Proposed Cell Count Normal Limits for Urines Processed on the Sysmex UF-100

- Determination by Retrospective Statistical Analysis of "Normalised" Urine Data -

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Obtaining a suitable population, in sufficient numbers, for a prospective reference range study is a significant challenge for most medical diagnostic laboratories. Previously published normal limits for cell counts performed using the Sysmex UF-100 fully automated urine cell analyzer appear to be limited in number and the suggested normal ranges differ dramatically among those reviewed. To obtain normal limits, rather than perform a prospective reference range study, a retrospective statistical analysis of "normalised" urine cell count data was performed and the method and results are presented here. The limits established compare well with some of those previously published. In lieu of a full and formal reference/normal range study, this approach and these results will be useful for laboratories using the Sysmex UF-100 system.

(Sysmex J Int 14 : 23-29, 2004)

Key Words Fully Automated Urine Cell Analyzer, UF-100, Normal Ranges, Urine Cell Counts

Received 24 December, 2003; Accepted 7 January, 2004

INTRODUCTION

Obtaining a suitable population, in sufficient numbers, for a prospective reference range study requires a significant amount of work, logistical organisation and involves regulatory or ethical considerations. Consequently embarking on such a study is often a significant challenge and a lengthy process for most medical diagnostic laboratories. To perform a study according to recommended methods, under controlled conditions, can be impractical, particularly for those where access to healthy, normal volunteers or patients is limited.

It is quite common for laboratories to adopt normal limits from other laboratories in their region or from those providing a similar service. Laboratories often use ranges that have been quoted in literature references or textbooks, but in some cases the normal ranges in use have been inherited and their original source is unknown.

There appears to be limited, and sometimes quite conflicting, published data for normal values for urine samples analysed by the Sysmex UF-100 fully automated urine cell analyzer. In this study, urine sample results, processed by a Sysmex UF-100, by a large community laboratory, were "normalised" and a statistical analysis performed retrospectively to obtain normal limits for adult males and females. The results obtained are comparable with some of those previously published.

MATERIALS AND METHODS

All data in this study was from urine samples processed routinely by the Microbiology department of Medlab Hamilton, Hamilton, New Zealand over several months. Medlab Hamilton is a medical diagnostic laboratory serving a large geographical area, receiving samples predominantly from patients in the community referred by their general practitioner.

Routine urinalysis

Routine analysis of urine samples in this laboratory includes: dipstick test strip analysis, cell count, culture and where necessary, antibiotic sensitivity. All urines are processed as soon as possible after receipt by the laboratory.

Dipstick analysis was performed using Combur[10] Test M test strips which were read by a Miditron M semi-automated urinalysis system (both from Roche Diagnostics GmbH). The parameters provided by dipstick analysis were; Specific Gravity (SG), pH, Leucocyte (Leuc), Nitrite (Nit), Protein (Prot), Glucose (Glu), Ketones (Ket), Urobilinogen (Ubg), Bilirubin (Bil), Erythrocytes (Ery) plus urine colour compensation.

Urine cell counts were performed on all samples. Medlab Hamilton utilises a Sysmex UF-100 fully automated urine cell analyzer, which incorporates flow cytometry along with impedance detection, to directly identify and count urine formed elements. The Sysmex

UF-100 provides cell counts (/μL) for White Blood Cells (WBC), Red Blood Cells (RBC), Epithelial Cells (EC), Casts (Cast), and Bacteria (Bact). Flags are provided to alert the laboratory to the presence of Pathological Casts (P. Cast), Small Round Cells (SRC), Yeast Like Cells (YLC), Crystals (X'TAL) and Sperm. The Bact count will be flagged if the criteria for the UF-100 UTI-flag is met. Three parameters determine the UTI-flag. They are:

- The average size of the bacteria (Fsc2 measurement)
- The number of bacteria
- The WBC count

A cross-check function within the UF-100 automatically reviews and identifies mismatches between dipstick and UF-100 results. If an increased number of RBC are present (≥10/μL), information is also provided on the size distribution (microcytic, normocytic or non-classified) of the RBC. Urine conductivity is also measured. The UF-100 will also asterisk parameters for review where the reliability of data is suspect due to poor discrimination between cell/particle types, abnormal conductivity or a very high total particle count.

In conjunction with the UF-100's in-built review flag system, the laboratory has additional criteria for when manual microscopic review needs to be performed to supplement or replace the UF-100 results. When this review criteria is met or there is insufficient sample for UF-100 analysis, a manual urine microscopy review and possibly manual cell count, is then performed using FAST-READ 102 disposable counting chambers (Hycor Biomedical Inc.).

All urine samples were routinely cultured onto Cystine Lactose Electrolyte-Deficient (CLED) agar using a 1μL disposable loop. The CLED agar plates were incubated aerobically overnight at 37°C and any bacterial growth recorded.

Results from these urinalysis procedures were captured by the Laboratory Information System (LIS). The Information Technology department of Medlab Hamilton extracted results for all urines processed from their LIS, over several months, into an excel-compatible format (.csv = comma separated value) and made this available for statistical analysis. For each sample in the resulting Microsoft Excel spreadsheet there were thirty-nine columns of information from the routine urinalysis testing performed, they included:

- Urine sample lab number
- Patient Sex (M or F)
- Patient Age in years
  (in months for patients under 2 years)
- Date of Analysis
- Dipstick Results – One column for each of the following: SG, pH, Leuc, Nit, Prot, Glu, Ket, Ubg, Bil, Ery
- UF-100 Routine Parameter Results - RBC, WBC, EC, Cast, Bact.
- Additional separate columns for UF-100 low reliability flagging (asterisks) and cross-check error flagging for each of the five counted cell types were included. In the low reliability flag column for Bact there was also an indication if the UTI flag had been triggered.
- UF-100 Additional Parameters - SRC, YLC, X'TAL, Sperm, Other
- UF-100 Other Flags and Information - Review Flag, Carryover Flag (indicates previous sample had very high total count), Error Flag (Sampling Error), RBC Info, Discrimination Errors and UTI-flag
- Culture Results - Quantity and name of organism isolated

Samples with any obvious abnormality or evidence of possibly unreliable UF-100 results were eliminated entirely. The remaining "normalised" samples therefore met all of the following criteria:

- No bacterial growth (whether significant or insignificant in quantity)
- No positive/abnormal parameter on urine dipstick
- No asterisks (low reliability flags/discrimination errors), Carryover flags, Sampling Error and/or RBC Info
- No Review flags for SRC, YLC, X'TAL, Sperm, Other particles, Abnormal conductivity and/or High total count
- No UTI-flag triggered
- No missing results (dipstick, UF-100 or culture)

The main focus of analysis was to produce normal limits for adult males and females. An arbitrary cut-off age for adulthood was made at 13years and older. The "normalised" data was then sorted according to sex (male or female) and age (≥13years and <13years) and the five main parameters (WBC, RBC, EC, CAST and Bact) separated out for analysis.

Data was also analysed for samples from patients under 13years of age but this will not be presented in this paper.

Statistical analysis

Microsoft Excel (Microsoft Corporation) and Minitab Release 14 Statistical Software - demo version (Minitab Inc.) were the software tools used to sort the data and determine the limits of the normal ranges.

The "normalised" result data was copied from Microsoft Excel into the Minitab program.

Using Minitab, initially histograms were produced for each of the five cell types for both males and females. An example is ***Fig. 1***, which shows the histogram/frequency distribution of EC counts for adult females.

All of the cell types produced a skewed (non-Gaussian) distribution, consequently logs were applied to the data, using functions incorporated in Minitab, and a histogram of the log data produced. ***Fig. 2*** shows the histogram/frequency distribution of the log EC counts for females which shows a normal distribution.

A Normality Test (Anderson-Darling Normal Probability Plot) function within Minitab was then applied to the log data, complete with 95% confidence interval markers. ***Fig. 3*** shows the Anderson-Darling Normal Probability Plot for log EC counts for females. The log numbers relative to the 2.5 and 97.5 percentiles were converted to the normal limits by taking the antilog of these log values. Not all cell types normal limits were able to be determined

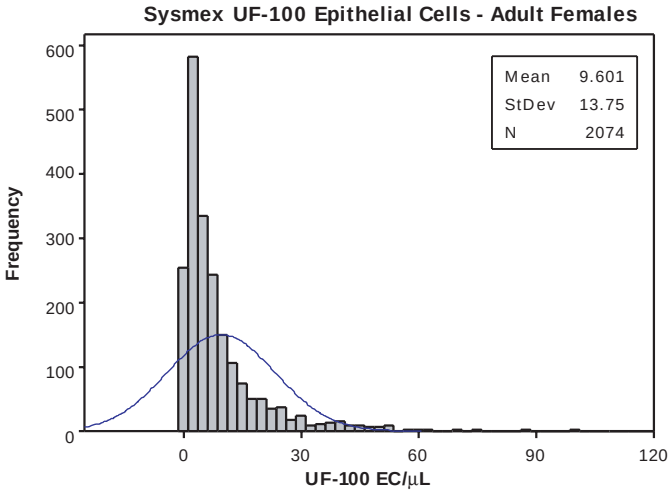

Fig. 1 *Histogram of UF-100 epithelial cell counts for females produced by Minitab (with normal curve indicated)*

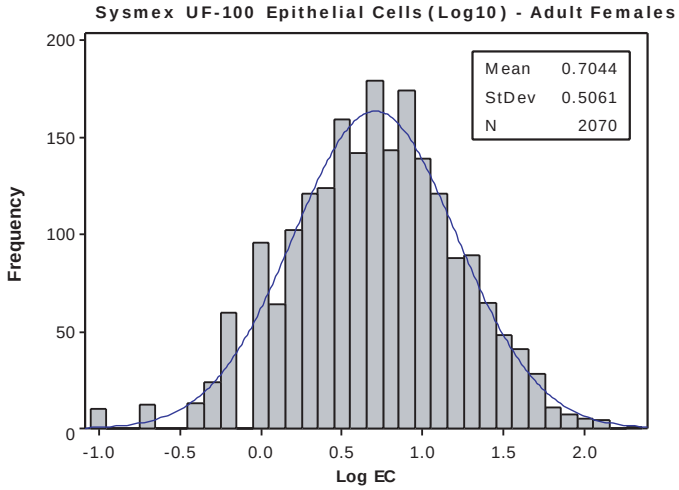

Fig. 2 *Histogram of UF-100 epithelial cell counts for females after conversion into Log10 (with normal curve indicated) Note normal (Gaussian) distribution of the data.*

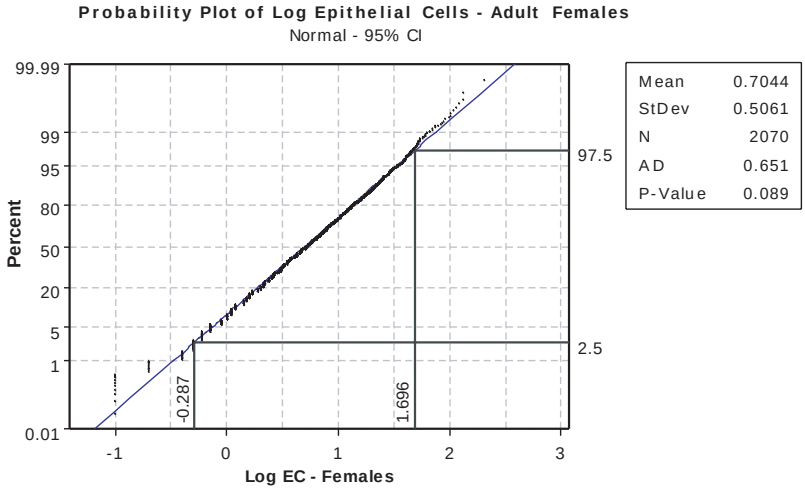

Fig. 3 *Anderson-Darling Normal Probability Plot for UF-100 Epithelial Cell counts for females after conversion into Log10 If data is perfectly normal, then the data points on the probability plot will form a straight line. The marked line forms an estimate of the cumulative distribution function for the population from which the data are drawn. Markers are shown for 2.5 and 97.5 percentiles (95% confidence intervals) with corresponding count value (in Log10). This data follows the line within confidence limits.*

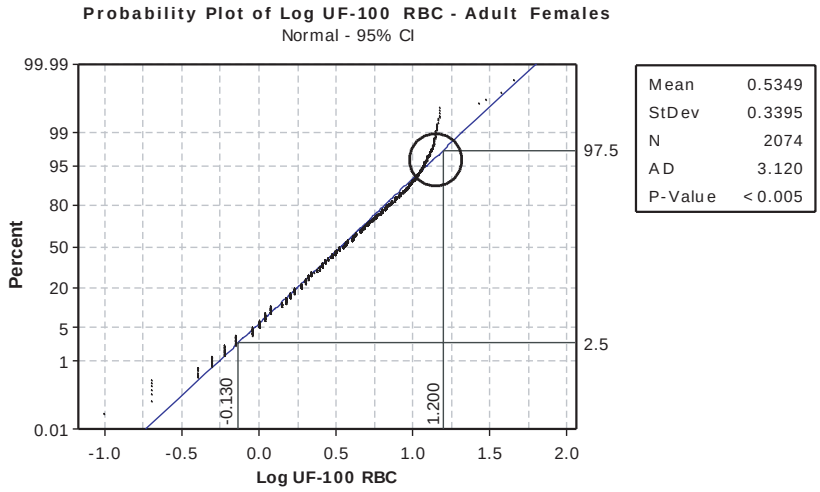

Fig. 4 *Anderson-Darling Normal Probability Plot for UF-100 RBC counts for females after conversion into Log10*
Markers are shown for 2.5 and 97.5 percentiles (95% confidence intervals) with corresponding count value (in Log10). As indicated, the data deviates from the "normal" line before reaching the 97.5% marker.

Table 1 *Statistical method used to determine the normal limits*

UF-100 Parameter	Sex	Statistical Method Used
WBC	Females	Anderson-Darling Probability Plot
	Males	Anderson-Darling Probability Plot
RBC	Females	2.5 and 97.5 percentiles
	Males	2.5 and 97.5 percentiles
EC	Females	Anderson-Darling Probability Plot
	Males	2.5 and 97.5 percentiles
Cast	Females	2.5 and 97.5 percentiles
	Males	2.5 and 97.5 percentiles
Bact	Females	Anderson-Darling Probability Plot
	Males	Anderson-Darling Probability Plot

using the Normality Test since their data did not follow the line of normality within the 2.5 and 97.5 percentiles. ***Fig. 4*** demonstrates the Anderson-Darling Normal Probability Plot for log RBC counts for adult females and the deviation of the data before reaching the 97.5% marker can be observed. When deviations were observed, the normal limits were established by an alternate method. In these cases (percentiles) were used (the value at 2.5% and 97.5% of the data in the Microsoft Excel spreadsheets was taken). ***Table 1*** indicates which method was used to determine the limits for each of the cell counts.

RESULTS

From a total of 21,866 urine sample results collected, 5,098 (23%) remained after the data was "normalised". The 5,098 included 767 patients below 13 years of age and these were removed. Of the remaining 4,331 samples from adults, results from 2,074 females (48%) and 2,257 males (52%) were analysed statistically and limits for WBC, RBC, EC, Cast and Bact counts determined.

Age distribution of females (normalised data) was 13 to 100 years with a mean age of 49 years and median age of 47 years, standard deviation of 21 years. Age distribution of males (normalised data) was 13 to 95 years with a mean age of 53 years, median age of 54 years and standard deviation of 18 years. Age distribution for females and males can be seen in ***Fig. 5*** and ***Fig. 6*** respectively. The normal limits obtained are shown in ***Table 2***.

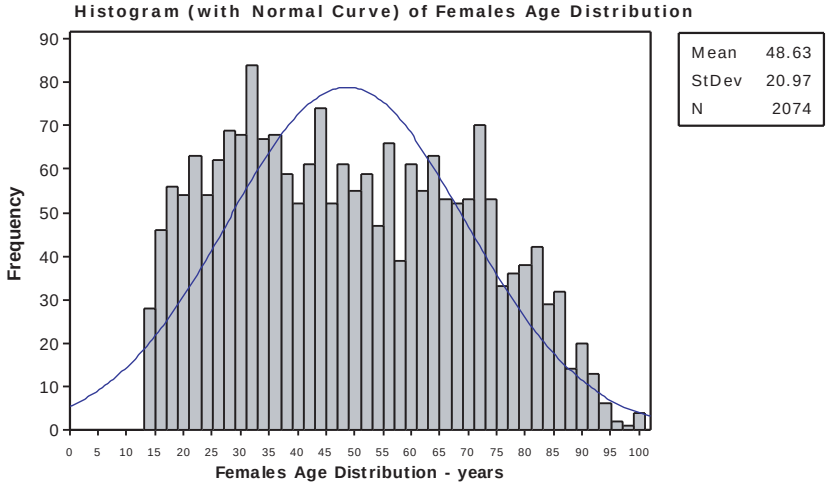

Fig. 5 *Age distribution of for adult females (after data was "normalised")*

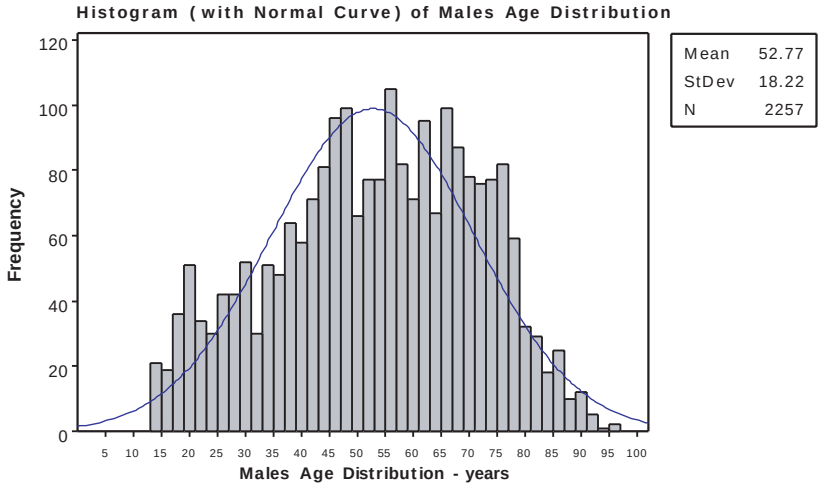

Fig. 6 *Age distribution of for adult males (after data was "normalised")*

Table 2 *Normal limits determined from statistical analysis of "normalised" data*

Group	n	WBC/μL	RBC/μL	EC/μL	CAST/μL	BACT/μL
Females ≥ 13years	2,074	0.6-31.5	0.7-13.3	0.5-49.7	<0.64	<6310
Males ≥ 13years	2,257	0.4-15.2	0.5-10.2	0.1-5.1	<0.89	<3733

DISCUSSION

As opposed to analysing the results of urine samples from a selected group of normal patients, the approach taken in this study was to take all available routine urine result information from a laboratory, extract the normal results and then analyse them statistically. A fair proportion of samples sent to the laboratory had been found to be normal according to their current criteria (negative dipstick, normal/low cell counts, no/insignificant bacterial growth). There were a large number of sample results available for analysis, and assuming that statistical analysis would eliminate or at least diminish the affect of any abnormal results which were not removed through the normalisation process, it was decided this approach would produce reasonable normal limits. By not pre-screening or pre-selecting the patients for the study, the samples would be typical of the population and samples routinely tested by this laboratory.

The normal limits established for most parameters are, perhaps surprisingly, similar to those from other published material for the UF-100, although not the same as those from any one study. ***Table 3*** provides examples of normal limits presented in previously published work.

There is a distinct difference in normal results between male and female urine samples in this and other investigations[2,3] particularly for WBC and EC counts. For this reason there is very poor agreement between this study and the limits determined by Regeniter, et al.[1] where no gender separation was performed.

The limits established here compare well with those from Hannemann-Pohl & Kampf[2] with the exception of RBC for females. There is no obvious reason why the RBC limit they established is significantly higher than other studies, including this one, particularly when they specifically selected healthy, non-menstruating females and used the 97.5 percentiles for the limit. And although they selected "healthy" individuals they only included samples if the dipstick and sediment microscopy were negative.

The limits established here compare reasonably well with those of György, et al.[3], particularly for females. For males, they established somewhat lower normal limits for WBC, RBC and EC. The volunteers in the György, et al.[3] study were from a much narrower age range; 20 to 56 years for females, 18 to 60 years for males. To check whether age was a factor in the difference in normal limit, the data was re-analysed using these age limits and is shown in ***Table 4***. Using the narrowed age limits made no significant difference to the normal limits established except for a slight reduction in the EC limit for males, despite the fact that there was a significant number of samples removed due to the change in age range. The difference in the limits between György, et al.[3] and those established here may be explained by other factors associated with the difference in patient selection/sample collection (György, et al.[3] used early morning, clean midstream urine from normal volunteers without a history of urinary disease, urinary infection, stone disease, or systemic hypertension) and the statistical methods used.

The Bact count limits established here were significantly

Table 3 *Published Sysmex UF-100 normal ranges/limits*

Source	WBC/μL	RBC/μL	EC/μL	CAST/μL	BACT/μL
Regeniter, et al. 2001[1]					
55 Females & 36 Males	<16	<14	<9	<2	<173
Hannemann-Pohl & Kampf 1998[2]					
Females (n = 82)	1-36	1-43	0-49	0-0.9	13-369
Males (n =119)	1-10	1-24	0-4	0-0.9	6-265
György, et al. 1998[3]					
Females (n = 118)	≤35.7	≤12.3	≤48.5	≤0.21	≤404
Males (n =94)	≤ 6.6	≤ 4.1	≤ 2.1	≤0.42	≤100
Sysmex Document 2001[4]					
Females (n =619)	<15.4	<17.6	<8.7	<0.62	<3,324
Males (n =1,332)	<10.4	< 9.9	<4.0	<0.89	<1,991

Table 4 *Normal limits determined using the same age limits as György, et al.[3]*

Group	n	WBC/μL	RBC/μL	EC/μL
Females (20 – 56 years)	1,146	0.6-32.0	0.7-13.0	0.5-48.8
Males (18 – 60 years)	1,345	0.4-14.9	0.5-9.7	0.1-3.9

different from those previously published with perhaps the exception of the Sysmex document[4]. New Bact detection was implemented from version 00-14 UF-100 software. This "new" type of detection is referred to as high sensitivity and this count also includes any bacteria present of smaller size. Bact counts using the "new" or high sensitivity detection will be significantly higher than systems using the low sensitivity detection. From version 00-15 software the operator can select which type of Bact detection they want to use (low or high sensitivity). The UF-100 in this study is on version 00-17 and was set to high sensitivity mode. This is probably not a significant issue since Bact is not usually a reported parameter and the results for Bact were included for interest only, but probably explains the large differences in Bact limits established by the various studies.

Dipstick reader sensitivity can be adjusted to be more or less sensitive to the presence of chemical constituents in the urine and this should be considered when using dipstick results in a "normalisation" or sample selection process in case there is a risk of excessive normals being excluded and abnormals included. It is not thought to have been an issue in this study.

This study included a large number of samples but there was no control in terms of initial selection of participants. However by using results collected over a large time period and from many different sources perhaps these results reflect the limits of normality in practice (taking into account variety of collection times and techniques).

By using retrospective analysis of data already produced, a considerable quantity of data is made available relatively easily. The exclusion criteria used in this study should have accounted for many of the possible abnormalities that patient selection may have otherwise eliminated. Statistical analysis tends to deal with those few, possibly pathological samples, which may have made it through the exclusion process. Statistical analysis has been made relatively quick and easy by the use of software programs such as Microsoft Excel and Minitab but there are many ways that data can be statistically analysed and this too can account for differences in the limits established in the various studies.

Rather than proposing this approach as a total replacement, when a more formal normal range study is required, this is likely to be a satisfactory method of establishing interim limits or a means of validating those in use or those that have been adopted from others, particularly if performing a proper reference range study poses some difficulties. For some laboratories urine cell count normal limits are used just as a guide rather than an exact reference for normality of results and in some cases they are not quoted or used at all. These normal limits are probably most useful to a laboratory, using the Sysmex UF-100, whose main focus is screening samples for urinary tract infection. Whether they are appropriate to be used as normal limits in the detection and management of all conditions or whether they will satisfy individual laboratories, regulatory and/or clinical requirements is not known.

ACKNOWLEDGEMENTS

Many thanks to Medlab Hamilton for supplying the urinalysis results and associated information and also to Shadi Ghadalla for his support and assistance with Minitab software and the statistical analysis of the data.

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4) *"Normal population reference range (upper limit)"UF-100, UF-50 Clinical Case Study 2. Issued by Scientific Division, Sysmex Corporation, Japan, 2001.*