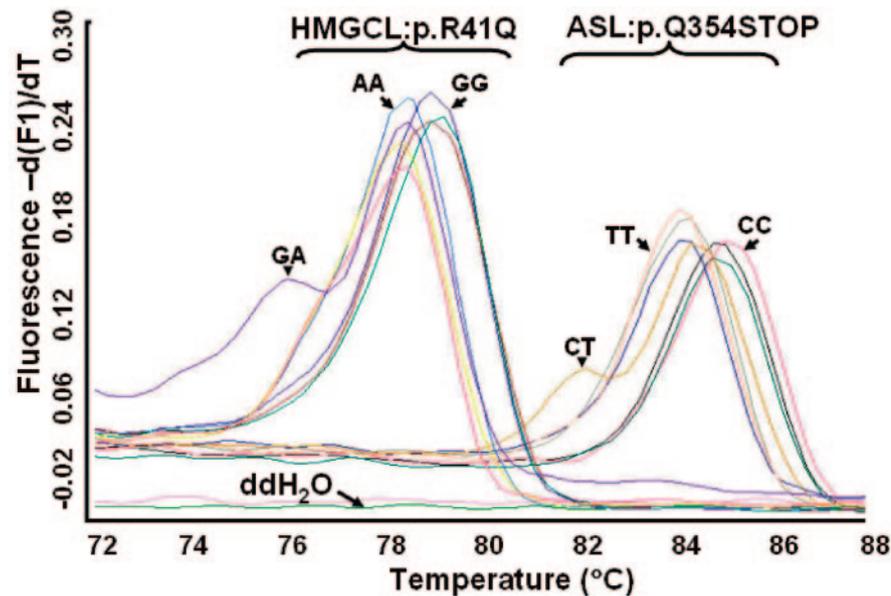Fig. 1. Derivative melting curve analysis for *HMGCL*:p.R41Q and *ASL*:p.Q354STOP.

LCGreen I assays were carried out in parallel. Triplicate representative traces are shown for each homozygous wild-type/variant genotype in each assay. One heterozygous genotype is also shown for each assay.

tion. These assays are readily applicable for small-scale analysis, inductive screening, and if necessary, for regional population studies. Caution must be exercised to limit false negatives by minimizing the gap between assay primers. Although specific base changes may be associated with melting signatures, confirmation requires sequencing of PCR products. We reduced the likelihood of false positives arising in this manner by ruling out the presence of sequence variants in the region being interrogated within the population under study. Nevertheless, in unknown populations, we recommend conducting sequencing in a limited number of individuals to verify the basis for melting curve profiles of the variants being screened.

References
1. Brusilow SW, Horwich AL. Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Bases of Inherited Disease, 8th ed. New York: McGraw-Hill, 2001; 1909–64.
2. Alsayed M, AlAhmed S, Alsmadi O, Khalil H, Rashed M, Imtiaz F, et al. Identification of a common novel mutation in Saudi patients with Argininosuccinic aciduria. J Inherit Metab Dis 2005;28:877–83.
3. Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. Semin Perinatol 1999;23: 183–93.
4. Faull K, Bolton P, Halpern B, Hammond J, Danks DM, Hahnel R, et al. Patient with defect in leucine metabolism [Letter]. New Engl J Med 1976;294:1013.
5. Ozand PT, Devol EB, Gascon GG. Neurometabolic diseases at a national referral center: five years experience at the King Faisal Specialist Hospital and Research Centre. J Child Neurol 1992;7(Suppl):S4–11.
6. Mitchell GA, Ozand PT, Robert M-F, Ashmarina L, Roberts J, Gibson KM, et al. HMG CoA lyase deficiency: identification of five causal point mutations in codons 41 and 42, including a frequent Saudi Arabian mutation, R41Q. Am J Hum Genet 1998;62:295–300.
7. Liew M, Pryor R, Palais R, Meadows C, Erali M, Lyon E, et al. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. Clin Chem 2004;50:1156–64.
8. Santalucia J. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. Proc Natl Acad Sci U S A 1998;95:1460–5.
9. Sugimoto N, Nakano S, Yoneyama M, Honda K. Improved thermodynamic parameters and helix initiation factor to predict stability of DNA duplexes. Nucleic Acids Res 1996;24:4501–5.

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Survivin and MKI67 mRNA Expression in Bladder Washings of Patients with Superficial Urothelial Cell Carcinoma Correlate with Tumor Stage and Grade but Do Not Predict Tumor Recurrence

To the Editor:

Seventy percent of patients with superficial urothelial cell carcinoma develop recurrences after transurethral resection of the primary tumor. Because recurrence prediction on the basis of tumor size, grade, and multiplicity is unreliable, all patients need regular bladder examination with cystoscopy. An accurate method to differentiate between patients with short or long recurrence-free periods might reduce the number of invasive cystoscopies in a subgroup of patients.

The gene *MKI67* (proliferation marker antigen identified by the monoclonal antibody Ki-67) and the antiapoptosis gene *survivin* are promising prognostic markers, because increased MKI67 and survivin mRNA concentrations indicate short recurrence-free periods (1–4). In addition to the presence of survivin mRNA in tumor tissue, we observed a relationship between early tumor recurrence and increased concentrations of survivin mRNA in bladder washings (5). Bladder washings are easily obtained during cystoscopy and generally reflect the molecular biological status of the urothelium (6,7).

To confirm our previous findings regarding survivin and to investigate the recurrence-predictive value of MKI67 mRNA, we collected 95 blad-
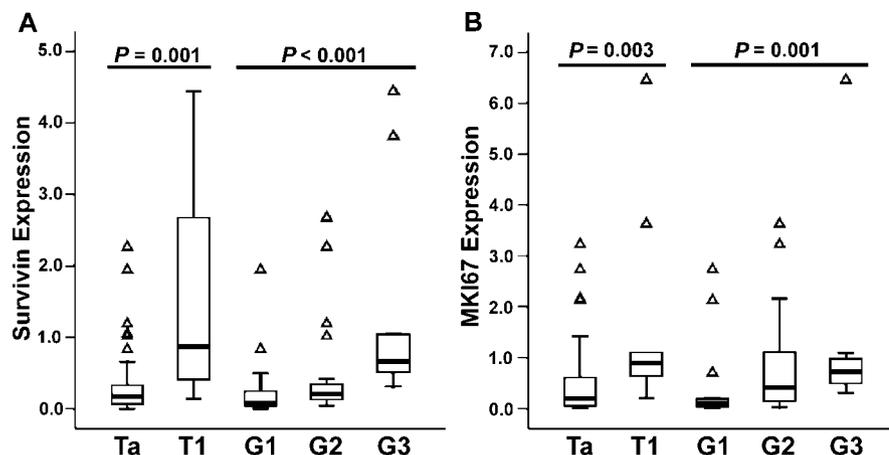
Fig. 1. Distribution of survivin (*A*) and MKI67 mRNA expression (*B*) in the 57 bladder washings.

The *boxes* represent the interquartile range. △ represent outliers. The median gene expression concentrations (*thick black horizontal bars*) are as follows (range in parentheses): survivin, Ta, 0.18 (0.002–2.27); T1, 0.87 (0.14–4.44); G1, 0.07 (0.002–1.95); G2, 0.21 (0.05–2.68); G3, 0.66 (0.31–4.44); MKI67, Ta, 0.20 (0.02–3.23); T1, 0.90 (0.21–6.5); G1, 0.10 (0.02–2.73); G2, 0.41 (0.02–3.63); G3, 0.72 (0.31–6.45). The survivin and MKI67 expression values were normalized with cyclophilin A and calculated using the formula $2^{-(Ct\ target\ -Ct\ cyclophilin\ A)}$.

der washings from a new cohort of 75 patients undergoing transurethral resection (TUR). Collection of the cells from the washings was performed as described previously *(5)*, except that the elaborate enrichment procedure for urothelial cells was omitted. RNA isolation and cDNA synthesis were carried out as described previously *(5)*. We investigated cDNA quality and quantity in all samples by measuring the mRNA expression of the housekeeping gene cyclophilin A with the cyclophilin A TaqMan® Endogenous Control reagent set (Applied Biosystems), according to the manufacturer's instructions. Expression data were analyzed with Sequence Detector, Ver. 1.9.1 (Applied Biosystems). We excluded 27 samples because the cyclophilin critical threshold (Ct) values were >34, indicating insufficient cDNA quality/quantity for gene expression analysis of survivin and *MKI67*.

We then evaluated pathology reports from the 68 remaining TUR cases and excluded 11 bladder washings because they were from patients who did not present with Ta or T1 urothelial cell carcinoma. The remaining group of 47 patients (57 washings) consisted of 36 men and 11 women (mean age, 67 years; range, 45–82 years; for additional data, see Table 1 in the Data Supplement that accompanies the online version of this letter at http://www. clinchem.org/content/vol52/issue7). During the follow-up period of 6 to 35 months, 29 patients suffered cancer recurrence, 12 within 1 month of evaluation. The recurrence-free period was recorded from the time of TUR.

We next measured survivin and MKI67 mRNA expression in the 57 bladder washings. The real-time quantitative-PCR conditions for survivin were as published previously *(3)*. MKI67 was measured with the MKI67 TaqMan Gene Expression Assay (Assay ID Hs00606991_m1; Applied Biosystems) according to the manufacturer's instructions. Survivin and *MKI67* gene expression data were normalized with the formula $2^{-(Ct\ target\ -\ Ct\ cyclophilin\ A)}$ *(8)*.

We investigated the correlation between survivin and MKI67 mRNA expression in the bladder washings with tumor stage and grade of the corresponding tissue, using the Mann–Whitney *U*-test (for stage) and the Kruskal–Wallis test (for grade). The mRNA expression of both genes showed a clear correlation with pathologic characteristics (Fig. 1).

To investigate the relationship between mRNA expression and the time to recurrence, we constructed Kaplan–Meier survival curves for patients with survivin or MKI67 mRNA expression below and above the median and used the log-rank test for comparison. Neither survivin nor MKI67 expression correlated with the recurrence-free period (*P* = 0.88 and 0.21, respectively). Of the variables tumor stage, grade, and multiplicity, only grade showed a relationship with the time to recurrence (*P* = 0.012).

In contrast to the present results, we previously had found a correlation between survivin mRNA expression in bladder washings and the recurrence-free interval *(5)*. In that study, bladder washings were enriched for urothelial cells to prevent interference of gene expression normalization by nonurothelial cells, especially leukocytes. In the present study, we omitted the elaborate enrichment step to simplify the procedure. This omission may have led to the loss of a correlation between survivin mRNA expression and the time to tumor recurrence. The lack of this correlation may also be related to the loss of a substantial number of bladder washings, which may have influenced the recurrence pattern of the patient cohort.

Despite the lack of a correlation with recurrence, we observed a correlation of survivin and MKI67 mRNA expression with the pathologic variables tumor stage and grade. Therefore, even in the absence of enrichment for urothelial cells, bladder washings provided an accurate profile of the molecular biological status of the urothelium. The independent prognostic value of tumor markers assessed in bladder washings needs further investigation.

References

1. Pich A, Chiusa L, Formiconi A, Galliano D, Bortolin P, Comino A, et al. Proliferative activity is the most significant predictor of recurrence in noninvasive papillary urothelial neoplasms of low malignant potential and grade 1 papillary carcinomas of the bladder. Cancer 2002;95:784–90.
2. Santos L, Amaro T, Costa C, Pereira S, Bento MJ, Lopes P, et al. Ki-67 index enhances the prognostic accuracy of the urothelial superficial bladder carcinoma risk group classification. Int J Cancer 2003;105:267–72.
3. Schultz IJ, Kiemeney LA, Witjes JA, Schalken JA, Willems JL, Swinkels DW, et al. Survivin mRNA expression is elevated in malignant urothelial

cell carcinomas and predicts time to recurrence. Anticancer Res 2003;23:3327–31.

4. Weikert S, Christoph F, Schrader M, Krause H, Miller K, Muller M. Quantitative analysis of survivin mRNA expression in urine and tumor tissue of bladder cancer patients and its potential relevance for disease detection and prognosis. Int J Cancer 2005;116:100–4.

5. Schultz IJ, Kiemeney LA, Karthaus HF, Witjes JA, Willems JL, Swinkels DW, et al. Survivin mRNA copy number in bladder washings predicts tumor recurrence in patients with superficial urothelial cell carcinomas. Clin Chem 2004;50:1425–8.

6. Acar H, Kilinc M, Yildirim MS, Kaynak M, Cenker A. Evaluation of chromosome 8 and 11 aneuploidies in washings and biopsy materials of bladder transitional cell carcinoma. Cancer Genet Cytogenet 2003;142:25–9.

7. Vet JA, Hessels D, Marras SA, van de Kaa CA, van der Poel HG, Michalides RJ, et al. Comparative analysis of p53 mutations in bladder washings and histologic specimens. Am J Clin Pathol 1998;110:647–52.

8. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C(T)}$ method. Methods 2001;25:402–8.

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Preanalytic Error Tracking in a Laboratory Medicine Department: Results of a 1-Year Experience

To the Editor:
Remarkable advances in technology, automation, and testing procedures have produced radical changes in laboratory organization, granting major precision and accuracy of test results. Nevertheless, errors occurring within the whole testing process still influence the quality of laboratory performance. There is heterogeneous information on the error rate within the whole laboratory testing process (from 0.1% to 9.3%) (1). Moreover, the frequencies and types of mistakes differ between one facility and another and between one time period and another (2). Process analysis has demonstrated that laboratory errors occur primarily in the preanalytic phase, influencing patient outcomes and costs (1–3). Compliance with systems of quality management, such as certification and accreditation, requires accurate procedures for identifying the processes that are more susceptible to errors (4).

The Laboratory Medicine Department of the University Hospital of Verona is a laboratory service providing stat and routine tests for clinical chemistry, hematology, coagulation, and immunology, serving an area with a population of 270 000 inhabitants. The laboratory serves a hospital with 750 beds and specialized care units. Inpatient phlebotomies are performed by clinical department staff, whereas blood specimens from outpatients are collected on site by laboratory personnel. After implementation of the new ISO accreditation guidelines, since October 2004 the laboratory staff has been trained to identify and systematically record each error encountered within the global testing process, particularly preanalytic mistakes. Venous blood samples for routine clinical chemistry (lithium heparin gel tubes), hematologic (EDTA tubes), coagulation (buffered sodium citrate tubes), electrophoresis, specific protein, and immunology testing (serum gel tubes) were considered unsuitable according to the following accepted criteria (5, 6): inappropriate volume (excess or deficit in the volume required to perform the analysis), wrong or missing patient identification, inappropriate container, visible hemolysis after centrifugation, clotting, and contamination from infusion route. The laboratory staff was provided with a notebook in which numbers of tubes and errors were recorded daily, and the quality referent of our laboratory systematically discussed and reviewed data on a weekly basis.

From October 2004 to September 2005, a total of 423 075 routine venous blood specimens (71 922 from outpatients; 17%) were received in the 5 more representative sections of our laboratory (130 806 for clinical chemistry testing, 113 699 for hematologic testing, 61 301 for coagulation testing, 59 403 for electrophoresis and specific protein testing, and 57 866 for immunology testing). According to the above specified criteria, 3154 (0.74%) preanalytic errors were identified and recorded in the 1-year observational period (Table 1). Errors were related to samples in which they were relevant. In agreement with earlier data (1, 7), we observed a significant difference in the error rate between inpatients and outpatients (0.82% vs 0.37%; χ^2 test, P <0.001). The most common mistakes could be traced to incorrect procedures for sample collection, including hemolysis and clotting. The prevalences of other types of errors were rather different between specimens collected from inpatients and outpatients. In particular, insufficient volume was a prevailing cause of unsuitable specimens for inpatient samples, whereas the prevalence of inappropriate containers was particularly high for outpatient specimens.

Current data on laboratory error rates are mixed (1). In a survey on outpatient phlebotomy success, most unsuitable samples resulted from hemolysis (18.1%), insufficient quantity (16.0%), and clotting (13.4%) (5). These data are comparable to those provided by additional investigations, which confirm that problems directly related to specimen collection are the first causes of preanalytic errors, especially hemolyzed, clotted, insufficient, and incorrect samples (1, 7–9). This investigation represents a logical extension of previous analyses focused on laboratory errors identified after collection of clinically questionable results in stat departments for a shorter period of time (2, 8) or involving limited areas of laboratory testing, such as clinical chemistry (7) and hematology (9).