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PORNPHAN NAWARUNGRAENG : ASSESSING FACTORS
AFFECTING THE MEASUREMENT OF LIPID ASSOCIATED SIALIC ACID IN
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Katopodis method for measuring gangliosides in the form of lipid-associated sialic acid (LASA) based on the partitioning of LASA between chloroform-methanol and aqueous phase followed by phosphotungstic acid precipitation and determining the concentration of LASA by resorcinol method. This method has gained widespread acceptance as a new tumor marker assay, but it is criticized for providing overestimates. Contamination of glycoproteins is postulated to be the cause. This study was carried out to identify factors that might contribute to assay outcome. During chloroform-methanol-water partitioning, contamination of glycoproteins was increased as the ratio of chloroform:methanol was lowered from 2:1 to 1:2. Difference in spectral absorption between gangliosides (540 nm) and glycoprotein, (e.g. IgG 630 nm) reduced the glycoprotein interference during assay. Phosphotungstic acid used in precipitation of LASA did not alter the absorption spectrum of LASA. Different recipes of chloroform:methanol seemed to extract different populations of gangliosides. A chloroform:methanol ratio 2:1 yields a population of gangliosides which were sensitive to periodate oxidation as opposed to the gangliosides extracted by lowering the chloroform:methanol to 1:2 ratio.

In conclusion, Katopodis method was technically valid. Though Katopodis method can indicate ganglioside levels that correctly differentiated the diseased from the non-diseased states, the best technique for producing accurate measurement has not yet been established. However, the assay sensitivity could be further enhanced by evaporating the lipid extract to dryness rather than by periodate oxidation. Since periodate oxidation caused break-down products of glycoproteins to interfere with assay outcome.