Evaluation of Sienna Cancer Diagnostics hTERT Antibody on 500 Consecutive Urinary Tract Specimens

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ABSTRACT

Introduction: Studies have detected telomerase activity in up to 90% of urothelial carcinomas (UC), and telomerase activity can be detected in urinary tract cytology (UTC) specimens, and indicating an increased risk of UC. hTERT is the catalytic subunit component of the telomerase ribonucleoprotein complex. The majority of UCs have mutations in the TERT promoter. We evaluated the performance of a commercially available antibody on 500 consecutive UTC specimens.

Methods: Unstained Cytospin™ preparations were created from residual urine specimens and were stained using the anti-hTERT Antibody (SCD-A7). Two algorithms were developed for concatenating the hTERT result and cytologic diagnosis: a "no indeterminates algorithm" in which a negative cytology and positive hTERT result is considered positive, and a "high specificity algorithm", in which a negative cytology and positive hTERT result is considered indeterminate (and thus negative for comparison to the gold standard).

Results: 410 specimens were considered to have interpretable hTERT stains; 297 of had sufficient follow up data to determine a gold standard diagnosis. The "no indeterminates algorithm" and "high specificity algorithm" yielded a sensitivity of 56.9% and 50.8%, a specificity of 68.5% and 89.2%, a positive predictive value of 56.9% and 33.6%, and a negative predictive value of 86.6% and 85.0%, respectively.

Conclusion: A positive hTERT result may identify a subset of patients with an increased risk of HGUC who may otherwise not be closely followed, while a negative hTERT ICC result is associated with a slight reduction in risk for HGUC.

MATERIALS AND METHODS

- Unstained Cytospin preparations created from leftover urine specimens
- Slides stained with anti-hTERT mouse monoclonal antibody (SCD-A7)
- Antibody interpretation by one board-certified cytopathologist (see figure for examples)
- Concurrent cystoscopy findings, concurrent and subsequent tissue biopsies, and subsequent UTC results considered gold standard for comparison
- Data analyzed with two algorithms, one "high specificity" and one "no indeterminates"

| Urine Cytology Result	hTERT ICC Result	Overall Urine Result
Atypical (AUC-US)	Negative	Negative
Atypical (AUC-US)	Positive	Positive
Negative (NUAM)	Negative	Negative
Positive (AUC-H or HGUC)	Positive	Positive
Positive (AUC-H or HGUC)	Negative	Positive
Negative	Positive	"Atypical" (Negative) or Positive*

Table 1. Final test interpretation for comparison to gold standard based on concatenation of urinary cytology specimen clinical diagnosis and hTERT result.

CONCLUSIONS

- hTERT antibody staining may help to identify a sub-population of patients with HGUC who could be missed by cytologic screening alone
- **Negative** hTERT staining is associated with a **decreased** risk of HGUC compared to cytology alone

Abbreviations: HGUC, high grade urothelial carcinoma; LGUC, low grade urothelial carcinoma; UTC, urinary tract specimen; NUAM, negative for urothelial atypia or malignancy; AUC-US, atypical urothelial cells of undetermined significance; AUC-H, atypical urothelial cells, cannot exclude HGUC.

RESULTS
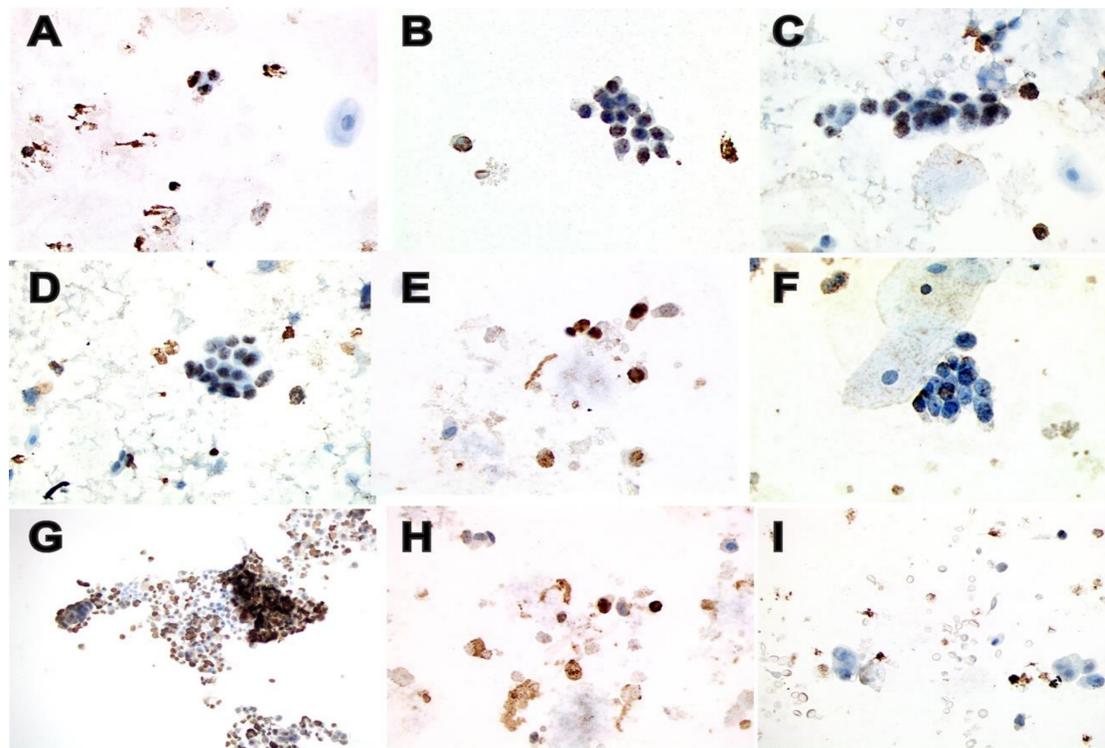
Figure 1. Examples of hTERT staining. A, A cluster of three cells (top center) stain **positive**; note the negative cell at the right side of the field. The corresponding diagnostic specimen was called "Negative for Urothelial Atypia or Malignancy"; the patient was subsequently found to have a low grade urothelial neoplasm. B, A cluster of atypical urothelial cells, the majority of which stain **positive**. The corresponding diagnostic specimen was called "Atypical Urothelial Cells of Uncertain Significance"; the patient was subsequently found to have high grade urothelial carcinoma. C-H, Additional specimens in which hTERT was interpreted to be **positive**. I, An example of a specimen containing high grade urothelial carcinoma cells in which hTERT was negative.
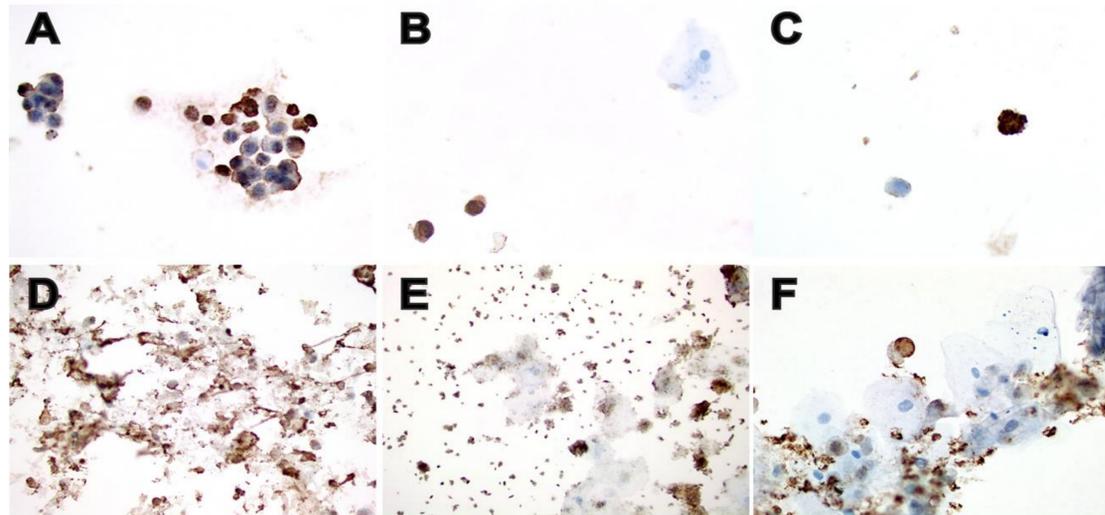
Figure 2. Examples of hTERT interpretation. A, Numerous malignant cells are seen, more than half demonstrating positive nuclear staining. The cells have recognizable and intact cytoplasm on the counterstain and are morphologically compatible with urothelial cells; thus it is appropriate to interpret them as hTERT positive. The presence of cytoplasmic staining in some of these cells does not invalidate the result. B, Two cells (lower left) have nuclear staining and recognizable, intact cytoplasm. The cells are morphologically compatible with urothelial cells; a mature squamous cell (upper right) acts as an internal negative control. C, A cluster of bacteria stain positively by hTERT and approximate the size of a urothelial cell nucleus. The stain was not interpreted as positive if a cell's cytoplasm was not clearly seen. D-F, Numerous positive-staining bacteria are present in the background and stuck to mature squamous cells. In cases where positive-staining inflammatory cells and/or bacteria obscured the preparation, the test was considered non-intepretable. F, Despite positive-staining bacteria at the bottom of the field, a positive urothelial cell is clearly seen in the center of the field. In such instances, cases could be interpreted as positive.

Cytology Alone

		Gold Standard			Test Statistics	
		Positive	Negative		Number	297
Urine Cyto	Positive	19	8	27	Sensitivity	29.2%
	Negative	46	224	270	Specificity	96.6%
					Likelihood Ratio for Positive Test (LR+)	8.48
		65	232		Likelihood Ratio for Negative Test (LR-)	0.73
					Positive Predictive Value (PPV)	70.4%
					Negative Predictive Value (NPV)	83.0%
					Diagnostic Accuracy	81.8%

Figure 3. Performance of cytology alone compared to the study's gold standard

High Specificity Algorithm

		Gold Standard			Test Statistics	
		Positive	Negative		Number	297
Cyto + hTERT	Positive	33	25	58	Sensitivity	50.8%
	Negative	32	207	239	Specificity	89.2%
					Likelihood Ratio for Positive Test (LR+)	4.71
		65	232		Likelihood Ratio for Negative Test (LR-)	0.55
					Positive Predictive Value (PPV)	56.9%
					Negative Predictive Value (NPV)	86.6%
					Diagnostic Accuracy	80.8%

Figure 4. Overall performance of cytology plus hTERT immunocytochemistry (ICC) compared to the study's gold standard. The "high specificity" algorithm considers a benign cytology specimen with a positive hTERT ICC result as indeterminate and thus as an overall negative result when compared to the gold standard.

No Indeterminates Algorithm

		Gold Standard			Test Statistics	
		Positive	Negative		Number	297
Cyto + hTERT	Positive	37	73	110	Sensitivity	56.9%
	Negative	28	159	187	Specificity	68.5%
					Likelihood Ratio for Positive Test (LR+)	1.81
		65	232		Likelihood Ratio for Negative Test (LR-)	0.63
					Positive Predictive Value (PPV)	33.6%
					Negative Predictive Value (NPV)	85.0%
					Diagnostic Accuracy	66.0%

Figure 5. Overall performance of cytology plus hTERT immunocytochemistry (ICC) compared to the study's gold standard. The "no indeterminates" algorithm considers a benign cytology specimen with a positive hTERT ICC result as an overall positive result for comparison to the gold standard.