SENSITIVITY AND SPECIFICITY OF THE TRAP ASSAY (TELOMERASE) FOR THE DETECTION OF BLADDER CANCER. COMPARISON WITH OTHER ASSAYS.

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INTRODUCTION: Telomerase maintains telomere integrity and an absence of telomerase is associated with telomere shortening and somatic cell aging. Many human cancers, including bladder cancer have been found to have increased telomerase activity and this activity in urine has been used as a marker for the detection of bladder cancer. Here we compare the diagnostic sensitivity of the telomerase assay with various other tests used for the detection of the bladder cancer and discuss the possible analytical pitfalls.

MATERIALS AND METHODS: Voided urine specimens (N=280) were obtained from 265 patients immediately before cystoscopy for telomerase (TRAP-assay), BTA-Stat (Bard Diagnostic) hemoglobin dipstick (Hb-DS), and UroVysion (Abbott Laboratories) analysis. Sensitivity was determined for these assays on 75 of the 265 patients whose biopsy results showed urothelial carcinoma and specificity on 80 of the 265 patients who had no history of urothelial cancer and a negative cystoscopy result.

RESULTS: From most sensitive to least sensitive, the overall sensitivity of UroVysion, BTA-Stat, hemoglobin dipstick and telomerase was 81%, 78%, 74% and 46% respectively. Each of the first 3 tests was significantly more sensitive than the telomerase assay (p<0.05). The specificity of UroVysion, telomerase, BTA stat and Hb-DS was 96%, 91%, 74% and 51% respectively. UroVysion and telomerase were significantly more specific (p<0.01) than the BTA-Stat and hemoglobin dipstick assays, and all of the assays were more specific than Hb-DS testing (p<0.001).

DISCUSSION: Two methods have generally been utilized to detect telomerase, the TRAP assay and an RT-PCR assay which detects either the RNA (hTR) or reverse transcriptase (hTERT) components of the human telomerase. In this study, telomerase testing (using the TRAP assay) had good specificity (91%) but poor sensitivity (46%). The sensitivity observed for telomerase testing was significantly lower than a previous study by our group (Ramakumar et al.). Also other studies in the literature have shown a wide range of sensitivities for this assay and perhaps this is the reason why this assay has not found much clinical utility. The disadvantage of the TRAP assay, is that it detects the activity of the telomerase enzyme and not just the mere presence of it and therefore has a decreased sensitivity due to enzyme lability (see Fig 1). The pitfall of testing voided urine for telomerase is two folds. First the urine contains many sloughed off dead and degraded cells that have either lost or diminished enzyme activity. These cells are further subjected to insult from various compounds that are present in the urine which further degrades the
enzyme. This, has been discussed in detail by Müller. The second possible explanation for the lower sensitivity of this assay may be a result of a difference in sample collection and processing techniques. In the first study by our group, urine samples for telomerase analysis were immediately placed on ice and quickly processed (<2 hours) for analysis while in the current study, samples were collected at ambient temperature and processed anywhere from 2-6 hours later. Therefore the lower sensitivity in the current study may be due to the fact that the enzyme lost activity before sample processing.

The discussion about the sensitivities and specificities of these newer assays is beyond the scope of this abstract. In conclusion, even though increased telomerase activity is present in a very high percentage of bladder cancer, the TRAP assay for detection of this activity in the voided urine specimens is not very reliable.

**Fig 1. TRAP-assay. Lanes 1-3 is positive showing variable enzymatic activity. Lane 4 is negative.**

**REFERENCES**