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'Aussie Normals': an *a priori* study to develop reference intervals in a healthy Australian population using the Beckman Coulter LH 750 Haematology Analyser as candidates for harmonised values

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Summary

Reference limits or intervals are important benchmarks or tools that help the clinician to distinguish between a result that is most likely to lie within a 'healthy' or diseased category. It has been suggested that a review of haematology reference intervals is long overdue. In this study we report on our findings for analytes routinely measured in a complete blood count (CBC) performed on the Beckman Coulter LH 750 analyser and an additional comparative study using the Beckman Coulter LH 750, the Sysmex XN and Abbott Sapphire. The results from the comparative study indicate that bias would not prevent harmonisation of reference intervals for these common haematology parameters. The results offered by the Aussie Normals study represent good candidates as the basis for harmonisation reference intervals.

Key words: Haematology; reference intervals; harmonisation; bias.

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INTRODUCTION

Reference limits or intervals are important physiological measures that allow the clinician to distinguish between a result that is most likely to lie within a 'healthy' category and a result that is more likely to lie within a 'disease' category.[1]

The development of reference intervals (RIs) is expensive, time-consuming and defining the population(s) being studied is a difficult task. Further problems arise because particular analysers used may have systematic biases for the estimation of individual analytes.[2–4]

Fraser and Hyltoff Petersen[5] suggest that in a homogeneous population, laboratories should ideally use the same RI. Where there is adoption of assays with metrological traceability, there should be equivalence in patient results. Harmonising RIs requires knowledge and understanding of analytical bias. Bias in a laboratory sense is a testing error that causes a systematic favouring of some outcome over others which could prevent reasonable and objective consideration of a clinical situation. The presence and magnitude of any bias can be obtained by comparing results obtained by the various analytical methods using shared patient's samples, samples from external quality assurance programs or inter-laboratory internal quality control programs.[6,7]

Results tend to show greater variability and method dependence when using 'artificial' material rather than fresh or frozen patient samples.[7] If using artificial material for analysis of bias, commutability should be determined. Without assessment of commutability it is not possible to determine whether biases observed are artifactual or genuine.[8,9]

It has been suggested that a review of haematology reference intervals is long overdue.[10]

The Aussie Normals study is an *a priori* study developed to determine reference intervals in healthy adult Australians. The study has previously reported reference intervals for common clinical chemistry analytes.[11] All volunteers reside in the Canberra region, Australian Capital Territory (ACT), Australia, and have completed a questionnaire that included questions associated with lifestyle such as known diseases and medications. Exclusion was based on conditions such as pregnancy, diabetes, renal or cardiovascular disease (CVD). We report on our findings for analytes routinely measured in a complete blood count (CBC) performed on the Beckman Coulter LH 750 analyser (Beckman Coulter, Australia).

An additional comparative study using the Beckman Coulter LH 750, the Sysmex XN (Roche Diagnostics, Australia) and Abbott Sapphire (Abbott Diagnostics, Australia) analysers was designed to determine if bias would prevent the Aussie Normals reference intervals from being candidates for use as harmonised reference intervals for the parameters commonly measured when undertaking a complete blood count (CBC) on these instruments.

MATERIALS AND METHODS

All studies using human blood samples were approved by one of the ACT Health Human Research Ethics Committee, the Australian National University Human Research Ethics Committee or the Australian Institute of Sport Research Ethic Committee and all volunteers gave written informed consent.

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Populations studied

How representative of Australia is the population of the ACT?

The Australian Bureau of Statistics Census Data demonstrate that the population of the Australian Capital Territory (ACT) is representative of the general Australian population in age distribution, ethnic origins, marital status, size of family, home ownership, language spoken at home or religion. The ACT has a higher percentage of public service and defense employment than other parts of Australia, and these data are supported by a higher percentage of people who term themselves 'professional'. Income levels are, on average, not significantly different to those in the rest of Australia though levels of education are, on average, higher in the ACT.[12]

Aussie Normals

The Aussie Normals study is a community-based multidisciplinary *a priori* study to determine reference intervals in a healthy Australian population. Potential volunteers were sourced through the Australian Electoral Roll and approached by letter of invitation The initial response rate was 9.6% which varied from 2.1% for subjects <30 years of age to 16.2% for those over 60 years. Those respondents undertook an initial interview to determine suitability for the study. A number of volunteers had significant medical conditions at the time of initial interview and were excluded from the reference interval study. The following conditions were grounds for exclusion from the study: pregnancy, diabetes, asthma requiring medication with oral steroids, any history of malignancy and other conditions involving significant systemic disease. We accepted individuals on anti-depressants medications and those over the age of 50 who are on statins and/or anti-hypertensives. We also accepted women who are on the oral contraceptive pill or hormone replacement therapy.

A cohort of 1856 volunteers was recruited. For this study 736 (386 female, 350 male) individuals from that cohort provided samples for CBC analysis. The age and gender frequencies are shown in Fig. 1 with the ages ranging from 19 to 90 years. During the course of the study three subjects were identified with clinical conditions and/or medications prescribed which required preclusion of all results from our final database for analysis. All volunteers were required to supply the contact details for their general practitioner who would receive a copy of all results generated.

Bias analysis

This study measured the CBC parameters for 280 patient samples (217 adult samples with one or more abnormal result, 50 adult samples with no abnormal results and 13 samples from subjects <19 years of age) on three commonly used analytical instruments, the Abbott Sapphire, Beckman Coulter LH 750 and the Sysmex XN.

Blood collection and handling

Blood samples for the Aussie Normals study were collected into K2EDTA collection tubes (Becton Dickinson, USA). These samples were analysed within 4 hours. On review, if the results generated by the Beckman Coulter LH 750 were outside the reference intervals used by the analysing laboratory, a blood film was prepared and analysed by an experienced morphologist. All results were provided to the volunteers' nominated medical officer.

Samples for the bias analysis were also collected into Becton Dickinson K2EDTA collection tubes, analysed on receipt into the laboratory using the Beckman Coulter analyser with further analysis undertaken by the on-site Sysmex XN and Abbott Sapphire instrumentation within 4 hours.

Haematological analysis

All analyses reported here used routine proprietary methods for those analysers. The analytes tested as part of the CBC were: haemoglobin (Hb), red cell count (RCC), mean corpuscular volume (MCV), haematocrit (Hct), red cell distribution width (RDW), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets, (Plt), mean platelet volume (MPV), white cell count (WCC), neutrophil count (Neut), lymphocyte count (Lymph), monocyte count (Mono), eosinophil count (Eos) and basophil count (Baso).

The analytical methods were controlled according to manufacturer instructions by preventative maintenance, function checks calibration and quality control. Analyses were undertaken only when analytical parameters were deemed acceptable in line with ISO15189 measurement of uncertainty principles.[13] The performance characteristics for the assays over the analysis period showed the routine measured and calculated CBC parameters meeting the desirable or optimal analytical goals based on biological variation with coefficients of variation (CVs) ranging from 0.5–2.7% for the 10 routine measured and calculated CBC parameters, 1.4% for neutrophil count, 2.5–4.5% for lymphocyte count, 3.2–4.6% for monocyte count and 5.3–7.1% for eosinophil count.

Statistical analysis

All Aussie Normals subjects were coded whilst testing to prevent study results being recorded into patient routine results files. The results were electronically transmitted from the Beckman analysers into a laboratory

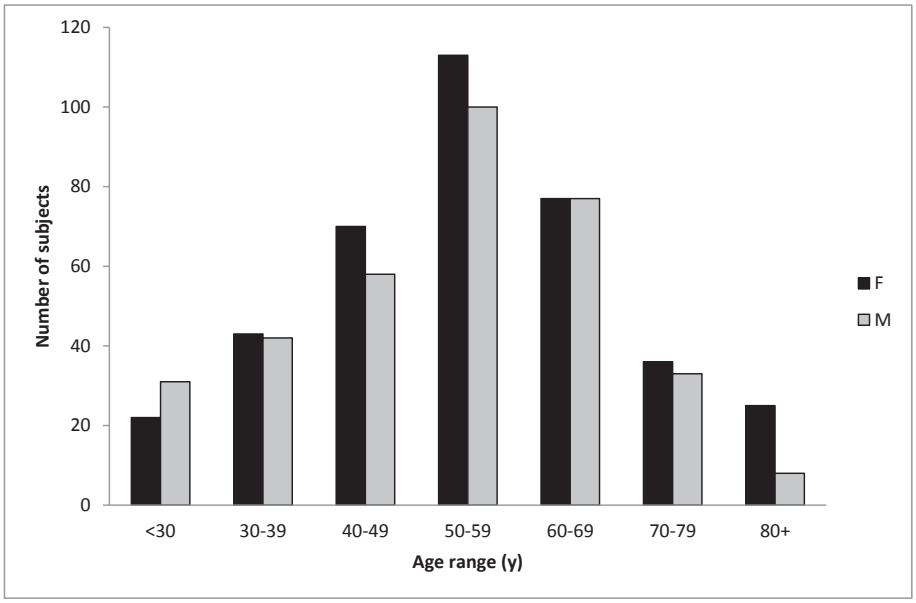

Fig. 1 Age and gender frequencies for Aussie Normals. Female, black; male, grey.

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