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¹School of Information Technology and Electrical Engineering, The University of Queensland, Brisbane, Queensland, Australia

²Department of Respiratory and Sleep Medicine, The Mater Children's Hospital, South Brisbane, Queensland, Australia

³Australasian Paediatric Endocrine Group, Mater Medical Research Institute, Brisbane, Queensland, Australia

Correspondence to

Dr Philip Terrill, School of Information Technology and Electrical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia; p.terrill@uq.edu.au

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Nocturnal oxygen saturation profiles of healthy term infants

Philip Ian Terrill,¹ Carolyn Dakin,² Ian Hughes,³ Maggie Yuill,² Chloe Parsley²

ABSTRACT

Objective Pulse oximetry is used extensively in hospital and home settings to measure arterial oxygen saturation (SpO₂). Interpretation of the trend and range of SpO₂ values observed in infants is currently limited by a lack of reference ranges using current devices, and may be augmented by development of cumulative frequency (CF) reference-curves. This study aims to provide reference oxygen saturation values from a prospective longitudinal cohort of healthy infants.

Design Prospective longitudinal cohort study.

Setting Sleep-laboratory.

Patients 34 healthy term infants were enrolled, and studied at 2 weeks, 3, 6, 12 and 24 months of age (N=30, 25, 27, 26, 20, respectively).

Interventions Full overnight polysomnography, including 2 s averaging pulse oximetry (Masimo Radical).

Main outcome measurements Summary SpO₂ statistics (mean, median, 5th and 10th percentiles) and SpO₂ CF plots were calculated for each recording. CF reference-curves were then generated for each study age. Analyses were repeated with sleep-state stratifications and inclusion of manual artefact removal.

Results Median nocturnal SpO₂ values ranged between 98% and 99% over the first 2 years of life and the CF reference-curves shift right by 1% between 2 weeks and 3 months. CF reference-curves did not change with manual artefact removal during sleep and did not vary between rapid eye movement (REM) and non-REM sleep. Manual artefact removal did significantly change summary statistics and CF reference-curves during wake.

Conclusions SpO₂ CF curves provide an intuitive visual tool for evaluating whether an individual's nocturnal SpO₂ distribution falls within the range of healthy age-matched infants, thereby complementing summary statistics in the interpretation of extended oximetry recordings in infants.

INTRODUCTION

Modern pulse-oximeters non-invasively measure and record high temporal resolution arterial blood haemoglobin oxygen saturations (SpO₂), making them a valuable clinical tool in a range of paediatric clinical applications. Published reference ranges have documented the normal ranges of summary measurements such as baseline SpO₂, mean SpO₂ and median SpO₂ values,^{1–9} with more recent studies expanding reference statistics to include ranges, nadirs, 5th and 10th percentiles of SpO₂ distributions and time below specific SpO₂ levels.^{10–11} However, many of these studies used devices which are no longer available in clinical practice or present selected parameters specific to particular models and averaging time settings. Furthermore, with

What is already known on this topic

- Extended recording of pulse oximetry can provide important information about the health of an individual relatively non-invasively in a hospital environment.
- SpO₂ reference data, stratified by sleep-state in term infants aged 2 weeks–24 months, are currently not available for modern high temporal resolution pulse-oximeters.
- Summary statistics, such as mean and medians, do not capture the overall distribution of extended SpO₂ recordings.

What this study adds

- This study provides reference SpO₂ statistics, stratified by sleep-state, for healthy term infants aged 2 weeks–2 years, using a current generation pulse-oximeter.
- Cumulative frequency reference-curves provide an intuitive visual tool for rapidly evaluating whether an individual's SpO₂ distribution is within a healthy range.
- Cumulative frequency plots complement summary statistics and visual inspection of raw data in the clinical interpretation of nocturnal SpO₂ recordings.

modern oximeters capable of recording individual data points for extended periods, it is highly desirable to develop normative measurements which quantify the overall SpO₂ distribution. This would be of particular value in the titration of supplemental oxygen requirements in infants with chronic neonatal lung disease^{12–15} and screening for obstructive sleep apnoea.¹⁶

One method of presenting SpO₂ data in a graphical, and therefore perhaps more readily interpretable manner, is the SpO₂ cumulative frequency (CF) curve. These are produced by some commercial oximetry software, and reference values for healthy preterm infants have been presented by Harigopal *et al.*¹⁷ and Ng *et al.*¹⁸ In this approach, the SpO₂ CF curve was generated for each infant by calculating the proportion of the recording spent at or below each discrete SpO₂ saturation level. CF reference-curves were then generated by calculating the 5th and 50th centiles at each SpO₂ value for the study population. Thus,

analogous to using a growth chart, by plotting the SpO₂ CF curve for a subject of interest over the reference-curves, it can be easily observed whether the infant's SpO₂ profile falls within the range expected for a healthy infant. This study thus aimed to present reference ranges for SpO₂ summary statistics and CF reference-curves for healthy term infants from the neonatal period to 2 years of age. The impact of manual artefact removal and sleep-state stratification on CF reference-curves and summary statistics are also investigated.

METHODS

Subjects and procedures

Complete details of the protocol have been described elsewhere.^{19–22} Recruitment was from antenatal clinics during the third trimester of an uneventful singleton pregnancy, with 34 healthy Caucasian infants (16 girls) enrolled following birth.

The intention of the initial study design was to collect normative data on various cardiorespiratory parameters, including oxygen saturation. The sample size was selected to ensure statistical power to detect maturational changes in key summary variables such as number of SpO₂ desaturation events, while being a feasible number in which detailed sleep studies could be performed. Infants were eligible if they were: born by normal vaginal delivery or planned caesarean section at term (38–42 weeks) with normal birth weight (10th–90th percentile),²³ Apgar score >7 at 5 min and were from a non-smoking household. Exclusion criteria included congenital or anatomical abnormality; sudden infant death syndrome sibling; supplemental oxygen requirement >5 min following delivery; or a strong maternal family history of atopy.

This prospective cohort was studied with full overnight polysomnogram within the Queensland Paediatric Sleep Service at the

Table 1 Participant characteristics and SpO₂ summary statistics calculated without manual artefact removal

Summary statistic	All		non-REM/QS		REM/AS		Wake	
2 weeks (N=30, 13 female)								
Age (days)	14±3							
Duration (min)	549.6	(396–1024)	153.9	(114–544)	203.8	(93–270)	156.0	(38–357)
Artefact-free duration (min)	503.0	(343–635)	151.2	(113–220)	193.9	(93–266)	117.6	(15–231)
SAT5 (%Hb)	95.0	(88–97)	96.0	(85–98)	95.0	(88–97)	92.0	(73–99)
SAT10 (%Hb)	96.0	(92–98)	97.0	(93–98)	96.0	(92–98)	96.0	(77–99)
Median SpO ₂ (%Hb)	98.0	(95–100)	98.0	(95–100)	97.5	(95–100)	98.0	(96–100)
Mean SpO ₂ (%Hb)	97.6	(95–99)	97.9	(95–99)	97.2	(95–99)	97.6	(93–100)
3 months (N=25, 10 female)								
Age (days)	97±9							
Duration (min)	534.5	(411–660)	249.5	(151–327)	189.5	(86–302)	75.5	(8–313)
Artefact-free duration (min)	492.9	(391–621)	249.5	(149–327)	189.5	(73–302)	60.6	(8–169)
SAT5 (%Hb)	97.0	(90–99)	98.0	(94–99)	97.0	(93–99)	85.0	(68–99)
SAT10 (%Hb)	98.0	(95–99)	98.0	(95–100)	98.0	(94–99)	91.0	(76–100)
Median SpO ₂ (%Hb)	99.0	(97–100)	99.0	(97–100)	99.0	(97–100)	99.0	(97–100)
Mean SpO ₂ (%Hb)	98.5	(97–100)	98.7	(97–100)	98.9	(97–100)	97.3	(92–100)
6 months (N=27, 10 female)								
Age (days)	198±6							
Duration (min)	498.6	(367–680)	270.6	(160–421)	147.0	(88–229)	91.5	(20–214)
Artefact-free duration (min)	462.5	(351–654)	262.0	(160–397)	140.0	(83–227)	66.7	(14–179)
SAT5 (%Hb)	97.0	(95–98)	97.0	(95–99)	97.0	(95–99)	96.0	(83–98)
SAT10 (%Hb)	97.0	(96–99)	98.0	(96–99)	98.0	(96–99)	97.0	(86–100)
Median SpO ₂ (%Hb)	98.0	(97–100)	98.0	(97–100)	99.0	(97–100)	99.0	(98–100)
Mean SpO ₂ (%Hb)	98.3	(97–100)	98.3	(97–100)	98.5	(97–100)	98.2	(96–99)
12 months (N=26, 10 female)								
Age (days)	371±14							
Duration (min)	499.0	(259–744)	272.3	(131–532)	128.3	(47–216)	72.0	(3–251)
Artefact-free duration (min)	442.8	(205–612)	267.1	(119–355)	121.9	(47–216)	43.4	(3–132)
SAT5 (%Hb)	97.0	(89–99)	97.0	(95–99)	97.0	(83–99)	95.5	(69–99)
SAT10 (%Hb)	98.0	(95–99)	98.0	(95–99)	98.0	(94–99)	97.0	(75–99)
Median SpO ₂ (%Hb)	99.0	(96–100)	98.5	(96–100)	99.0	(96–100)	99.0	(97–100)
Mean SpO ₂ (%Hb)	98.4	(96–100)	98.4	(96–100)	98.5	(96–100)	98.2	(95–100)
24 months (N=20, 5 female)								
Age (days)	725±8							
Duration (min)	486.3	(323–658)	337.8	(240–512)	112.8	(41–188)	37.5	(0–155)
Artefact-free duration (min)	481.5	(220–582)	306.7	(181–385)	103.9	(38–188)	28.9	(0–138)
SAT5 (%Hb)	97.5	(96–99)	98.0	(96–99)	98.0	(95–99)	97.0	(73–98)
SAT10 (%Hb)	98.0	(96–99)	98.0	(96–100)	98.0	(96–99)	97.0	(75–100)
Median SpO ₂ (%Hb)	99.0	(97–100)	99.0	(97–100)	99.0	(97–100)	99.0	(97–100)
Mean SpO ₂ (%Hb)	98.7	(97–100)	98.7	(97–100)	98.8	(97–100)	97.9	(94–100)

All summary statistics are calculated from SpO₂ data without manual artefact removal. Data are presented for the entire recording (All), and stratified according to sleep-states non-REM, REM and wake for children at 3 months and older; and quiet sleep (QS), active sleep (AS) and wake for children at the neonatal study. SAT5, 5th percentile SpO₂ value; SAT10, 10th percentile SpO₂ value. Values are presented as median (range) or mean±SD. REM, rapid eye movement.

Mater Children's Hospital in the neonatal period (day 5–20) and then at 3, 6, 12 and 24 months of age. Scheduled studies were conducted if the infant was well and free of inter-current illness (on physician examination). In the event of a viral respiratory illness, studies were rescheduled to at least 2 weeks after clinical resolution of the illness. A polysomnographic sleep-study was conducted using an EMBLA acquisition system (Embla N7000 system, Natus Medical Inc) and Somnologia software (Somnologia V3.3.2 Build 1559). As part of this montage, arterial oxygen saturation was measured using a Masimo Radical (software-build 4.1, Masimo Corporation, Irvine, California, USA) pulse-oximeter with 2 s averaging and 1 Hz temporal resolution. All procedures were completed between March 2006 and July 2009, and were compliant with the Thoracic Society of Australia and New Zealand recommendations for paediatric sleep laboratories.²⁴

Each study was manually scored by a trained, experienced scorer, with sleep-staging conducted using Anders criteria at the neonatal study²⁵ and the AASM manual for scoring sleep for infants aged 3 months and over,²⁶ as recommended by Grigg-Damberger *et al.*²⁷ This study was approved by the Mater Health Services Human Research Ethics Committee (Number 952C).

Data analysis

The nocturnal SpO₂ recording for each infant, at each of the study ages, was exported from Somnologia into the European Data Format (.edf) and then imported into MATLAB (Mathworks, Natick, Massachusetts, USA) for further analysis. To account for small analogue acquisition calibration errors

(<0.5%) when digitally sampled at the polysomnogram system, SpO₂ values were rounded to the nearest integer percentage value. The Masimo Radical oximeter has sophisticated artefact detection algorithms which mark artefactual periods by setting SpO₂=0% and, consequently, samples of data less than 25% after rounding were removed from further analysis.

The SpO₂ cumulative distribution function (CDF) describes the proportion of the recording spent at or below each integer SpO₂ value, that is, for a recording of SpO₂ samples *S*, and integer SpO₂ values (*x*):

$$CF(x) = P(S \leq x). \quad (1)$$

For each infant, at each of the five study points, a CF curve was generated using the method described by Harigopal *et al.*¹⁷ Reference CF reference-curves were then generated by identifying the minimum, 25th percentile, median, 75th percentile and maximum value across the study population at each discrete SpO₂ value to generate CF reference-curves representing the expected range of values for healthy infants at the specified age. In addition, the mean, median, 5th and 10th percentiles of SpO₂ values (SAT5 and SAT10) were calculated for each study.

Secondary analysis included the stratification of results according to the sleep-states of wake, non-rapid eye movement (REM) and REM (wake, quiet sleep and active sleep for infants at 2 weeks);^{27–29} the manual identification and removal of movement artefacts in the SpO₂ channel (defined as changes in SpO₂ corresponding to movement noted on video recording)

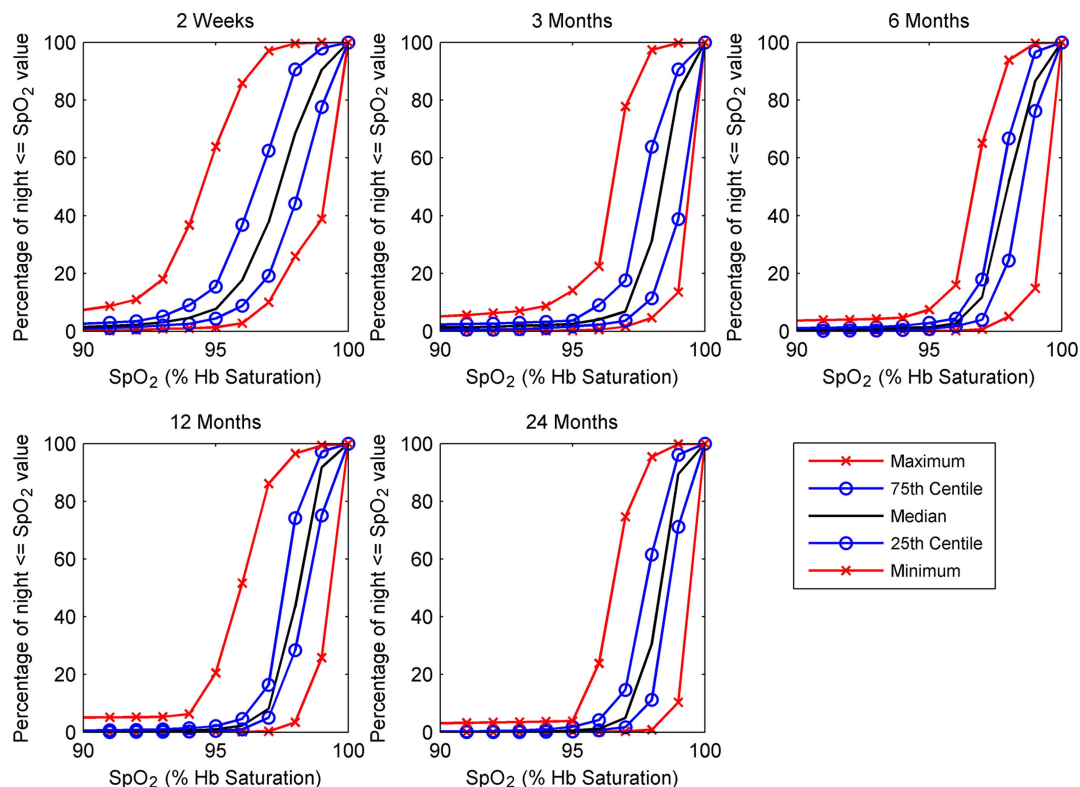


Figure 1 Cumulative frequency (CF) reference-curves of SpO₂ profiles for a population of healthy term infants aged 2 weeks, 3, 6, 12 and 24 months. CF reference-curves are derived from the nocturnal SpO₂ recordings of 30, 25, 27, 26 and 20 infants at 2 weeks, 3, 6, 12 and 24 months of age, respectively, in a longitudinal follow-up study. Results are presented for the whole nocturnal recording, using CF plots calculated from oximetry data without manual artefact removal. The CF plot for an individual shows the percentage of a recording spent at, or below a particular SpO₂ value, while the CF reference-curve shows the range of percentage time spent below a particular SpO₂ value in the healthy term population. For example, it may be seen that in healthy infants at 2 weeks of age, between 0% and 60% of a recording may be spent with an SpO₂ of 95% or less. Notably, the CF reference-curves shift right by 1%–2% between 2 weeks and 3 months, where they remain stable up to 24 months.

which were not automatically detected; and comparison of CF reference-curves to those previously presented for healthy preterm children.¹⁷ Wilcoxon-Sign-Rank tests were used to compare summary statistics in sleep-state, age and artefact rejection comparisons. p Value <0.01 was considered statistically significant. In the event of a missing study for an infant at a particular age, that infant was excluded from statistical tests in relevant age comparisons.

RESULTS

Of the 34 children enrolled, 31, 25, 27, 26 and 20 participants completed studies at 2 weeks, 3, 6, 12 and 24 months, respectively (see online data supplement 1.1, including figure S1). One study at 2 weeks had less than 60 min of artefact-free SpO₂ data and was removed from analysis. Summary statistics of the SpO₂ profiles calculated without manual artefact rejection for the infant population are presented in table 1. As expected, mean duration and percentage of study time of REM decreased with maturation, while non-REM percentage of study time increased. SAT5 was lower during wake than non-REM and REM at 2 weeks and 3 months (p <0.01) and SAT10 for the same comparison at 3 months (p <0.01). There was an increase in median values of SAT5 and SAT10 between 2 weeks and 3 months of age for pooled non-REM and REM stratifications (p <0.01). SAT5 and SAT10 increased during wake between 3 and 6 months (p <0.01). The Masimo Radical pulse-oximeter was generally effective in identifying artefact (82.5%, 66.3%, 80.1%, 93.3% and 90.0% of total manually scored artefacts in infants at 2 weeks, 3, 6, 12 and

24 months, respectively). Complete summary statistics for analysis with manual artefact rejection are presented in the online data supplement 1.2. Analysis with manual artefact rejection did not change the population median by >2% for any summary statistics for pooled sleep-states or in REM or non-REM, but during wake did result in a statistically significant increase of median SAT5 at 2 weeks, 3 and 12 months (p <0.01) and a notable increase in the lower bound of the range for SAT5 and SAT10 (up to 23%).

Figure 1 shows the population minimum, 25th, 50th and 75th percentiles and maximum SpO₂ CF reference-curves generated for the study population at each of 2 weeks, 3, 6, 12 and 24 months of age. Plots are shown for the full nocturnal recording without manual artefact removal. The CF reference-curves shift right by 1% between 2 weeks and 3 months, where they remain stable up to 24 months. The same CF reference-curves overlaid with reference-curves generated using manual artefact removal are presented in figure 2. There is no qualitative change to median or minimum population curves, but the maximum curve shows a reduced percentage of the night spent below 94% saturation. Tabulated data for these SpO₂ CDF nomograms and those generated with sleep-state stratifications are presented in online data supplement 1.3, while commonly used summary statistics derived from these (percentage of night with SpO₂ below 92% and 95%) are tabulated in online data supplement 1.4.

Figure 3 compares the CF reference-curves presented in figure 1 with those presented in literature for healthy preterm infants at the same corrected age of 2 weeks post-term.¹⁷ The CF reference-curves for the preterm infant group are shifted to

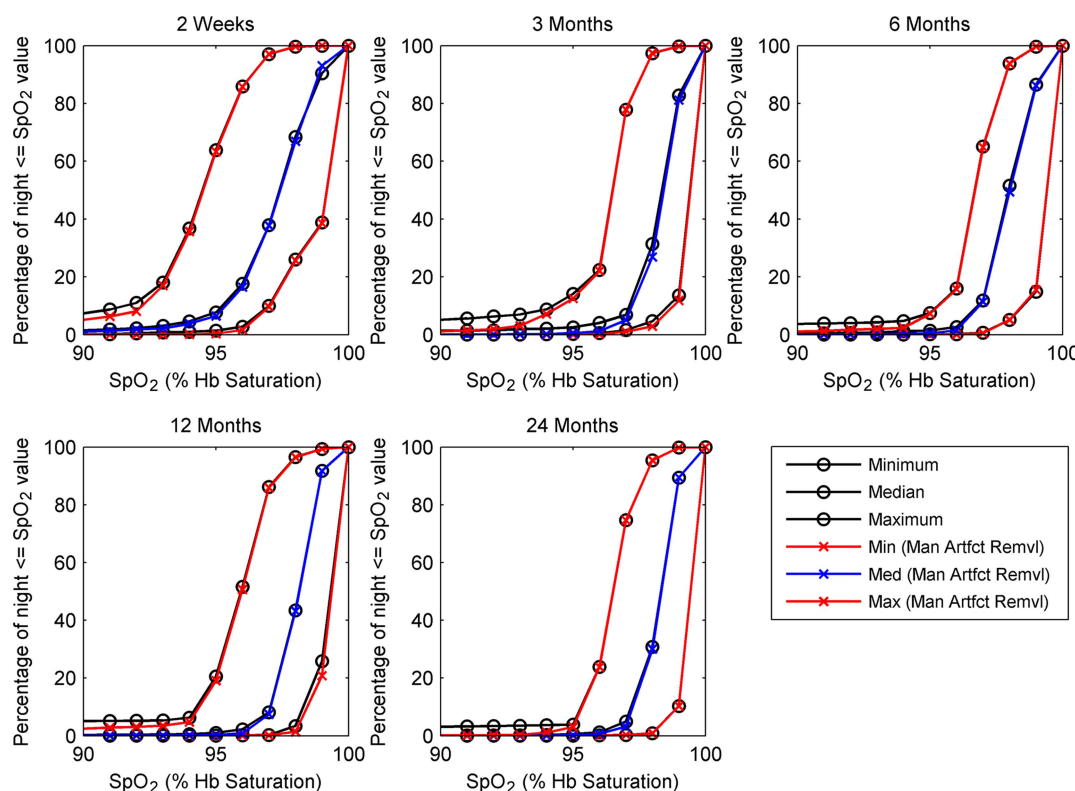


Figure 2 SpO₂ cumulative frequency (CF) reference-curves for healthy infants aged 2 weeks–24 months, generated with and without manual artefact removal. CF reference-curves are shown for SpO₂ data from the whole nocturnal recording. At SpO₂ values greater than 95%, there is no discernible deviation between nomogram curves generated with and without manual artefact removal. At lower SpO₂ values, the maximum curve for data without manual artefact removal remains higher than the respective nomogram curve with manual artefact removal, indicating that artefact manually removed tends to be at lower SpO₂ values. Max (Man Artfct Remvl), population maximum reference-curve generated with manual artefact removal; Med (Man Artfct Remvl), population median reference-curve generated with manual artefact removal; Min (Man Artfct Remvl), population minimum reference-curve generated with manual artefact removal.

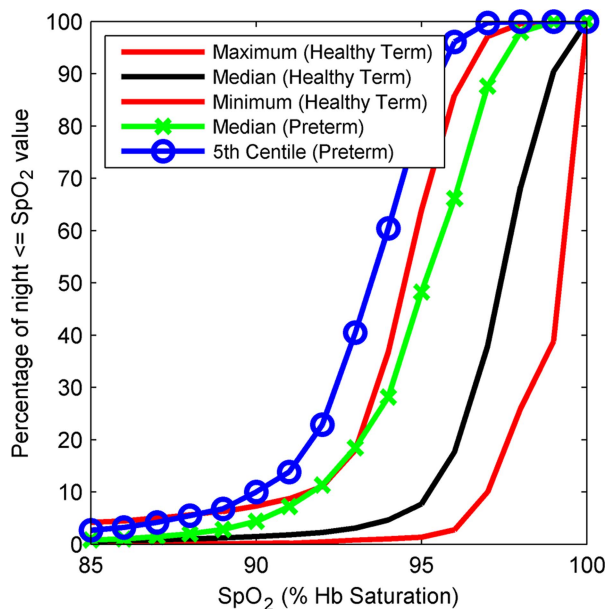


Figure 3 SpO₂ cumulative frequency (CF) reference-curves for healthy term infants and healthy preterm infants. CF reference-curves for healthy infants at 2 weeks, overlaid with the reference-curves presented by Harigopal *et al*¹⁷ for healthy preterm infants (median gestational age at birth of 33 weeks; median age at study of 2 weeks). The reference-curves for the preterm infant group are shifted to the left relative to the term infant group (median curve shifted approximately 3%–4% SpO₂). Physiological differences in the oxy-haemoglobin dissociation curve, use of different pulse-oximeter models and the extent of manual artefact removal may account for these differences.

the left relative to the healthy infant group (median curve shifted approximately 3%–4% SpO₂).

DISCUSSION

The objective of this study was to present reference ranges of nocturnal SpO₂ recordings from a prospective cohort of healthy term infants at 2 weeks, 3, 6, 12 and 24 months of age using both conventional summary statistics and CF reference-curves. To our knowledge, this is the first study to present reference ranges recorded using a Masimo Radical pulse-oximeter with 2 s averaging in a prospective cohort of healthy term infants from 2 weeks to 24 months. This study was conducted in a sleep-laboratory and, as such, secondary analyses investigated the impact of sleep-state and manual artefact identification on reference ranges.

The SpO₂ summary statistics presented for entire nocturnal recordings are generally consistent with the literature despite the recognised variations in recorded SpO₂ values between different oximeter makes and models.³⁰ In particular, the population range of mean and median nocturnal SpO₂ values was between 95% and 100% over the first 2 years.^{2 5 6 31 32} Median SAT5 was 95% at 2 weeks which supports existing studies that show healthy term neonates have nocturnal SAT5 values as low as 95%¹⁰ and these lower end SpO₂ values tend to increase and stabilise over the first few months of life.³¹ These results provide important normative data using a current generation high temporal resolution pulse-oximeter. While this is the first study to present CF reference-curves for healthy term infants, these curves have previously been presented in literature for a cohort of healthy preterm infants.¹⁷ It was noted in the results that there was a left shift of 3%–4% in the median curve for preterm infants indicating that SpO₂ values are lower in the preterm group. Physiological differences in the oxy-haemoglobin

dissociation curve, use of different pulse-oximeter models³⁰ and the extent of artefact removal may account for these differences.

Sleep-state stratification confirmed earlier work that there is no clinically significant difference (>2%) in summary measures of SpO₂ between REM and non-REM sleep-states,¹ and this was reflected in CF reference-curves (see online data supplement 1.3). Although we found that SAT5 and SAT10 were lower during wake than sleep at 2 weeks and 3 months, this may be related to the additional likelihood of movement artefact during wake which was not detected by the automatic algorithm. While results indicated that Masimo Radical's artefact identification was good, manual artefact removal during wakefulness resulted in an increase in summary statistics (particularly SAT5) and deviation in CDF's (observed as lower percentage of time spent below 90%). No difference was found in either SpO₂ summary statistics or SpO₂ CDF nomograms for pooled data, REM or non-REM stratifications following manual artefact rejection. These results suggest that clinical utility would not be limited by the inability to classify sleep-stage, although there would be benefit to manually excluding obvious movement artefacts during wake from analysis (see online data supplement 1.5 for further discussion).

The CF reference-curve has previously been proposed as a useful clinical tool for interpreting extended SpO₂ recordings due to its ability to present information about the SpO₂ distribution in an intuitive graphical manner.^{17 18} Indeed, SpO₂ CF curves for individuals can be produced with some commercial oximetry and polysomnogram software, and may be readily generated using standard spreadsheet software (see online data supplement 2 provides a brief tutorial). Yet, to date, CF reference-curves for healthy term infants have been lacking. This study thus provides important reference data to allow this method to be translated into wider clinical practice. To illustrate the potential clinical utility of CF reference-curves, figure 4 displays the CF curves for an infant with chronic neonatal lung disease at 3 months gestational corrected age during an oxygen titration study superimposed on the CF reference-curves for infants at 3 months. It can be observed that when the infant is in room air, the CF is shifted to the left of the normative range indicating that SpO₂ values are lower than may be expected in healthy infants. However, when the same infant is placed in supplemental oxygen at 0.25 L/min, the CF curve is within the healthy range, indicating normal SpO₂ distribution. The CF curve alone does not discriminate between a large number of short desaturations or a smaller number of longer desaturations and, as such, best clinical utility would be achieved by using CF curves to complement summary statistics and visual inspection of large time-scale trend graphs. As with all diagnostic tests, results need to be interpreted in the wider clinical context.

There are a number of limitations in this study. First, participants were continuously supervised by an experienced sleep technologist to ensure that all sensors remained correctly attached, likely resulting in fewer artefacts than expected in a general clinical environment. While this provides the scientific advantage of improved data quality, it may limit generalisability to other clinical environments. Second, generalisability is also potentially restricted to the pulse-oximeter used in this study. However, it is a commercially available model and is commonly used in hospital settings. Finally, this was a resource-intensive sleep-laboratory based prospective cohort follow-up study, and consequently results may also be limited by a relatively small sample size. This may have particular impact on maximum and minimum CF reference-curves, and these should be interpreted as the maximum and minimum in this sample, rather than the expected limits for the wider population. Future work should

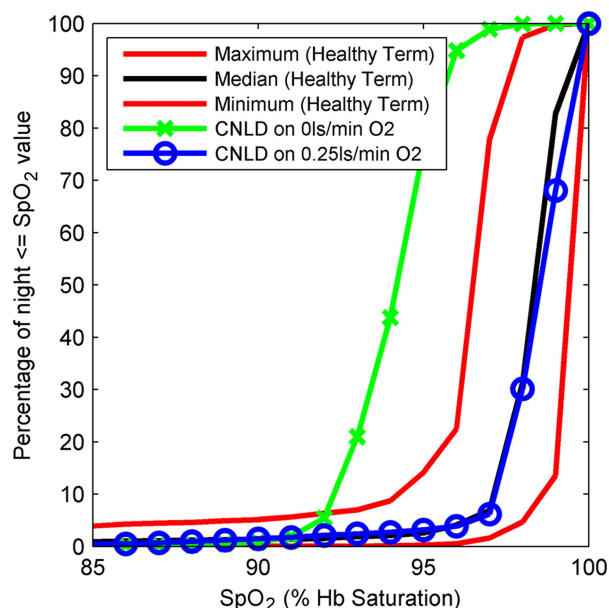


Figure 4 Effect of supplemental oxygen on an infant with chronic neonatal lung disease at 3 months corrected age during an oxygen titration study. Cumulative frequency (CF) plots for an infant with chronic neonatal lung disease at 3 months gestational corrected age, plotted over the SpO₂ CF reference-curves for healthy infants at 3 months. It can be observed that when the infant is in room air, the CF plot is shifted to the left of the normative range indicating that SpO₂ values are lower than may be expected in healthy infants. However, when treated with 0.25 L/min of supplemental oxygen, the CF plot is within the healthy range indicating normal SpO₂ distribution.

expand these results by studying a larger sample in a more generalised clinical environment.

In conclusion, this paper presents reference ranges for healthy term infants aged from 2 weeks to 24 months of nocturnal SpO₂ recordings using a Masimo Radical pulse-oximeter with 2 s averaging. The SpO₂ CF reference-curves provide an intuitive visual tool for evaluating whether an individual infant's nocturnal SpO₂ distribution falls within the range of healthy age-matched infants. This reference range is potentially applicable to extended oximetry studies conducted in a range of generalised care settings and may be a useful tool in monitoring and setting appropriate supplemental oxygen levels for infants with respiratory and other diseases.

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Contributors Conception and design: PIT, CD; Analysis and interpretation: PIT, CD, IH, MY, CP; Drafting the manuscript for important intellectual content: PIT, CD, IH.

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Competing interests None.

Ethics approval The Mater Hospital Human Research Ethics Committee.

Patient consent Written, informed consent was provided by the parent/guardian of the children participating in this study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The SpO₂ cumulative frequency reference-curves presented in the manuscript are available in tabulated form in the online data supplement.

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Nocturnal Oxygen Saturation Profiles of Healthy Term Infants

Online Data Supplement

Philip Ian Terrill¹, Carolyn Dakin², Ian Hughes³, Maggie Yuill², Chloe Parsley²

¹ School of Information Technology and Electrical Engineering, The University of Queensland, Brisbane, Queensland, Australia

² Dept. Respiratory and Sleep Medicine, The Mater Children's Hospital, South Brisbane, Queensland, Australia

³ Australasian Paediatric Endocrine Group, Mater Medical Research Institute, Brisbane, Queensland, Australia

ONLINE MATERIAL 1:

1.1 Loss to follow-up in prospective cohort study:

Figure S1 shows a flowchart which documents the loss to follow-up in the prospective cohort follow-up study. In summary, 34 children were enrolled, and 31, 25, 27, 26 and 20 participants completed studies at 2 weeks, 3, 6, 12 and 24 months respectively. One study at 2 weeks of age was discarded from analysis because less than 60 minutes of artefact free SpO₂ data was available.

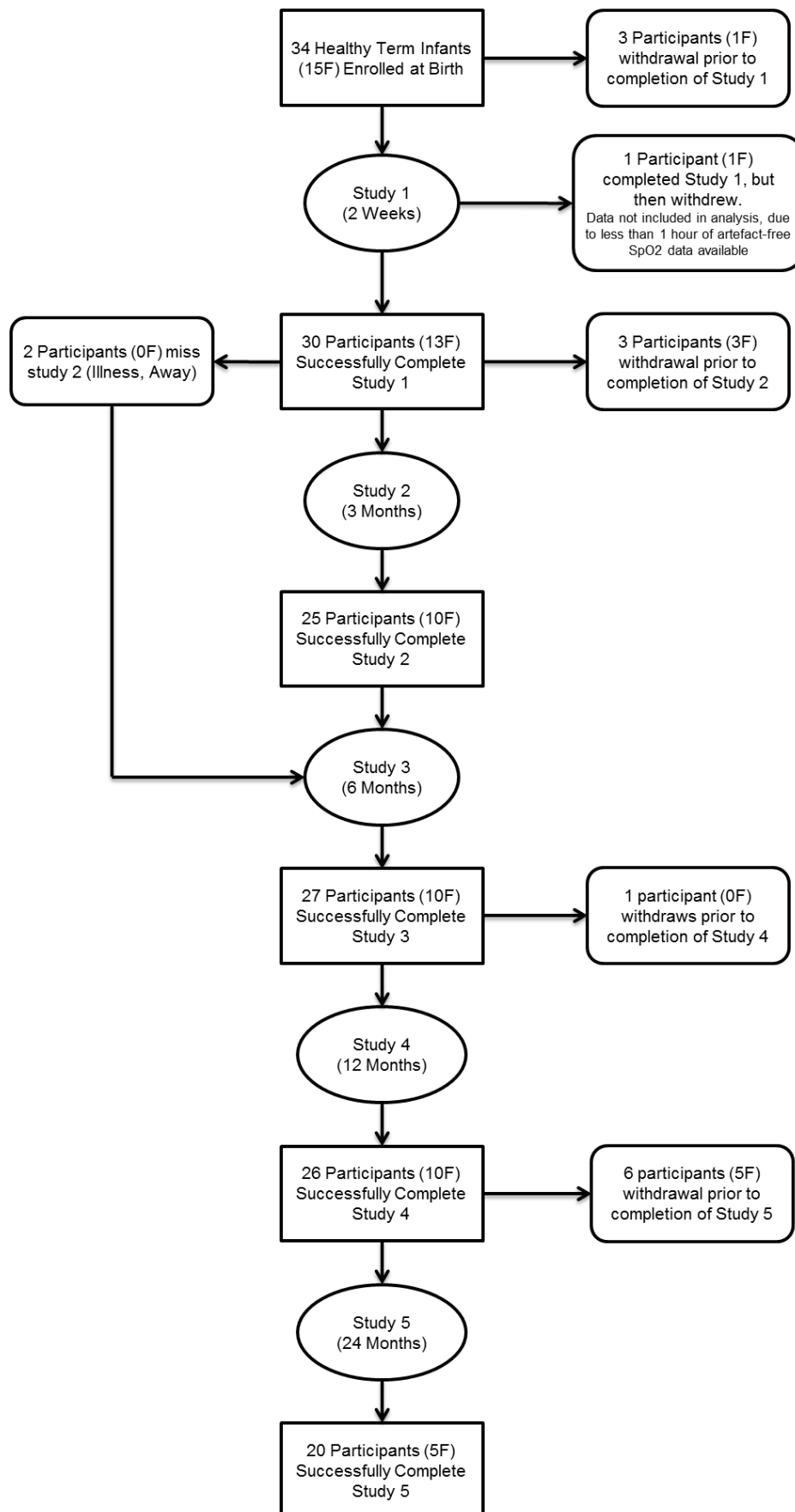


Figure S1: Flow-chart documenting loss to follow-up in the prospective cohort study. Many of the study withdrawals were prior to the completion of the first 2 studies, due to the difficulty undertaking full overnight polysomnograms so close to birth, and the attendant adjustment difficulties for families.

1.2 Impact of Manual Artefact Removal on SpO2 Summary Statistics:

Supplementary Table S.I displays the summary statistics of the SpO2 profiles for the infant population with manual artefact rejection. Median and range across the infant population are presented for SpO2 recording duration, artefact free SpO2 recording duration and the SpO2 metrics: SAT5 (5th percentile value across the recording); SAT10 (10th percentile value across the recording); median value, and mean value. Key trends associated with maturation, and sleep state stratification do not vary from those presented in Table I of the main paper. Relative to data with no manual artefact rejection, analysis with manual artefact rejection did not change the population median by >2% for any summary statistics for pooled sleep-states or in REM or non-REM, but did result in an decrease of median SAT5 at 2 weeks, 3 and 12 months ($P < 0.01$ with Wilcoxon-Sign-Rank Test) and a notable increase in the lower bound of the range for SAT5 and SAT10 (up to 23%) during wake. A tabulated version of these results, and those contained in Table I of the main document is available in the online material file “SpO2_Summary_Statistics.xlsx”.

Table S.1 – Summary of statistics of nocturnal SpO2 recording calculated with manual artefact removal

Summary Statistic	All	non-REM/QS	REM/AS	Wake
2 Weeks (N=30, 13 Female)				
Age (days)		14±3		
Duration (mins)	549.6 (396-1024)	153.9 (114-544)	203.8 (93-270)	156.0 (38-357)
Artefact Free Duration (mins)	489.4 (334-629)	150.2 (112-219)	191.6 (92-258)	108.3 (7-222)
SAT5 (%Hb)	95.0 (90-97)	96.0 (85-98)	95.0 (88-97)	95.0 (75-99)
SAT10 (%Hb)	96.0 (93-98)	97.0 (93-98)	96.0 (92-98)	96.0 (79-99)
Median SpO2 (%Hb)	98.0 (95-100)	98.0 (95-100)	97.5 (95-100)	98.5 (96-100)
Mean SpO2 (%Hb)	97.8 (95-99)	97.9 (95-99)	97.3 (95-99)	97.9 (94-100)
3 Months (N=25, 10 Female)				
Age (days)		97±9		
Duration (mins)	534.5 (411-660)	249.5 (151-327)	189.5 (86-302)	75.5 (8-313)
Artefact Free Duration (mins)	483.1 (351-620)	248.9 (149-327)	188.2 (72-301)	46.9 (7-137)
SAT5 (%Hb)	97.0 (94-99)	98.0 (94-100)	98.0 (93-99)	97.0 (91-99)
SAT10 (%Hb)	98.0 (95-99)	98.0 (95-100)	98.0 (94-99)	98.0 (94-100)
Median SpO2 (%Hb)	99.0 (97-100)	99.0 (97-100)	99.0 (97-100)	99.0 (98-100)
Mean SpO2 (%Hb)	98.9 (97-100)	98.8 (97-100)	98.9 (97-100)	99.1 (98-100)
6 Months (N=27, 10 Female)				
Age (days)		198±6		
Duration (mins)	498.6 (367-680)	270.6 (160-421)	147.0 (88-229)	91.5 (20-214)
Artefact Free Duration (mins)	456.9 (350-610)	259.1 (159-396)	139.7 (83-226)	60.3 (14-161)
SAT5 (%Hb)	97.0 (95-98)	97.0 (95-99)	97.0 (95-99)	97.0 (90-99)
SAT10 (%Hb)	97.0 (96-99)	98.0 (96-99)	98.0 (96-99)	98.0 (94-100)
Median SpO2 (%Hb)	99.0 (97-100)	98.0 (97-100)	99.0 (97-100)	99.0 (98-100)
Mean SpO2 (%Hb)	98.5 (97-100)	98.4 (97-100)	98.5 (97-100)	98.6 (97-99)
12 Months (N=26, 10 Female)				
Age (days)		371±14		
Duration (mins)	499.0 (259-744)	272.3 (131-532)	128.3 (47-216)	72.0 (3-251)
Artefact Free Duration (mins)	439.5 (205-611)	266.6 (118-354)	119.7 (47-214)	41.5 (3-130)
SAT5 (%Hb)	97.0 (95-99)	97.0 (95-99)	97.0 (93-99)	97.0 (83-99)
SAT10 (%Hb)	98.0 (95-99)	98.0 (95-99)	98.0 (94-100)	97.0 (88-99)
Median SpO2 (%Hb)	99.0 (96-100)	98.5 (96-100)	99.0 (97-100)	99.0 (98-100)
Mean SpO2 (%Hb)	98.6 (96-100)	98.5 (96-100)	98.6 (96-100)	98.5 (96-100)
24 Months (N=20, 5 Female)				
Age (days)		725±8		
Duration (mins)	486.3 (323-658)	337.8 (240-512)	112.8 (41-188)	37.5 (0-155)
Artefact Free Duration (mins)	476.8 (216-580)	305.1 (178-383)	103.2 (38-188)	27.3 (0-134)
SAT5 (%Hb)	98.0 (96-99)	98.0 (96-99)	98.0 (95-99)	97.0 (95-100)
SAT10 (%Hb)	98.0 (96-99)	98.0 (96-100)	98.0 (96-99)	98.0 (96-100)
Median SpO2 (%Hb)	99.0 (97-100)	99.0 (97-100)	99.0 (97-100)	99.0 (97-100)
Mean SpO2 (%Hb)	98.8 (97-100)	98.8 (97-100)	98.8 (97-100)	98.7 (97-100)

All summary statistics calculated from SpO2 data with manual artefact removal. Data is presented for the entire recording (All), and stratified according to sleep states non-REM, REM and Wake for children at 3months and older; and Quiet Sleep (QS), Active Sleep (AS) and Wake for children at the neonatal study. SAT5 - 5th percentile SpO2 Value; SAT10 - 10th percentile SpO2 Value. Values are presented as Median (Range) or Mean±SD.

1.3. Impact of Age, Manual Artefact Removal and Sleep State Stratifications on SpO2 Cumulative Distribution Function Nomograms:

The file “Complete_SpO2_CF_ReferenceCurves.xlsx” contains complete tabulated data for SpO2 cumulative frequency (CF) reference-curves at each of the study ages (2 weeks, 3, 6, 12 and 24 months). Reference-curves are presented for CF plots generated from raw data with, and without manual artefact rejection. In addition to results for the entire nocturnal data recordings (“All”), results are also presented for the sleep state stratifications: REM sleep (Active sleep for infants at 2 weeks); non-REM sleep (quiet sleep for infants at 2 weeks); and wake. This tabulated data is presented to allow other researchers to present their own data in this format and to compare with this reference dataset of healthy infants. Microsoft Excel plot functions may be utilised to replicate the style of the nomogram plots presented in Figure 1 and Figure 2 of the main document.

As reported in the main paper, The CF reference-curves shift right by 1% (both with and without manual artefact rejection) between 2weeks and 3 months, where they remain stable up to 24 months. For any given age group, there is no appreciable difference in data generated from the REM and non-REM sleep stratifications, with or without manual artefact rejection. However, it is observed that data generated from wake stratifications show CF reference-curves shifted to the left of those for REM and non-REM sleep states (indicating a greater proportion of the night at lower SpO2 values). Furthermore, the max and 75th percentile nomogram curves in particular are shifted to the left for no manual artefact rejection relative to manual artefact rejection. The relatively low percentage of the total nocturnal recordings spent in wake for most infants (Table I of main paper) manifests in CF reference-curves for entire nocturnal recordings which show no appreciable difference to those for REM or non-REM stratifications with the exception of the max curve, which is shifted to the left by 1-3%. This also explains the observation in Figure 2 of the main document showing that only the lower end of the max curve is appreciably affected by manual artefact rejection.

The higher proportion of wake with lower SpO2 values, and the greater sensitivity to manual artefact rejection may be explained by the likely increase in movement artefacts during wake periods relative to sleep periods. The periods of wake available for analysis are highly variable from individual to individual,

highlighted by the range of analysed wake duration of 3-251 minutes at 12months of ages (Table I). In the method of translating individual CF plots to reference-curves, equal weighting is given to each individual and as such outlier points, particularly manifesting in the max nomogram curve, may arise due to an individual with a disproportionately large proportion of artefact in a relatively short period of wake.

1.4. Additional Summary Statistics

Table S2 presents additional SpO2 summary statistics: percentage of the night below SpO2=92%, and percentage of the night below SpO2=95%. It is noted that values for both statistics are low during REM and non-REM sleep, with values higher during wake. Manual artefact removal reduces values. Indeed, where manual artefact removal has been performed, SpO2 values below 95% occupy less than 1% of the night by three months of age.

Table S.2 – Additional Summary statistics for nocturnal SpO2 recordings

Summary Statistic	All	non-REM/QS	REM/AS	Wake
2 Weeks, no manual artefact removal				
% Time<92%	1.8 (0.2-8.7)	0.1 (0.0-7.2)	0.8 (0.1-9.5)	4.6 (0.2-24.7)
% Time<95%	4.6 (1.0-36.7)	1.0 (0.0-32.3)	3.5 (0.2-38.9)	8.3 (0.3-29.3)
2 Weeks, manual artefact removal				
% Time<92%	1.3 (0.0-6.3)	0.1 (0.0-7.2)	0.6 (0.0-9.4)	2.0 (0.0-20.9)
% Time<95%	3.7 (0.1-35.7)	0.9 (0.0-32.2)	3.1 (0.1-37.7)	4.3 (0.1-25.4)
3 Months, no manual artefact removal				
% Time<92%	1.4 (0.0-5.6)	0.1 (0.0-0.6)	0.1 (0.0-3.2)	10.1 (0.0-39.6)
% Time<95%	2.1 (0.1-8.7)	0.2 (0.0-5.5)	0.5 (0.0-11.5)	12.1 (0.0-43.9)
3 Months, manual artefact removal				
% Time<92%	0.0 (0.0-1.4)	0.0 (0.0-0.5)	0.0 (0.0-3.0)	0.0 (0.0-5.5)
% Time<95%	0.2 (0.0-7.1)	0.0 (0.0-5.4)	0.3 (0.0-11.1)	0.1 (0.0-10.4)
6 Months, no manual artefact removal				
% Time<92%	0.5 (0.0-3.9)	0.0 (0.0-0.5)	0.1 (0.0-2.0)	2.7 (0.0-18.8)
% Time<95%	1.2 (0.1-4.7)	0.1 (0.0-3.4)	0.6 (0.0-2.4)	3.4 (0.0-21.2)
6 Months, manual artefact removal				
% Time<92%	0.1 (0.0-1.3)	0.0 (0.0-0.6)	0.1 (0.0-1.1)	0.0 (0.0-6.1)
% Time<95%	0.2 (0.0-2.4)	0.0 (0.0-3.0)	0.3 (0.0-1.9)	0.3 (0.0-12.1)
12 Months, no manual artefact removal				
% Time<92%	0.3 (0.0-5.1)	0.0 (0.0-3.5)	0.1 (0.0-5.9)	1.8 (0.0-16.9)
% Time<95%	0.6 (0.0-6.3)	0.1 (0.0-3.6)	0.4 (0.0-14.8)	3.1 (0.0-22.0)
12 Months, manual artefact removal				
% Time<92%	0.0 (0.0-2.8)	0.0 (0.0-0.2)	0.1 (0.0-1.7)	0.0 (0.0-16.8)
% Time<95%	0.2 (0.0-4.7)	0.0 (0.0-2.3)	0.2 (0.0-13.5)	0.4 (0.0-21.3)
24 Months, no manual artefact removal				
% Time<92%	0.2 (0.0-3.2)	0.1 (0.0-2.1)	0.0 (0.0-0.6)	0.6 (0.0-20.2)
% Time<95%	0.3 (0.0-3.7)	0.2 (0.0-2.3)	0.2 (0.0-3.8)	1.4 (0.0-21.6)
24 Months, manual artefact removal				
% Time<92%	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.2)	0.0 (0.0-0.4)
% Time<95%	0.1 (0.0-1.2)	0.0 (0.0-0.9)	0.1 (0.0-3.7)	0.0 (0.0-2.8)

Data is presented for the entire recording (All), and stratified according to sleep states non-REM, REM and Wake for children at 3months and older; and Quiet Sleep (QS), Active Sleep (AS) and Wake for children at the neonatal study. Values are presented as Median (Range).

1.5. Application of the SpO2 Cumulative Frequency Reference-Curves to the Clinical Environment:

It is important to critically evaluate the applicability of the CF reference-curve method to a clinical environment where robust sleep staging and manual artefact rejection cannot be performed. These detailed results, as well as the trends observed in Figure 2 of the main document suggest that provided the individual is sleeping throughout most the recording, suggest that sleep staging and manual artefact identification is not necessary. However, where there are significant wake periods, there would be clear benefits to excluding obvious movement artefact from analysis. In a clinical management scenario, an individual's SpO2 CF curve falling entirely to the right of the maximum reference-curve is probably able to be reasonably identified as being within the healthy range. However, an individual who's CF plot entirely, or partially, falls to the left of the maximum nomogram curve will warrant further investigation to identify the explanation for this variation from the healthy reference range – whether this be movement artefact, identification of pathology, or referral for further, more detailed diagnostic tests (i.e. full overnight polysomnogram).

ONLINE MATERIAL 2:

Whilst the SpO₂ CF plots, and the derived CF reference-curves presented here were generated using a customized MATLAB program, SpO₂ CF plots are able to be generated using standard spread-sheet software. In order to facilitate other researchers to present SpO₂ data in the CF plot format, we provide a brief tutorial here on how to generate a CF plot using Microsoft Excel 2010 Spreadsheet software. This tutorial assumes some familiarity with MS Excel. There are many excellent tutorials available online to assist readers which may not be familiar with Excel. The completed spread-sheet file “Generating_CFplot_Excel_Example.xlsx” is available online.

This example implementation is intended for research purposes only, and should not be used itself or in conjunction with the CF reference-curves as a clinical diagnostic tool.

Step 1:

Download, or export the extended SpO₂ recording into a text file format. If the SpO₂ data has been recorded on a Masimo pulse oximeter, you will need software to copy this data to a PC. Software available to do this includes the Masimo Trendcom Download Software, and the independent product “Download 2001” and the more recent version, “Visi-Download” (<http://www.stowood.co.uk/Visi-Download.html>) from Stowood. In a sleep laboratory environment, where nocturnal oximetry is recorded on a commercial polysomnogram system, the oximetry channel is generally able to be exported as a text file. The example below is an extended SpO₂ recording on a Masimo Radical, downloaded using “Download 2001” version 2.8.0, Stowood.

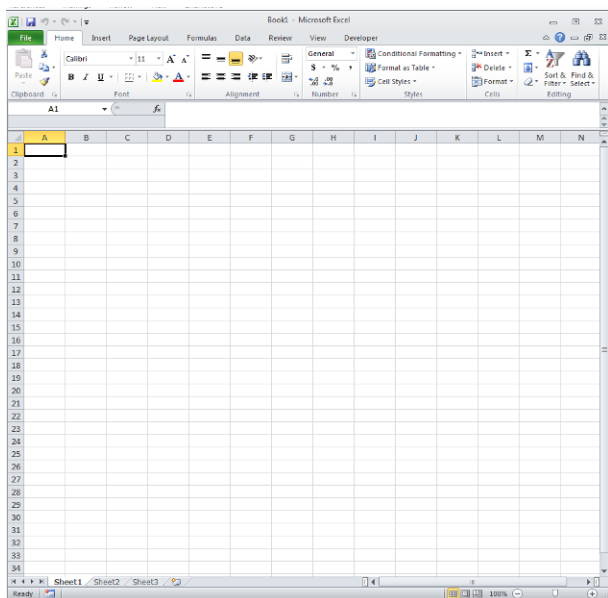
Open the text file containing the SpO₂ recording:

```
SpO2_CDF_Example.txt - Notepad
File Edit Format View Help
Date of Study: 04/12/2008 5:01:53 PM
Chan 1: Saturation
Chan 2: Pulse
17:01:53 0.0000 0.0000
17:01:55 0.0000 0.0000
17:01:57 0.0000 0.0000
17:01:59 0.0000 0.0000
17:02:01 0.0000 0.0000
17:02:03 0.0000 0.0000
17:02:05 0.0000 0.0000
17:02:07 0.0000 0.0000
17:02:09 0.0000 0.0000
17:02:11 0.0000 0.0000
17:02:13 0.0000 0.0000
17:02:15 0.0000 0.0000
17:02:17 0.0000 0.0000
17:02:19 0.0000 0.0000
17:02:21 0.0000 0.0000
17:02:23 0.0000 0.0000
17:02:25 0.0000 0.0000
17:02:27 0.0000 0.0000
17:02:29 0.0000 0.0000
17:02:31 0.0000 0.0000
17:02:33 0.0000 0.0000
17:02:35 0.0000 0.0000
17:02:37 0.0000 0.0000
17:02:39 0.0000 0.0000
17:02:41 0.0000 0.0000
17:02:43 0.0000 0.0000
sampled at every 2 seconds
sampled at every 2 seconds
```

This particular recording contains 3 header rows detailing the format of the data recording, and then 3 data columns – a time-stamp, a column of SpO2 saturation data, and a column of pulse rate data. Samples are provided every 2 seconds (0.5Hz) across the recording.

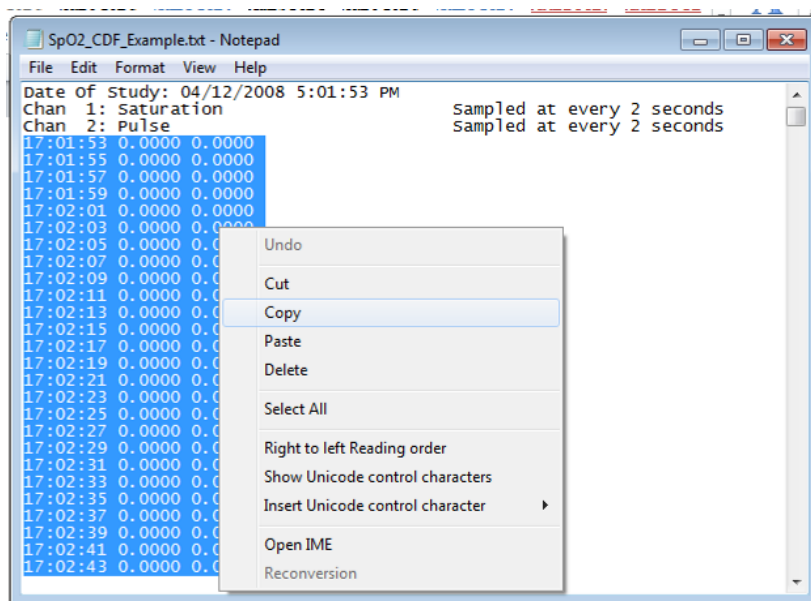
Step 2:

Open a blank workbook in Microsoft Excel 2010:



Step 3:

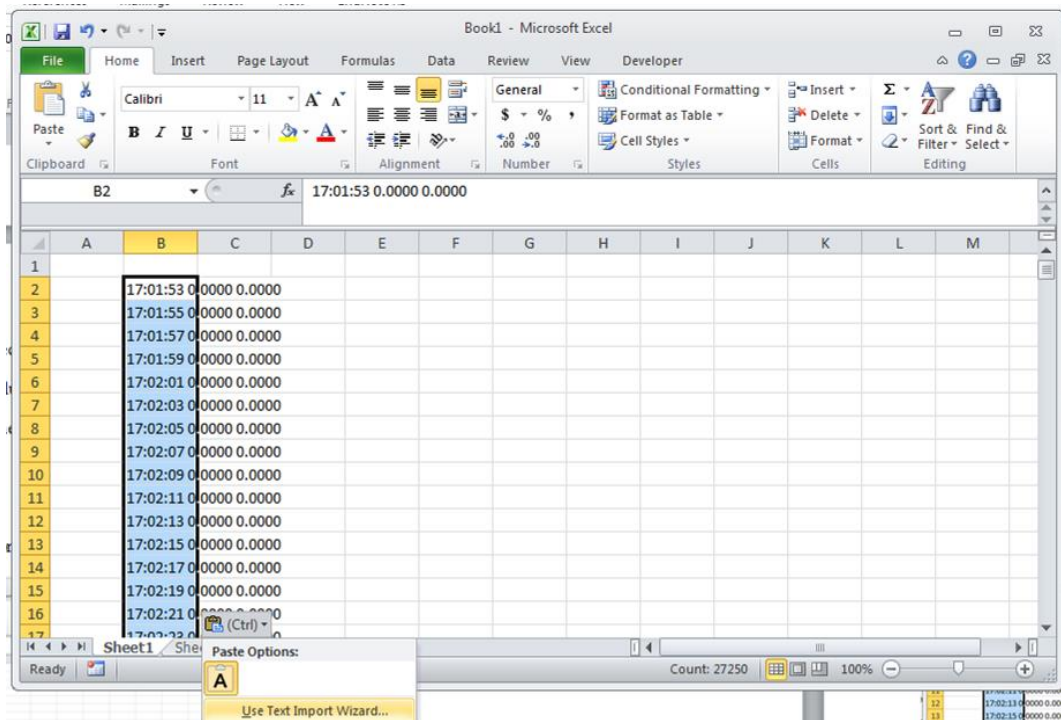
Highlight and Copy the columated data in the text file:



Note, in this example, we have copied from the first row of the columated data (i.e. we have not selected the first three header rows).

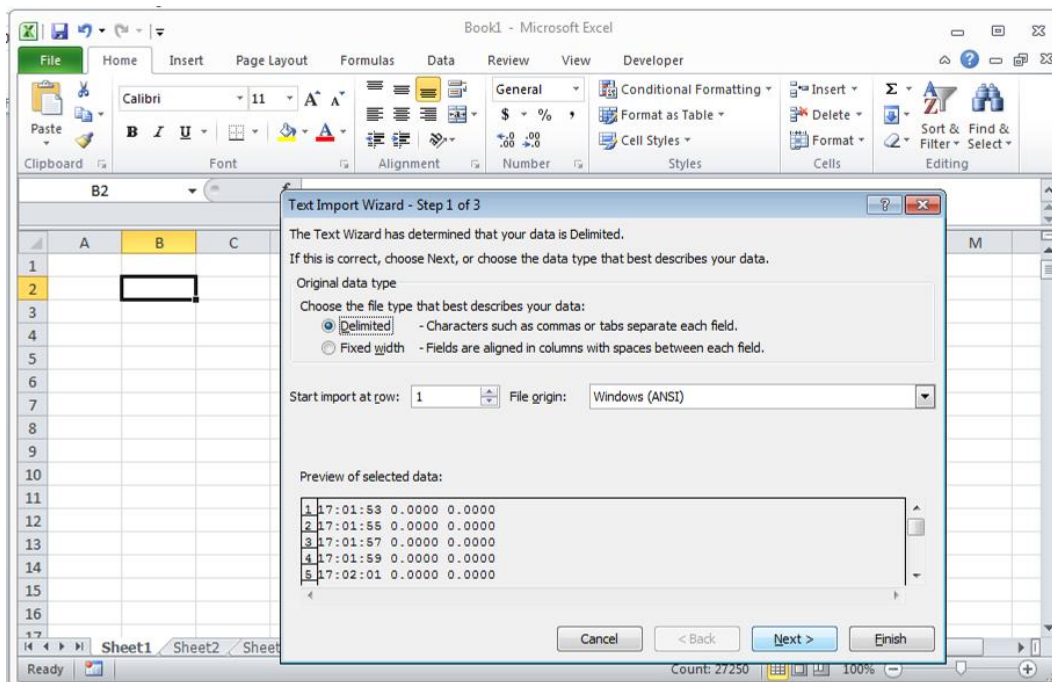
Step 4:

Select a cell in the Excel workbook, and paste the data. The data will appear, but all three columns from the text file will appear in a single column of the spread-sheet. Click the "Ctrl" button on the screen, and select "Use Text Import Wizard":



Step 5:

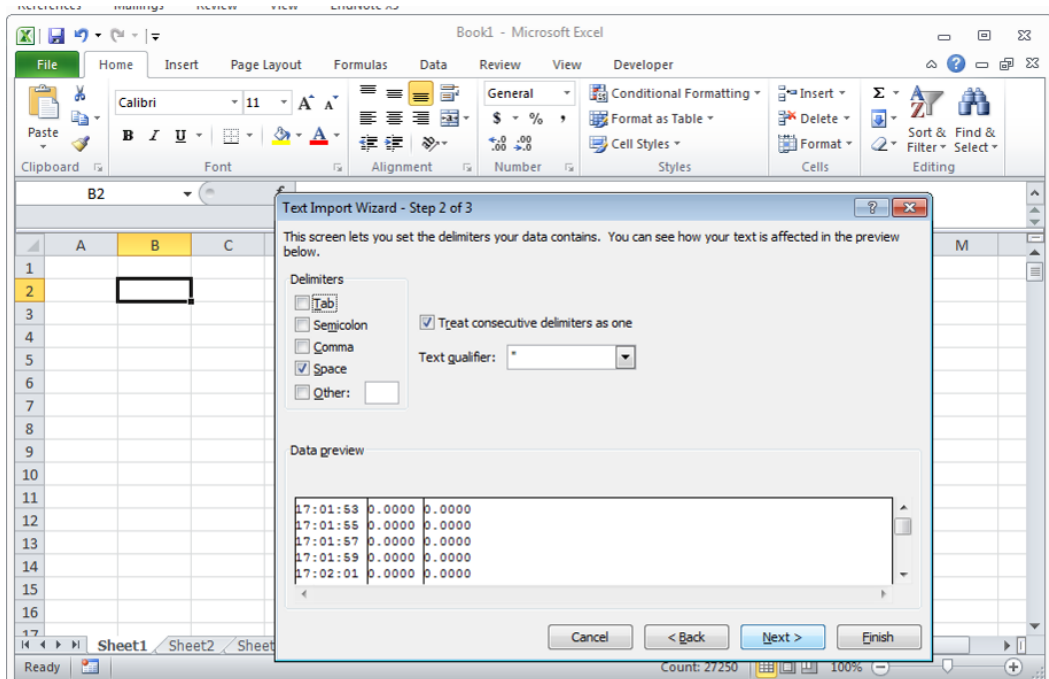
A dialog box will appear. Select “delimited” in “original data type, and then click “next”:



Step 6:

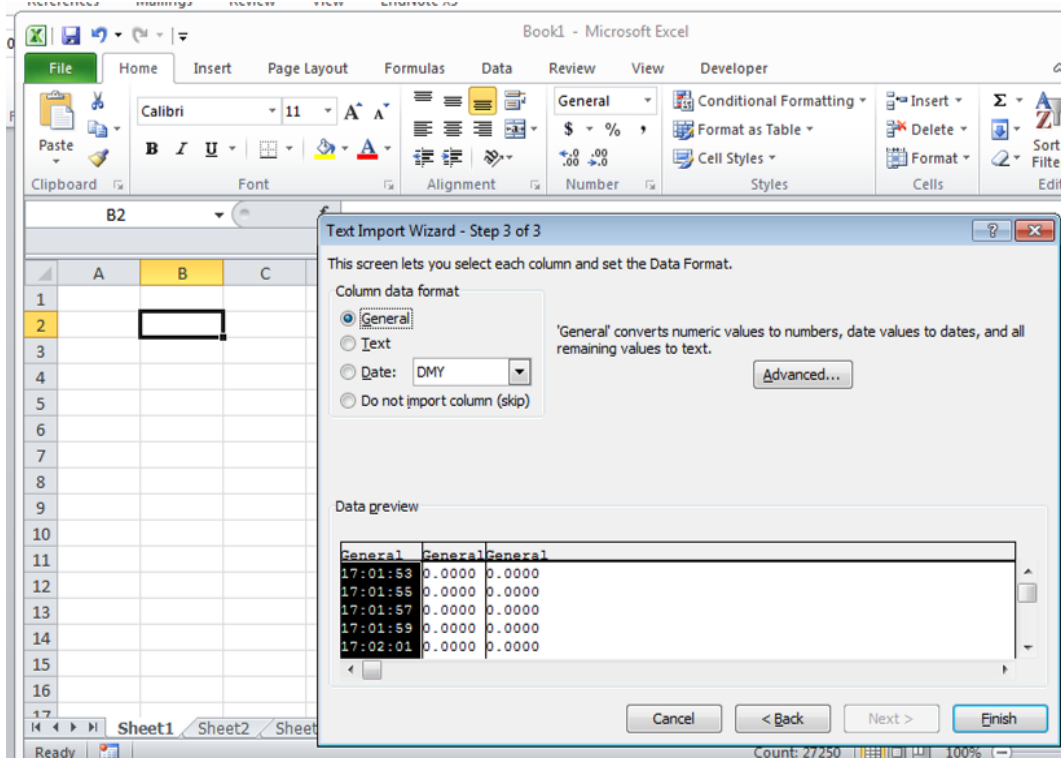
This screen allows you to select a Delimiter character, which tells Excel what defines the separation between the columns of data. The specific character may vary depending on the specific format of the text file (which may vary depending on the download software). In this example, the delimiter is a “Space” (i.e. the character separating the rows is a space). In the data preview section of the dialog box, it can be seen that the vertical lines divide the data correctly into columns.

Once formatted correctly, click “Next”.



Step 7:

The next dialog screen allows the selection of the data format for a particular column. In this case, the default “general” is sufficient. Click “finish”.



Step 8:

The data will now appear in three columns of the spread-sheet. We can now label the columns so that we can keep track of what they are:

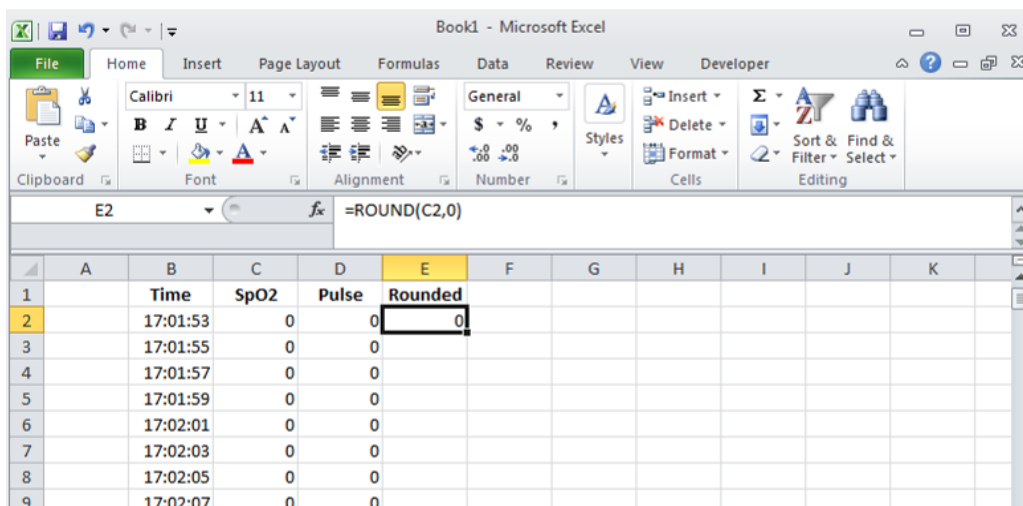
The screenshot shows the Microsoft Excel interface with the following data in the spreadsheet:

	A	B	C	D	E	F	G	H	I	J	K
1		Time	SpO2	Pulse							
2		17:01:53	0	0							
3		17:01:55	0	0							
4		17:01:57	0	0							
5		17:01:59	0	0							
6		17:02:01	0	0							
7		17:02:03	0	0							
8		17:02:05	0	0							
9		17:02:07	0	0							
10		17:02:09	0	0							
11		17:02:11	0	0							
12		17:02:13	0	0							
13		17:02:15	0	0							
14		17:02:17	0	0							
15		17:02:19	0	0							
16		17:02:21	0	0							
17		17:02:23	0	0							

Step 9:

We will now do some simple pre-processing, replicating the process described in the methods section of the paper to exclude artefactual samples. The first step of this process was to round SpO2 values to the nearest integer to account for small rounding errors associated with analogue acquisition at the polysomnogram unit. The data in this example was downloaded directly from the oximeter unit using visi-download, and consequently, does not contain any rounding errors. However, we include this step here for generality.

Select cell E2, and enter: “=ROUND(C2,0)”. This rounds the value in cell C2 to the zero decimal places (i.e. to the nearest whole integer:



	A	B	C	D	E	F	G	H	I	J	K
1		Time	SpO2	Pulse	Rounded						
2		17:01:53	0	0	0						
3		17:01:55	0	0							
4		17:01:57	0	0							
5		17:01:59	0	0							
6		17:02:01	0	0							
7		17:02:03	0	0							
8		17:02:05	0	0							
9		17:02:07	0	0							

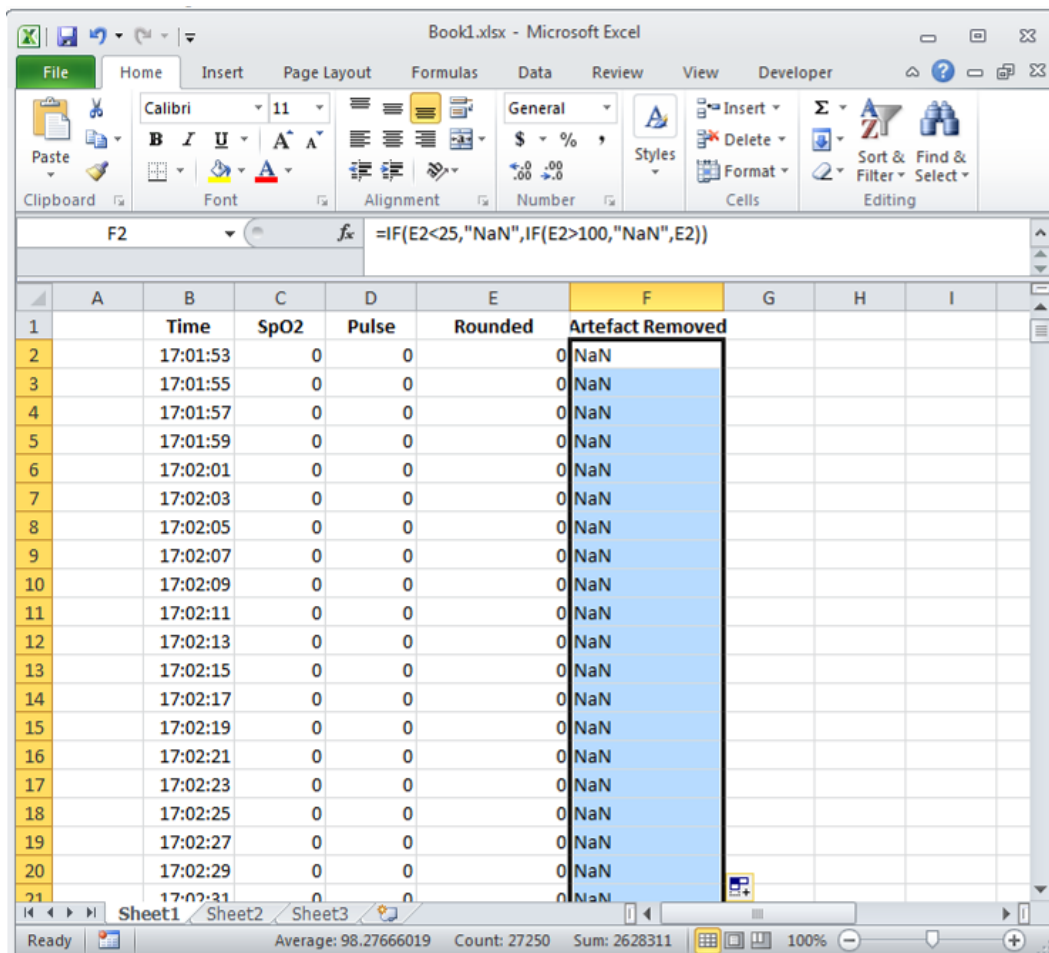
This formula can now be “filled” down column E to apply this rule to all SpO2 samples. This can be done by double dragging the bottom right corner of cell E2 down to the bottom row, or simply double clicking the bottom right hand corner of cell E2.

[illegible]

Step 10:

The next step is to identify, and exclude any artefactual samples from analysis. As described in the methods section of the paper, we apply the rule that SpO2 samples greater than 100, or less than 25 are defined as artefact.

Click on cell F2, and enter: “=IF(E2<25,"NaN",IF(E2>100,"NaN",C2))”. This uses a nested “IF” statement to determine whether the value in cell E2 (the rounded sample) is less than 25, or greater than 100, and if so, enters the text “NaN” (meaning non a number). Otherwise (i.e. the value in cell E2 is a valid sample), it copies the value in cell E2 to cell F2. Fill this formula to the final row of data.

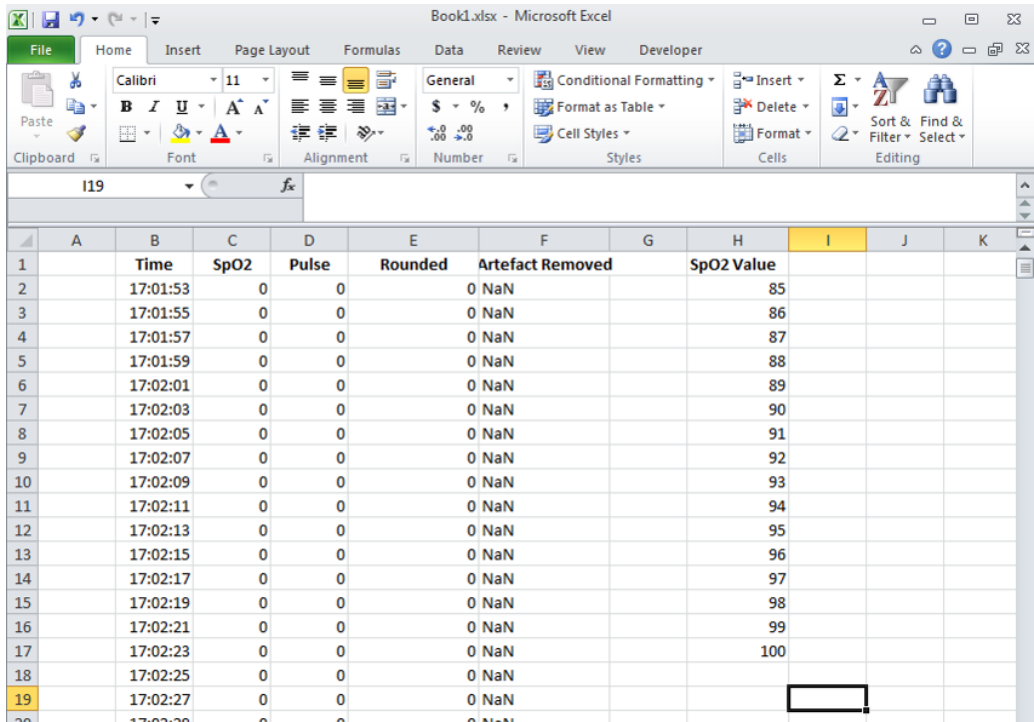


You will observe that values of SpO2 which are equal to zero (indicating that the pulse oximeter was likely not connected to the patient at this stage) have now been marked as NaN's.

<div> <div>Paste</div> <div>Clipboard</div> <div>Font</div> <div>Alignment</div> <div>Number</div> <div>Styles</div> <div>Format</div> <div>Cells</div> <div>Sort & Find & Filter</div> <div>Editing</div> </div>										
F2					=IF(E2<25,"NaN",IF(E2>100,"NaN",E2))					
	A	B	C	D	E	F	G	H	I	
333		17:12:55	0	0	0	NaN				
334		17:12:57	0	0	0	NaN				
335		17:12:59	0	0	0	NaN				
336		17:13:01	0	0	0	NaN				
337		17:13:03	0	0	0	NaN				
338		17:13:05	0	0	0	NaN				
339		17:13:07	98	144	98	98				
340		17:13:09	98	146	98	98				
341		17:13:11	98	148	98	98				
342		17:13:13	94	150	94	94				
343		17:13:15	95	154	95	95				
344		17:13:17	97	153	97	97				

Step 11:

With pre-processing completed, we can now generate the cumulative frequency curve. Start by entering the values 85 through to 100 in a new column:



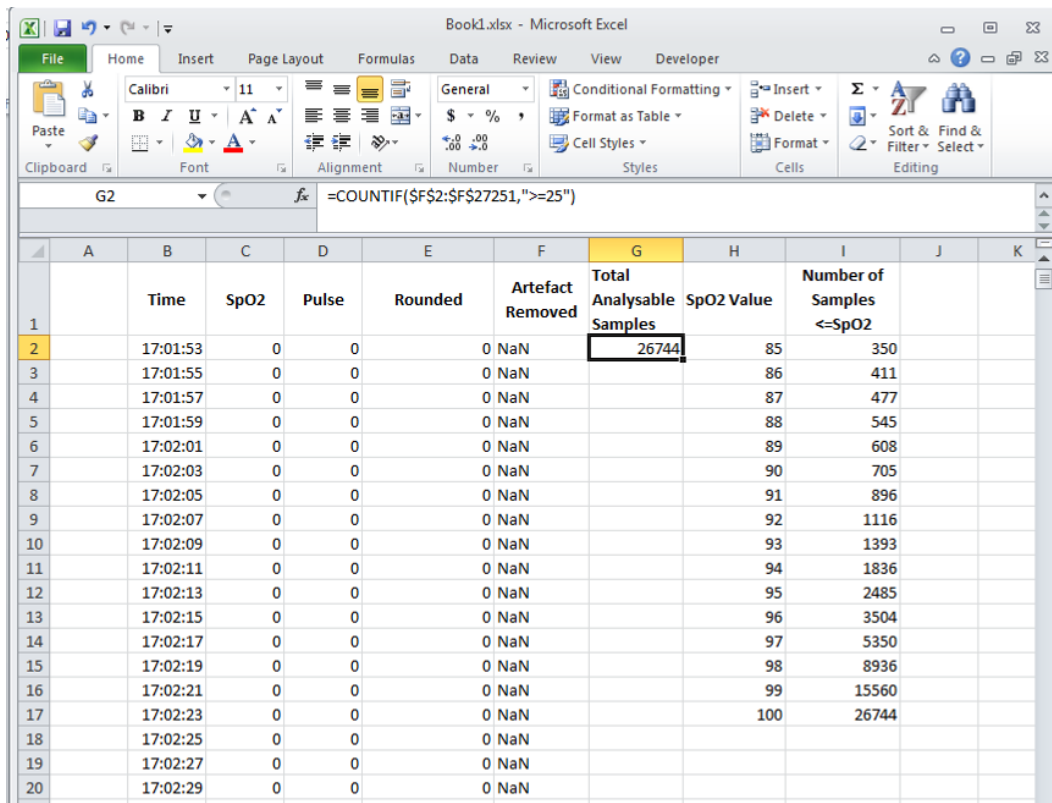
	A	B	C	D	E	F	G	H	I	J	K
1		Time	SpO2	Pulse	Rounded	Artefact Removed		SpO2 Value			
2		17:01:53	0	0	0	NaN		85			
3		17:01:55	0	0	0	NaN		86			
4		17:01:57	0	0	0	NaN		87			
5		17:01:59	0	0	0	NaN		88			
6		17:02:01	0	0	0	NaN		89			
7		17:02:03	0	0	0	NaN		90			
8		17:02:05	0	0	0	NaN		91			
9		17:02:07	0	0	0	NaN		92			
10		17:02:09	0	0	0	NaN		93			
11		17:02:11	0	0	0	NaN		94			
12		17:02:13	0	0	0	NaN		95			
13		17:02:15	0	0	0	NaN		96			
14		17:02:17	0	0	0	NaN		97			
15		17:02:19	0	0	0	NaN		98			
16		17:02:21	0	0	0	NaN		99			
17		17:02:23	0	0	0	NaN		100			
18		17:02:25	0	0	0	NaN					
19		17:02:27	0	0	0	NaN					

Now, for each SpO2 value (85-100), we want to count the number of samples less than, or equal to that value. Select cell I2, and enter the formula: “=COUNTIF(\$F\$2:\$F\$27251,"<="&H2)”. This counts the number of values in the selection F2:F27251 (column F) which are less than or equal to the value entered in cell H2 (SpO2=85%). Fill this formula down column I:

Book1.xlsx - Microsoft Excel										
<div> <div>FileHomeInsertPage LayoutFormulasDataReviewViewDeveloper</div> <div> <div> <div>Paste</div> <div>Clipboard</div> </div> <div> <div>Calibri11</div> <div> <div>B</div> <div>I</div> <div>U</div> <div>A</div> </div> <div>Font</div> </div> <div> <div> <div></div> <div></div> </div> <div> <div></div> <div></div> </div> <div>Alignment</div> </div> <div> <div>General</div> <div> <div>\$ %</div> <div>0.00 0.00</div> </div> <div>Number</div> </div> <div> <div>Conditional Formatting</div> <div>Format as Table</div> <div>Cell Styles</div> </div> <div> <div>Insert</div> <div>Delete</div> <div>Format</div> </div> <div> <div>Σ</div> <div>Sort & Find & Filter & Select</div> </div> <div> <div>Editing</div> </div> </div> </div>										
fx =COUNTIF(\$F\$2:\$F\$27251,"<="&H2)										
	A	B	C	D	E	F	G	H	I	J
		Time	SpO2	Pulse	Rounded	Artefact Removed		SpO2 Value	Number of Samples <=SpO2	
1										
2		17:01:53	0	0	0	NaN		85	350	
3		17:01:55	0	0	0	NaN		86	411	
4		17:01:57	0	0	0	NaN		87	477	
5		17:01:59	0	0	0	NaN		88	545	
6		17:02:01	0	0	0	NaN		89	608	
7		17:02:03	0	0	0	NaN		90	705	
8		17:02:05	0	0	0	NaN		91	896	
9		17:02:07	0	0	0	NaN		92	1116	
10		17:02:09	0	0	0	NaN		93	1393	
11		17:02:11	0	0	0	NaN		94	1836	
12		17:02:13	0	0	0	NaN		95	2485	
13		17:02:15	0	0	0	NaN		96	3504	
14		17:02:17	0	0	0	NaN		97	5350	
15		17:02:19	0	0	0	NaN		98	8936	
16		17:02:21	0	0	0	NaN		99	15560	
17		17:02:23	0	0	0	NaN		100	26744	
18		17:02:25	0	0	0	NaN				
19		17:02:27	0	0	0	NaN				
20		17:02:29	0	0	0	NaN				

Step 12:

Recalling that the cumulative frequency curve is defined as the proportion of the night spent below each SpO2 value, we now need to scale the number of samples below each SpO2 value by the total number of analysable samples. To determine the total number of analysable samples, we can again use the “COUNTIF” function. Select cell G2 and enter the formula: =COUNTIF(\$F\$2:\$F\$27251,">=25"). This counts all numerical values in column F (i.e. does not count NaN's). This should be equal to the value in cell I17.



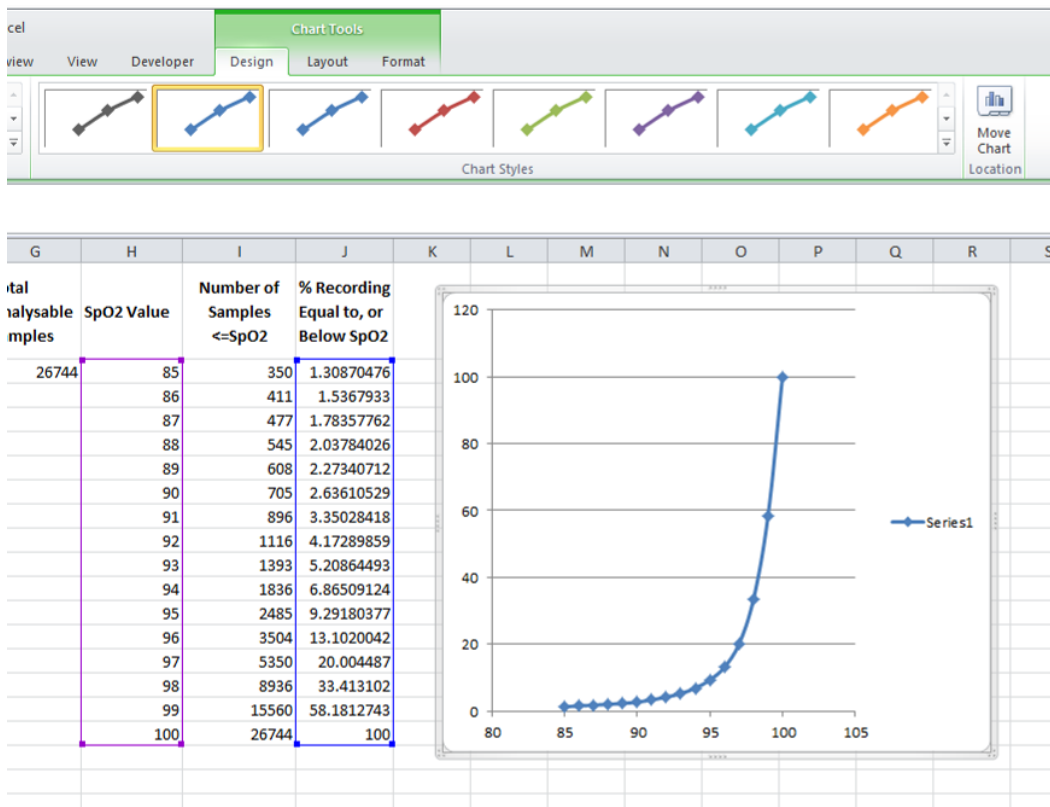
	A	B	C	D	E	F	G	H	I	J	K
		Time	SpO2	Pulse	Rounded	Artefact Removed	Total Analysable Samples	SpO2 Value	Number of Samples <= SpO2		
1											
2		17:01:53	0	0	0	NaN	26744	85	350		
3		17:01:55	0	0	0	NaN		86	411		
4		17:01:57	0	0	0	NaN		87	477		
5		17:01:59	0	0	0	NaN		88	545		
6		17:02:01	0	0	0	NaN		89	608		
7		17:02:03	0	0	0	NaN		90	705		
8		17:02:05	0	0	0	NaN		91	896		
9		17:02:07	0	0	0	NaN		92	1116		
10		17:02:09	0	0	0	NaN		93	1393		
11		17:02:11	0	0	0	NaN		94	1836		
12		17:02:13	0	0	0	NaN		95	2485		
13		17:02:15	0	0	0	NaN		96	3504		
14		17:02:17	0	0	0	NaN		97	5350		
15		17:02:19	0	0	0	NaN		98	8936		
16		17:02:21	0	0	0	NaN		99	15560		
17		17:02:23	0	0	0	NaN		100	26744		
18		17:02:25	0	0	0	NaN					
19		17:02:27	0	0	0	NaN					
20		17:02:29	0	0	0	NaN					

The values in column I can now be scaled by the total number of samples. In Cell J2 enter the formular:

“=100*I2/\$G\$2”, and down column J:

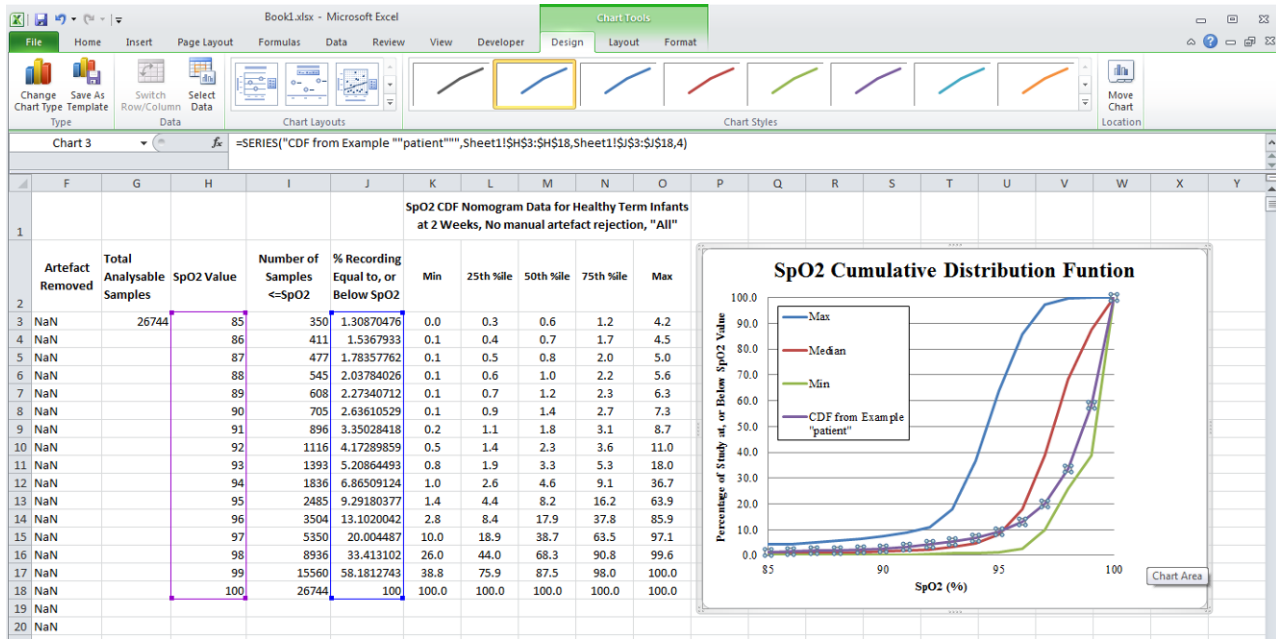
Book1.xlsx - Microsoft Excel										
File Home Insert Page Layout Formulas Data Review View Developer										
Clipboard Font Alignment Number Styles Cells Editing										
J2 =100*I2/\$G\$2										
	A	B	C	D	E	F	G	H	I	J
		Time	SpO2	Pulse	Rounded	Artefact Removed	Total Analysable Samples	SpO2 Value	Number of Samples <=SpO2	% Recording Equal to, or Below SpO2
1										
2		17:01:53	0	0	0	NaN	26744	85	350	1.30870476
3		17:01:55	0	0	0	NaN		86	411	1.5367933
4		17:01:57	0	0	0	NaN		87	477	1.78357762
5		17:01:59	0	0	0	NaN		88	545	2.03784026
6		17:02:01	0	0	0	NaN		89	608	2.27340712
7		17:02:03	0	0	0	NaN		90	705	2.63610529
8		17:02:05	0	0	0	NaN		91	896	3.35028418
9		17:02:07	0	0	0	NaN		92	1116	4.17289859
10		17:02:09	0	0	0	NaN		93	1393	5.20864493
11		17:02:11	0	0	0	NaN		94	1836	6.86509124
12		17:02:13	0	0	0	NaN		95	2485	9.29180377
13		17:02:15	0	0	0	NaN		96	3504	13.1020042
14		17:02:17	0	0	0	NaN		97	5350	20.004487
15		17:02:19	0	0	0	NaN		98	8936	33.413102
16		17:02:21	0	0	0	NaN		99	15560	58.1812743
17		17:02:23	0	0	0	NaN		100	26744	100
18		17:02:25	0	0	0	NaN				
19		17:02:27	0	0	0	NaN				
20		17:02:29	0	0	0	NaN				
21		17:02:31	0	0	0	NaN				

The plot of column H vs. Column J will now give us our SpO2 cumulative frequency curve. Using the “Scatter Plot”:



Step 13:

In isolation, with no reference data for comparison, it is difficult to ascertain whether this cumulative frequency curve matches the normal healthy range. We can copy the reference nomogram data from the table described in Online Material 2 (Complete_SpO2_CF_ReferenceCurves.xlsx) – in this case, reference CF reference-curves for infants at 2 weeks, for the entire nocturnal recording, without manual artefact rejection.



We can now observe that the SpO2 CF curve of this particular individual fits safely within the range expected in health infants at 2 weeks of age.