UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF A SINGLE-STEP PLASMA IRON DETECTION METHOD

LIM WAI FENG

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

August 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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August 2017

Chairman : Lai Mei I, PhD
Faculty : Medicine and Health Sciences

Iron deficiency anaemia (IDA) is the most common cause of anaemia worldwide that affects almost two billion people in many developing countries including Malaysia. The gold standard for identifying iron deficiency is a direct test of bone marrow iron. Bone marrow aspiration is too invasive in nature for routine use. Therefore, peripheral whole blood and serum/plasma are used to assess iron status through haematological and biochemistry tests, respectively. Iron status quantification can be limited in some areas due to high cost and lack of access to these analysers. Therefore, a low-cost and efficient technique was designed to detect iron status using human plasma. Currently, no single definitive diagnosis can assess iron status effectively except bone marrow iron. Although ferritin is a common practice, it can be confounded by inflammation and required haemoglobin level to detect iron deficiency anaemia. To devise a technique for field studies, plasma iron (PI) was chosen due to its simplicity without involving multiple steps like ferritin. Firstly, a recipe to rapidly induce iron release from human plasma was identified, comprising 720 mM citric acid, 20 mM ascorbic acid, 100 mM thiourea and 3 mM ferrozine. Next, a total of 190 samples were collected, i.e. 10 inflammation (Infla), 31 iron deficiency with and without anaemia (IDwwoA), 114 normal iron (NI) and 35 iron overload (IOL), respectively. These samples were subjected to PI screening using the freeze-dried version of the concocted recipe. By comparing the current technique (termed Prototype_PI) to autoanalyser (termed Cobas_PI), Prototype_PI and Cobas_PI across all samples were ranged from 148.3-2744.4 µg/L and 184.0-2918.0 µg/L, respectively with 72.1-157.4% of recoveries. Only nine samples were found to be beyond 80-120% of the acceptance range. Both methods correlated well with a Spearman rho coefficient of 0.967. In Passing-Bablok analysis, both methods did not differ by any constant or proportional error but has random error, with residual standard deviation (RSD) of 61.5 µg/L across all samples. The Bland-Altman's limit of agreement (LoA) was -239.7 to 104.8 µg/L with a mean difference of -67.5 µg/L. Concordance (CCC) and intraclass correlation coefficient (ICC) of 0.980 and 0.994, respectively, indicating a good agreement between two methods. Across iron status, each group indicated good agreement with values more than 0.9 for Spearman rho coefficient, CCC and ICC. LoAs were -156.3 to 65.6 µg/L (IDwwoA), -225.8 to 86.5 µg/L (NI) and -336.0 to 129.3 µg/L (IOL) with a mean difference of -45.35 µg/L, -69.65 µg/L and -103.32 µg/L, respectively.

Similarly, neither constant nor proportional error found across iron status, indicating random error contributed to the difference between both methods. As compared to Cobas_PI, Prototype_PI has a sensitivity of 87.5% (91.7%) and a specificity of 97.1% (96.8%) in diagnosis of IDwwoA in male (female), respectively. However, the ability of Prototype_PI to diagnose IOL in male (female) was reported to have lower sensitivity, i.e. 71.4% (male) and 80.8% (female) but 100% specificity, respectively. By comparing to ferritin level, both Prototype_PI and Cobas_PI found to have moderate sensitivity and specificity. This project concluded that Prototype_PI could screen PI successfully as comparable to Cobas_PI for diagnosis of IDwwoA but less accurate in IOL screening. Further justification has to be done by performing double-blind study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN KAEDAH PENGESANAN ZAT BESI PLASMA DENGAN SATU LANGKAH

Oleh

LIM WAI FENG

Ogos 2017

Pengerusi : Lai Mei I, PhD
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Anemia kekurangan zat besi merupakan anemia yang paling biasa di seluruh dunia dan melibatkan hampir dua bilion orang dan kebanyakan negara-negara membangun termasuk Malaysia. 'Gold standard' untuk mengesan kekurangan zat besi adalah ujian terus atas zat besi di sumsum tulang. Namun begitu, Aspirasi sumsum tulang adalah terlalu invasif untuk penggunaan rutin. Oleh itu, darah periferi dan plasma telah digunakan untuk mengukur tahap zat besi, iaitu melalui ujian hematologi dan biokimia masing-masing. Kuantifikasi tahap zat besi terhad di beberapa daerah kerana kos yang tinggi dan kekurangan akses terhadap autoanalyser. Oleh itu, satu kaedah yang berkos rendah dan teknik yang berkesan telah dicipta untuk mengukur tahap zat besi dalam plasma manusia. Masa kini, tiada satupun diagnosis muktamad yang dapat mengukur tahap zat besi secara berkesan kecuali pengukuran zat besi di sumsum tulang. Walaupun feritin merupakan amalan biasa, dan tahapnya akan meningkat disebabkan oleh mana-mana gejala meradang dan memerlukan tahap hemoglobin untuk mengesan anemia kekurangan zat besi. Untuk merangka teknik untuk bidang kajian, besi plasma (PI) telah dipilih kerana ia lebih mudah tanpa melibatkan pelbagai langkah seperti feritin. Satu resipi untuk mendorong pembebasan zat besi dari plasma manusia dengan cepat telah dikenalpasti, termasuk 720 mM asid sitrik, 20 mM asid askorbik, 100 mM thiourea dan 3 mM ferrozine. Seterusnya, sejumlah 190 sampel telah dikumpul iaitu 10 individu yang beradang (Infla), 31 individu yang kekurangan zat besi dengan dan tanpa anemia (IDwwoA), 114 individu yang mempunyai zat besi tahap biasa (NI) dan 35 individu yang mempunyai bebanan zat besi (IOL) masing-masing. Sampel-sampel ini telah disaringkan dengan menggunakan resipi yang telah dibeku dan kering. Dengan membandingkan teknik semasa (Prototip_PI) dengan autoanalyser (Cobas_PI), Prototip_PI dan Cobas_PI berjulat dari 148.3-2744.4 µg/L dan 184.0-2918.0 µg/L ke atas semua sampel masing-masing dan mempunyai pemulihan sebanyak 72.1-157.4%. Hanya terdapat 9 sampel yang terkeluar daripada julat yang dibenarkan, iaitu 80-120%. Kedua-dua kaedah ini berkorelasi baik dengan koefisyen spearman rho iaitu 0.967. Dalam analisis Passing-Bablok, tiada sebarang ralat konstant atau berkadar didapati dalam kedua-dua kaedah tetapi mempunyai ralat rawak dengan sisa sisihan piawai sebanyak 61.5 µg/L ke atas semua sampel. Had persetujuan dengan analisis Bland-Altman adalah -239.7 ke 104.8

µg/L dengan perbezaan purata sebanyak -67.5 µg/L. Konkordans (CCC) dan pekali korelasi intraclass (ICC) adalah sebanyak 0.980 dan 0.994 masing-masing, menunjukkan persetujuan yang baik antara kedua-dua kaedah tersebut. Setiap kumpulan tahap zat besi menunjukkan persetujuan yang baik dengan nilai melebihi 0.9 untuk koefisyen spearman rho, CCC dan ICC. Had persetujuan dengan analisis Bland-Altman adalah -156.3 ke 65.6 µg/L (IDwwoA), -225.8 ke 86.5 µg/L (NI) dan -336.0 ke 129.3 µg/L (IOL) dengan perbezaan purata sebanyak -45.35 µg/L, -69.65 µg/L dan -103.32 µg/L masing-masing. Serupanya, tiada ralat konstant ataupun berkadar didapati tetapi ralat rawak yang menyumbang kepada perbezaan antara kedua-dua kaedah. Berbanding dengan Cobas_PI, Prototip_PI mempunyai tahap sensitiviti sebanyak 87.5% (91.7%) dan spesifisiti sebanyak 97.1% (96.8%) dalam diagnosis IDwwoA untuk lelaki (perempuan) masing-masing. Namun begitu, kemampuan Prototip_PI untuk diagnosis IOL dalam lelaki (perempuan) dilaporkan mempunyai sensitiviti yang rendah iaitu 71.4% (lelaki) dan 80.8% (perempuan) tetapi mempunyai spesifisiti 100% masing-masing. Dengan berbanding dengan tahap feritin, kedua-dua Prototip_PI dan Cobas_PI didapati mempunyai tahap sensitiviti dan spesifisiti yang sederhana. Projek ini menyimpulkan bahawa Prototip_PI mampu membuat penyaringan PI dengan berjaya untuk diagnosis IDwwoA tetapi kurang memuaskan dalam diagnosis IOL berbanding dengan Cobas_PI. Justifikasi yang selanjutnya seharusnya dilakukan dengan melaksanakan kajian dalam sampel secara rawak.

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I certify that a Thesis Examination Committee has met on 3 August 2017 to conduct the final examination of Lim Wai Feng on her thesis entitled "Development of a Single-Step Plasma Iron Detection Method" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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3D	Three-dimensional
AAS	Atomic absorption spectrophotometer
AsC	Ascorbic acid
AUC	Area under curve
BHT	2,6-bis[hydroxy(methyl)amino]-1,3,5-triazine
BPY	2,2'-bipyridine
BSA	Bovine serum albumin
c-SBF	Conventional-SBF
CA	Citric acid
CAD	Computer-aided-design
CCC	Lin's concordance correlation coefficient
cHP	Commercially available human plasma
CI	Confidence interval
Cobas_PI	Plasma iron measured from Cobas
CRP	C-reactive protein
CRM	Certified reference material
CV	Coefficient variation
DFO	Desferoxamine
DI	Deionised water
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
EqaSF	Equine spleen apoferritin
EqSF	Equine spleen ferritin
FdwC	Freeze-dry with prior concentrating
FdwoC	Freeze-dry without prior concentrating
FER	Ferene
$FMNH_2$	Reduced flavin mononucleotide
FMN	Flavin mononucleotide
FRC	Fragmented red cells
FRN	Ferritin
FRZ	Ferrozine
Hb	Haemoglobin
HCT	Haematocrit
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HuaTF	Human apotransferrin
HuLF	Human liver ferritin
HuTF	Human transferrin
HyHCl	Hydroxylamine hydrochloride
i-SBF	Ionised-SBF
I_0	Incident radiant energy
ICC	Intraclass correlation coefficient
ICP-MS	Inductively coupled plasma mass spectrometry
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDE	Iron deficiency erythropoiesis
IDwwoA	iron deficiency with and without anaemia
Infla	Inflammation

IOL	Iron overload
IRF	Immature reticulocyte fraction
LDR	Light-dependent resistor
LED	Light-emitting diode
LG	Logarithm-transformed
LoA	Limit of agreement
LOD	Limit of detection
LOQ	Limit of quantitation
LR-	Likelihood ratio negative
LR+	Likelihood ratio positive
m-SBF	Modified-SBF
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
MCVr	mean reticulocyte volume
MES	2-(N-Morpholino)ethanesulfonic acid, 4-Morpholineethanesulfonic acid
MFR	Middle-fluorescent reticulocytes
Mops	3-Morpholinopropanesulfonic acid
NA	Not available
NI	Normal iron
noPC	Without pathlength correction
NPV	Negative predictive value
NRBCs	Nucleated red blood cells
OFR	Out of range
op-amp	Operational amplifier
PEG	Polyethylene glycol
PCDI	Pathlength correction with deionised water
PCr-SBF	Pathlength correction with revised-simulated body fluid
PCV	Packed cell volume
PI	Plasma iron
POC	Point-of-care
PPV	Positive predictive value
Prototype_PI	Plasma iron measured from Prototype
r-SBF	Revised-SBF
RBC	Red blood cell
RCF	Red cell flag
RDW	Red cell distribution width
RDWI	Red cell distribution width index
ReSD	Relative standard deviation
Ret-Hb/CHr/Ret-Y	Reticulocyte haemoglobin content
Ret-Hbe	reticulocyte haemoglobin equivalent
RMI	Reticulocyte maturity index
ROC	Receiver operative curve
RSD	Residual standard deviation
SBF	Simulated body fluid
SD	Standard deviation
SDT	Sodium dithionite
SI	Serum iron
sTfR	Soluble transferrin receptor
sTfR-F index	Ratio of transferrin receptor to ferritin

Tf	Serum transferrin
TfR	Transferrin receptors
TIBC	Total iron binding capacity
TRIS	Tris(hydroxylmethyl)aminomethane
TU	Thiourea
WBC	White blood cells
WHO	World Health Organization
ZPP	Zinc protoporphyrin
%HYPO	Percentage of hypochromic red cells
%HYPOr	Percentage of hypochromic reticulocytes
%T	Percentage of transmittance
%TSAT	Transferrin saturation

CHAPTER 1

INTRODUCTION

1.1 Anaemia

A single red blood cell (RBC) consists of millions of haemoglobins, which function as an important oxygen-carrier from the lungs to the tissues in our bodies. Haemoglobin comprises of two α- and two β-globin chains; each binding to a haem group. The haem group consists of a cyclic protoporphyrin ring and an iron core (**Figure 1.1**) (Hoffbrand et al., 2006).

Anaemia is described as a condition when RBCs are not supplying sufficient oxygen throughout the body for the body's normal physiological need (Hoffbrand et al., 2006; WHO, 2011). The three main causes of anaemia are contributed by (1) key micronutrient deficiencies for RBC synthesis, such as iron, folate, vitamin B_{12} and vitamin A deficiencies; (2) inherited conditions that affect haemoglobin structure or function, such as α- or β-thalassaemia and sickle cell anaemia; and (3) infectious diseases that cause intravascular haemolysis, such as malaria, hookworm and schistosomiasis (WHO, 2007; Miller et al., 2013; Pasricha, 2014). Anaemia is a public health problem, mainly attributed by iron deficiency anaemia (Hoffbrand et al., 2006; WHO, 2001; WHO, 2015).

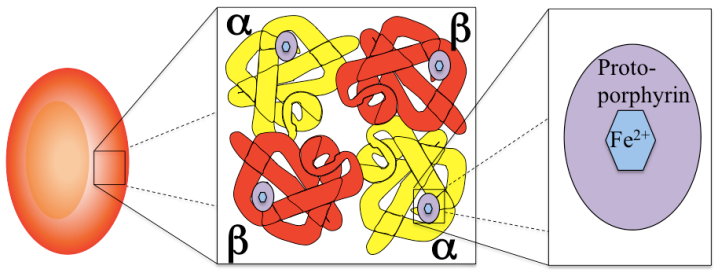
Structure	**Red blood cell**	**Haemoglobin**	**Heme**
			
Content	• millions of haemoglobin	• two α-globin chains • two β-globin chains	• iron • protoporphyrin
Deficiency /faulty	• anaemia	• α-thalassaemia • β-thalassaemia	• iron deficiency anaemia • sideroblastic anaemia

Figure 1.1: Red blood cell structure. Adult red blood cells (RBCs) consist of millions of haemoglobins: two α-globin chains (shown in orange colour) and two β-globin chains (shown in red colour); each globin has a heme group made of a protoporphyrin ring (shown in purple colour) and an iron core (shown in blue colour). Reduced production of globin chains and iron leads to a low haemoglobin content in the RBCs resulting in anaemia.

1.2 Iron deficiency anaemia

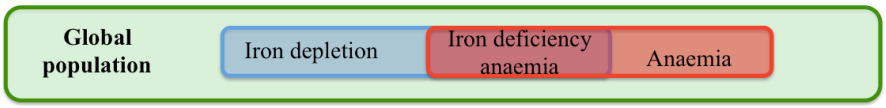
Iron deficiency anaemia (IDA) is the most common cause of anaemia worldwide due to a deficient iron supply to produce functional haemoglobin (Hoffbrand et al., 2006; WHO, 2001). Causes of IDA are dietary iron deficiency, iron malabsorption, chronic blood loss from gastrointestinal bleeding, the maternal and perinatal period of iron deficiency anaemia and certain infectious diseases like malaria and hookworm infestations (Miller et al., 2013).

High-risk groups that are most vulnerable to iron deficiency are infants, adolescents, women of reproductive age, pregnant and breastfeeding women, postmenopausal women, elderly people in terms of physiological demand (age and gender-related factors); vegetarians in terms of dietary habit; patients with chronic renal failure undergoing haemodialysis and receiving erythropoietin in terms of pathological demand, individuals in resource-poor area in terms of socioeconomic influences and others (Hoffbrand et al., 2006; Provan, 2013). Impairment of oxygen delivery in iron deficiency anaemia may lead to weakness, lethargy, dyspnoea, unusual headaches, taste disturbances, difficulty in concentration, poor work capacity and productivity as well as decreased cognitive performance and physical development, are of major concerns (Kassebaum et al., 2014; Provan, 2013).

1.3 Prevalence of iron deficiency anaemia

To date, there are no direct global estimates for iron deficiency, instead a comprehensive global estimate of anaemia based on haemoglobin level, has often been used as a proxy indicator of iron deficiency (WHO, 2001; WHO, 2015; Pasricha, 2010). Anaemia is a public health problem that affects almost two billion people globally, in both non-industrialised and industrialised countries, with 50% of the anaemic causes can be attributed by iron deficiency anaemia (IDA) (**Figure 1.2**) (WHO, 2001; WHO 2011). The prevalence of global anaemia was 32.9% (2010) and 29.4% (2011); respectively (Kassebaum et al., 2014; Pasricha, 2014; WHO, 2015). In Malaysia, almost two million women of reproductive age are anaemic (McLean et al., 2009).

Population coverage by anaemia prevalence surveys							
WHO region	Children aged 6-59 months	Children aged 5-14 years	Non-pregnant women aged 15-49 years	Pregnant women aged 15-49 years	Men aged 15-59 years	Elderly aged ≥60 years	All
	Coverage in percent and number of countries in each grouping						
Africa (46)	74.6 (26)	13.2 (8)	61.4 (23)	65.8 (22)	21.9 (11)	0.0 (0)	40.7
Americas (35)	76.7 (16)	47.1 (9)	56.2 (13)	53.8 (15)	34.3 (2)	47.6 (1)	58.0
South-East Asia (11)	85.1 (9)	13.6 (3)	85.4 (10)	85.6 (8)	4.1 (2)	5.2 (1)	14.9
European (52)	26.5 (12)	9.3 (3)	28.0 (12)	8.3 (4)	14.1 (3)	8.0 (2)	22.9
Eastern Mediterranean (21)	67.4 (11)	15.5 (6)	73.5 (11)	58.7 (7)	27.5 (6)	3.2 (3)	84.3
Western Pacific (27)	90.4 (10)	83.1 (7)	96.9 (13)	90.2 (8)	96.2 (10)	93.3 (6)	13.8
Global (192)	76.1 (84)	33.0 (36)	73.5 (82)	69.0 (64)	40.2 (34)	39.1 (13)	48.8
The global prevalence of anaemia							
	Percent and 95% confident interval						
Prevalence of anaemia (%)	47.4 (45.7-49.1)	25.4 (19.9-30.9)	30.2 (28.7-31.6)	41.8 (39.9-43.8)	12.7 (8.6-16.9)	23.9 (18.3-29.4)	24.8 (22.9-26.7)
Population affected (number million)	293 (283-303)	305 (238-371)	468 (446-491)	56 (54-59)	260 (175-345)	164 (126-202)	1620 (1500-1740)



(Modified from World Health Organization, 2001; 2005)

Figure 1.2: The surveys of global prevalence of anaemia in WHO region with respect to the number of countries and percentage of population involved. Anaemia is the most common nutritional disorder in the world, mainly contributed by iron deficiency anaemia. Affected groups are children, pregnant women, all women, men and elderly (Modified from WHO, 2001; WHO, 2015).

In 2011, it is estimated that almost 800 million children and women suffered from anaemia globally, including 43% of children, 38% of pregnant women, 29% of non-pregnant women and 29% of all women of reproductive age (**Figure 1.3-1.6**) (WHO, 2015). Additional meta-analysis on the effect of iron supplementation is an approach to estimate the attribution of iron deficiency to the prevalence of anaemia, based on haemoglobin level (WHO, 2015). These analyses indicated that iron supplementation did improve around 42% of anaemic children and 50% anaemic women; respectively (WHO, 2015).

It is a serious public health problem whereby essential control strategies have to be implemented (WHO, 2001). In 2012, World Health Organization (WHO) has endorsed the second global nutrition target, in which by the year 2025, 50% reduction of anaemia in women of reproductive age (pregnant and non-pregnant women), i.e. from prevalence of anaemia 29.4% (2011) to 14.7% (2025) (WHO, 2014; WHO, 2015). **Figure 1.7-1.8** showed the latest prevalence and number cases of anaemia globally using WHO Nutrition Tracking Tool (WHO, 2016).

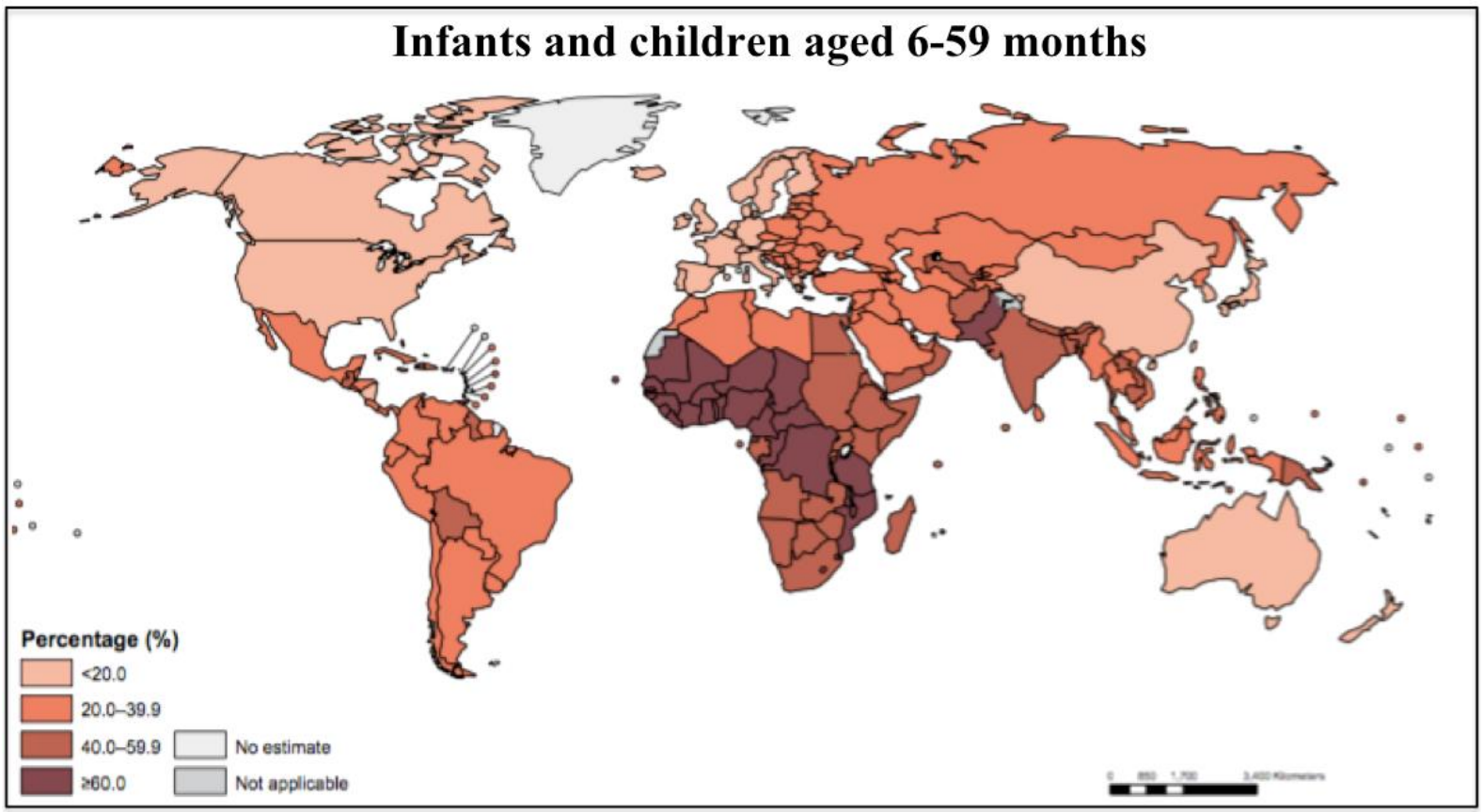


Figure 1.3: The global prevalence of anaemia in high-risk group of infants and children aged 6-59 months (Adapted from WHO, 2015).

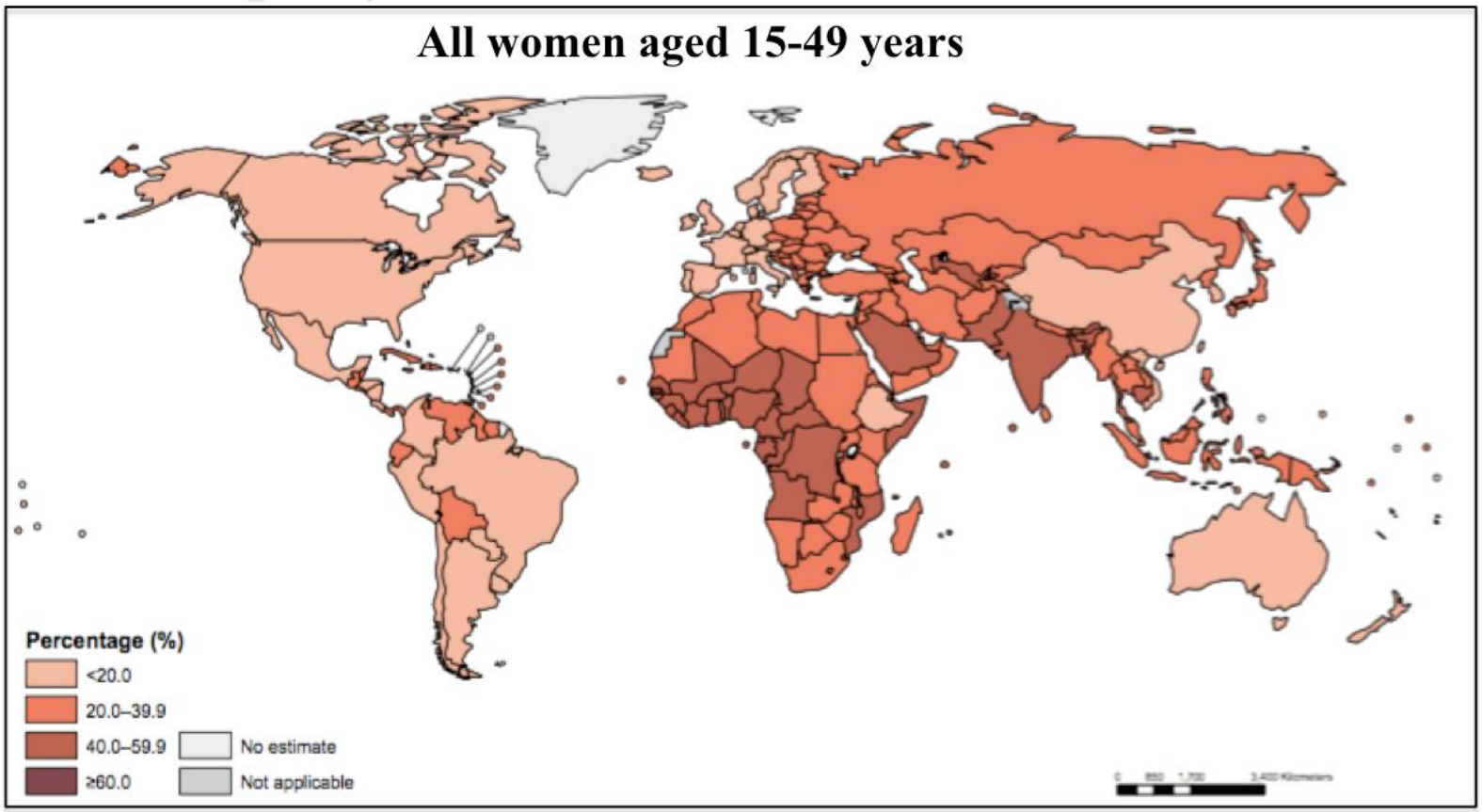


Figure 1.4: The global prevalence of anaemia in high-risk group of all women aged 15-49 years (Adapted from WHO, 2015).

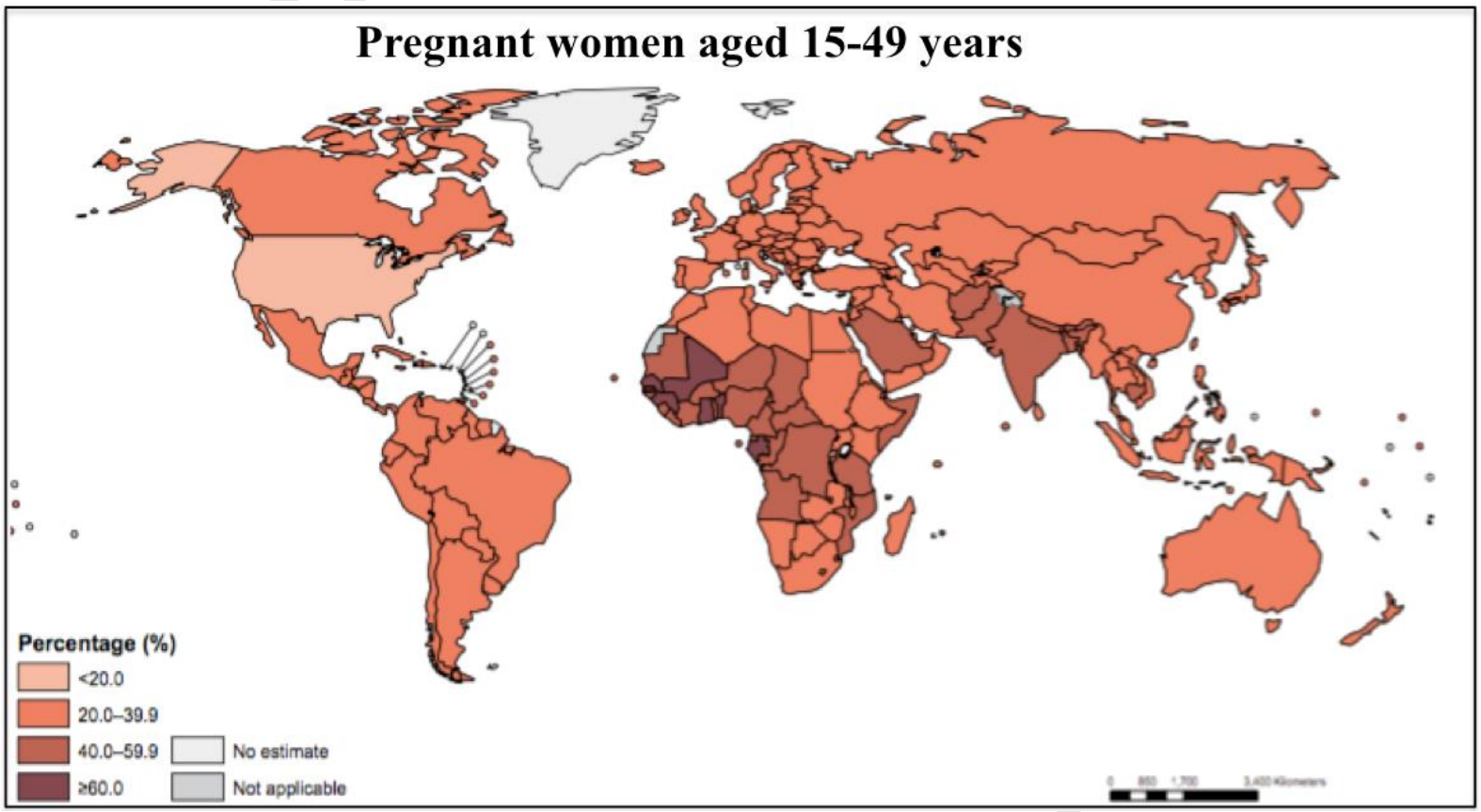


Figure 1.5: The global prevalence of anaemia in high-risk group of pregnant women aged 15-49 years (Adapted from WHO, 2015).

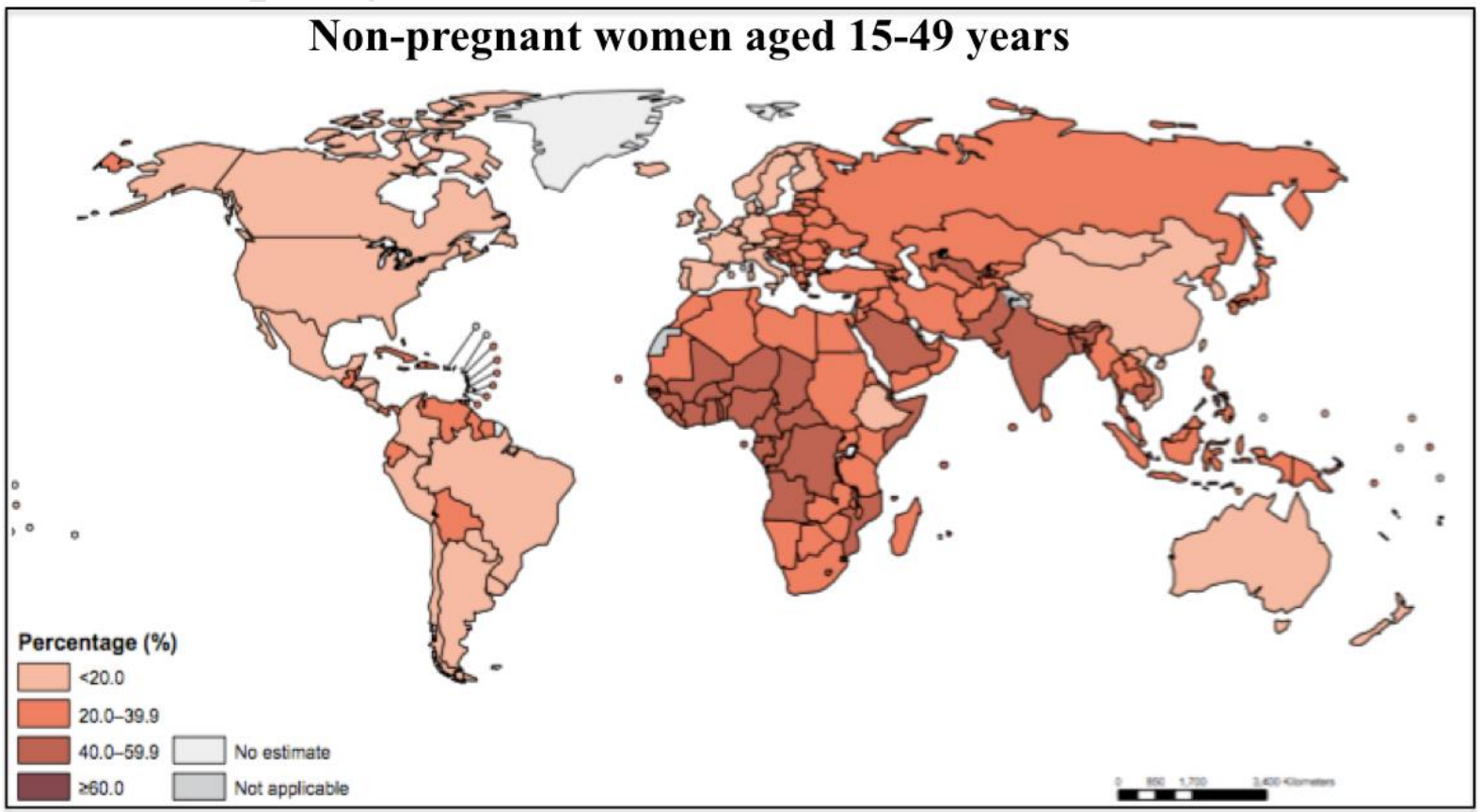


Figure 1.6: The global prevalence of anaemia in high-risk group of non-pregnant women aged 15-49 years (Adapted from WHO, 2015).

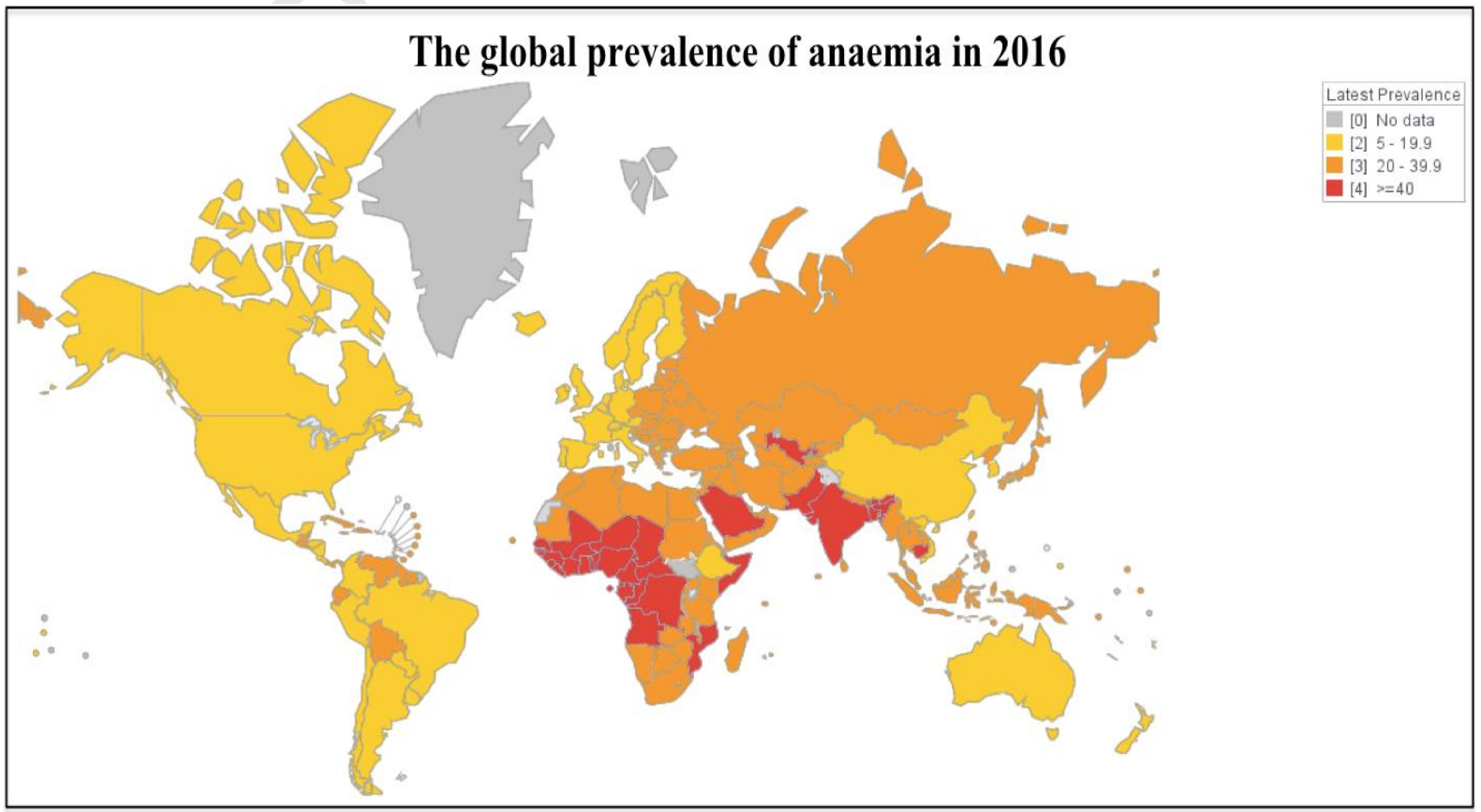


Figure 1.7: The global prevalence of anaemia in 2016 (Adapted from http://www.who.int/nutrition/trackingtool/en/, accessed on 2nd March 2016).

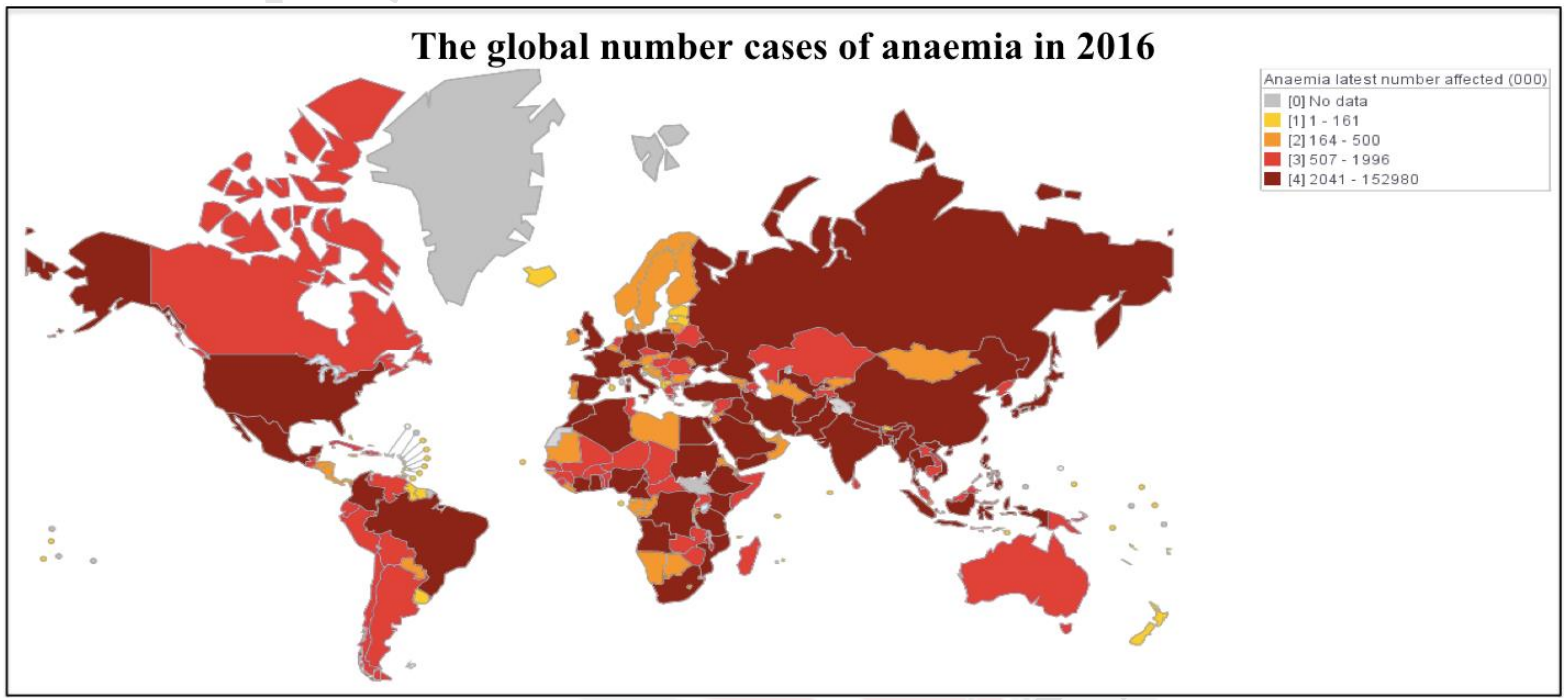


Figure 1.8: The global number cases of anaemia in 2016 (Adapted from http://www.who.int/nutrition/trackingtool/en/, accessed on 2nd March 2016).

1.4 Problem statement

Although there have been many efforts to improve nutritional well-being to eradicate iron deficiency anaemia, the condition is still common globally (WHO, 2001; WHO, 2005). Since the prevalence of iron deficiency is referred to the prevalence of anaemia based on haemoglobin levels, other causes for non-anaemic iron deficiency could have been easily underestimated (WHO, 2001; WHO, 2005).

Iron deficiency anaemia is characterised by smaller RBCs (microcytic) and reduced haemoglobin content (hypochromic) with pencil cells and target cells in the blood film and reduced mean cell volume (MCV) and mean cell haemoglobin (MCH) in red cell indices (Hoffbrand et al., 2006; Lewis et al., 2007; Provan, 2013). However, other diseases such as thalassaemia, chronic anaemia and sideroblastic anaemia have similar clinical symptoms; i.e. microcytic hypochromic anaemia in terms of blood film and red cell indices. Therefore, misdiagnosis is easy unless iron status is assessed.

To effectively fight iron deficiency anaemia, there is an urgent need to have better information in assessing iron status of populations, especially in rural areas (WHO, 2001; WHO, 2005). However, among currently available iron parameters, not one parameter alone can be used to confirm iron deficiency. Instead a combination of several indicators is needed for a definite conclusion (WHO, 2005).

Serum/plasma ferritin is commonly used to assess the body's iron status (Haskin et al., 1952). However, the serum/plasma ferritin test might only be available in some areas and have to be quantified using a biochemistry autoanalyser or enzyme-linked immunosorbent assay (ELISA). This assay requires trained staff for blood collection and assay runs. Not only that, the sample quality may be compromised during transportation and the results take a while to be released. Thus far, there is no low-cost and efficient iron tool for iron deficiency anaemia screening in market yet, especially in field studies. Furthermore, to target rural area, a simple iron tool might be useful, accompanying with simple blood collection (WHO, 2005).

Serum/plasma ferritin is an acute phase protein, rising with any inflammatory state. However, studies have shown that iron available in the ferritin could clearly distinguish those with iron overload from those with elevated ferritin due to inflammation, as the iron level is not affected by inflammation (Herbert et al., 1997).

Serum/plasma iron is abundant in blood plasma, which includes all iron, especially from transferrin-bound iron apart from ferritin-bound iron. Transferrin-bound iron is readily released in acidic condition while iron release from ferritin is not clear (Iron Panel of the International Committee for Standardization in Haematology, 1990). It was reported that there was only 25% of iron release from ferritin of >1000 μg/L using serum iron method according to the modifications to the Iron Panel of the International Committee for Standardization in Haematology (ICSH) reference method (Iron Panel of the International Committee for Standardization in Haematology, 1990).

1.5 Hypothesis

The hypothesis of this project is that the concocted recipe is able to detect plasma ferritin iron and plasma iron spontaneously in individuals with iron deficiency, normal iron status and β-thalassaemia individuals with iron overload as a result from blood transfusion and increased iron absorption from the intestine.

1.6 Objectives

The general objective is to develop a single-step plasma iron detection method.

The specific objectives are:

1. to identify a suitable method to rapidly detect iron from human plasma.
2. to quantify the iron status from a selected cohort of subjects.
3. to calculate the sensitivity and specificity of the screening test.

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