at 42°C in a microaerophilic atmosphere. Plates were read at 24 and 48 hours, and positive results were reported on the basis of colonial appearance and microscopic morph- 
ology. Both laboratories participate in the UK NEQAS scheme for faecal specimens. The study period comprised the whole of 1988 and 1989, and 3287 specimens were processed in parallel.1 The results are shown in the table. The isolation rate of Campy- 
lobacter was significantly better (p < 0.001) from specimens processed promptly. There are several variables here, but our culture method works satisfactorily for fresh specimens in both laboratories, and the most obvious reason for the difference is the delay in culturing specimens at the large laboratory.

Storage and transport of Campylobacter jejuni has been studied by Monfort et al,1 who found that the organism survives in animal faeces for at least three hours at 4°C but for less than two hours at 25°C; holding atmos- 
phere had no effect on isolation rates. Our experience is that Campylobacter sp survive for over a week at 4°C in Stuart’s transport medium. The organism may be killed by toxic metabolites, and transport at ambient temper- 
ure may be the real problem.

Thus centralisation would result in a sig- 
ificant false negative rate, whereas culture at the small laboratory would yield more positive results a day earlier. Other alter- 
atives could include using transport medium (which would entail handling each specimen almost as much as direct culture) or enrichment (which would add another day to the delay already experienced); both would add to the cost. Our observations also have implications for provision of services or storage of specimens at weekends and Bank holidays.

CJ BUTT Yeovil District Hospital, Yeovil, Somerset MCJ WALE Department of Microbiology, City Hospital, Hucknall Road, Nottingham NG5 1PB

Flow cytometric DNA in ectopic pregnancy

The estimated incidence of ectopic preg- 
nancy varies from 1 in 84 to 1 in 230 intrauterine pregnancies.1 There are several maternal factors associated with ectopic implantation, such as hormonal changes in the tube involved. These factors are probably more important than the less well document- ed factors inherent in the embryo. According to various reports, the range of abnormal embryogenesis associated with ectopic preg- 
nancy varies from 0% to 63%-6.4 It is dif- 
ficult to ascertain from the information available whether this abnormal embryogenesis is due to increased incidence of chromosomal aberrations. The actual incidence of chromosomal anomalies in ectopic pregnancy remains largely obscure as specimens are rarely available for karyotyping analysis, or karyotype analysis cannot be carried out for technical reasons.

Flow cytometric DNA analysis is rapid and 
relatively simple but can only show relatively gross DNA size changes in cell suspensions prepared from paraffin wax embedded solid tissues. The DNA contents of indi- 
vidual chromosomes ranges from 4.3% (chromosome No 1) to 0.8% (chromosome No 21) when expressed as a percentage of total autosomal DNA.4 Thus in ideal conditions with fresh tissues DNA difference of at least 8-10% would be required to detect two mixed cell populations by DNA flow cytometry.4 This means that DNA flow cytometry would only show DNA aneuploidy if a substantial amount of the analysed cells lose or acquire several chromosomes.

We performed flow cytometric DNA analysis of 42 cases; the selected cases of conceptual mass removal was from the fallopian tubes. The histological material consisted of varying amounts of trophoblastic tissue with or without gestational sac and embryo, and of maternal blood. Single cell suspensions for flow cytometric analysis were prepared as described by Schutte et al5 with a few modifications. Quantitative DNA analysis was performed using an EPICS-C (Coulter Electronics, Hialeah, Florida, USA) flow 
cytometer. For each DNA histogram up to 10 000 nuclei were scanned. The mean co- 
efficient of variation (CV) of 8-2% was 
attained (range 4-0-12-0). The DNA index (D1) for each case was calculated. The peak having the lowest channel number was con- 
sidered to represent diploid cells and to have a DI of 1-0. DNA hypoploid populations might not have been identified due to the definition of the G0/G1 peak used and due to the use of dewaxed samples characterised by excessive cell debris. The DNA Index (D1) of a given aneuploid peak was calculated by dividing its channel number by that of the diploid cells in the same sample. The peak was considered when the G2/M peak consisted of more than 15% of the total cell count.

Tubal implantation was associated with aneuploidy in 14 (33%) cases. The DNA indices of the aneuploid peaks ranged from 1-4 to 2-17. Eight (19%) cases showed karyotype changes in the near diploid region. Six cases were tetraploid. Triploid cases (D1 1-4-6) were not recorded.

The results of this study support earlier 
reported overall incidences of cytogenetic anomalies in ectopic pregnancy.1 It is quite possible that chromosomal aberrations contribute significantly to the incidence of ectopic gestation, especially when the tubes seem to be grossly normal or ectopic preg- 
nancy is recurrent. The prevalence of DNA tetraploidy was high. No triploidy—that is, 
DNA aneuploidy associated with partial mole 
morphology—was detected.

Flow cytometric DNA analysis can be of 
positive use in the evaluation of ectopic pregnancies. The advantage of flow cytometric DNA analysis over conventional cytogenetics is the simplicity of the method. At the moment DNA flow cytometry is not as sensitive as cytogenetic techniques for detect- 
ing small chromosomal changes. Neverthe- 
less, it can provide interesting but approxi- 
mate information on the DNA content of 
the cells under study and thus on the role of karyotype abnormalities in ectopic pregnancy.

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FLOW CYTOMETRIC DNA ANALYSIS

Monocyte esterase deficiency: familial or environmental?

A recent report by Markey et al presents some intriguing results concerning monocyte esterase deficiency and malignant neoplasia.1 They found a significant deficiency in 0.8% of blood donors, in 3-9% of patients with malignancies, and in 1-7% of patients with non-malignant diseases. Their method for measuring monocyte esterase deficiency was the Technicon Hemolag D automated white cell differential counter. This system uses the presence of esterase in monocytes as a means of distinguishing monocytes from neutro- 
philis. The Hemolag D has been in use world- 
wide since 1974, but has mostly now been phased out by the more recent Technicon H- 
6000 and H-1 systems. These current systems do not use esterase staining, and thus make the study performed by Markey et al difficult to repeat. Two other semiautomatized methods for the measurement of monocyte esterase offer possible substitutes. Markovic et al reported an image processing system which measures the esterase of blood cell smear preparations.2 My laboratory recently reported an adaptation of the Hemolag D chem- 
isty for use with an analog DNA analysis system.3 Our method, like that in use with the Hemolag D, stains cells in suspension using a whole blood sample.

The observations by Markey et al, while intriguing, do require further experimenta- 
tion to test their hypothesis that “severe diminution in monocyte esterase activity, which occurs in an inheritable form and is relatively common, may at least in a minor
way, predispose to certain malignant neo-
plasms. Although these authors attempt to
exclude external causes for suppression of
monocyte activity by comparing patients with
malignancy with other patients, this may not
be sufficient as a control. Monocyte esterase
is easily inhibited by several pharmacological
and environmental factors. Organophosphate,
used as a component in insecticides, are
potent inhibitors of monocyte esterase.4
Monocyte esterase has been shown to be a
useful measure of occupational exposure to
organophosphates in plastics manufacturing.3
The excess in monocyte esterase deficiency in
patients with cancer observed by Markey et al
could be due to differences in drug exposure
compared with patients who do not have cancer.
Following is a summary of familial studies in
only 11 esterase negative patients, of which
five had cancer and six other conditions,
out of a total of 90 esterase negative patients.
Attributing esterase deficiency to a specific
occupational exposure is definitely not known
as the cause in most patients in their study.
Their results are certainly important, however,
and could lead to further studies which might elucidate
these questions.

Dr Markey comments:
The Haemalog D automated differential
white cell counter, producing as it did, a
monocyte count on esterase stained cells and a
monocyte count on peroxidase stained cells
(see table on each channel) was an excellent system for
identifying monocyte esterase deficiency. It is indeed unfortunate
that subsequent improvements to its technology
involved relinquishing the esterase channel.
The semi-automated method of Ross et al., however, which involves the manual
staining for esterase of whole blood samples
and counting the stained cells on the current
Technicon H1 system, should permit confirm-
ation of positive results. A peroxidase
monocyte count by the H1 must also be recorded
for each sample because a low esterase
percentage count can occur due to many other
reasons. An acquired deficiency in esterase
deficiency will not be a low recording on the esterase
channel because it is accompanied by a normal count
on the peroxidase channel.
We do not believe that the excess of esterase
deficient subjects among cancer patients can be
due to differences in drug exposure because
treatment regimens for different malignant
diseases are reasonably standard for
patients treated in this hospital and the
radiotherapy centre, yet only a small propor-
tion of any malignant disease group manifes-
ted the anomaly. Moreover, of the group of
patients with carcinoma, six had not had any
chemotherapy or radiotherapy (primary diag-
nostic beds), while six had (oncology specialist
beds). Neither have we any evidence that
our subjects' deficiency was acquired as a
result of exposure to organophosphates.
Levine et al (brought to us by Ross) did not
report any follow up of their esterase deficient
subjects after they had been removed from
the plastics production process, but one may
reasonably expect recovery of monocyte
esterase by developing monocytes when
organophosphates disappear from the blood
stream, as occurred within 14 days of acute
organophosphate poisoning in the case reported
by Oehmichen et al.5 Fifty of our subjects
had confirmatory samples taken over 14 days
(mean 17 weeks) following the initial
Haemalog D sample and many have had
repeat samples since then. None has reverted
to positivity. Moreover, we have no evidence of exposure to organophosphates, such as has
been reported by Levine et al. On the con-
trary, we have evidence of familial deficiency
in nine of 11 families studied (and in one
family of these studied in the interim period).
At this time we therefore feel that the balance
of our evidence is in favour of an inheritable
basis for the deficiency in our subjects.
As to our hypothesis that esterase
deficiency may predispose to malignant
neoplasia, we note that esterase negative monocytes do not respond to lac-
toferroin stimulation, with an increase in
cytotoxicity for K562 cells, using a modified
assay for measurement of monocyte cytotoxic-
ity;5 esterase positive monocytes respond
vigorously (unpublished observations).
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Dr Cronin et al comment:
We thank Dr Crocker for his interest in our
small study. Essentially we agree with his
comments. The point of our paper was that
although we did find a statistical difference
between the two groups studied (follicular
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overlap was such that AgNOR counts had
no discriminant value as a diagnostic test in
the group of patients we examined. As
stated in our paper, we confined our study to
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