



Fig. 2. Knowledge about medication.

of the drugs they were taking, most of the respondents were uninformed. In contrast to their more-than-adequate level of communication about their patients' illnesses, most practitioners seemed to avoid counselling patients on the effects and side-effects of therapy. It is hoped that this study will increase awareness of the need for effective counselling on drug effects and side-effects.

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## Iron deficiency anaemia in healthy South African women despite iron fortification

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**To the Editor:** The prevalence of iron deficiency in a South African urban environment is probably in keeping with European and USA findings of around 10%. Although our results present the prevalence of iron deficiency anaemia among urban females, a more detailed study that includes ferritin levels is needed for confirmation. Awareness of and attention to screening for iron deficiency remain essential for improving the quality of life and productivity of women in South Africa.

### Method and findings

We used data from a study on the prevalence of HIV infection among health care workers in South Africa,<sup>1</sup> to evaluate and revalidate the current automated full blood count reference ranges for the Gauteng region. A striking finding was the large number of samples that had to be excluded from the statistical analysis because of the presence of anaemia.

Samples were obtained from 631 HIV-negative adult females working at the Helen Joseph and Coronation Hospitals in Gauteng. The demographics were representative of the urban population in South Africa.

The current National Health Laboratory Service (NHLS) full blood count reference range for adult females in the Gauteng region for haemoglobin (Hb) is 12.1 - 16.3 g/dl, haematocrit (HCT) 0.37 - 0.49 l/l, and mean cell volume (MCV) 79.1 - 98.9 fl. Using these reference ranges, the criteria for possible iron deficiency were defined as a combined Hb level below 12 g/dl, an HCT below 0.37 l/l, and an MCV of less than 79 fl. Approximately 10% of the 631 participants had microcytic



hypochromic red cell indices, which is in keeping with possible iron deficiency. One participant showed the macrocytic indices and thrombocytopenia associated with megaloblastic anaemia. Another had an elevated red cell count with microcytic hypochromic indices suggestive of a haemoglobinopathy. A further 5% had haemoglobin levels below 12 g/dl and HCTs <0.37 l/l, but normal MCV.

Of the subjects with possible iron deficiency (10% overall), 19% were aged 20 - 30 years, 14% 40 - 50 years, 7.6% 30 - 40 years and 7.5% >50 years. Few local data are available on the prevalence of anaemia in 'normal' non-pregnant urban women. A prevalence of 10% in non-pregnant coloured women was reported from the Cape Peninsula in 1994.<sup>2</sup> Local studies on pregnant women reported a prevalence of iron deficiency anaemia (IDA) of 9 - 12% in 2000 that contributed to 7.3% of perinatal deaths and 4.9% of maternal deaths. An estimated 0.4% of all deaths in South Africa in 2000 could be attributed to iron deficiency.<sup>3</sup>

## Discussion

Our conservative estimate of the prevalence of anaemia among 'healthy' South African adult females in Gauteng is 15%, with 10% of females showing features suggestive of IDA. This probably underestimates the prevalence in South Africa, as the study was conducted among economically active, apparently healthy females in an urban environment.

IDA remains a global health care problem affecting an estimated 2 billion people, both in developing and First-World countries.<sup>4</sup> The Global Burden of Disease project in 2000 concluded that there was an urgent need to develop effective and sustainable interventions in the control of IDA. Europe and the USA introduced iron fortification of flour in 1998, and the prevalence of anaemia decreased from 30% to 10%. Food fortification was identified as a possible strategy for preventing iron deficiency in South Africa in 1975.<sup>5</sup> Although opinions on fortification still vary,<sup>6</sup> fortification of maize and wheat flour was legislated in South Africa in 2003. At the time, South Africa also received a US\$2.8 million grant from the World Health Organization (WHO)-led Global Alliance for Improved Nutrition (GAIN) to support a food fortification programme

for 3 years. Successful management of IDA has also been achieved through the UN World Food Program (WFP) by cereal fortification in Afghanistan, Angola and Zambia and bio-fortification strategies in China.<sup>7,8</sup> These efforts underline the importance of food fortification as an effective strategy against nutritional iron deficiency.

Although our figures for possible IDA were lower than those in Japan (22.3%), Israel (24%) and India (20%), their laboratory methods for assessing the condition were more detailed, and the population socio-demographics are different. In addition, it was assumed that most women in our study consumed iron-fortified foodstuffs.

The laboratory infrastructure for screening and diagnosing anaemia is widely available throughout South Africa in the private and public sectors. The cost of basic screening (haemoglobin – Board of Healthcare Providers (BHF) rate R13.20) is low. However, most women only have their haemoglobin levels tested (and are incidentally diagnosed as having IDA) on admission to a hospital or antenatal clinic, or when donating blood. In South Africa, with the current prevalence of IDA and in the context of the high prevalence of HIV, clinicians and primary health care professionals should consider basic screening of haemoglobin more frequently. This could be extended further at the primary health care level by a basic 'dipstick' method.

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## Oral fluid detection of hepatitis B vaccine-induced antibodies can improve vaccination programmes

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**To the Editor:** About 387 million people (over 5% of the global population) are chronically infected with the hepatitis B virus (HBV).<sup>1</sup> The World Health Organization (WHO) plans to contain the burden of HBV infections by means of universal vaccination programmes for infants and adolescents. The vaccine may be almost universally available to children within the next few years.<sup>2</sup> A major drawback of monitoring immunity to the hepatitis B vaccine or HBV infection is the need for a blood specimen to detect hepatitis B surface antibodies (anti-HBs). Collecting blood is by needle, and is often painful and traumatic for babies, children and adults with poor venous access, so non-invasive sampling would be an ideal alternative.

Several oral fluid collection devices exist.<sup>3-7</sup> The OraSure (OraSure Technologies Inc., USA), a non-invasive cotton pad impregnated with gelatine, salts (sodium chloride, citric acid, sodium benzoate, potassium sorbate, and sodium hydroxide to give pH 6.5) and deionised water is approved for use in humans by the USA's Food and Drug Administration (FDA) and is licensed for collection of oral mucosal transudate (OMT) for anti-HIV testing.<sup>8</sup> As OMT is a serous fluid rich in immunoglobulins (IgG, IgM and IgA), it is possible to test for antibodies induced by infections of public health importance such as hepatitis A virus (HAV), HBV, hepatitis C virus (HCV),<sup>7,9,10</sup> human papillomavirus type 16<sup>11</sup> and many others.<sup>6</sup> Samples can also be used to investigate the presence of hepatitis B surface antigen.<sup>12</sup>

We aimed to assess the OraSure device for collection of OMT samples; modify a serum-based commercial enzyme-linked immunosorbent assay (ELISA) kit (Murex anti-HBs ELISA, Murex Biotech Limited) for the detection of anti-HBs

from OMT specimens; and evaluate the suitability of OMT as an alternative to blood for detecting anti-HBs induced by the hepatitis B vaccine.

### Methods

A total of 67 paired serum and OMT specimens were collected from vaccinated health care workers (HCWs) at Dr George Mukhari Hospital, Medunsa complex. In addition, 5 HCWs previously identified as having undetectable anti-HBs despite hepatitis B vaccination were used as negative controls. An OMT sample and  $\leq 3$  ml of blood (using a 5 ml syringe and Vacutainer tubes) were collected after obtaining consent from each HCW. Blood samples were collected, processed and stored as previously described.<sup>13</sup> To obtain OMT samples, the OraSure pad was placed for 2 minutes between the lower gum and inner cheek. The osmotic action of the pad draws antibodies from the mucosal tissues. Antibodies move from the capillaries to interstitial fluid and across the mucosa. The collection device is immediately placed into a preservative solution, where it remains stable for up to 21 days when stored at 20 - 37°C, or at room temperature. All serum specimens were tested for anti-HBs with automated AUSAB IMx assay<sup>13</sup> and manual Murex anti-HBs ELISA, following manufacturers' instructions. The OMT specimens were tested only with Murex ELISA, after in-house experiments to modify and optimise the assay for detection of antibodies from oral fluid (results not shown).

### Results

The AUSAB IMx assay (used as the gold standard) accurately determines anti-HBs to approximately 1 mIU/ml. Using this assay, the sera of 65 (97.0%) of 67 hepatitis B-vaccinated HCWs were found to be anti-HBs positive, and 2 (3.0%) were anti-HBs negative (Table I). None of the 5 controls tested positive for anti-HBs with AUSAB IMx assay. Testing with the AUSAB IMx assay therefore identified 65 seropositive for anti-HBs, and 7 negatives. The 65 anti-HBs positives had anti-HBs titres ranging from 1.2 to >1 000 mIU/ml.

The anti-HBs results from the AUSAB IMx assay were compared with those obtained from the serum samples tested with Murex anti-HBs ELISA (Table IA). The Murex assay detected anti-HBs in 64 (98.5%) of the 65 positive sera, and in none of the 7 negative sera, leading to one false negative (the anti-HBs titre of this sample was 1.2 mIU/ml, which was below the cut-off value ( $\geq 5$  mIU/ml) for the Murex assay). The Murex assay therefore yielded a sensitivity of 98.5% (i.e. 64/65) and specificity of 100% (i.e. 7/7).

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**Table I. Detection of anti-HBs from serum and OMT samples and assay validation results using the AUSAB IMx as gold standard assay**

	Positive	Negative	Total	Sens	Spec	PPV	NPV
A: AUSAB IMx sera v. Murex sera assay							
Positive	64	0	64				
Negative	1	7	8	98.5	100	100	87.5
Total	65	7	72				
B: AUSAB IMx sera v. Murex OMT assay							
Positive	65	2	67				
Negative	0	5	5	100	71.4	97.0	100
Total	65	7	72				

Sens = sensitivity (true positive/true positive + false negative); Spec = specificity (true negative/true negative + false positive); PPV = positive predictive value (true positive/true positive + false positive); NPV = negative predictive value (true negative/true negative + false negative).

The anti-HBs results from the AUSAB IMx assay were compared with the Murex ELISA on OMT samples. All 67 OMT specimens from vaccinated HCWs were reactive for anti-HBs using the Murex assay, including the 2 identified as anti-HBs negative by the AUSAB IMx assay. Therefore, the OMT-based testing resulted in 2 false positives (Table IB). However, all 5 OMT specimens from the control group were non-reactive for anti-HBs. The Murex assay on OMT samples showed a sensitivity of 100% and specificity of 71.4%.

## Discussion

In keeping with previous reports,<sup>5,10</sup> we demonstrated that anti-HBs can be detected from oral fluid. The quality of sampling is not affected by common oral pathologies, recent food intake, cigarette smoke, dentures, drugs causing dry mouth, or HIV status.<sup>14</sup> Although different oral fluid collection devices are commercially available,<sup>3-6</sup> a limitation is that most assays for detection of antibodies are optimised and licensed for use with blood or serum samples. Further investigations are needed to optimise the sensitivity and specificity of these assays to accurately detect and quantitatively measure antibodies from oral fluid samples. Oral diagnostic testing of antibodies is particularly attractive for epidemiological and surveillance studies in developing countries, and has been used to determine antibody status for rubella in Ethiopian children<sup>15,16</sup> and antibodies to diphtheria-tetanus-pertussis (DTP) in British children,<sup>6</sup> and can monitor hepatitis B immunisation programmes in babies or adolescents, as most developing countries are now introducing hepatitis B vaccine into their national immunisation programmes. Technologies such as oral diagnostic testing can enable developing countries to introduce new and under-utilised vaccines and monitor their impact with ease.

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