Technical method

Microcomputer program for cell counting

I CLARK,* OHB GYDE, D HOLTOM
*Department of Haematology, East Birmingham Hospital, Birmingham, and *Wolfson Research Laboratories, Queen Elizabeth Medical Centre, Edgbaston, Birmingham*

Mechanical registers or pencil and paper were used for many years to keep the score for accurate recording of differential cell counts. These have recently been replaced by electromechanical or microprocessor assisted devices, which often include facilities additional to straightforward counting. Prices inevitably have risen because the number of sales is small in relation to the development costs. This has caused difficulties where budgets are inadequate

even for the replacement of worn out equipment. The present availability of a range of inexpensive microcomputers, aimed at the home user, has, however, provided a solution. We have written and put to use a program in BASIC which converts a standard home computer and portable television set into a counter suitable for a haematology laboratory. The choice of instrument for this single purpose is based on reliability, ease of use, durability, and price. The latter, together with the additional cost of a small television set, should be less than that of a mechanical tabulator.

The screen displays basic instructions above two rows of boxes which display the counts. Each box is identified with the name of a cell and a letter corresponding to a key on the computer keyboard. The cell counts are entered manually but terminated automatically when a predetermined total is reached. Additional features may be incorporated without difficulty. The count may be stopped at any point, and the absolute numbers of the individual cells are calculated by the program after entering the total cell concentrations. Non-white cells can be

Accepted for publication 10 April 1985

Fig. 1 Counting in progress.

Technical method

identified and totalled separately. This allows any red cells counted to be subtracted from the cell total and hence to correct the cell concentration to white cells only. Subdivisions of cell types may be entered. Neutrophils may be categorised as hypersegmented or normal. The hypersegmented cells may be displayed separately as a percentage of all the neutrophils. If this number exceeds a given threshold a warning statement is shown. Depending on the facilities of the computer, colour may be employed to highlight the results. Sound is helpful in signalling the ending of a count or the use of incorrect keys. The program listing has been liberally annotated with descriptive remarks (REMs) to help explain the functions of the sections that follow them. This should assist comprehension and the making of any required changes.

Four computer assisted stations using the program have been working in this laboratory for over a year (Figure). The chosen keyboards have proved their reliability after thousands of depressions. Our claim

that the program was written with a view to easy modification has been verified as related improvements have followed; these include an offspring marrow differential program with automatic calculation of myeloid to erythroid ratios. The facility to adapt the system easily has led to greater departmental interest in computing and has resulted in entirely new uses for the equipment being suggested and implemented.

A copy of the program incorporating all the features described is available for the BBC microcomputer. This machine is more versatile and expensive than the others but has found a place in educational establishments and may already be found in laboratories. The same program will also run without modification on the cheaper Electron, which is smaller but slower to respond. An earlier version was prepared for the Newbrain and this was successfully modified for the Spectrum. It is likely therefore that this BASIC program could be adapted successfully to many more machines in this range.

```

10REM --- White cell differential counts
20REM
30REM --- Ian Clark, W.R.L., 15/1/85
40REM
50REM --- Shift display down 1 line for TV use
60*TV 255,1
70REM --- Turn off keyboard auto repeat
80*FX11,0
90DIM Abs(12),Nc%(12),Key%(12),Lab$(12),Oc%(12)
100MODE 1
110REM --- Sort out colours, counts etc.
120PROCStart
130PROCInit
140REM --- Top of main loop which counts and displays a set of differentials
150REPEAT
160PROCBoxes
170PROCCount
180PROCCalc
190UNTIL 0
200REM --- Bottom of loop
210END
220REM
230REM --- All procedures below here
240REM
250REM -----
260REM --- Draw a box (i.e. a keycap)
270REM
280DEF PROCBox (X,Y,S$,K$)
290XS=150:YS=200
300MOVE X,Y
310DRAW X,Y+YS
320DRAW X+XS,Y+YS
330DRAW X+XS,Y
340DRAW X,Y
350MOVE X+10,Y+YS-20
360GCOL 0,Labs:REM --- Change colour for labels
370PRINT S$;:REM --- Display cell type

```

```

380MOVE X+10,Y+40
390PRINT K$;REM --- Display corresponding key character
400GCOL 0,Box;REM --- Change back for next box
410ENDPROC
420REM
430REM -----
440REM --- Draw keycap layout i.e. main first screen display
450REM
460DEF PROCBoxes
470REM --- Set up colours for each run
480COLOUR Bgnd:COLOUR Text:GCOL 0,Bgnd:GCOL 0,Box
490CLS
500MOVE 200,1000
510VDU 5
520PRINT "Differential Counting";
530MOVE 200,950:DRAW 870,950
540@ % = 10:REM --- Set numeric format to default
550VDU 4:PRINT TAB (1,6);"Enter counts below, maximum set at ";STR$(Cmax%)
560PRINT TAB(1,8);"Hit RETURN to stop at lower value"
570PRINT TAB(1,10);"NEUTS with 5+LOBES are entered as HYPE"
580VDU 5
590FOR J=0 TO 1
600FOR I=0 TO 5
610Kn=1+I+J*6:Nc%(Kn)=0:Oc%(Kn)=0:Abs(Kn)=0.
620PROCBox(180*I+90*J+20,20+(1-J)*250,Lab$(Kn),CHR$(Key%(Kn)))
630NEXT I
640NEXT J
650MOVE 1100,450
660GCOL 0,Labls
670PRINT "Total"
680ENDPROC
690REM
700REM -----
710REM --- Initialise arrays, etc
720REM
730DEF PROCInit
740DATA "A";"Myel"
750DATA "S";"Prom"
760DATA "D";"Blas"
770DATA "F";"Hype"
780DATA "G";" "
790DATA "H";"NRbc"
800DATA "Z";"Neut"
810DATA "X";"Lymf"
820DATA "C";"Mono"
830DATA "V";"Eosn"
840DATA "B";"Baso"
850DATA "N";"Meta"
860FOR I=1 TO 12
870READ Key$,Lab$(I)
880Key%(I)=ASC(Key$)
890NEXT I
900VDU 19,2,4,0;REM --- Change yellow to blue
910Won=0
920ENDPROC
930REM
940REM -----
950REM --- Count diffs
960REM
970DEF PROCCount
980Tot%=0:Otot%=0
990GCOL 0,0

```

Technical method

```

1000REM --- Only update display when keyboard buffer empty
1010IF (Tot%>Otot% OR Nc%(6)>Oc%(6)) AND ADVAL(-1)=0 THEN PROCUpdate
1020REM --- Display warning if display lags behind counts
1030IF ADVAL(-1)>0 AND Won=0 THEN PROCWarn(1)
1040IF ADVAL(-1)=0 AND Won=1 THEN PROCWarn(0)
1050REM --- Stop early if RETURN hit
1060K%=INKEY(1000):IF K%=13 AND Tot%>0 THEN PROCUpdate:PROCSound:ENDPROC
1070IF K%>90 THEN K%=K%-32:REM --- Upper or lower case
1080Inc%=INSTR("ASDFGHZXCVBN",CHR$(K%)):IF Inc%=0 OR Inc%=5 THEN VDU 7:GOTO 1010
1090Nc%(Inc%)=Nc%(Inc%)+1
1100IF Inc%<>6 THEN Tot%=Tot%+1:REM --- Neuc reds don't count to total
1110IF Tot%=Cmax% THEN PROCUpdate:PROCSound:ENDPROC
1120GOTO 1010
1130REM
1140REM -----
1150REM --- Display updated counts
1160REM
1170DEF PROCUpdate
1180@a%=&10:REM --- Standard numeric format
1190FOR Iv%=1 TO 12
1200IF ADVAL(-1)>0 AND Tot%<Cmax% THEN Iv%=12:GOTO 1280
1210IF Oc%(Iv%)=Nc%(Iv%) THEN 1280
1220Num$=RIGHT$ (" "+STR$(Nc%(Iv%)),3)
1230Y=380:X=0:Ic=Iv%:IF Iv%>6 THEN Y=Y-250:X=90:Ic=Iv%-6
1240X=120+X+(Ic-1)*180
1250MOVE X,Y:VDU 127,127,127:REM --- Erase previous string
1260PRINT Num$;
1270Oc%(Iv%)=Nc%(Iv%)
1280NEXT Iv%
1290MOVE 1220,380
1300VDU 127,127,127
1310PRINT STR$(Tot%);
1320Otot%=Tot%:IF Won=1 THEN PROCWarn(0)
1330ENDPROC
1340REM
1350REM -----
1360REM --- Calculate absolutes and display
1370REM
1380DEF PROCCalc
1390REM --- Flush keyboard
1400VDU 4:*FX21,0
1410PRINT TAB(1,12);"Calculate absolutes? (Y/N D:N) ";
1420A=INKEY(0):IF A=-1 THEN 1420
1430IF A=13 THEN ENDPROC
1440A$=CHR$(A):IF A$="Y" OR A$="y" THEN 1470
1450IF A$="N" OR A$="n" THEN ENDPROC
1460GOTO 1420
1470PRINT TAB(1,14);"Enter white cell count: ";
1480INPUT Wbc:OWbc=Wbc
1490IF Wbc<=0 THEN ENDPROC
1500Neuts=Nc%(7):Hsegs=Nc%(4):HS=0
1510REM --- Correct WBC for nucleated red cells
1520Wbc=Wbc*(1-(Nc%(6)/(Tot%+Nc%(6))))
1530FOR I=1 TO 12
1540IF I<>4 THEN 1570
1550IF Hsegs=0 THEN Nc%(4)=0:GOTO 1600
1560HS=(Hsegs*100)/(Hsegs+Neuts)
1570IF I=7 THEN Nc%(7)=Nc%(7)+Hsegs
1580REM --- Calculate absolute counts
1590Abs(I)=Wbc*Nc%(I)/Tot%
1600NEXT I
1610CLG:REM --- Clear screen, display diffs

```

```

1620MOVE 200,1000:VDU 5:GCOL 0,Labls
1630PRINT " White cell differentials";
1640MOVE 200,950:DRAW 980,950
1650VDU 4:GCOL 0,0
1660PRINT TAB(1,3);"Count stopped at ";STR$(Tot%);" (excl. NRbc's)"
1670@ %=&01020206:REM --- Numeric format to 2 decimal places
1680PRINT TAB(1,5);"Original white cell count:";OWbc
1690PRINT TAB(1,7);"Corrected white cell count:";Wbc:N=1
1700FOR I=7 TO 12:PROCDisp(I,N):N=N+1:NEXT I
1710FOR I=1 TO 6:PROCDisp(I,N):N=N+1:NEXT I
1720@ %=&10
1730IF Nc%(6)>0 THEN PRINT TAB(0,21); "Nucleated red cells: "; STR$(Nc%(6))
1740@ %=&01020206
1750IF HS=0 THEN 1770
1760PRINT TAB(0,26);"Hypersegmented neut: ";STR$(HS);" %"
1770IF HS<=5 THEN 1830
1780COLOUR Box:REM --- If colour, highlight above result
1790PRINT TAB(0,24);"Right Shift!"
1800VDU 7
1810PRINT TAB(0,25);"-----"
1820COLOUR 0
1830PRINT TAB(0,30);"Hit any key to re-run";
1840A=INKEY(32000)
1850ENDPROC
1860REM
1870REM -----
1880REM --- Display an absolute result
1890REM
1900DEF PROCDisp(I,N)
1910IF I=4 OR I=6 THEN N=N-1:ENDPROC
1920IF Lab$(I)=" " THEN N=N-1:ENDPROC
1930Row%=11+((N-1) DIV 3)*2
1940Col%=13*(((N-1) MOD 3))
1950COLOUR Vals:REM --- If colour, highlight non-zero results
1960IF Abs(I)=0 THEN COLOUR 0
1970PRINT TAB(Col%,Row%);Lab$(I);";";
1980Res$=STR$(Abs(I))
1990IF LEN(Res$)=6 THEN 2010
2000Res$=" "+Res$:GOTO 1990
2010IF Nc%(I)<>0 THEN PRINT Res$ ELSE PRINT
2020ENDPROC
2030REM
2040REM -----
2050REM --- Make a noise when count reached
2060REM
2070DEF PROCSound
2080REM --- (lifted from Beeb User Guide)
2090SOUND &1010,-15,50,10
2100SOUND 2,-15,200,1
2110SOUND &102,-15,100,10
2120ENDPROC
2130REM
2140REM -----
2150REM --- Display title, sort out monitor type, colours etc.
2160REM
2170DEF PROCStart
2180PRINT TAB(13,10);"Differentials"
2190Bgnd=131:Box=0:Text=0:Labls=0
2200Vals=0:Novals=0
2210PRINT TAB(2,20);
2220PRINT " Monochrome/Colour display? (M/C D:M)";
2230A=INKEY(32000):IF A=-1 THEN 2230

```

Technical method

```

2240IF A=13 THEN 2290
2250A$=CHR$(A)
2260IF A$="M" OR A$="m" THEN 2290
2270IF A$<>"C" AND A$<>"c" THEN 2230
2280Box=1:Labls=2:Vals=2
2290PRINT TAB (2,23);"Enter max. count: (default=100) ";
2300INPUT Cmax%
2310IF Cmax%=0 THEN Cmax%=100:ENDPROC
2320ENDPROC
2330REM
2340REM -----
2350REM --- Display/erase warning message re display status
2360REM
2370DEF PROC Warn(Onoff)
2380VDU 4
2390IF Onoff=0 THEN 2420
2400PRINT TAB(3,15);"**** Counts display incomplete ****":Won=1
2410VDU 5:ENDPROC
2420PRINT TAB(37,15);
2430FOR Q=1 TO 35:VDU 127:NEXT Q
2440Won=0:VDU 5:ENDPROC

```

Editorial note

We are not sure we like publishing programs in the above format, but we are bowing to increasing pressures—tell us if it is helpful or not!

A cassette containing this program ready for use may be obtained from the department of haematology of the East Birmingham Hospital.

Letters to the Editor

Effect of *Bacteroides fragilis* on the phagocytic killing of *Escherichia coli*

We read with interest the article by Vel *et al*¹ published recently in your journal. Having re-examined the inhibitory effect of *Bacteroides fragilis* on the in vitro killing of *Escherichia coli* by human polymorphonuclear leucocytes, the authors report that such impairment occurred only when the *E coli* strain required intact complement for opsonophagocytosis. We believe this paper to be a useful contribution to the growing volume of published work on the potential for synergistic interaction between organisms in infection.

In their discussion of results Vel *et al* state that we² were unable to show any impairment of clearance of *E coli* from peritoneal cavities of mice after intraperitoneal inoculation of the coliform in the presence of any one of 10 strains of *B fragilis*. The authors suggest that the *E coli* strain we used might have been one of those not requiring complement for phagocytosis and killing.

Evidence that the above suggestion is not applicable to our findings comes from two sources. Firstly, in experimental studies described by Finlay-Jones *et al*³ complement components required for

phagocytosis and killing of *Proteus mirabilis* by mouse neutrophils in vitro were absorbed by *E coli*. That strain is the one used by Reznikov *et al*.² Secondly, in phagocytic killing assays the *E coli* strain in question is not killed either in the absence of serum or in the presence of heated serum (Table). Moreover, opsonisation of that *E coli* strain for phagocytic killing by mouse neutrophils will proceed in the presence of Mg⁺⁺ and EGTA but not edetic acid.

We regret that the above information does not account for the discrepancy between results derived in vitro¹ and those derived in vivo.² In the meantime we await the appearance of the article by Verweij-

van Vught *et al*,⁴ which describes a study in which, as indicated by Vel *et al*,¹ the *E coli* strain requiring complement for phagocytic killing in vitro also produced subcutaneous abscesses in mice because its clearance was impaired by the accompanying *B fragilis*. Differences between the latter results and ours² may relate to the host defence mechanisms operative at different sites.

JJ FINLAY-JONES

M REZNIKOV

Clinical Microbiology Unit,

School of Medicine, Flinders University, and

Flinders Medical Centre,

Bedford Park, South Australia, 5042, Australia

Requirement of serum for phagocytosis and killing of *Escherichia coli* by murine neutrophils*

Serum in phagocytic assay†	No of experiments	Phagocytic killing at 120 min‡
None	7	-0.45 ± 0.16
Heated NMS (50°C, 30 min)	5	0.06 ± 0.19
Heated NMS (56°C, 30 min)	6	-0.09 ± 0.10
NMS	7	2.51 ± 0.44

*Methods for preparing neutrophils and performance of the phagocytic killing assay are given elsewhere.³ To calculate phagocytic killing the change in the viable count in each tube during the 2 hour phagocytic killing assay was determined, and the value for the tube containing neutrophils was subtracted from that of the relevant tube without neutrophils.¹

†NMS = normal mouse serum.

‡Units are log₁₀ CFU/ml (mean ± SEM). A negative value means that growth of the organism was better in the presence of neutrophils than in their absence.