of congenital malformations in the developed world (17). Because neopterin concentrations sensitively detect acute viral infections, one possible application of neopterin measurements would be the detection of unrecognized infections such as those by CMV (18, 19). In immunocompromised adults, neopterin determinations are already a valuable tool in addition to CMV antibody determinations for estimation of the severity of CMV infection (20).

In our study, the only newborn with a symptomatic CMV infection had a cord-blood neopterin concentration that was slightly above the 95th percentile of the healthy control group. Nevertheless, the number of CMV-infected newborns was too low for definite conclusions. Further investigations with a higher number of CMV-infected newborns will be necessary to clarify the potential diagnostic impact of neopterin determinations in cord blood for estimation of severity of CMV infection.

The cord-blood neopterin concentrations of CMV-PCR-positive newborns in general were in the high end of values for the general population but were not extraordinarily high. Therefore, the differences between healthy newborns with neopterin concentrations at the low or high end of the scale remain unclear, and one can only speculate whether other infectious agents or metabolic differences are involved or whether such differences may be related to the maturation status of newborns. Higher neopterin concentrations have also been described in preterm infants compared with term infants (11).

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References

Bias and Random Error in Retinol Measurements

Laboratories in Countries with Populations with Mild to Severe Vitamin A Deficiency, Paul J.M. Hulshof,1* Jitske T. Brouwer,1 Jan Burema,1 and Clive E. West1,2 (1 Division of Human Nutrition and Epidemiology, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands; 2 Division of Gastroenterology, University Medical Centre Nijmegen, 6500 HB Nijmegen, The Netherlands; * author for correspondence: fax 31-317-483342, e-mail paul.hulshof@staff.nutepi.wau.nl)

Vitamin A deficiency is common in many developing countries. Population-based surveys suggest that as many as 175 million children of preschool age are vitamin A-deficient (1). Especially in sub-Saharan Africa, severe subclinical deficiency (retinol values ≤0.70 μmol/L) often exceeds 30%. In these countries, laboratories should be able to measure the biochemical indicators of nutritional status to acceptable standards. The aim of the present study was to determine the proficiency of selected laboratories, particularly in Africa, in measuring retinol in serum.

The study was performed between September 1999 and February 2000. Laboratories in Africa with the capability to measure vitamin A in serum were located with help from WHO headquarters and country offices, UNICEF headquarters, and through our contacts. Sixteen of the 35 invited laboratories were able to participate in the study: 12 within Africa [in Ethiopia, South Africa (4 laboratories), Tanzania, Zambia, Gabon, Ghana, Egypt, and Morocco], and 4 outside Africa [in Guatemala, Indonesia (2 laboratories), and Vietnam]. Participants were offered a total honorarium of US $200. All laboratories claimed that they could measure retinol in serum. The
prevalence of subclinical vitamin A deficiency among preschool children in these countries is 30–40% (2).

Two concentrations of serum were used: a high-concentration serum (undiluted serum with a target of 1.75 μmol/L retinol) and a low-concentration serum (0.60 μmol/L retinol), which was prepared by diluting one volume of the high-concentration serum with two volumes of an aqueous solution containing 0.15 mol/L sodium chloride and 0.45 mmol/L bovine serum albumin. Each participating laboratory was provided with four samples of each concentration (eight samples in total), with a random number being assigned to each sample. Laboratories were asked to store the samples at −20 °C until time of analysis and to designate one analyst to perform the analysis within 1 month after arrival of the samples. Laboratories measured the retinol concentrations, using in-house analytical procedures, on 2 separate days, with a maximum time interval of 5 days between the 2 measurement days. On each measurement day, two low- and two high-concentration retinol serum samples were measured.

The organizing laboratory in the Division of Human Nutrition and Epidemiology, Wageningen University, analyzed the high- and low-concentration serum samples from both storage conditions before and after preparation, during the experimental period, and after the experimental period. Samples were extracted according to the method of Cantilena and Nierenberg (3). HPLC analysis was performed according to Craft et al. (4), except that gradient elution was applied to allow simultaneous measurement of retinol and carotenoids in one run. Analysis of Standard Reference Material (SRM) 968b (fat-soluble vitamin and cholesterol in human serum; NIST, Gaithersburg, MD) was performed during the experimental period.

Statistical analyses, including tests for variance homogeneity (Levene) and normality of mean values distribution (Kolmogorov–Smirnov–Lilliefors), were performed using SPSS (Ver. 7.5.3; SPSS Inc.). Procedures for removal of outlying data (Grubbs, Dixon, Cochran, and extreme ranges tests), outlined in ISO 5725-2, were followed (5).

All results reported to the organizing laboratory were checked with the participants. Data from the organizing laboratory are included in the results (Table 1). Most laboratories used methodologies based on HPLC except for laboratories 3 and 8, which used colorimetric and spectrofluorometric methods, respectively. Mean results for the low-concentration serum retinol samples ranged from 0.04 to 17.55 μmol/L (trimmed mean, 1.84 μmol/L; n = 9) and for the high-concentration samples from 0.04 to 22.21 μmol/L [trimmed mean (outliers removed), 0.62 μmol/L; n = 9] and for the high concentration ranged from 0.07 to 17.55 μmol/L (trimmed mean, 1.84 μmol/L; n = 10). Outlying mean values were observed for three laboratories (laboratories 1, 3, and 6) for both the low and high concentrations. Outlying within-laboratory variability was observed for seven laboratories for the low concentration (laboratories 2, 3, 6, 8, 11, 13, and 15) and for six laboratories for the high concentration.

### Table 1. Retinol concentrations in serum for each participating laboratory.

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*Outlying mean value based on Grubbs or Dixon test (P < 0.05).

*Borderline outlying within-laboratory variability based on Cochran test or the test for extreme ranges (P < 0.05).

*Outlying mean value based on Grubbs or Dixon test (P < 0.05).

*Outlying within-laboratory variability based on Cochran test or the test for extreme ranges (P < 0.05).
(laboratories 2, 3, 5, 6, 8, and 15). The results for eight laboratories (laboratories 4, 7, 9, 10, 12, 14, 16, and 17) revealed no outlying mean values or outlying within-laboratory variability for either low or high serum retinol. For the trimmed data, repeatability and reproducibility CVs were 3.8% and 14%, respectively, for the low concentration and 2.4% and 13%, respectively, for the high concentration.

This proficiency study has brought to light two types of variability: (a) differences in concentrations measured between laboratories, and (b) within-laboratory variation. Outlying mean values were observed for three laboratories (laboratories 1, 3, and 6) for both concentrations (Table 1). Poor analytical methodology is probably the main reason that these laboratories produced values that were either too low (laboratory 1) or too high (laboratories 3 and 6). Laboratory 1 used acid propanol to deproteinate the serum samples, which may have contributed to the low values reported because retinol is unstable in an acid environment (6). Laboratory 3 used the Carr–Price colorimetric method (7) with antimony trichloride as chromogen, which is known to produce spurious high values for stored samples (8). Laboratory 6 measured absorbance at 265 nm instead of 325 nm, which is the absorbance maximum for vitamin A, although this may not explain the high values reported.

The reasons for poor repeatability (high within-laboratory variability) are much more difficult to determine. Poor repeatability was observed for laboratories 2, 3, 5, 6, 8, 11, 13, and 15 for either one or both concentrations of serum retinol. In particular, laboratories 2, 3, 5, 6, 8, and 15 produced scattered results. Assuming a within-subject variability for serum retinol of 15% (9), then a desirable precision would be ≤7% (0.50 CVw). All trimmed laboratories (outlying laboratories removed) met this precision criterion.

Traceability of the organizing laboratory to SRM 968b was shown by calculating z-scores from the measured concentrations in the SRM (10). These z-scores were −1.1, 2.2, and 1.5 for the low, medium, and high retinol concentrations, respectively. Thus, it can be concluded from this study that the mean results of the trimmed laboratories would be close to the certified value if a certification system was in place.

In conclusion, approximately one-half of the participating laboratories were unable to measure serum retinol concentrations to acceptable standards based on the strict statistical evaluation procedures used in this proficiency study. Because data quality is an integrated result of the methodology (status of analytical methods, validation procedures, and analytical quality control), machine (maintenance of instrumentation, support, state of the art, and calibration), men (skills, training, and experience of staff and technicians), manipulation of data (calculation, mode of expression, and documentation), and materials used (purity of standards and reagents, and sample collection and treatment), collectively referred to as the “5 Ms”, it is difficult to point to a specific cause of the poor performance. However, laboratories may be able to improve repeatability through monitoring and controlling within-laboratory variation by analyzing internal control pools and using standard quality-control techniques, such as Shewhart or Westgard control charts (11). Bias can be reduced by participating in interlaboratory trials and by analyzing external reference materials with a certified concentration of the analyte of interest (12–14). In addition, standardization of the preanalytical stage (sampling, sample preparation, transport, and storage) may be necessary to reduce the variability in test results.

This study would not have been possible without the cooperation of the staffs of the participating laboratories. We thank Bruno de Benoist (WHO, Geneva) for providing us with addresses of laboratories in Africa, and our colleagues Peter van de Bovenkamp, Pieter Versloot, and Jan Harryvan for preparation of the serum samples. This project was funded by UNICEF (New York); we are grateful to Werner Schultink for advice.

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