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THE EFFECT OF EXTRACTION PRECEDURE ON THE ENZYMEIMMUNOASSAY OF 17-HYDROXYPROGESTERONE IN DRIED BLOOD SPOT

萃取步驟對血片檢體中17羥助孕酮酶免疫分析之影響

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Measurement of 17-hydroxy-progesterone (17-OHP) in dried blood spots is now accepted as a reliable test for mass screening of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiences. However, it has been observed that there are some interferences by water-soluble steroids in the direct enzymeimmunoassay (EIA) which causes a high screen false positive rate. In order to improve the specificity of our screening program, we evaluated an extraction method with diethyl ether to eliminate the effects of those water-soluble steroids.

In our study, the within-run and between-run coefficients of variance of the extraction EIA at three 17-OHP concentrations ranged from 5.3% to 16.3% (n=20), and 9.8% to 18.1% (n=10), respectively. The linearity was good (r=0.9998) within the analytical range. The detection limit was determined to be 1.06 ng/ml in blood, and the range of the recovery rate was between 94.9% and 96.6%. Compared with the direct method, the cross reactivity was lower in the extraction method. Also, anticoagulants (EDTA and heparin) and bilirubin was found not to have any significant interference with the extraction assay. There was significant difference ($p < 0.01$) between the 17-OHP concentrations of neonates measured by extraction and direct assays (3.5±1.4 ng/ml blood vs. 13.3±7.2 ng/ml blood). This was also true for the children and adults samples. On the contrary to the direct method, the concentrations of 17-OHP measured by extraction method in pre-term (n=200) and term neonates (n=2,430) showed no difference at α=0.01, and their reference range in dried blood spots was estimated to be 1.4-7.8 ng/ml blood (n=200). As indicated in our result, the extraction method was more specific and may serve as a preliminary confirm test for the neonatal CAH mass screening.

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