

NATIONAL LABORATORY HANDBOOK

Survey report on Reference Intervals for the Full Blood Count in the Republic of Ireland

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Introduction and Background

The full blood count (FBC) is the most frequently requested test in laboratory haematology worldwide. It provides a large amount of vital clinical information rapidly and therefore is integral to modern medical practice, required on a 24/7 basis. It has evolved from methods that derived cell counts from manual microscopy and haemoglobin estimation by comparison of a solution of the patient's blood to a depth of colour index, through automated cell counts using electrical impedance technology and spectrophotometry from the 1950s, to latest generation analysers using multiple technologies including flow cytometry to produce an extended blood count. This typically now includes a white cell differential, fluorescent or immuno-fluorescent platelet count, automated reticulocyte and nucleated red blood cell counts.

A "normal" or "reference" range is required for the interpretation of quantitative biological measurements such as the parameters of the FBC ^(1, 2, 3). A "reference" range, or reference interval is the more correct term because a result within such an interval can still be pathological in a particular subject; furthermore the method for establishing a reference interval requires the definition of a reference population ^(1,2). The "reference" individuals who make up such a population are selected according to defined criteria and come from a reference population of individuals who meet those criteria ^(1,2). In order to establish upper and lower limits for a reference interval, a sufficiently large and representative sample of reference interval is required. The conditions for sample collection and testing must be standardised. The data obtained by testing the reference sample is used to derive the reference interval by statistical analysis provided the sample is sufficiently large. If the parameters being tested fit a Gaussian or symmetric distribution, a 95% reference interval can be calculated using the arithmetic mean plus or minus 1.96 standard deviations.

The majority of published guidance ^(1,2,3) recommends that each laboratory should ideally establish its own reference interval using its own techniques and automated analyser(s), particularly because of the variation that can occur between different analysers that may use different analytical technologies. Some specifically state that a laboratory should not use the reference values given in a textbook ⁽³⁾. However it is also recognised that the establishment of a reference interval locally can be a difficult and expensive procedure, beyond the resources of some individual laboratories. It presents challenges particularly in the definition of, and in gaining access to a suitable reference population. The ideal reference population, should comprise individuals that are truly representative of the healthy local population,

spanning the full age range that makes up the desired population such as an adult population. It is clearly difficult to establish what can be considered "healthy" and instead criteria such as absence of known medical conditions likely to affect the FBC, smoking, diet, alcohol intake, pregnancy and degree of physical activity must be used. In addition, some attempts to establish local reference ranges have inadvertently been affected by "selection bias" if the chosen reference population does not adequately reflect the local healthy population; for example the use of hospital staff or blood donors can tend to narrow the population sample's age range and is also thought to include a higher proportion of highly motivated and health-aware individuals than is typical of the wider healthy population ⁽³⁾. The additional difficulties inherent in gaining access to samples from a paediatric reference population are obvious. For these reasons, many laboratories use reference intervals from the published literature or from other hospitals rather than trying to establish their own local reference interval. The parameters that make up the FBC are known to vary significantly depending on age, gender and to a much lesser degree on ethnicity (for example where lower neutrophil counts than are typical in a Caucasian population can be normal in individuals of African origin). Separate reference intervals are therefore required for males, females, and for multiple age ranges from birth and throughout childhood. It is not usual or practical for separate FBC reference intervals to be used for different ethnic groups, although the laboratory can add appropriate comments to results issued where necessary.

In recent years, there have been pressures on pathology laboratories to harmonise units of measurement and also reference intervals in the interests of uniformity of patient result output. This is considered desirable in the context of the emergence of hospital networks and common Laboratory Information System (LIS) providers, in order to limit the complexity of data handling and to avoid confusion for the patient and clinicians using the laboratory service. Such pressures, however, can run contrary to the scientific principles that should ideally be used in the establishment of reference intervals according to best practice described above.

The purpose of the survey carried out was to investigate the status of reference intervals used for selected haematology tests including the FBC in the Republic of Ireland.

Methods

A data collection worksheet (DCW) was issued to 42 public laboratories in the Republic of Ireland hospitals in 2016, via laboratory managers. The DCW requested information concerning the following assays: Full Blood Count (FBC) including white cell differential, Reticulocytes, Erythrocyte Sedimentation rate (ESR) and Haematinic assays (Vitamin B12, Folate and Ferritin). Responders were asked to provide reference interval information concerning upper and lower limits, source, critical high and low limits, review high and low limits and whether a minimal retesting interval (MRI) is used for each test. They were also asked to include age and gender of the subject, specimen type, tube type, analyser, test method and units of measure for each test.

<u>Note:</u> This report focuses only on the reference interval data for the FBC data, primarily for the adult range, although comments are made on the sources used for paediatric ranges. Reports on the data for the other haematology tests will follow.

Results

Results of the survey of Republic of Ireland Laboratories 2016

Returns were received from 29 laboratories for Haematology, of which 23 or 79.3% are accredited for FBC testing. Supplementary questions were later asked from selected responders for additional information where required, for example where the responding laboratory had not indicated the source of their reference range. A large amount of data was gathered. The initial analysis focussed particularly on the upper and lower reference limits results for the FBC in adults, and on the laboratory's stated source for these reference limits. The numerical data for the limits themselves was then examined to verify that they matched the stated source, where this was available. The stated sources of the reference interval used are summarised in Tables 1 and 2 and are illustrated graphically in Figure 1 below.

Summary of Sources	No. of Labs	% of Total
Literature - Dacie & Lewis, Practical Haematology, any edition (See details of		
edition number in Table 2 below).	16	55.2
Other Literature	2	6.9
Derived Locally (In-house)	3	10.3
From Other Hospital	5	17.2
From Great Ormond Street Hospital (GOSH) (Paediatric reference ranges)	2	6.9
Recommended by analyser manufacturer	1	3.4
TOTALS	29	100

Table 1. Stated sources used for Reference Interval for the Full Blood Count in adults, among the 29 Republic of Ireland laboratories who responded

Figure 1. Stated sources used for Reference Interval for the Full Blood Count in adults, among the 29 Republic of Ireland laboratories who responded



Table 2. Stated sources used for Reference Interval for the FBC in adults – edition number of the most frequently cited publication used

Source of reference Interval - Edition of Dacie & Lewis, Practical haematology cited	No. of Labs	% of all Laboratories
Dacie & Lewis, Practical Haematology, 10 th Edition (2006)	12	41.4
Dacie & Lewis, Practical Haematology, 4 th Edition (1968)	1	3.4
Dacie & Lewis, Practical Haematology, 7 th Edition (1991)	1	3.4
Dacie & Lewis, Practical Haematology, 9 th Edition (2001)	1	3.4
Dacie & Lewis, Practical Haematology, 11 th Edition (2011)	1	3.4

Note: The reference intervals suggested for the FBC in adults is almost identical between editions 9, 10 and 11 of this textbook; only the upper reference limit for the platelet count differs between edition 9 and the other two editions by 10×10^9 /Litre. ^(1,11,12). See also appendix 3 below.

Verification of the stated source of Reference Interval – Variations found

The sources of reference intervals stated by responding laboratories were verified by checking the upper and lower reference limits they provided against the published upper and lower limits in their stated source for each parameter of the FBC, where possible. This revealed that there were some variations, in some respondents for certain parameters between their reference interval and the stated source. Where these variations were found, this was brought to the attention of the responding laboratories concerned. They were given the opportunity to check for errors in their data returns and were asked to comment on the variations where they were genuine. These variations have been categorised as either minor variations or significant variations for the purpose of summary and reporting of the data. Minor variations were defined as variations from the stated source in only between one and four FBC parameters; significant variations were defined as changes made to the reference limits for five or more parameters. In most cases, the minor variations consisted of changes to only one or two parameters and/or very small changes to the published reference limits, for example a change in the lower limits for Eosinophils and Basophils from 0.02 x 10¹²/Litre to 0.00 x $10^{12}/L$, and/or a change in the upper limit only for platelets from 410 x $10^{9}/L$ to 400 x 10⁹/L.

Those laboratories that had introduced significant variations commented that their reference limits were initially based on the literature but were adjusted by local decision; or that the published limits were felt to be too narrow; or changes were requested by the consultant haematologist and/or approved by the consultant haematologist. It was found that in all six laboratories where significant variations had been introduced, the reference limits had been broadened for most parameters compared to the published limits.

The numbers of laboratories that had introduced such variations from their stated source and the categorisation of the variation are illustrated in Figure 2 below.



Figure 2. Local laboratory variations made to adult reference intervals derived from literature

Comment on Laboratories who obtained their Reference Interval from another Hospital

A total of five responding laboratories stated the source of their reference interval was from another hospital. Four of these cited a large teaching hospital in their region as the source, of which two cited the same hospital who had used Literature as its source (Dacie & Lewis 10th Ed. with local variations), while another two cited separate large teaching hospitals who had each derived their reference interval in-house. The fifth laboratory did not state which other hospital their intervals were derived from. For the purpose of this report, this group has been categorized as regards source of reference interval as "Other hospital" for the reasons that (a) it cannot be assumed that the reference intervals used in the smaller hospitals have remained synchronised with the source hospital, if the latter changed their reference interval since the information was first transferred, and (b) it would be misleading to categorise those hospitals who took their interval from larger hospitals who had derived them in-house, as also having derived them in-house themselves.

How Adult Reference Intervals Compare across Responding Laboratories

It is useful to directly compare the various adult reference intervals in use graphically, grouped by RI source, to illustrate differences according to source as well as the degree of difference. The upper and lower limits of reference intervals for haemoglobin are shown in figure 3 below. This illustrates that the intervals derived from the most commonly used literature source, where no local variation was introduced, are not only identical as they should be, but also span the narrowest range. The intervals derived from every other source for females include this interval but are broader, both at the upper and lower limits. The picture is similar for the male reference intervals except that, for the most part, the intervals derived from other sources appear shifted upwards, in that they have a higher upper but an identical lower limit to the literature-derived group.

It is interesting that the intervals derived in-house are broader for females at both limits while two labs also have a higher upper limit for males compared to the most used literature sources, however these intervals are not quite as wide as where local variations from the published range were introduced. The picture is similar for RBC, WBC and Platelet counts which are illustrated in appendix 2, except that the RI limits show less variation for WBC and Platelet counts and most of the variation is at the upper limit, with little variation of the lower limits regardless of source. It should be noted that the intervals used by labs that cited another hospital as their source are copies of these intervals also plotted on the graphs

Figure 3. Haemoglobin Adult Reference Intervals grouped by RI Source

(Rows 1 – 34 Female, Rows 37-69 Male)



Dacie & Lewis Editions 9, 10, 11 quoted adult Haemoglobin ranges: Male 130 – 170 /Female 120 – 150 g/L

Female	RI Source	Male
Rows 1 – 2	GOSH (Red Colour)	Rows 37 – 38
Rows 4 – 5	B. Bain (Amber Colour)	Rows 40 – 41
Rows 7 – 12	D&L Eds. 9,10,11 with Var. (Dark Blue Colour)	Rows 43 – 45, 47,48
Rows 13 – 20	D&L Eds. 9,10,11 No Var. (Mid Blue Colour)	Rows 46, 49 – 55
Rows 21	D&L Ed. 4 No Var. (Dark Red Colour)	Rows 56
Rows 22	D&L Ed.7 No Var. (Purple Colour)	Rows 57
Rows 24 –26	In House (Pale Blue Colour)	Rows 59 – 61
Rows 28	Manufacturer (Green Colour)	Rows 63
Rows 30 – 34	Other Hospital (Yellow Colour)	Rows 65 – 69

Cell counter manufacturer and model used by responding laboratories

The responding laboratories stated which cell counter manufacturer and model they currently use. However, this does not have great relevance to the reference intervals in use because of the fact that only 3 out of 29 laboratories derived their own reference interval inhouse and one laboratory used the manufacturer's recommended interval. Nevertheless, this is useful information in regard to the cell counter models used and may be important in determining a future national strategy for the FBC reference interval. It is given in appendix 1 below.

Discussion

- The survey findings show that a minority of laboratories (3 laboratories or 10.3% of respondents) derived their own local reference interval for the FBC, which is generally considered best practice. The majority have used other sources, primarily published literature. This may be due to a variety of factors, including the difficulties inherent in obtaining samples from a truly representative reference population, described in the introduction above.
- The majority of other sources include published literature, of which 55.2% have used one or other edition of Practical Haematology edited by Dacie & Lewis. The great majority of these (12 of 16 laboratories) have cited the 10th edition, published in 2006.
- A minority of respondents (6 laboratories or 20.7%) that cited a literature source for their reference range, had on closer examination introduced significant variation by changing the reference interval they used for certain parameters. In all of these laboratories, the intervals had been broadened for most parameters when compared to the original published reference intervals. Some of these laboratories commented that they had made these variations in collaboration with other laboratories in their region, sometimes when the same consultant haematologist had responsibility for the clinical service in that region.
- In regard to paediatric reference intervals, two paediatric hospitals that responded cited Great Ormond Street Hospital London (GOSH) as the source of their reference interval. However, one such hospital commented that the reference interval from GOSH may be influenced by its patient population which is purely tertiary referral, in contrast to the population seen by Irish paediatric hospitals. Other hospitals having a significant paediatric as well as adult service cited the same source for their paediatric reference intervals, as distinct from the source of their adult reference interval. The sources stated by all hospitals for paediatric ranges all derived from either other hospitals or from the literature, for understandable reasons. This could be treated more fully in a separate report. It should be noted here that the single reference intervals from paediatric hospitals plotted in the graphs above, are for children over 16 years of age.
- An analysis of the spread of reference intervals according to source shows that the most commonly used intervals from the literature are generally the narrowest intervals in use, while those from other sources are broader and most broad where local variations to published sources were used. The differences are significant for some key parameters notably haemoglobin and

RBC count, while less so for WBC and Platelet counts where the lower limits in use are almost identical regardless of source, while upper limits vary.

Conclusions

- Historical best practice recommends that each laboratory should determine its own reference interval for laboratory analyses such as the FBC which should reflect the local reference population and the type of analyser used. However, there are inherent difficulties associated with implementing this practice, especially for smaller laboratories.
- Current thinking in pathology considers that given increased information sharing and transfer plus the emergence of regional laboratory networks, differences in reference intervals should be avoided, where possible.
- The survey has shown that there is a certain degree of homogeneity already in the Republic of Ireland due to the practice that has been used to set a reference interval (i.e., the published literature), albeit that this is not generally considered ideal practice.
- The fact that the majority of laboratories used published literature for the source of their reference intervals means that, by definition, these intervals are not specific to a particular cell counter, nor are they population-specific for Ireland or any Irish region.
- There were local variations in a minority of these laboratories for some parameters, however we don't currently have detailed information as to how these variations to the published limits were decided. It is worth considering that the published reference limits that were initially used were in most cases themselves derived from a variety of other published sources ^(1, 4-9).

Future Directions

It could be considered in view of the survey findings that a good degree of agreement and commonality currently exists in regard to the normal reference intervals in use for the FBC in adults in Republic of Ireland hospitals. The "most used" reference interval is derived from the same textbook publication series, Dacie and Lewis Practical Haematology, primarily the 10th edition but also single uses of the 4th, 7th, 9th and 11th editions. The authors of this report have confirmed that these reference intervals are almost identical between editions 9, 10 and 11 of this textbook (only the upper reference limit for the platelet count differs between edition 9 and the other two editions by 10 x 10⁹/Litre) ^(1,11,12). This is shown in appendix 3 below. The 14 out of 29 responding laboratories that cited one of these three editions as the source of their reference interval constitute 48.2% of the total. If one excludes the 6 laboratories that introduced significant variation to these published ranges, the 8 remaining laboratories constitute 27.5% of the total who are using almost identical reference intervals. It is interesting however that the graphic representation of the ranges shown in Figure 3 and in appendices 2a to 2c below, show that for the clinically important parameters Haemoglobin concentration, WBC, RBC and Platelet counts, the broader reference intervals where variation from the published source was introduced locally are sometimes closer to the inhouse derived reference intervals used in other hospitals.

It must also be remembered that, as stated in the conclusions, reference intervals derived from published literature are not stated to be either instrument specific or population specific and certainly not for any Irish population. They could also be said to be out of date since

many Irish laboratories have updated their cell counter systems to models that did not exist when the reference intervals they are using were derived. Therefore an attempt could be made to establish new reference intervals for the Republic of Ireland, which would at least provide more up-to-date information to help evaluate the reference intervals now in use. Such an exercise would also provide local reference population information not available to date, and be constructed to assess the various cell counter platforms and technology currently in use. This might take the approach that, given the inherent difficulties involved in establishing a representative reference population, a collaborative exercise across many laboratories could be attempted which could take account of the various cell counter technology used. Alternatively, a data mining exercise might be feasible, that could use FBC data for carefully selected patients across many hospitals derived from local Laboratory Information Systems, provided sufficiently detailed information regarding patient clinical details as well as age and gender can be obtained for patient data selection into a suitable reference population. The feasibility of either approach would need to be investigated carefully and a detailed implementation plan drawn up.

In regard to variation found between cell counter analysers that can influence a reference interval, it is interesting to note some data made available to the authors by the Irish EQA scheme (IEQAS) ⁽¹⁰⁾. Over the last five years, IEQAS carried out several FBC surveys for their Irish participants using fresh blood, which should minimise artefactual effects often seen with commercially-prepared EQA material normally used by EQA schemes. The three major cell counter manufacturers and models studied were Abbott CellDyn series, Sysmex XE and XN series and Siemens Advia. The largest differences between manufacturers were seen in the platelet count; however these are unlikely to be of clinical significance except at very low counts. Differences in red cell parameters and white cell count were smaller. Such information could be used to help inform a new reference interval setting exercise for the Republic of Ireland.

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Appendices

Appendix 1. Manufacturer and model of cell counters in use in responding laboratories at the time of the 2016 survey

Manufacturers / Analysers					
Manufacturer	Analyser N Family	lodel	or	Number of Laboratories	
Siemens (20.7%)*	Advia 120/2120i/2	120i2		6	
Abbott (17.2%)*	Cell Dyn Ruby				
	Cell Dyn Sapphire			2	
Sysmex (62.1%)*	Sysmex XE Analys	sers		4	
	Sysmex XN Analysers			12	
	Sysmex XS Analysers			1	
	Sysmex XT Analys	sers		1	
Total				29	

*Percentage of Users who returned Data

Appendix 2. Graphical plots of Reference Intervals in use for RBC Count, WBC count and Platelet count, grouped by source.

See under results section for a commentary on the graphs below.

Appendix 2a. RBC Count Reference interval by source



(Rows 1 – 34 Female, Rows 37-69 Male)

Dacie & Lewis Editions 9, 10, 11 quoted RBC ranges: Male 5.0 \pm 0.5/Female 4.3 ±0.5 x 10 $^{12}/L$

Female	RI Source	Male
Rows 1 – 2	GOSH (Red Colour)	Rows 37 – 38
Rows 4 – 5	B. Bain (Amber Colour)	Rows 40 – 41
Rows 7, 8, 10, 12 –20	D&L Eds. 9, 10, 11 No Var. (Mid Blue Colour)	Rows 43,44,46,48 - 55
Rows 9, 11	D&L Eds. 9, 10, 11 with Var. (Dark Blue Colour)	Rows 45, 47
Rows 21	D&L Ed. 4 No Var. (Dark Red Colour)	Rows 56
Rows 22	D&L Ed.7 No Var. (Purple Colour)	Rows 57
Rows 24 –26	In House (Pale Blue Colour)	Rows 59 – 61
Rows 28	Manufacturer (Green Colour)	Rows 63
Rows 30 – 34	Other hospital (Yellow Colour)	Rows 65 – 69



Appendix 2b. WBC Count Reference interval by source

Dacie & Lewis Editions 9, 10, 11 quoted adult WBC range: 7.0 \pm 3.0 x 10 $^{9}/L$

Row	RI Source	
Rows 1 – 2	GOSH `	(Red Colour)
Rows 4 - 6	B. Bain	(Amber Colour)
Rows 8 – 13, 21	D&L Eds.9, 10, 11 With Var.	(Dark Blue Colour)
Rows 14 –20	D&L Eds. 9, 10, 11 No Var.	(Mid Blue Colour)
Rows 22	D&L Ed. 7 No Var.	(Purple Colour)
Rows 23	D&L Ed.4 No Var.	(Dark Red Colour)
Rows 25 – 27	In House	(Pale Blue Colour)
Rows 29	Manufacturer	(Green Colour)
Rows 31 – 36	Other hospital	(Yellow Colour)



Appendix 2c. Platelet Count Reference interval by source

Dacie & Lewis Ed. 9 quoted Platelet range: 150 – 400 x 10⁹/L: Dacie & Lewis Eds. 10 & 11 quoted Platelet range: 150 – 410 x 10⁹/L

RI Source	
GOSH `	(Red Colour)
B. Bain	(Amber Colour)
D&L Eds. 10,11 With Var.	(Dark Blue Colour)
D&L Eds. 10 Var. Use Ed.9RIs	(Mid Pink Colour)
D&L Ed.10. No Var.	(Dark Grey Colour)
D&L Ed. 9	(Pale Brown Colour)
D&L Ed.11 No Var.	(Mid Grey Colour)
D&L Ed.4 No Var	(Dark Brown Colour)
D&L Ed.7 No Var	(Purple Colour)
In House	(Pale Blue Colour)
Manufacturer	(Green Colour)
Other hospital	(Yellow Colour)
	RI Source GOSH B. Bain D&L Eds. 10,11 With Var. D&L Eds. 10 Var. Use Ed.9RIs D&L Ed.10. No Var. D&L Ed.9 D&L Ed.11 No Var. D&L Ed.4 No Var D&L Ed.7 No Var In House Manufacturer Other hospital

Appendix 3. Reference Intervals for the FBC in adults in published literature (Dacie and Lewis Practical Haematology 9th ed., 10th ed. and 11th ed.) ^(1, 11, 12)

FBC parameter	Unit of	Gender	10 th Edition	9 th Edition	11 th Edition
	Measurement		(2006) (most	(2001)	(2011)
			used)		
WBC	x 10 ⁹ /L		4.0-10.0	As 10 th Ed.	As 10 th Ed.
Neutrophils	x 10 ⁹ /L		2.0-7.0	As 10 th Ed.	As 10 th Ed.
Lymphocytes	x 10 ⁹ /L		1.0-3.0	As 10 th Ed.	As 10 th Ed.
Monocytes	x 10 ⁹ /L		0.2-1.0	As 10 th Ed.	As 10 th Ed.
Eosinophils	x 10 ⁹ /L		0.02-0.5	As 10 th Ed.	As 10 th Ed.
Basophils	x 10 ⁹ /L		0.0201	As 10 th Ed.	As 10 th Ed.
RBC	x 10 ¹² /L	Male	4.5-5.5	As 10 th Ed.	As 10 th Ed.
		Female	3.8-4.8	As 10 th Ed.	As 10 th Ed.
Haemoglobin	g/L	Male	130-170	As 10 th Ed.	As 10 th Ed.
-	_	Female	120-150	As 10 th Ed.	As 10 th Ed.
PCV or Hct	L/L	Male	0.40-0.50	As 10 th Ed.	As 10 th Ed.
		Female	0.36-0.46	As 10 th Ed.	As 10 th Ed.
MCV	fL		83-101	As 10 th Ed.	As 10 th Ed.
MCH	pg		27-32	As 10 th Ed.	As 10 th Ed.
MCHC	g/L		315-345	As 10 th Ed.	As 10 th Ed.
RDW (CV)	%		11.6-14.0	As 10 th Ed.	As 10 th Ed.
RDW (SD)	fL		39-46	As 10 th Ed.	As 10 th Ed.
Platelet count	x 10 ⁹ /L		150-410	150-400	As 10 th Ed.
Reticulocyte count	x 10 ⁹ /L		50-100	As 10 th Ed.	As 10 th Ed.