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**-Method validation and establishment-
Retinol assessment out of capillary and venous
dried blood spots and respective plasma from
Indonesian preschool children**



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5 Summary

Micronutrient deficiency is a major public health problem in developing countries. As micronutrients are concerned, vitamin A deficiency is the one with the highest significance, because insufficient plasma levels cause blindness and promote frequent and severe infectious diseases. Thus, targeted intervention programs have to be implemented and vitamin A status needs to be assessed to identify populations at risk and to evaluate prophylactic programs. Conventional monitoring usually involves analyses of serum retinol derived from venous blood. Venipuncture, however, is demanding to both, the affected child and the responsible nurse.

In this diploma thesis dried blood spot technique with finger prick has been investigated and further improved. It prevents the threatening drawing of venous blood and guarantees the reduction in personnel costs and equipment expenses, since superseding the centrifugation and freezing of the blood samples.

In this study, separation performance was enhanced compared to studies by Craft *et al.* (2000), utilizing acetonitrile/water (85/15 by vol) as the mobile phase, thus signals for retinol and the internal standard could be separated more sharply.

Retinol concentration in dried blood spots declined gradually at around 20% during the first two weeks of storage at room temperature and remained constant for at least three months. Based on the constant reduction in retinol concentration of about 20%, accurate adjustment of dried blood spots to plasma is made possible.

According to the experiments, a concentration of 20 mg BHT/mL in 85% acetonitrile was necessary to prevent losses in retinol out of dried blood spots.

Results of the testing of different shaking techniques and shaking durations were not definite. The longer the shaking of dried blood spot samples, the lower their retinol yield, presumably due to degradation of retinol during shaking. Manual horizontal shaking for 30 seconds was chosen for better coefficients of variation and physical convenience.

Applying the improved method during validation in Indonesia, resulted in a precision of 5.10%, consequently being within the desired range.

The recovery rate was about 80% and was remarkably improved by the utilization of the entire dried blood spot compared to the recovery rate of about 55% achieved with the punch technique by Craft *et al.* (2000). This discrepancy of about 25% is due to an uneven distribution of retinol and cell compounds in the matrix. Retinol concentration is highest in the margin of the spot.

Dried blood spots technique was aimed at retinol assessment in field studies, practicability therefore needed to be verified under such conditions. For comparison, capillary and venous dried blood spots and respective plasma were collected simultaneously from 34 Indonesian children in the Semarang district, Indonesia. To obtain dried blood spots, aliquots of blood were applied to filter paper; the remaining blood was centrifuged to obtain plasma.

Bivariate correlation and agreement was best for capillary plasma and venous plasma ($R^2 = 0.83$; $\text{Lin} = 0.90$). Vital paired samples: capillary dried blood spots and venous plasma yielded less meaningful correlation and agreement ($R^2 = 0.67$; $\text{Lin} = 0.60$). Still, the equality of the dried blood spot technique and the traditional method can be adhered to, since low coefficients were possibly due to the low sample size and the narrow range of analysed retinol concentrations. The dried blood spot technique was shown to be promising in regard to a less traumatic collection procedure, cost and equipment efficiency.

In this diploma thesis manual pipetting with an Eppendorf pipette was used to quantify the amount of blood applied onto the filter paper. Under field conditions, this is less convenient and the assumption of a mean value of hematocrit to adjust the plasma content in dried blood spots increased the uncertainty of the method. In the future, both could be overcome by determining the sodium content of dried blood spots since sodium is directly proportional to the plasma content and therefore correlates very well with the retinol content. Finally, the development of retinol dried blood spots standard material as currently in the market for L-Tyr and L-Phe, could increase the reliability of the measurement of retinol in dried blood spots.