Table 1: Characteristics of cases evaluated in the hTERT immunoassay

Sex	M/F	53/48	
Voided urine specimens		62	61.4%
Instrumented specimens		39	38.6%
Positive for malignancy		29	28.7%
	Urothelial carcinoma	25	
	Squamous cell carcinoma	3	
	Small cell carcinoma	1	
Negative for malignancy		39	38.6%
Atypical/undetermined		33	32.7%
Biopsy correlation available		56	55.5%

perature. Slides were counterstained with hematoxylin, dehydrated, and mounted for microscopical examination. Optimization of conditions was performed with sections of normal colon (hTERT-positive at the base [13] of the crypts) (Figure 1A) and tissue bladder biopsies with urothelial carcinoma (Figure 1B). Negative control slides included a blank control and omission of primary antibody.

Assessment of hTERT immunostaining

The entire hTERT stained slide was examined for immunostaining of hTERT. Positivity for hTERT expression was evaluated by two independent observers (WK, SG). Nucleolar urothelial cell positivity was used for evaluation of utility in diagnosis. Positivity in non-urothelial cells such as inflammatory cells, lymphocytes, microorganisms, or contaminating debris was duly noted and recorded. The availability of corresponding tissue bladder biopsy material, which is the gold standard for diagnosis or exclusion of urothelial carcinoma, enabled the determination of the sensitivity and specificity of hTERT immunostaining for the detection of bladder cancer. The positive and negative predictive values were determined as follows: *positive predictive* value is equal to the number of truly positive cases identified/total number of cases that tested positive × 100%, *negative predictive value* is equal to the number of truly negative cases identified/total number of cases that tested negative × 100%.

Table 2: Comparison of hTERT immunoassay results with cytological diagnosis.

	hTERT		
Cytological diagnosis	Positive	Negative	Total
Positive	27	2	29
Negative	3	36	39
Atypical	19	14	33
Total	49	52	101

Results

Optimization of the hTERT immunostaining technique, as described in Materials and Methods, revealed that specific hTERT expression was nucleolar in both proliferatively active normal cells, and in tumor cells. In normal colon crypts, staining intensity was concentrated in the base of the crypt (Figure 1A), as previously observed [13], where the basal cells with proliferative capacity reside. In excised bladder tumor tissue, the expression of hTERT was clearly evident in the multiple nucleoli of neoplastic cells (Figure 1B). Notably, some morphologically normal urothelia adjacent to the tumor border also expressed hTERT, but the immunoreactivity diminished and was lost in normal cells more distal to the tumor (Figure 1B). Diffuse nuclear staining accompanied nucleolar positivity in a few examples, but we did not observe any cytoplasmic staining. Lymphocytes were reactive to hTERT antibody, as were yeast when present (Figure 1C). Yeast is a common contaminant in clinical urine sampling and is known to contain the telomerase enzyme, so the presence of yeast can pose a serious confounding problem in urine-based assays. Using the advantage of histological identification facilitated using a hematoxylin counterstain, we classified patient samples as positive when the presence of specific nucleolar staining was evident in urothelial cells. Patient demographics and tumor characteristics of the samples evaluated are presented in Table 1. The 101 cases came from 53 male patients and 48 female patients. The average age of the patients were 62 years (ranges: 30–91 years). The majority of the specimens were voided urine specimens (61.4%). The majority of the urothelial carcinoma cases were grade III (13 cases) and grade II (8 cases), and one case grade I. The 39 non-malignant cases included diagnoses of urolithiasis, cystitis glandularis and cases with no detectable lesion. We also evaluated 33 cases classified as atypical by cytological examination. Tissue bladder biopsies were available in 56 cases for histological and cytological correlation.

All types of urothelial cancer expressed hTERT, including transitional cell carcinomas of low grade, high grade, carcinoma in situ (CIS), squamous cell carcinoma (Figures 1D–1G), small cell carcinoma and metastatic adenocarcinoma. Of the 29 cases cytologically diagnosed as malignant and confirmed by biopsy, 27 (93%) exhibited nucleolar hTERT expression (Table 2). One case of high-grade urothelial carcinoma and one case of squamous cell carcinoma did not display hTERT expression. Among the cytologically classified non-malignant cases, transitional cell hTERT expression was detectable in only 3 (<1%) of the 39 samples evaluated. One of the 3 false-positive cases was from a patient with confirmed diagnosis of urolithiasis (Figure 1J). The other 2 cases have no corroborative evidence of bladder disease to date. There were 56 cases (malignant, benign and atypical cytologically) on which