only certain aspects of performance would be compared, but as far as possible under standard conditions involving identical criteria, test slides and protocols. We therefore wish to emphasise that this was not, nor was it intended to be, a clinical trial of automatic systems. A working group chaired by one of us (ADC) was charged with drawing up the test protocols and putting them into effect. We report here the results of the study, involving four systems and carried out between November 1989 and June 1990.

Material and Methods

The study protocol was based on that approved at the annual CAACG workshop in September 1989, though inevitably certain changes were found to be necessary in the course of the study.

Machines

Manufacturers of four commercial instruments, widely-available in Europe and having both metaphase-finding and karyotyping facilities, were invited to nominate a test site at which one of their regular production models was in routine use. Each site was requested to make available an operator and an instrument for 4–5 consecutive working days, at a time to be arranged with the Trial Supervisor (GK). Tests were carried out on the following instruments and in the following order:

(1) Ibas (Kontron Bildanalyse GmbH, Breslauer Strasse 2, D-8057, Eching/Munich, Germany) at Policlinico, Modena, Italy from 30th October to 3rd November 1989; this is a regional service laboratory using Q-banding as standard, but also occasionally G-banding;

(2) Miamed (Ernst Leitz Wetzlar GmbH, Ernst-Leitz-Strasse, P.O. Box 2020, D-6330 Wetzlar 1, Germany) at Laboratorio TOMA Busto Arsizio, Varese, Italy from 6th to 10th November 1989; this is the largest private prenatal diagnostic clinic in Italy, and uses Q-banding as standard;

(3) Magiscan (Joyce-Loebl Ltd., Dukesway, Team Valley, Gateshead, NE11 0PZ, U.K.) at Rigshospitalet, Copenhagen from 13th to 17th November 1989; this is the largest prenatal diagnostic laboratory in Denmark, and uses G-banding as standard; it has been closely involved in the development of the Magiscan system;

(4) Cytoscan (Image Recognition Systems Ltd., 720 Birchwood Boulevard, Birchwood, Warrington, WA3 7PX, U.K.) at British Nuclear Fuels, Sellafield, U.K. from 4th to 7th June 1990; the laboratory is primarily concerned with aberration scoring for radiological monitoring; however, the operator in this instance was provided by the manufacturer, and was experienced in analysing G-banded preparations; note that, as a result of problems in arranging a test site, the Cytoscan test took place some 6 months later than the others.

All machines included fully-automatic metaphase-finding and, except for Ibas, produced a list of found objects ranked in order of 'analysability'. The karyotyping

Table 1

Summary of system features

	Cytoscan	Ibas	Magiscan	Miamed
Karyotyping (see below)	1	4	2	3
Metaphase quality ranking		–		
Artefact rejection as separate option	–	–	–	
Zoom		–		?
Resolution	768 × 575	780 × 580	512 × 512	512 × 512
Startup time (s)	106	145	45*	76
Microscope	inverted	standard	standard	standard

Karyotyping can be classified as follows: 1. Automatic karyotyping (based on size, axis, centromere position and G-bands) followed by interactive correction of misclassified chromosomes. 2. As 1., but preceded by interactive identification of all centromeres. 3. Automatic presorting of chromosomes by size, followed by fully interactive classification. 4. As 3., but with additional interaction for correct p-q orientation.

Startup time: cold start, including loading main program/menu but excluding slide mounting.

* Denotes figure supplied by manufacturer.

and other facilities available on each machine are summarised in Table 1. A special feature of Ibas was a screen display of found objects in batches of 16, and of Miamed was a computer-controlled objective changer ($\times 10$, $\times 40$, $\times 100$) allowing automatic artefact rejection at $\times 40$ magnification.

Personnel

During the tests each machine was controlled by the nominated operator, under the direction of the Trial Supervisor, who was responsible for ensuring, as far as possible, uniformity of procedures and of evaluations of individual metaphase spreads and karyotypes.

Material

The types of tissue included in the tests, and their sources, are indicated in Table 2. For each tissue, 4 slides were prepared from each of 5 (4 in the case of bone marrow) separate individuals/cultures representative of the range of material routinely processed by the source laboratory concerned. All slides were routine G-banded preparations. Each test site received for training purposes a set of 5 slides (4 for bone marrow) containing one of the slides from each individual/culture, and a set of photographs indicating the Trial Supervisor's evaluations regarding the suitability for analysis of representative samples of cells from each type of tissue. Additionally (for each tissue) one slide was prepared from each of two further individuals/cultures to serve as a test set. The same test set was used at all sites.

Training and Testing

The training slides and photographs, and a copy of the procotol, were distributed to each site at least 3 working weeks before the start of the test. This period was provided for the purpose of adapting each instrument to the types of material to be tested. In the test phase itself, no adjustments of system parameters were permitted during scanning. Both the metaphase-finding and karyotyping abilities of each system were evaluated.

Test Procedure: Metaphase-Finding

The rectangular scan area on all slides was defined by four England finder coordinates. Four 'sparse' slides (2 bone marrow and 2 chorionic villi, short term) were evaluated by 'Method A', in which the Supervisor had previously recorded the England finder coordinates of all metaphases that he considered analysable by the same criteria that he had used to evaluate the metaphases photographed for the training phase. At the

Table 2

Number of slides

	Training					
*Tissue	Cytoscan	Ibas	Magiscan	Miamed	Test	Total
1.	5	5	5	5	2	22
2.	5	5	5	5	2	22
3.	5	5	5	5	2	22
4.	5	5	5	5	2	22
5.	5	5	5	5	2	22
6.	4	4	4	4	2	18
Total	29	29	29	29	12	128

* Tissue and source: 1. Amnion (Daker, London). 2. C.V. (long-term culture) (Jacobs, Salisbury). 3. C.V. (short-term culture) (Hoovers, Amsterdam). 4. Lymphocytes (Beverstock, Leiden). 5. Fibroblasts (Sachs, Rotterdam). 6. Bone marrow (Ross, Edinburgh).

end of each test scan of these slides, he reviewed the list of all objects found, classified them as 'metaphase' or 'non-metaphase' and, by converting England finder coordinates to system coordinates, matched them to the pre-recorded list. All slides (i.e. 2 each from fibroblast, lymphocyte, amniotic and long-term chorionic villus cultures, as well as the four 'sparse' slides) were evaluated by 'Method B', in which the Supervisor reviewed and classified each object found as 'good analysable', 'average analysable', 'poor analysable', 'non-analysable' or 'non-metaphase'. The performance of the system was then determined from a listing of the classes of all objects reviewed (both methods) and according to whether they were in the pre-recorded list (Method A only). Note that a proper false negative rate could be obtained only by Method A. All timings were recorded by the Supervisor while the operator controlled the machine.

Test Procedure: Karyotyping

Karyotyping was carried out on 10 pre-selected metaphases on the same test slides as used for metaphase-finding, including at least one metaphase per tissue. The selected metaphases contained short to medium length chromosomes; four contained overlapping chromosomes, and most contained touching ones; two had abnormal karyotypes. Timings and numbers of operator interactions were recorded by the Supervisor while the operator controlled the machine. The final karyotype had to be approved by the Supervisor before completion of the test and production of a hard copy. Note that since the karyotyped metaphases were pre-selected the normal selection procedures (e.g. chromosome counting) were not included in the test, *except* in the case of the Magiscan, where manual chromosome counting is a necessary step in karyotyping.

Results

Metaphase-Finding

Results are expressed in terms of false-positive rates (Table 3), false-negative rates

Table 3

Metaphase-finding: false-positive rates (FPR) by Method B

		Cytoscan			Ibas			Magiscan			Miamed		
Tissue	Slide	Obj	Meta	FPR	Obj	Meta	FPR	Obj	Meta	FPR	Obj	Meta	FPR
Lymphocyte	LYMPH.VII.I	416	416	0.0	493	443	10.1	398	363	8.8	375	362	3.5
Lymphocyte	P870482	82	81	1.2	123	82	33.3	98	87	11.2	96	84	12.5
Fibroblast	FIBRORD129	26	26	0.0	350	229	34.6	219	217	0.9	178	177	0.6
Fibroblast	FIBRORD116	17	14	17.6	42	25	40.5	13	10	23.1	28	28	0.0
Amniotic	AMNION1973	33	33	0.0	48	44	8.3	31	29	6.5	48	46	4.2
Amniotic	AMNION1959	25	22	12.0	66	37	43.9	45	36	20.0	123	58	52.8
C.V. (long)	CVL-FINA	15	14	6.7	139	69	50.4	130	99	23.8	86	57	33.7
C.V. (long)	CVL-FINB	0	0	–	61	17	72.1	30	23	23.3	20	14	30.0
Mean		76.8	75.8	5.4a	165.3	118.3	31.6a	120.5	108.0	13.5a	119.3	103.3	15.3a
Bone marrow	H3871	15	10	33.3	73	7	90.4	46	7	84.8	174	11	93.7
Bone marrow	H2139	29	29	0.0	85	19	77.6	16	8	50.0	14	11	21.4
C.V. (short)	CV-Pt8	45	16	64.4	163	9	94.5	98	18	81.6	29	14	51.7
C.V. (short)	CV-Pt10	24	17	29.2	171	12	93.0	183	26	85.8	34	5	85.3
Mean		28.3	18.0	31.7	123.0	11.8	88.9	85.8	14.8	75.6	62.8	10.3	63.0

'Obj' denotes number of objects found in scan area. 'Meta' denotes number of metaphases found in scan area, according to Supervisor. 'FPR' = 100 × (1 − Meta/Obj). a: omitting CVL-FINB.

(Table 4; bone-marrow and short-term CVS only), and timings and numbers of relocations (Table 5). Terminology used in Tables 3–5 is explained below.

Scan area (Table 5): The area scanned varied between machines to a small extent because systems could not scan a half field; in cases of an inexact match an extra field was added to ensure that the complete area was included.

Objects (Table 3): All objects found in the scan area, as determined from the complete list produced by the machine; in cases where the 'artefact-rejection' option was invoked (Miamed only), the final list was used.

Metaphases (Table 3): Includes all qualities of metaphase; although the total was determined for the bone marrow and CV-short slides, it was not used there for calculating false-negatives (see below), and in most cases was greater than the number of metaphases pre-recorded by the Supervisor; if a system found extra metaphases (as in Table 4, column 'Add'), they were not added to the pre-recorded list as this would have introduced a bias.

False positives (Table 3): All non-metaphases recorded by each system in the scan area; it is influenced by settings such as lower cut-off of figure-of-merit (Magiscan) and artefact rejection (Miamed).

False negatives (Table 4): All pre-recorded analysable metaphases in the scan area that were missed by each system (Method A, bone marrow and CV-short only); all were manually pre-recorded and assessed for quality in conformity with the photographs supplied to each operator.

Scan time, Tscan (Table 5): Measured from the actual start of the scan, and not including set-up time (defining scan area etc.); the separate autofocus step (Magiscan) was included; scan times were influenced by settings such as power of objective, focus frequency and field overlap, which often varied from scan to scan and were chosen by the operator; for certain slides, as shown in Table 5, the Miamed timings included 'preview' time and/or artefact rejection; 'preview' time is the time taken to examine a number of operator-selected metaphases, perform a detection test on them and adjust the finder parameter settings accordingly; it has been added to the scan time since it forms part of the routine procedure of the system, though not allowed under the present protocol.

Table 4

Metaphase-finding: false-negative rates (FNR) by Method A

			Cytoscan				Ibas				Magiscan				Miamed			
Tissue	Slide	Anal	Loc	FN	FNR	Add	Loc	FN	FNR	Add	Loc	FN	FNR	Add	Loc	FN	FNR	Add
Bone marrow	H3871	9	6	3	33.3	4	5	4	44.4	2	5	4	44.4	2	3	6	66.7	0
Bone marrow	H2139	19	15	4	21.1	2	12	7	36.8	5	8	11	57.9	0	9	10	52.6	1
C.V. (short)	CV-P18	10	7	3	30.0	6	6	4	40.0	0	8	2	20.0	0	7	3	30.0	0
C.V. (short)	CV-P10	18	14	4	22.2	2	8	10	55.6	1	14	4	22.2	1	3	15	83.3	2
Total		56	42	14		14	31	25		8	35	21		3	22	34		3
Mean FNR					26.7				44.2				36.1				58.2	

'Anal' denotes number of manually pre-recorded analysable metaphases in scan area. 'Loc' denotes number of ditto located by system in scan area. 'FN' denotes number missed by system in scan area. 'FNR' = 100 × FN/Anal. 'Add' denotes additional analysable metaphases found in scan area that were not pre-recorded.

Table 5

Metaphase-finding: timings and numbers of relocations by Method B

Tissue	Slide	Area mm²	Cytoscan			Ibas			Magiscan			Miamed		
			Tscan	T20	N20	Tscan	T20	N20	Tscan	T20	N20	Tscan	T20	N20
Lymphocyte	LYMPH.VII.I	276	89	56	20	1022	36	21	378	68	20	545	54	21
Lymphocyte	P870482	276	87	58	24	674	41	34	335	49	23	393	23	25
Fibroblast	FIBRORD129	84	82	56	20	2286	36	27	1314	40	20	3250ef	21	20
Fibroblast	FIBRORD116	84	71	41	24	1357	–	42	926	54	26*	737f	16	26
Amniotic	AMNION1973	99	104	39	20	909	40	24	371	38	20	1037ef	12	21
Amniotic	AMNION1959	99	103	58	29	1152	39	56	600	45	22	648	16	44
C.V. (long)	CVL-FINA	100	85	51	21	720	–	63	479	57	23	1814ef	27	33
C.V. (long)	CVL-FINB	100	84	–	–	600	–	72*	368	36	24	844f	–	40*
Mean values			88	51.3a	22.6a	1090	38.4b	38.1a	596	48.4a	22.0a	1159	24.1a	27.1a
				T05	N05		T05	N05		T05	N05		T05	N05
Bone marrow	H3871	348	82	13	8	828	13	–	613	20	12	465e	5	79
Bonemarrow	H2139	348	83	14	9	948	10	–	429	16	10	482ef	6	8
C.V. (short)	CV-Pt8	348	62	15d	6	931	10	91	507	16	5	617f	3	13
C.V. (short)	CV-Pt10	348	57	12d	5	810	9	37	518	15	12	600ef	5	27
Mean values			71	13.5	7.0	879	10.5	64.0c	516	16.8	9.8	465	4.8	31.8

'Tscan' denotes scan time per unit area (s/cm²); includes autofocus (Magiscan) but not setting up. 'T20', 'T05' denote times to relocate 20 or 5 objects respectively (seconds) and, for the Ibas system, includes image capture time. Where fewer than 20 objects were relocated, T20 was estimated from the mean time per object. T05 was estimated as T20/4 or, where fewer than 20 objects were relocated, from the mean time per object. 'N20', 'N05' denote numbers of objects reviewed to relocate 20 or 5 analysable metaphases respectively. Where fewer than 20 analysable metaphases were relocated, N20 was estimated from the mean number of objects per relocated metaphase (indicted by '*'). a: excluding CVL-FINB. b: excluding CVL-FINB, CVL-FINA and FIBRORD116. c: excluding H3871 and H2139. d: these figures were obtained from a separate scan. e: includes pre-view time (Miamed) – see text. f: includes artefact rejection (Miamed) – see text.

T20, T05 (Table 5): Time to relocate, centre and focus (at high magnification), but not evaluate, the first 20 (or 5) objects in the list; for the Ibas system, image capture of each object was included.

N20, N05 (Table 5): Number of objects relocated in order to find 20 (or 5) analysable metaphases from the list, either ranked (Cytoscan, Magiscan, Miamed) or unranked (Ibas).

Karyotyping

Results are expressed in terms of timings, both automatic and interactive, and numbers of operator interactions at each stage (Table 6). The karyotyping process in the four systems varied in organisation and degree of automation due to differences in hardware and software configurations. In order to obtain comparable stages the different steps were split or combined in various ways (see legend of Table 6). Each system required operator interaction in both the pre-karyotyping (S01–S03, S10) and karyotyping (S05, S11) stages, and the separation between these stages was clear in all cases. In all systems except Magiscan the selection of metaphases for karyotyping was clearly distinguished from the segmentation (pre-karyotyping) stage. In Magiscan, manual chromosome counting was an integral part of segmenting a metaphase. Thus, instead of correcting machine-proposed centromere positions and chromosome axes (as in earlier versions), the operator pointed to the centromeres of all chromosomes. This was faster than the earlier procedure, and was also a useful aid in deciding which metaphases to karyotype. The other systems had an initial automatic count in the prekaryotyping stage which was later adjusted during the interactive separation of touching and overlapping chromosomes. Although each system required interaction at the prekaryotyping stage, each had additional or unique interactive steps. For example, Ibas required extra operator interactions either to recover the smaller chromosomes 21 and 22 which sometimes disappeared, or to remove dirt. Also, the lack of a zoom facility in Ibas frequently made it necessary for the operator to recapture stray chromosomes from outside the field. There was increasing operator interaction (i.e. diminishing automation) for karyotyping in the order: Cytoscan, Magiscan, Miamed, Ibas (Table 1). The automatic part of Ibas karyotyping used chromosome size and axis to present chromosomes on the screen as a row of vertically oriented objects of decreasing size, irrespective of p/q orientation, leaving the final classification to the operator. This is perhaps best described as automatic presorting with interactive karyotyping. The Miamed used chromosome size, axis and centromere position to present chromosomes in a karyotype, but since it did not use banding information, it too is best described as interactive karyotyping. For these reasons, and because the Ibas and Miamed operators were not experienced in G-band classification, the numbers of interactions for these machines were not recorded (S11, Table 6). Cytoscan and Magiscan both used G-band information for classification and are therefore best described as automatic karyotyping systems, with operator correction of misclassified chromosomes. The explicit need to point to each centromere with Magiscan (so as to reduce the number of subsequent karyotype corrections) puts it between Ibas/Miamed and Cytoscan on the scale of automation. Note that, with the exception of Magiscan, we have not included the time taken for chromosome counting.

Discussion

Metaphase Finding

On relatively rich slides, it is important to

Table 6

Karyotyping: timings (in s) and numbers of interactions for 10 metaphases

Stage	Description	Cytoscan			Ibas			Magiscan			Miamed		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
S01.	Setup	39.0*	24	71	60.8*	14	340	–	–	–	–	–	–
S02.	Image capture and init. chrom. segmentation	43.4	28	55	6.0*	4	8	23.1	14	39	16.8*	9	41
S03.	Operator interaction for chrom. separation	68.4	0	185	132.7	14	339	73.1	37	111	110.1	12	360
S04.	Init. automatic karyogram creation	8.7	7	13	34.9	23	51	31.6	29	36	30.9*	19	59
S05.	Karyogram amendment by operator	69.5	23	160	375.4*	249	607	43.9	18	85	396.0*	273	731
S06.	Chromosome straightening	25.7	17	38	–	–	–	–	–	–	–	–	–
S07.	Chromosome enhancement	12.2	7	18	–	–	–	–	–	–	–	–	–
S08.	Production of hard copy	110.0	110	110	63.0	63	63	65.0	65	65	72.0	72	72
S09.	Total karyotyping (machineoperator)	204.7	123	272	593.6*	449	880	180.4	135	228	521.2*	351	851
S10.	Number of corrections at Stage S03	6.6	0	16	8.0	1	19	49.0	46	54	13.9	1	29
S11.	No. of operator interactions at Stage S05	11.1	7	19	–	–	–	11.5	5	22	–	–	–

* Based on 5–9 metaphases only.

S01. Cytoscan: includes setting magnification and illumination, focussing and thresholding. Ibas: includes enhancing contrast manually, thresholding and capturing chromosomes in adjacent fields (if nec.). S02. Cytoscan: includes operator time for typing specimen identifier. S03. Ibas: includes time to recover missing chromosomes and remove dirt. Magiscan: includes time to point to all centromeres. S04. Ibas displayed a row of size-ordered chromosomes, not a full karyogram. S05. Note that the Ibas and Miamed operators were not experienced in karyotyping G-banded preparations; also the former was not aware that timings were being recorded at this stage. S06. Straightening of entire karyogram; recorded only for Cytoscan. S07. Recorded only for Cytoscan. S08. Depends on hard copy size, but this was similar for all systems. S09. Includes Stages S02–S05 only. S10. Separating two chromosomes was counted as 1 interaction, except for Magiscan where a single stroke of the light pen was able to separate several chromosomes simultaneously. Ibas: includes recovering missing chromosomes. Magiscan: includes counting chromosomes by pointing to centromeres.

be able to select good metaphases rapidly and without many false-positives. However, the false-positive rates given in Table 3 are not a good measure of relative performance, since they depend critically on arbitrary machine settings. For example, although the Cytoscan had the lowest FPR, it also found the lowest mean number of metaphases on the 'Method B' slides (lymphocyte, fibroblast, amniotic and CV-long), and in fact found no objects at all on one slide. This presumably reflects a more rigorous selection of objects than for the other machines, but may also be partly due to the fact that the long thin scan patterns on some of these slides were not ideally suited to the linear array type of scanner used by this machine. A better indicator of false positives is the number of objects required to relocate 20 (N20 for Method B slides) or 5 (N05 for Method A slides) analysable metaphases (Table 5). By this measure the Cytoscan and Magiscan both performed better than the other machines, and considerably so for the Method A slides. The high N20 and N05 values of the Ibas presumably result from the lack of metaphase quality ranking. However, the relative advantage of the Cytoscan and Magiscan in this respect was partly offset by the fact that both machines took longer on average to relocate each object than did the Ibas and Miamed. For certain applications such as aberration scoring, requiring large numbers of objects to be reviewed quickly, the observed relocation time for the Cytoscan and Magiscan (2–3 seconds per object) could have a serious effect on overall throughout. Furthermore, this would seem to be an aspect of performance where considerable improvements are realistically achievable. Table 5 also confirms the very fast scan time of the Cytoscan system. On relatively sparse slides, where every suitable metaphase may be needed for accurate diagnosis, achieving a low false negative rate assumes greater importance. Table 4 indicates that the Cytoscan had the best performance in this respect (though not significantly better than the Magiscan; $\chi^2_1 = 1.5$, $P > 0.2$) and found the largest number of 'additional' analysable metaphases on the Method A slides. Incidentally, the existence of these extra metaphases confirms that there is an appreciable false negative rate even for skilled human operators, a fact that should be borne in mind when comparing the latter with automatic systems.

Karyotyping

Although we attempted to break down the karyotyping process on different machines into roughly comparable stages (Table 6), this proved to be difficult because of the varying procedures required by each machine. In the present state of the art, karyotyping is highly interactive even on the most 'automatic' machines, and therefore very dependent on the operator's skill. With the Ibas and Miamed machines, for example, it is impossible to make allowance for the operator's lack of familiarity with G-banded preparations in comparing the times taken for karyotype amendment (stage S05). On the other hand, this should not have had a major influence on the time taken for initial chromosome separation, and it therefore seems fair to conclude that the Cytoscan and Magiscan were the most efficient at this stage (S03). It is interesting to note that, although the Magiscan operator pointed to all centromeres with the light pen at stage S03, the time taken was only slightly greater than for the Cytoscan, and the time for subsequent karyotype amendment (stage S05) was considerably less. It should also be noted that the time taken to select metaphases for karyotyping, omitted in this study, is a significant component of the total time to complete a patient diagnosis. In this respect, therefore, systems for which this step is claimed to be particularly fast (e.g. Magiscan) may have an advantage which

we have not attempted to quantify here. It is worth noting that this step is carried out on Cytoscan by looking down the microscope, but on all other systems by looking at the screen.

General Comments

Differing software/hardware features often result in extra program options, but some of these may in fact be essential in order to overcome the deficiencies of a particular system. For example, for metaphase-finding the highly sensitive Miamed classifier requires an additional 'artefact-rejection' option and a pre-scan metaphase detection test. The display of found objects in batches of 16 on the Ibas system is a useful option, but probably of no advantage if metaphase ranking is available.

In a rapidly-changing field of technology, a study such as this can provide only a snapshot of the state of the art at a particular time, and in respect of certain limited aspects of performance. A fuller test would need to examine performance on a much wider range of material, including different stains and degrees of chromosome contraction. However, the difficulty and expense of even the present limited evaluation suggest that this is unlikely to be done. System improvements are continually being introduced, and potential users should obviously acquaint themselves with recent developments, as well as considering the many other aspects of machine performance not covered here (Lundsteen & Martin 1989), before committing themselves to a particular system. The latter include such factors as 'user-friendliness', flexibility in dealing with different materials, ability to retrain on new types of preparation, efficiency of reporting and record-keeping, reliability and serviceability, hard copy quality and many others. We hope, however, that the present study gives an idea of both the relative and absolute capabilities of some current systems, as well as indicating possible areas of improvement to their developers.

Acknowledgements

Funding was provided by the Commission of the European Communities through the Medical and Health Research Program Project No. II.1.1/13. The study would not have been possible without the support of many colleagues. In particular, we would like to thank: *For the supply of test materials:* Geoffrey Beverstock, Michael Daker, Jan Hoovers, Pat Jacobs, Claes Lundsteen, Fiona Ross, Eva Sachs; *For arranging, permitting and/or assisting with the site testing:* Paola Battaglia, Antonio Forabosco, Tommy Gerdes, G. Johannsen, John Keat, Claes Lundsteen, Jan Maahr, Frederico Maggi, Malcolm Plant, Massimo Scaruso, Marina Di Segni, Martin Sippel, G. Solieri, Nic Tinson, Sarah Wall, British Nuclear Fuels plc, Image Recognition Systems Ltd., Joyce-Loebl Ltd., Kontron Bildanalyse GmbH, Leitz Italiana, Wild Leitz. *For discussion and constructive criticism:* Pat Cooke, Jose Garcia-Sagredo, Aime Geneix, Erik Granum, Paul Malet, Peter Pearson, Denis Rutovitz.

References

Finnon, P., D. C. Lloyd & A. A. Edwards (1986). An assessment of the metaphase finding ability of the Cytoscan 110. *Mut. Res.* **164**, 101–108.

Lundsteen, C., T. Gerdes, J. Maahr & J. Philip (1987). Clinical performance of a system for semiautomated chromosome analysis. *Am. J. Hum. Genet.* **41**, 493–502.

Lundsteen, C. & A. O. Martin (1989). On the selection of systems for automated cytogenetic analysis. *Am. J. Med. Genet.* **32**, 72–80.

Philip, J. & C. Lundsteen (1985). Semiautomated chromosome analysis. *Clin. Genet.* **27**, 140–146.

Shippey, G., A. D. Carothers & J. Gordon (1986).

Operation and performance of an automatic metaphase finder based on the MRC fast interval processor. *J. Histochem. Cytochem.* **34**, 1245–1252.

Zahed, L., M. Murer-Orlando & M. Bobrow (1989). The application of automated metaphase scanning to direct preparations of chorionic villi. *Pren. Diagn.* **9**, 7–17.

Address:

Dr A. D. Carothers
M.R.C. Human Genetics Unit
Western General Hospital
Crewe Road
Edinburgh EH4 2XU
U.K.