

A Microwell-Based Galactose Oxidase-Schiff's Assay for Thomsen-Friedenreich Antigen in Diagnosis of Lung Cancer

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Introduction. Thomsen-Friedenreich (TF/T) antigen (D-galactose- β -[1 \rightarrow 3]-N-acetyl-D-galactosamine; GalGalNAc) is a pan-carcinoma marker expressed in aberrantly-glycosylated mucins on the epithelial surface of cancer cells. TF antigen has been detected with plant lectins, monoclonal antibodies and the galactose oxidase-Schiff's (GOS) sequence. In the latter, GO oxidizes the C6 hydroxyls of the disaccharide to aldehydes which form a magenta-colored adduct with Schiff's reagent. The presence of marker is scored visually or quantified by measurement of the color attributes, hue or chroma. The GOS test has shown clinical utility as a screening procedure for colorectal cancer with specimens of rectal mucus in a solid-phase assay. Adaptation of this test to a liquid-based format would facilitate detection of soluble or secreted marker in fluid samples obtained by minimally- or non-invasive methods. Objective. To 1) assess the feasibility of a microwell GOS assay using a polymeric, high molecular-weight glycan as the galactose oxidase substrate and surrogate tumor marker, and 2) conduct an initial evaluation of its clinical performance with lung sputa.

Methods. We used guar, a galactomannan containing relatively high galactose content (approximately 30%), to develop and characterize the assay. Sputa were obtained from healthy volunteers, patients with benign lung disease (BLD) and patients diagnosed with lung cancer.

Results. The oxidized product of a solution of guar yields a magenta-colored derivative upon reaction with Schiff's that is similar to TF antigen and has an absorption maximum at 550 nm. Under the assay conditions, color development with Schiff's peaked around 30' and showed dose and time dependence with sequential addition of GO. At a GO activity of 2.5 U/100 μ L reaction volume and 30' stationary incubation at ambient temperature, the assay exhibited a linear dependence on guar concentration (0-25 μ g) with estimated detection limit of < 1 μ g polymer (< 2 nmoles galactose). Intra- and inter-assay imprecision were < 10 and < 15%, respectively. We tested the GOS assay on sputa from a small sample of 20 subjects comprised of 5 normal volunteers, 1 smoker, 2 patients with BLD and 12 with cancer (8 Stage I, 4 Stage II). Median absorbance values increased with severity of disease from normal subjects to patients with Stage II cancer. The difference between the patients with cancer and all others was statistically significant ($p = 0.04$). Analysis by the receiver-operator characteristic showed the discriminatory capability of the test (AUC = 0.78) in this limited data set. At a decision cutpoint intended to optimize both statistics, sensitivity was >80% and specificity >60%. Most notably, the test detected 7 of 8 early-stage cancers.

Conclusions. The traditional histological and more recent membrane-based (rectal mucus) GOS assay can be successfully formatted to test specimens directly in liquid phase. The data on sputa suggest that GOS has potential as a diagnostic test for lung cancer and that continued refinement of the procedure and large-scale testing are warranted. Furthermore, this simple procedure could find application in the diagnosis of other cancers from which fluids (breast) or mucus (colorectal, cervical, prostate) secretions can be obtained.

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