ICSH recommendations for the analysis of red cell, white cell and platelet size distribution curves: I General principles

From the International Committee for Standardization in Haematology

PREAMBLE
This document has been prepared as a proposed ICSH Standard by the ICSH Expert Panel on Cell Counting and Sizing.* It is intended to provide a reference method for analysis of cell size distribution curves. Comments on the draft protocol are invited. They should be submitted to the ICSH Secretariat, c/o Dr SM Lewis, Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, UK.

INTRODUCTION
Recommendations for the standardisation of cell size analysis are necessary since the use of many different techniques by researchers and manufacturers has led to an inability to compare results. This document describes the general principles. Later documents will deal with the mathematical analysis of the data and the methods of sample preparation.

The analysis of cell size distribution curves is an example of the analysis of reference values; the principles and terminology to be used will be those adopted by ICSH and IFCC (ICSH, 1981). Their protocol can be used for a population of reference individuals or a population of measurements on one individual; cell size measurements are an example of the latter.

REQUIREMENT FOR FITTING REFERENCE DISTRIBUTIONS
Some workers have suggested that observed cell size data should be used to calculate a mean and SD (and possibly skewness and kurtosis) directly and that, by implication, there is no requirement for fitting reference distributions. This suggestion would be perfectly valid if the cells of interest were uncontaminated with other cells or debris. In practice, cell samples are usually contaminated—for example, small platelets with debris and large platelets with small red cells (Fig. 1), and upper and lower thresholds have to be set to delineate the cells of interest. These thresholds are inevitably arbitrary, excluding some cells of interest and including others which are not. Any mean and SD based on all of the particles between the thresholds will, therefore, be misleading.

The presence of more than one population within the cell type of interest also makes a simple mean and SD misleading (Fig. 2).

Therefore, ICSH recommends that attempts should be made to fit theoretical distributions to cell size data rather than calculating a simple mean and SD.

PROCESS FOR FITTING REFERENCE DISTRIBUTIONS
Reference distributions should be fitted by an iterative process as shown in Fig. 3. To fit a distribution it may be necessary to follow around the flow chart several times, possibly selecting on each occasion different:
(a) size ranges for fitting.
(b) method for fitting.

![Figure 1](http://jcp.bmj.com/)

FIG. 1 Contamination (---) of the platelet size distribution curve (-----) by debris or red cells produces a summation curve (-----) on which an upper threshold and a lower threshold may be located. These thresholds exclude some cells of interest and include others which are not.
ICSH recommendations for analysis of cell and platelet size distribution curves

(c) size range for checking goodness of fit,
(d) method for checking goodness of fit.

Once one distribution appears to have been fitted adequately this can be subtracted and attempts made to fit further distributions to the residuum. The analysis stops when the experimenter decides that no residuum of practical significance remains.

Fig. 2 Cell populations A and B with differing means and SDs represent the two components of the overall cell size distribution curve. The overall curve cannot be interpreted unless the components are considered individually.

Fig. 3 An algorithm summarising the approach to the analysis of cell size distribution curves. With complex curves, as in Fig. 2, it may be necessary to follow round the chart several times. prev = previous, subtr = subtract.

Fig. 4 A comparison of normal (-----) and lognormal curves (-----) both having a median of 8 and a CV of 0.05.

SELECTION OF REFERENCE DISTRIBUTION TO BE FITTED

Whilst many workers will wish to fit their own selected reference distribution, ICSH recommends the use of a lognormal distribution since it has major advantages:
(i) it provides ready comparison of variation in different sizes—for example, diameter and volume, for same cells;
(ii) it is expected to occur in growth situations (red and white cells) and in fragmentation (platelet production);
(iii) there will be non-prediction of negatively sized cells;
(iv) it approximates closely to the normal distribution when the CV<13% (Fig. 4).

Details of methods of fitting will be given in a subsequent publication together with worked examples.

RESULTS TO BE PRESENTED

The following should be presented (either graphically, numerically or possibly both):
(a) the observed cell size distribution curve
(b) each of the one or more fitted distribution curves
(c) the sum of the curves in (b)
(d) the residuum, i.e. (a) minus (c)
(e) the goodness of fit of (c) to (a)

The constants of each of the individual fitted distribution curves should be given. When the suggestion to fit lognormal distributions has been followed, the reference distribution is completely determined by three values:

\[ \mu = \text{the mean of the logarithms of the cell size}; \]
\[ \sigma = \text{the SD of the logarithms of the cell sizes}; \]
\[ N = \text{the number of cells}. \]
The International Committee for Standardization in Haematology

From these may be obtained:
- Modal size, \( \exp (\mu - \sigma^2) \);
- Median size, \( \exp (\mu) \);
- Mean size, \( \exp (\mu + [\sigma^2/2]) \);
- CV of size, \( \text{approx } \sigma \).

Examples of data presentation will be given in the subsequent publication.

REFERENCE LIMITS

These are taken as sizes beyond which stated fraction of the reference values lie; see paragraph 6.6.1 of "The theory of reference values." Thus, for cell sizing one has:
- size of largest cell, \( \exp (\mu + 2 \sigma) \) for central
- size of smallest cell, \( \exp (\mu - 2 \sigma) \) \( \approx 95.4\% \) of cells.

Alternatively, limits can be fixed by study of healthy individuals, for example, by selecting a size so that 95% of healthy individuals have < 2.5% cells larger than stated size. Any cells larger than this size may be considered macrocytic, and in a similar way, microcytic can be defined. Thus, one has:

\[
\begin{align*}
\% \text{ macrocytic cells} & \quad \text{Given } \mu \text{ and } \sigma \text{ and size limits for macrocytes or microcytes.} \\
\% \text{ microcytic cells} & \quad \text{calculate difference between } \mu \text{ and size limit, divide by } \sigma \text{ and refer to tables of areas under the normal distribution.}
\end{align*}
\]

REFERENCE INTERVALS AND THE MEASUREMENT OF ANISOCYTOSIS

The size range may be defined in the same way as a reference interval: size of largest cell minus size of smallest cell.

This is unsatisfactory since anisocytosis is best expressed in \( \sigma \). Thus, the reference interval should be a logarithmic interval which is equivalent to a ratio of linear measurements—that is, size ratio = size of largest cell/size of smallest cell = \( \exp(4\sigma) \).

Reference