Original Article

Validity and correspondence of non-invasively determined hemoglobin concentrations by two trans-cutaneous digital measuring devices

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Hemoglobin (Hb) concentration is the central diagnostic indicator for anemia, including nutritional anemia. The objective of this study was to compare the Hb values determined by two portable, non-invasive devices across a wide Hb spectrum against formal laboratory measurements, and with each other. Eighty Guatemalan adults (40 highland men, 40 lowland pregnant women) provided venous blood for formal Hb colorimetric determination. Hb was also registered sequentially on the Rad-87[TM] pulse CO-Oximeter with Rainbow Set technology (Masimo) and Haemospect® (MBR Optical Systems) by non-invasive skin-probe contact procedures as per manufacturers' instructions. Whole blood Hb concentrations ranged from 7.8 to 18.5 g/dL (mean, 12.9±2.3 g/dL and median, 13.3 g/dL). Corresponding descriptive statistics were: range, 9.6 to 16.2 g/dL; mean, 12.1±1.5 g/dL; and median, 11.9 g/L, respectively, with the Rad-87[TM] (nail bed). They were: range, 8.7 to 15.8 g/dL; mean, 12.7±1.8 g/dL; and median, 13.0 g/dL for the Hemospect® for forearm contact. They were: range, 9.1 to 17.5 g/dL; mean, 13.2±2.1 g/dL; and median, 13.4 g/dL for palm contact. The Pearson correlation coefficient of venous blood Hb with the former device's Hb values was r=0.59 (*p*<0.001), and r=0.94 (*p*<0.001) and r=0.90 (*p*<0.001) with those of the latter device at the palm and forearm, respectively. The inter-site Lin coefficient was r=0.84. Sensitivity and specificity were variable across devices, depending on Hb cut-off and measurement procedures. With Hb cut-off values of <12.0 g/dL for adult, non-pregnant women and <13.0 g/dL for adult men, the Haemospect® device's performance here would provide adequate potential for screening purposes.

Key Words: hemoglobin, anemia, non-invasive technology, diagnostic screening, Guatemala

INTRODUCTION
The quest to target oral iron prophylaxis within iron-deficient individuals instead of the population-wide universal supplementation originally proposed as an international norm, has recently intensified.[1] This comes in the wake of the adverse consequences, including excess mortality among iron-replete children receiving routine iron and folic acid supplementation, on the Zanzibari island of Pemba; an area with severe transmission for *Falciparum* malaria.[2] These observations combine with the findings of Lind *et al* [3] in Indonesia, that iron supplementation adversely affects growth in children with an initial state of iron repletion, and are further corroborated by prior observations of parallel effects cited by Idjradinata in Indonesia,[4] Majumdar in India [5] and Dewey in Sweden and Honduras.[6] The notion of rapid individual screening within a population to identify individuals without anemia, and if possible, those specifically without iron deficiency anemia (IDA), in order to exempt them from iron supplementation has gained adherents [1,7] and, for malarial areas, has been codified in the Lyon Consultancy recommendations of the WHO, published in response to the experience in Pemba.[8-10]

Any such screening strategy, however, requires a series of fundamental elements. It must be accurate to detect subnormal and normal hematological status at the *individual* level, while being rapid, cost-effective and culturally-acceptable. Experience with portable field instruments for Hb determination with a relatively modest cost-per-test and reasonable diagnostic sensitivity and specificity has been reported.[11,12] Such approaches, however, involve the sampling of capillary blood.

This is painful and risks rejection in certain cultural settings, while running a finite risk of spreading blood-born virus infections if adequate precautions in handling are not strictly applied. A series of non-invasive (blood-free) approaches using contact probes to the skin, nail bed,

or mucosal surfaces have been reported over the last two decades,[13-16] but only in experimental or hospital-based settings.

We have recently taken the same technology, which Rabe *et al* [16] explored among 85 infants (24–41 wks of age) in a hospital setting, into a simulation of field conditions and challenges that might face its application in populations surveys under tropical conditions.[17] The explicit mandate from the WHO to implement hematological screening as part of any iron supplementation intervention in a malaria area was our guiding inspiration. We have undertaken tandem field-testing of diagnostic reliability in two non-invasive, and presumably culturally-acceptable, Hb-measuring devices in rural areas of western Guatemala. We present here the respective performance findings and inter-device comparisons.

MATERIALS AND METHODS
Study design and subjects
The study completed was a cross-sectional survey of Guatemalan adults to evaluate two non-invasive methods of measuring Hb against the conventional whole blood test. Sampling included pregnant subjects living on the coastal plain and men residing in the high highlands with the aim to include an extreme contrast in altitudes (240 vs. 2600 meters above sea level) to maximize the range of Hb values that would be attained. Diagnostic thresholds for determining anemia were based on WHO age- and sex-specific criteria for individuals at sea level with adequate correction for different altitudes.[10,18]

Eighty subjects from two different regions participated in this study. Forty adult males, ages 18 and older, were recruited from the surrounding areas of San Francisco el Alto, in Guatemalan's highland region of Totonicapán. Members of the Rancho de Teja, Chiutuy II, and Chitokche communities were asked to participate at their respective "centros de convergencia" or local meeting centers. Forty pregnant women were recruited from the lowland region of Retalhuleu. Women attending the health centers of San Sebastián and Retalhuleu proper for routine prenatal consults were considered for participation.

Residents of the community who presented at the local health center or post on the day of data collection were eligible to take part in the study. Exclusion criteria included patients with a history of adverse reaction to venous blood extraction, those with chronic diseases, and pregnant women who had had blood drawn within the previous four weeks.

The study was approved by the Human Studies Committee of the Center for Studies of Sensory Impairment, Aging, and Metabolism. Discussion of potential risks, benefits, and confidentiality was included in a written informed consent form given to all participants. All subjects voluntarily consented to participation. Complete hematological test results, including a brief explanation of normal and abnormal Hb results and instructions for follow-up, were also provided to all subjects following data collection and those with abnormal findings were referred to the local health centers for follow-up.

Equipment training by the manufacturers
The principal and exclusive operator of the devices (CC) received orientation and training from technical representatives from Masimo Corporation and MBR Optical Systems in Germany, who were also consulted for technical support from Guatemala as required. The Rad-87[TM], designed by the Masimo Corporation of Irvine, CA, USA, is a portable, bench-mounted device, weighing approximately 908 g. A finger-clip probe is applied to the finger to send light through the nail bed. This resulted in mild sensitivity to ambient light and decreased performance when skin temperature was lower. The device can be directly connected to an AC power source, or operated off battery power. Digital read time took up to 10 minutes, which is problematic with a relatively short battery life when used in repeated field trials.

The Haemeospect® device, of the generation used here, was produced by MBR Optical Systems GmbH & Co KG of Wuppertal, Germany. It is a handheld device, weighing approximately 544 g. A pen-like probe is based on transcutaneous reflection spectroscopy and was used at either the palm of the non-dominant hand, directly below the index finger, or in the center of the corresponding antecubital area. Moderate light sensitivity decreased device performance, and temperature did not appear to have a significant effect on functionality. The Haemeospect® runs exclusively off battery power, which lasts several hours before requiring recharging. Usually digital read time is approximately one minute, although official results could not be recorded until a later time when values had been recalculated by the manufacturer. This was a transient problem, which was solved in the later generations of the device as will be discussed in further detail in the subsequent section.

Procedures
Each subject's Hb was recorded using all three methods: one venous blood test, and non-invasive (probe contact) digital readings from each of the two devices. Using the conventional, invasive method, a local laboratory technician drew 3 mL of blood from the antecubital vein of the non-dominant arm. Samples remained in cold storage until they were delivered to the laboratory in Quetzaltenango the same day, where they were analyzed using the automated HumaCount – Human device [Corporación Analíticos, S. A. Guatemala]. The device was routinely calibrated using the Spectrophotometer Vital Scientific Microlab 300 [Human Gesellschaft für Biochemica und Diagnostica GmbH, Wiesbaden, Germany].

Hemoglobin concentrations were subsequently estimated using two distinct, non-invasive devices, which provided digital readings. The Rad-87[TM] with Rainbow Set technology [Masimo Corporation] was used to measure Hb, by placing a finger clip on the nail bed of the non-dominant ring finger. Two different sized finger-clips were used for each subgroup of participants; an adult clip for males, and a child clip for females. Subjects' hands were covered either with a dark sock (men) or a small, plastic sleeve (women) to reduce possible interference from ambient light. Any excess debris, such as dirt or nail polish, was removed from the nail and nail bed prior to the clip's application. Participants were asked to place their hand on a flat surface and keep it still for the duration of the test. In order to explore the possibility of a

shorter scanning time on the nail bed, Hb readings were also recorded at 3 and 5 minutes, marked from the time that a value for total Hb first appeared on the digital display.

The Haemospect® [MBR Optical Systems GmbH & Co. KG] was used in parallel to the Rad-87 ™. Any excess debris was removed from the skin prior to taking the reading, if necessary. Using the pen-like sensor, gentle contact was made on the palm of the dominant hand, immediately below the index finger. The probe remained in contact until the automated device produced a digital reading. A second measurement was taken on the forearm of the same arm. As the device is programmed to request a repeated measurement if the reading cannot be properly processed by the program, additional measurements often became necessary before a valid output was produced.

Once into trial conditions, we noted that constituent readings were not provided on the first attempt for both instruments, so we made repeat measurements as needed to provide a secure reading. Moreover, early in the field experience it became apparent that the readout on the Hb digital display of the Haemospect®, as calculated by the internally installed software, interpreted the recorded spectra with limited reliability. Therefore, the spectra collected in the field were processed by an iteratively improved software version with a more powerful calculator at the site of the Haemospect® manufacturer, in Wuppertal, Germany. The field-recorded electronic spectra were transmitted to Germany with a single, patient identifier. Whole blood values were not known to the manufacturers during the study to blind the evaluation process; both values were aligned by the field researchers back in Guatemala. Hence, within this paper the term Hb "readings" represents those values appearing on the digital displays of the devices and recorded on site in Guatemala and Hb "registrations" are those values processed from the spectral output remotely by the manufacturer.

### Data handling and statistical analysis

The two trials were conducted simultaneously, and all readings recorded manually at the time of testing and later inputted into Microsoft Excel 2002, Version 10 for Windows [Microsoft Corporation, Redmond, WA, USA]. In addition, all Haemospect® data were directly uploaded to a computer at the end of the examination day using the data transfer software. Subjects were identified using a single patient identifier, which corresponded to complete patient information in a separate database. Results from the laboratory blood test were labeled using only the patient's identifier at time of testing and entered into the same database following analysis.

Descriptive statistics, including arithmetic mean, standard deviation, median, inter-quartile range, and maximum and minimum values, were calculated for each of the three measurement methods using SPSS version 17 [Statistical Package for the Social Sciences, Chicago, IL, USA]. Statistics were based upon the pooled 80 subject sample and subdivided by gender. The Pearson product moment, Spearman Rank Order, and Lin Concordance coefficients were used to determine regression correlations using SPSS version 17 and the NIWA Statistical Calculator.[19,20] World Health Organization diagnostic criteria for anemia were used to generate sensitivity, specificity, and positive and negative predictive values of non-invasive readings versus the reference standard, or whole blood exam. Reference standards were based on the values for sea level, which indicate anemia at <11.0 g/dL for infants and pregnant women, <11.5 g/dL for children under five, <12.0 g/dL for non-pregnant women, and <13.0 g/dL for adult men.[10]

RESULTS
Characteristics of the reference standard (whole blood) values
Figure 1 displays the distribution of Hb values as
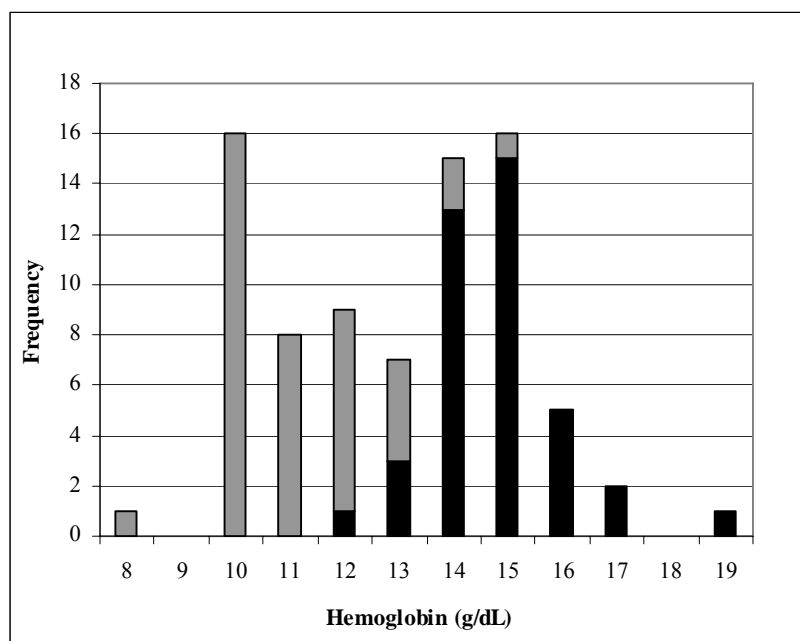
Figure 1. The histogram distribution of hemoglobin values of the complete sample of 80 subjects, as derived from a cutaneous, whole blood sample. Dark gray bars represent pregnant, female subjects, recruited from the coastal plain, while black bars represent adult male subjects, residing in the highland regions.

Table 1. A comparison of means and distributions of hemoglobin values from alternative measurement methods

Hb measurement method	Hemoglobin concentrations (g/dL)						
	mean	SD	min	25%	50%	75%	max
Whole blood (n=80)	12.9	2.3	7.8	10.7	13.3	14.8	18.5
Rad-87^{TM}, nail bed (n=80)	12.1	1.5	9.6	11.1	11.9	13.0	16.2
Haemospect$^®$, palm (n=70)	13.2	2.1	9.1	11.6	13.4	14.9	17.5
Haemospect$^®$, forearm (n=60)	12.7	1.8	8.7	11.2	13.0	14.4	15.8

determined by the conventional, invasive blood test. Gender sub-groups are illustrated among the entire 80 subject sample. The mean whole blood hemoglobin value was 12.9±2.3 g/dL, with a median value of 13.3 g/dL (interquartile range 4.1 g/dL). Using the conventional method, values covered the desired range of extremely anemic to plethoric values; ranging from 7.8 to 18.5 g/dL. The non-invasive devices did not discriminate as wide a range, but were able to produce readings as low as 8.7 and as high as 16.2 g/dL (Table 1).

As expected, male subjects had higher Hb values (12.2–18.5 g/dL), as compared to females (7.8–15.1 g/dL). The mean whole blood value among highland men was 14.7±1.2 g/dL whereas that of coastal, lowland women was 11.1±1.4 g/dL. The median value among female subjects was lower than the WHO reference limit for anemia for first and third semester of pregnancy (10.7 versus 11.0 g/dL, respectively). The median value for men residing in the highlands was higher than the WHO cut-off for adult males (13.0 g/dL versus 14.8 g/dL). It should be noted that, these WHO criteria are valid at sea level for all forms of anemia, whether of nutritional (eg iron-deficiency, etc.) or non-nutritional (eg malaria, hemoglobinopathy, etc.) form.[10]

Digital readings using the Rad-87^{TM} versus the whole blood standard

The Rad-87^{TM} device by Masimo Corporation captured a fairly large range of Hb values (9.6–16.2 g/dL). However, with a mean value of 12.1±1.5 g/dL and median of 11.9 g/dL, most readings tended to fall into a narrower range than those detected by the whole blood exam. A comparison of measurements using the invasive and non-invasive methods can be seen in Table 1. The Pearson correlation was r=0.59 between readings after 10 minutes with the Rad-87^{TM} and the whole blood (invasive) method, demonstrating a moderate, yet positive, direct relationship (Figure 2, panel A). With respect to the 40 readings from females, the proxy group for the low range of Hb concentration, and the 40 readings from highland males, the proxy for the high range, the respective Pearson correlations fell to r=0.30 and r=0.67, respectively.

At the lower cut-off values for discerning anemia, the Rad-87^{TM} was somewhat sensitive, but considerably improved at the 12.0 and 13.0 g/dL cut-off thresholds. Though sensitivity was relatively high at the aforementioned thresholds (90% and 92%, respectively), diagnostic discrimination at the extremely plethoric end of the Hb spectrum did not fair as well. Specificity, however, improved drastically to 99% and 100% for values above 16.0 and 17.0 g/dL, respectively. At the 11.0 g/dL cut-off criteria, specificity was also fairly high (86% across all subjects), but decreased as the thresholds approached 13.0

g/dL. Sensitivity, specificity, positive and negative predictive values of all subjects and subdivided by gender, as a proxy for the higher and lower range of Hb concentration, are outlined in Table 2. Among female subjects only, sensitivity and specificity were both highest at 13.0 g/dL (92% and 100%, respectively). Whereas sensitivity was 0% within only male subjects when evaluating plethoric values, specificity was extremely high (97% and 100% at 16.0 and 17.0 g/dL, respectively).

Repeated measures protocol with the Rad-87^{TM}

Given the long (10-min) period of recording over the nail bed required for a Rad-87^{TM} reading, we performed recordings at 3- and 5-min into the scanning in all 80 subjects. As compared with the 10-min reading as the reference value, the Lin Concordance coefficients for the 5-min reading were r=0.89 and for the 3-min reading, r=0.84. Despite the high concordance, when values at the shorter recording intervals were reflected into sensitivity and specificity parameters, there was a slight reduction compared with the 10-min values in Table 2, (data not shown).

Digital readings using the Haemospect$^®$ versus the whole blood standard

The Haemospect$^®$ was able to attain viable registrations from only 70 of the 80 subjects sampled (38 men, 32 women) on the palm, and from 60 sampled (31 men, 29 women) on the forearm. The registration data provided were for Hb values that did not capture the entire range of concentrations in the total sample as shown in Figure 1. Rather, for the 70 available palm registrations from the 80 original Haemospect$^®$ readings, the venous blood Hb references values ranged from 9.1–17.5 g/dL, with a mean of 13.2±2.1 g/dL and a median of 13.4 g/dL. Among the 60 available first-pass forearm registrations available from the 80 readings taken, the corresponding descriptive statistics were: 8.7–15.8 g/dL, 12.7±1.8 g/dL and 13.0 g/dL. Despite the discordances for both sites at the extremes of the reference Hb distribution, values were extremely close to the central tendency for whole blood Hb values (Table 1). This is further reflected in the strong, positive Pearson correlations found between the Haemospect$^®$ and venous blood test Hb values of r=0.94 for palm r=0.90 for forearm (Figure 2, panels B and C). Disaggregated by gender subgroups, the respective correlation coefficients were reduced in values, again with the females in the low range having the lower r values (data not shown). Finally, a Pearson coefficient was r=0.86 from the 55 registrations for the palm and forearm site in the same subjects (Figure 3, panel A). The Lin Concordance coefficient was r=0.84 for the same association (data not shown).

Table 2. Sensitivity, specificity and predictive accuracy of digital readings of hemoglobin concentration at different diagnostic cut-off criteria for anemia classification

cut-off values g/dL	Rad-87[TM], nail bed								Haemospect[®], palm								Haemospect[®], forearm							
	n=80				n=40				n=70				n=70[†,‡]				n=60				n=70[†,‡],			
	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV
<11.0[†]	50	86	58	82	50	78	73	56	67	98	92	89	67	93	92	68	58	96	78	90	58	88	78	75
<11.5[†]	72	78	60	86	72	67	78	59	67	100	100	88	67	100	100	61	93	93	82	98	93	79	82	92
<12.0[†]	90	69	62	92	90	64	87	70	88	98	96	94	88	86	96	67	100	92	88	100	100	92	88	100
<13.0[†]	92	44	61	86	92	100	100	50	97	100	100	93	93	100	100	50	97	97	97	97	96	100	100	50
>16.0[‡]	0	99	0	94	0	97	0	87	60	95	50	97	60	91	50	94	0	100	nc	97	0	100	nc	97
>17.0[‡]	0	100	nc	98	0	100	nc	95	100	96	100	96	100	100	100	100	nc	100	nc	100	nc	100	nc	100

SEN = sensitivity, SPE = specificity, PPV = positive predictive value, NPV = negative predictive value
[†] Female subpopulations of n=40 (Rad-87[TM] – nail bed), n=32 (Haemospect[®], palm), and n=29 (Haemospect[®], forearm) are used to calculate values in right-hand columns.
[‡] Male subpopulations of n=40 (Rad-87[TM] – nail bed), n=38 (Haemospect[®], palm), and n=31 (Haemospect[®], forearm) are used to calculate values in right-hand columns.
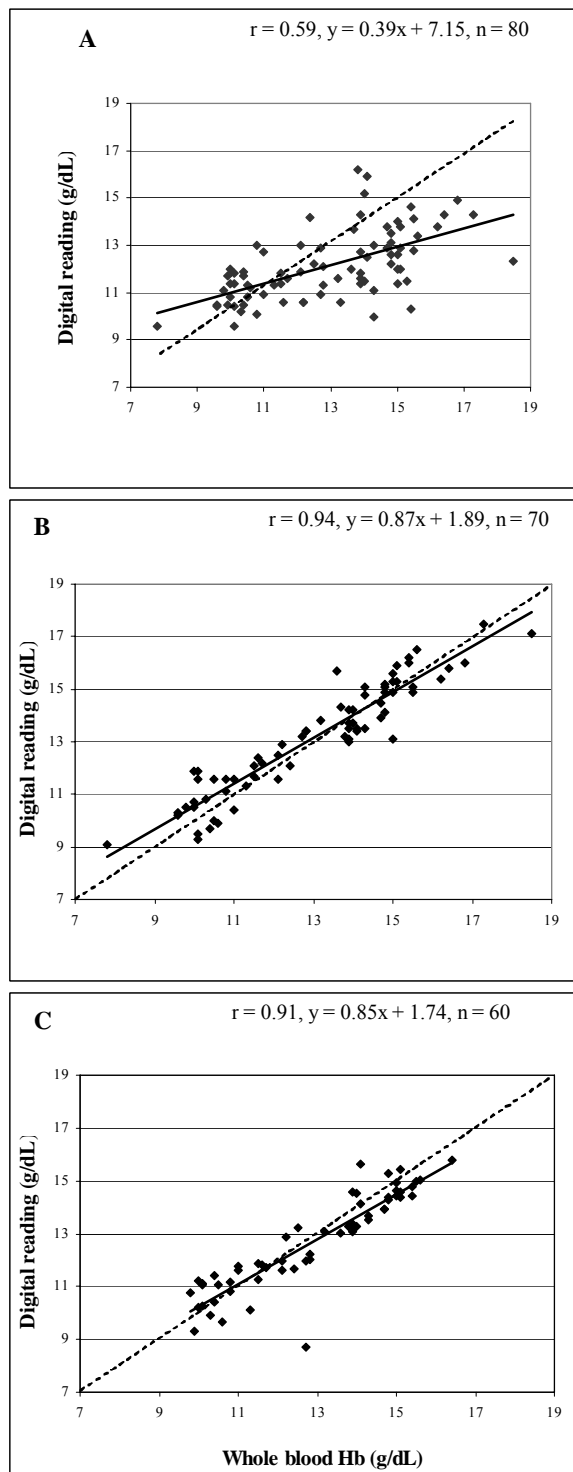
Figure 2. Scatterplots representing paired values of digital outputs and whole blood measurements of Hb in g/dL. The solid line represents the best-fit trend line for each diagram, while the discontinuous, diagonal line represents the 45° line of identity. Panel A displays paired values from all 80 subjects of readings taken using the Rad-87™ and the invasive blood test. The Pearson correlation coefficient is r=0.59. Panel B displays paired values from the 70 subjects who produced a valid palm registration with the Haemospect®. The Pearson correlation coefficient is r=0.94. Panel C displays paired values of the 60 subjects who produced a valid forearm registration with the Haemospect®. The Pearson correlation coefficient is r=0.91.

As shown in Table 2, the Haemospect® performed very well among diagnostic indicators at the mid-range cut-off

values of 11.5, 12.0, and 13.0 g/dL, both at the palm of the hand contact site and on the forearm. For the palm site, the sensitivity was 67% at the two lowest reference cut-offs, rising to 88% at the sea-level criterion for anemia in women (12.0 g/dL) and to 97% for the cut-off for men (13.0 g/dL). Specificities were all above 90%. With application of the probe to the forearm, specificity was 96% at the lowest threshold of 11.0 g/dL, but inferior to the palm values at the higher cut-offs, with specificities all remaining above 93%. Lack of extremely high Hb values made predictive accuracy difficult to determine in this range, but as with the Rad-87™, specificity was high for values above 16.0 and 17.0 g/dL (100%) and sensitivity 0% or indeterminable. Within the female subpopulations, sensitivity values reflected the numerical values for the entire 60 subject sample, and specificity fell off only slightly reduced at the lowest cut-off values.

Repeated measures observations with the Haemospect®
The internal controls of the Haemospect® apparatus signaled, on the first forearm readings (F1), the need for taking a second (F2) reading on a subset of 66 (83%) the applications at that site. A total of 39 of these second forearm readings (F2) became available registrations for analysis. The Lin Concordance coefficient for the paired forearm registrations was r=0.93. The Pearson correlation for this subset of data with the whole blood Hb value was r=0.94 (data not shown graphically).

Correspondence of outputs of Rad-87™ vs Haemospect®
Although neither device was a gold standard measure of Hb in this design, for completeness, we calculated the cross-device correlation coefficients series for paired data available from both machines. The respective Pearson and Lin Concordance coefficients' values for Rad-87™ nail bed readings in the same 70 subjects in whom we had corresponding Haemospect® palm registrations were r=0.55 and r=0.32, respectively (Figure 3, panel B). The respective coefficients for Rad-87™ nail bed readings in the 60 subjects, using the first (F1) forearm registration were r=0.40 and r=0.37 (Figure 3, panel C).

DISCUSSION
As outlined above, the motivation for this study was to bring more options to the field screening for anemia in at-risk populations in which issues of cultural acceptance, cost, and safety would limit even capillary blood extraction as a viable option. In this study, both classes of non-invasive Hb recording devices exhibited acceptable technical performance under field conditions, insofar as they booted up on all occasions and provided the expected digital reading. Although capable of battery operation, electricity was available in all study sites, and the Rad-87™ was mainly operated while charging with AC current. The Haemospect® was used exclusively on battery power. Such portable, painless field devices are capable of serving as effective alternatives to repeated blood extractions in a large-scale, field setting with periodic population surveys or monitoring the response to an anti-anemia intervention.

The Haemospect®, in particular, provided an almost instantaneous result upon contact at the non-pigmented

A   r = 0.86, y = 0.98x + 0.47, n = 55

B   r = 0.55, y = 0.39x + 7.08, n = 70

C   r = 0.40, y = 0.31x + 8.19, n = 60
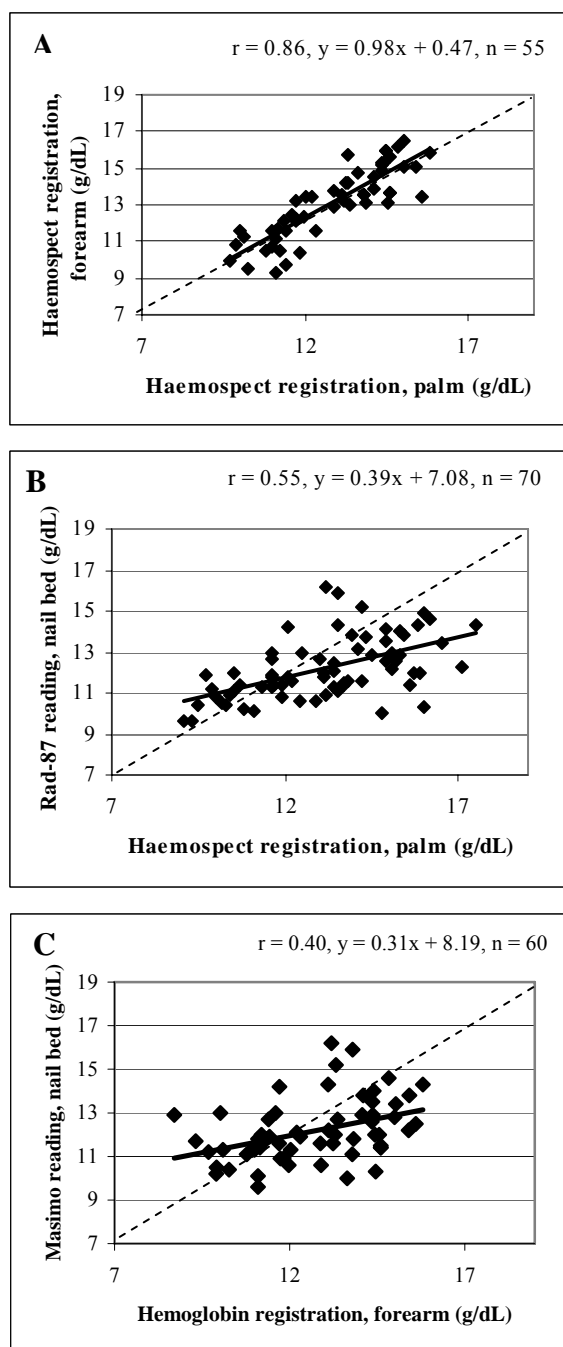
Figure 3. Scatterplots representing paired values of digital outputs from two non-invasive devices. The solid line represents the best-fit trend line for each diagram, while the discontinuous, diagonal line represents the 45° line of identity. Panel A represents paired values of registrations from the Haemospect® taken at the palm and forearm sites among the 55 subjects who provided viable readings at both sites. The Pearson correlation coefficient is r=0.86. Panel B represents paired values of the digital outputs from the Haemospect® palm site and the Rad-87[TM] nail bed. The Pearson correlation coefficient is r=0.55. Panel C represents paired values of the digital outputs from the Haemospect® forearm site and the Rad-87[TM] nail bed. The Pearson correlation coefficient is r=0.40.

(palm) or pigmented (forearm) areas of the skin. Though slight technical improvements are needed in future models to facilitate immediate output of the final reading, the speed of this apparatus is desirable in field settings, par-

ticularly with children as subjects, a group of central interest. Furthermore, both sites provided comparable outputs. The slope of the comparison regression equations of >0.85 for palm and forearm applications, is only a short way from the ideal of 1.0 of perfect response correspondence.

The Rad-87[TM] was slower in producing a digital result; requiring 90 sec of warm-up plus the 10 min of signal accumulation required to obtain a standard reading. With cleaning and changing of subjects, this hindered the ability to process more than 5 subjects in an hour, and would potentially be problematic for a young child. However, the good correspondence of the benchmark Hb value after 10-min accumulation with the shorter 5- and 3-min periods augers well for a simplification of the Rad-87[TM] in this aspect of its application.

If the task were only a matter of assessing the median Hb of a population with a wide array of values across the range constituted for this field evaluation, both contact sites for the Haemospect® (palm and forearm) would have performed adequately throughout the inter-quartile range; the mean, SD, 25th, 50th, and 75th percentiles were generally within 0.5 g/dL of the reference value of the whole blood laboratory Hb analyses. All three modes (nail bed, palm, and forearm) were less accurate at the upper and lower extremes of the measured Hb distribution (Table 1). The fact that registries information from the Haemospect® readings was selectively lost from the extremes of the actual Hb distribution could be a distorting factor in assessing true diagnostic prowess. This has important consequences. The low sensitivity at the lowest Hb cut-off has implications for applications in young children and pregnant women, the population sectors of greatest public health interest for anemia; both have a sea level cut-off value for anemia diagnosis of <11.0 g/dL. Only two-thirds or fewer of the subjects with anemia of that degree would be detected at any of the three anatomic sites across our two devices. This did not improve substantially until the <11.5 g/dL cut-off with the Rad-87[TM] nail bed readings and the Haemospect forearm registries, or until <12.0 g/dL for the palm contact. The specificity rates for the Rad-87[TM] never exceeded 80%, except when sensitivity was 50% (Table 1).

Non-invasive Hb screening was better in reports by other authors. In a study done under laboratory conditions with a non-invasive, sublingual contact-probe system visualizing the microcirculation, 100% sensitivity and specificity were obtained when simultaneously compared with whole blood.[13] From the presentation of the data by Rabe, we can impute that they had sensitivities and specificities greater than 95% in a pediatric hospital setting in England.[16] The transition from research center to the field survey situation transformed this superior diagnostic performance of the latter toward substantially less competent anemia detection in both our previous exploration, using identical sampling protocol and procedures,[17] and again with a second round in the field. We attribute the transport and possibly variable atmospheric conditions as factors in the inferior correspondence with whole blood, as compared with the Southampton experience.[16]

What we can affirm at the least is that the underlying technology for correct spectral analysis has been largely

perfected in the current software developed by the manufacturer of Haemospect®. Reliance on remote reading, however, would render the procedures employed here as cumbersome in the extreme for population survey work and totally non-applicable for clinical assessment. The promise of the Haemospect® software's capabilities for field use will only be successfully realized with the internal programming of the device, to register an immediate and valid reading in the digital display. Even then, further work is needed to determine which site – palm or forearm – has the most relative advantages to be the preferred site for routine recording, and through which ranges of the spectrum of human Hb concentrations.

A blood-free approach has inherent advantages in acceptability, safety and, theoretically, cost-of-analysis over the field-friendly capillary-blood screening methods reported in the past, but how does it compare with respect to relative accuracy? Hemocue®, a portable photometer that uses 10 µl of capillary blood in a cuvette, is generally regarded as the most field friendly Hb screening involving whole blood. Morris et al [11] in Honduras compared Hb determined in a Hemocue® versus the same blood measured by automated methods. They found a Pearson coefficient of r=0.98. Simulating capillary blood measured by Hemocue® in a population with a median Hb concentration of <13.0 g/dL and the anemia cut-off at <12.0 g/dL the reliability and accuracy characteristics of Hemocue® predicted 80% sensitivity and 95% specificity. Between standard (venous blood by automated method) and capillary, Neufeld et al [12] in Cuernavaca, Mexico, compared capillary-sampled blood by Hemocue® with venous blood by automated methods in children, finding a Pearson coefficient of r=0.83. Using the non-altitude adjusted <11.0 g/dL cut-off criterion for anemia, they reported 84% sensitivity and 93% specificity. Comparing these characteristics in Mexico and Honduras to our scenario with sensitivity at the <12.0 g/dL cut-off, we even have slightly better sensitivity and specificity when using the Haemospect® at the palm site (Table 2). Admittedly, the cut-off in Mexico was that of <11.0 g/dL; so, we concede that much work is still needed on the non-invasive devices to address that degree of anemia. With respect to Pearson correlations coefficients, ours were r=0.94 (palm) and r=0.90 (forearm).

Within the spirit of the Lyon mandate [8] to protect those not in need of iron to avoid the exposure, the application to the palm of the Haemospect® fall on the correct side of this balance with specificity consistently exceeding sensitivity. Although selecting all but 2 true non-anemics from iron interventions would be accomplished, at sea-level with this application, 33 out of 100 children with a potentially iron-responsive anemia would be left out of the intervention with reliance on the current palm site with Haemospect®. Hence, before application in sea-level populations with high anemia rates goes forward with either apparatus or any skin site, analytical software will require improvement to extend its diagnostic correspondence range well below 11.0 g/dL.

The non-invasive screening approach looks to be an alternative to a formal laboratory determination of Hb concentration, the estimation of a packed-cell volume (hematocrit) with a microcentrifuge, or the estimation of Hb

with the Hemocue® cuvette approach; specifically for Guatemala, as well as major inhabited regions of the Andean region, Himalayan Asia, and eastern and southern Africa with *highland* populations. Dirren et al showed that conventional extrapolations for the effect of lower oxygen tension at altitude apply to children.[18] Thus, the sea-level equivalent of <11.0 g/dL rises to become <12.0 g/dL at 2000 m above sea-level. At that altitude, the Haemospect® palm application only misses 12 of every 100 anemic subjects for intervention, and only 2 of 100 would inappropriately be intervened. With the forearm contact, no anemia is missed, and only 8 unnecessary dosings are given per 100 non-anemic individuals. This first assumes that the engineering required to get a faithful transformation of the spectral interpretation to a valid reading on the digital screen of the hand-held instrument is successfully applied. These conclusions also assume, of course, that all anemia would be of iron-deficiency origin, which may not necessarily be the situation. Indeed, a complementary non-invasive approach to assess a biomarker for iron status, such as percutaneous screening for circulating zinc protoporphyrin concentrations, would be needed to complement Hb for a rapid assessment of iron-deficiency anemia.

On the anemia screening side, however, the Haemospect® technology would be projected to have roughly equivalent diagnostic error to the Hemocue® [11] in a highland child or pregnant woman population, with all the advantages of the exclusion of any blood extraction or handling, from the testing situation. Perhaps for use at altitude, some of the technology we tested is ready for survey and diagnostic application.

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Original Article

Validity and correspondence of non-invasively determined hemoglobin concentrations by two trans-cutaneous digital measuring devices

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兩種經由皮膚的電子測量儀器以非侵入性評估血紅素濃度的效度及一致性

血紅素(Hb)濃度是貧血的重要診斷指標，包含營養性貧血。此研究目的為比較兩種具廣範圍 Hb 光譜、易攜帶且非侵入性的儀器所測量的 Hb 值，並與常規檢驗法比較。80 名瓜地馬拉成年人(40 名來自高原的男性，40 名為平地的懷孕女性)提供靜脈血液，以常規比色法測量血紅素。並以兩種儀器，Rad-87^{TM} (Masimo)和 Haemospect$^{®}$ (MBR Optical Systems)以非侵入性的皮膚探針連續記錄 Hb，程序如公司的使用說明。全血的血紅素濃度範圍是 7.8-18.5 g/dL(平均值為 12.9±2.3 g/dL；中位數為 13.3 g/dL)。相對應的描述性統計為：Rad-87^{TM} (指床)測出值範圍是 9.6-16.2 g/dL；平均值為 12.1±1.5 g/dL；中位數為 11.9 g/dL。Hemospect$^{®}$ 前臂測量值範圍 8.7-15.8 g/dL；平均值 12.7±1.8 g/dL；中位數 13.0 g/dL。Hemospect$^{®}$ 手掌測量值範圍是 9.1-17.5 g/dL；平均值 13.2±2.1 g/dL；中位數 13.4 g/dL。靜脈血測出的血紅素與 Rad-87^{TM} 所測的值，皮爾森相關係數是 r=0.59 (*p*<0.001)，與 Haemospect$^{®}$ 分別在手掌及前臂的測量值，相關係數是 r=0.94 (*p*<0.001)及 r=0.90 (*p*<0.001)。不同部位測量值間的 Lin 係數 r=0.84。不同儀器的敏感度及特異度受血紅素的切點及測量程序的影響。當血紅素切點設定為未懷孕女性<12.0 g/dL，成人男性<13.0 g/dL，Haemospect 儀器的表現，顯示可能適宜做為貧血篩檢的工具。

關鍵字：血紅素、貧血、非侵入性技術、診斷篩檢、瓜地馬拉