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## Modified UroVysion scoring criteria increase the urothelial carcinoma detection rate in case of equivocal urinary cytology

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## 1 TITLE

2 Modified UroVysion scoring criteria increase the urothelial carcinoma detection rate in case  
3 of equivocal urinary cytology

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23 **RUNNING TITLE**

24 UroVysion and equivocal cytology

25

26 **KEYWORDS**

27 Cystoscopy; cytology; FISH; urothelial carcinoma; UroVysion

## 28 **ABSTRACT**

### 29 Aim

30 UroVysion<sup>®</sup> is a four-target fluorescence in situ hybridisation (FISH) technique to detect  
31 urothelial carcinoma (UC) in urinary cytology. The aim of this retrospective study is to  
32 investigate the UC detection rate of a modified UroVysion test in patients with equivocal  
33 urinary cytology. The modification comprised the addition of a cytological pre-screening  
34 technique and different evaluation criteria.

### 35 Methods & results

36 A thin layer slide was prepared of the residual urine sample of 82 patients with equivocal  
37 urinary cytology, pre-stained and pre-screened to confirm the presence of atypical urothelial  
38 cells. The same slide was used for the UroVysion test and scored according to different  
39 evaluation criteria. Results were compared with the outcome of cystoscopic and histologic  
40 findings.

41 UroVysion detected 68% of the UCs when the manufacturer's evaluation criteria were  
42 applied. In case of altered evaluation criteria, the sensitivity increased to 81% when at least  
43 one copy number change of a probe target was considered as a positive test. The specificity  
44 only decreased from 84% to 82%.

### 45 Conclusions

46 Our data suggest that the sensitivity of the UroVysion test can be increased with the addition  
47 of a cytological pre-screening technique prior to the UroVysion test and a modification of the  
48 UroVysion evaluation criteria.

49 **TEXT**

50 **INTRODUCTION**

51 Bladder cancer is the seventh most common cancer worldwide. It accounts for about 3.2% of  
52 all cancers, mostly urothelial carcinomas (UC). The highest prevalence of bladder cancer is  
53 observed in countries of the European Union and North America [1].

54 Bladder cancer is mostly detected by cystoscopy combined with urinary cytology. Cystoscopy  
55 is very successful in detecting low-grade and high-grade papillary tumours but may fail to  
56 identify carcinoma in situ (CIS) [2]. Cytology is a sensitive and specific examination for both  
57 high-grade papillary and flat lesions, but is less sensitive for detecting low-grade papillary  
58 lesions. Both techniques are thus complementary. Because cystoscopy is an invasive  
59 procedure and urinary cytology has a low sensitivity for low-grade tumours, additional tests  
60 emerged for the diagnosis of urothelial carcinoma (UC) on urine samples [3].

61 UroVysion<sup>®</sup> (UV (Abbott Molecular, Des Plaines, IL, USA)) is one of the most common used  
62 commercial UC markers [3]. It is a multi-target, multi-colour fluorescence in situ  
63 hybridisation (FISH) assay that detects aneusomy of chromosomes 3, 7 and 17, and loss of  
64 9p21. UV has an overall sensitivity of 72% and specificity of 83% [4], moreover, it remains  
65 sensitive in the follow-up of patients under Bacillus Calmette-Guérin (BCG) treatment [3, 5-  
66 7]. Recent studies suggested the use of more flexible evaluation criteria for the UV test  
67 leading to an increase of the sensitivity of the UV test.[8-9].

68 When the UV test was introduced at our department, we added a pre-screening technique to  
69 the test. A FISH test is normally performed on another slide than the original slide used for  
70 cytological screening. This second slide may not always contain the atypical cells present in  
71 the previous cytological slide. In order to prevent false negative results and to confirm the  
72 presence of atypical cells also in the second slide, the UV test was preceded by a pre-staining  
73 and cytological screening of this slide (figure 1).

74 In the current study we retrospectively investigated the sensitivity of the UV test in  
75 combination with the pre-screening method for the detection of UCs in patients with  
76 equivocal urinary cytology. Additionally, the impact of different evaluation criteria [8-9] on  
77 the sensitivity of the UV test was examined.

78

## 79 MATERIAL AND METHODS

### 80 **Patient population**

81 In this study we retrospectively included patients with equivocal urinary cytology for which  
82 UV tests and follow up examinations were performed. Data were collected from December  
83 2005 till May 2009 (Table 1). Equivocal cytology was defined as cytology with a diagnosis  
84 that was neither clearly negative nor clearly positive for malignancy. The study group  
85 contained patients in the follow-up for UC, who all received intravesical chemotherapy or  
86 BCG, as well as patients without a history of UC. Cystoscopy and histology were used as  
87 'gold standards' to evaluate the presence of UC. The histology was evaluated and graded in  
88 accordance with the 1999 World Health Organization classification. The tumour stage was  
89 assigned according to the TNM classification, 6<sup>th</sup> edition. Cystoscopy was regarded as  
90 positive in case of an unequivocal tumour mass. In this study patients without information  
91 about a consecutive cystoscopy or biopsy, as well as patients with FISH specimens lacking  
92 the atypical cells were not included.

93

### 94 **Urinary cytology**

95 The urine samples were fresh voided (n=63), obtained through bladder washings (n=8),  
96 bladder (n=7) or ureter (n=4) catheterizations and collected in 50% ethanol. They were poured  
97 in a 50 ml conical centrifuge tube and centrifuged at 1000 rpm during 10 min. The cell pellet  
98 was added to 5 ml Cytosrich<sup>®</sup> Blue (BD Diagnostics TriPath, Burlington, NC, USA). Slides for

99 cytologic evaluation were made using the thin layer technique (BD Diagnostics Prepstain®  
100 slide processor, Burlington, NC, USA) and stained with the Papanicolaou stain.

101

## 102 **UroVysion FISH**

### 103 *Pre-screening technique*

104 A second thin layer preparation was stained with haematoxylin for cytological pre-screening.  
105 Atypical cells were scored using a Leica DM5000B microscope in bright field mode and their  
106 location annotated by coordinates. Cases with slides that contained no or a too low number of  
107 atypical cells **to successfully score the UV evaluation criteria (see further)** were not included  
108 in the study. The pre-screening technique was tested prior to the study and resulted in good  
109 quality of hybridization on the pre-stained specimens.

### 110 *FISH*

111 After cytological pre-screening, the slides were heated at 65°C for 30 min and immersed in a  
112 pepsin solution (500 mg/l, 10mM HCl), for 7 min. After washing in phosphate-buffered saline  
113 (PBS) cells were postfixed in 1% formaldehyde with 20mM MgCl<sub>2</sub> in PBS for 5 min,  
114 dehydrated in 70%, 96% and 2 X 100% ethanol and air dried. Six µl UroVysion probe  
115 mixture, containing probes for the chromosome 3, 7 and 17 centromeric regions and the 9p21  
116 locus labelled with SpectrumRed, SpectrumGreen, SpectrumAqua and SpectrumGold  
117 respectively, was administered under a coverslip of 18 x 18 mm. Cellular and probe DNA  
118 were denatured at 73°C for 2 min in a ThermoBrite® system (Abbott Molecular, Des Plaines,  
119 IL, USA). Posthybridisation washings were made in 0.4x SSC/0.3% 4-  
120 nonylphenolpolyethyleneglycol (NP-40, Abbott Molecular, Des Plaines, IL, USA) for 2 min  
121 at 73°C. The slides were rinsed in 2x SSC/0.1% NP-40 at room temperature for 2 min, air  
122 dried , and counterstained with 4,6-diamidine,2-phenylindole dihydrochloride (DAPI)  
123 solution. Next, the slides were examined using the Leica DM5000B microscope with



124 appropriate fluorochrome filter sets and evaluation included the atypical cells at the annotated  
125 coordinates. The UV test was considered positive if 1) four or more cells out of a minimum of  
126 25 morphologically abnormal cells demonstrated polysomy in two or more chromosomes (3,  
127 7 and 17) in the same cell and/or if at least 12 morphologically abnormal cells demonstrated  
128 homozygous loss of 9p21 (= UV1 criteria, according to Abbott Molecular); or 2) at least one  
129 chromosomal gain (3, 7 or 17) was detected in four or more cells out of a minimum of 25  
130 morphologically abnormal cells and/or if at least 12 morphologically abnormal cells  
131 demonstrated heterozygous or homozygous loss of 9p21 (= UV2 criteria).

132

### 133 **Statistics**

134 The chi-square test was used to evaluate the relation between the test results (UV and  
135 cystoscopy) and the presence of UC. All tests were 2-tailed with  $p < 0.05$  considered  
136 statistically significant. Statistical analysis was performed with SPSS<sup>®</sup> for Windows, version  
137 15.0.

138

### 139 **RESULTS**

140 Table 1 shows the patient characteristics including the number of the cystoscopic  
141 examinations, performed simultaneously with or within three months after the urine  
142 collection, and histologic evaluations, executed within an average of four months. Thirty-one  
143 out of the 82 patients revealed a UC on histology and / or cystoscopy. In seven out of 31  
144 patients with UC no biopsy was taken because of clearly presence of tumour on cystoscopy.  
145 In six of these patients, there was already a prior history of UC.

146 The application of the UV1 criteria resulted in an overall sensitivity of 68% . Using the UV2  
147 criteria the sensitivity increased to 81%, while the specificity only decreased from 84% to  
148 82%. Very high sensitivity scores were realised when combining UV and cystoscopy (Table

149 2).

150 Sensitivity and specificity of the UV test in follow-up patients, who all received intravesical  
151 chemotherapy or BCG, were 75% and 84%, respectively, using the UV1 criteria, in contrast  
152 with 88% and 81%, applying the UV2 criteria. For patients without a history of UC, UV1  
153 criteria resulted in a sensitivity and specificity of 60% and 84%, respectively, compared to  
154 73% and 84% in case of applying UV2 criteria.

155 Cystoscopic evaluation alone had a sensitivity of 65%: 11 out of 62 patients with negative  
156 cystoscopies revealed a UC on biopsy. In 7/11 cases the UC was located in the upper urinary  
157 tract (UUT). The other four cases were three CIS lesions and one grade 3 UC of the bladder.  
158 Out of 20 UCs detected on cystoscopy, the UV test was negative in six or seven cases,  
159 depending on using the UV2 or UV1 criteria.

160 When applying the UV1 criteria, UV detected 0/3 grade 1 UCs and only 1/5 grade 2 UCs,  
161 while cystoscopy was positive in 3/3 grade 1 UCs and 3/5 grade 2 UCs (Table 3). For grade 3  
162 lesions and carcinoma in situ (CIS) UV was positive in respectively 10/12 and 4/4 lesions,  
163 while cystoscopy detected six grade 3 lesions and only one CIS. It is important to mention  
164 that of the cystoscopically missed malignancies, all two grade 2 lesions and 5/6 grade 3  
165 lesions were located in the UUT. Regarding the relationship between tumour stage, UV and  
166 cystoscopy, there are no significant differences between the detection rates of UV and  
167 cystoscopy (Table 3). Exceptions are T3 tumours where none of the lesions were detected by  
168 cystoscopy, however these tumours were UUT tumours.

169 Of the 31 patients with a proven UC, chromosomal alterations were found in 25 cases. Gain  
170 of chromosome 3 (18/31), 7 (19/31) and/or 17 (15/31) was noted more frequently than  
171 heterozygous (4/31) or homozygous (9/31) deletions of locus 9p21. Interestingly, all CIS  
172 lesions had a gain of chromosome 3, 7 and 17. Five patients had chromosomal alterations that  
173 did not fit the UV1 criteria: one patient with an isolated gain of chromosome 7, three patients

174 with an isolated heterozygous deletion of 9p21 and one patient with an isolated heterozygous  
175 deletion of 9p21 combined with a solitary gain of chromosome 3.  
176 In the patients with UUT tumours, the UV test was positive in 5/7 cases with the UV1 criteria  
177 and in 7/7 cases with the UV2 criteria. Chromosomal alterations in the UUT tumours were not  
178 significantly different from the bladder UCs.

179

## 180 DISCUSSION

181 Currently, cystoscopy and urinary cytology are routinely used for the detection of tumours in  
182 the urinary tract. However, on cytology, it is sometimes difficult to discriminate between  
183 reactive and malignant atypical urothelial cells. Urothelial cells can show reactive atypia due  
184 to instrumentation, inflammation, infection, calculi, radiotherapy and intravesical BCG or  
185 chemotherapy [10-14]. Atypical findings are problematic because they raise concerns with the  
186 patient and physician about the possible presence or recurrence of cancer [15]. The UV test,  
187 however, can be a helpful additional tool in those cases [16, 17].

188 The present study evaluates the combination of the UV test with a cytological pre-screening  
189 on the same slide. This method allows us to link cytological observed cellular atypia with  
190 chromosomal alterations. It can be difficult to select the atypical cells in a FISH specimen in  
191 the case of equivocal urine cytology. The pre-screening technique however can facilitate this.

192 When gain of at least one chromosome (3, 7 or 17) and/or heterozygous or homozygous  
193 deletion of 9p21 (UV2 criteria) were interpreted as a positive UV test, 81% of the  
194 malignancies were detected compared to 68% when applying the manufacturer's criteria  
195 (UV1 criteria) with only a slight decrease in specificity (2%). Our data indicate that in case of  
196 equivocal urinary cytology the use of more flexible criteria for UV positivity substantially  
197 increases the number of detected UCs. This suggests that the current scoring criteria may be  
198 too restrictive since some urothelial carcinomas have chromosomal abnormalities (gains of

199 single chromosomes or heterozygous 9p21 deletions) that are undetected by the  
200 manufacturer's criteria.

201 Until now, no study has compared the current manufacturer's UV criteria with more flexible  
202 criteria in urine specimens of patients with equivocal cytology by using a cytological pre-  
203 screening technique in combination with the UV test. Ferra et al [9] also compared more  
204 flexible evaluation criteria, i.e. any cytogenetic abnormality regardless of the number of cells  
205 is a positive result, with the manufacturer's criteria in equivocal urinary cytology cases. With  
206 a sensitivity of 68% and 83% by using respectively the manufacturer's criteria and the  
207 flexible criteria, their results are almost identical to ours. Nevertheless, their specificity  
208 dropped from 40% to 22%, while we maintained a specificity of more than 80%. This  
209 difference might be explained by the pre-screening technique we used, as it allows us to better  
210 allocate the exact location of the atypical cells before application of the UV test, thereby  
211 excluding that tetrasomic cells, such as umbrella cells or dividing cells, are scored positive [8,  
212 18]. Also the number of the present atypical cells and cells with cytogenetic abnormalities  
213 needs to be high enough to maintain a high specificity.

214 Furthermore, in a recently published analysis of 14 studies involving 2477 UV tests (using  
215 UV1 criteria) the pooled sensitivity and specificity was 72% and 83% [4]. The overall  
216 prevalence of UCs in the examined patient groups was 35%, almost identical to the 36% in  
217 our study. In these studies the UV test was not only performed on equivocal urine specimens,  
218 but also on cytologic clear negative or positive cases. The fact that the sensitivity and  
219 specificity scores of these studies are comparable with our UV1 data, indicates that the pre-  
220 screening technique we use to trace atypical cells, leads to a more effective application of the  
221 UV test.

222 The UV test in our series detected 7 UUT tumours, most of them in spontaneously voided  
223 urine specimens. This finding suggests that the UV test can be helpful in cases of equivocal

224 cytology and negative findings on cystoscopy, which is in agreement with other studies [19-  
225 21].

226 Furthermore, our results show that the UV test and cystoscopy are complementary techniques,  
227 achieving extremely high sensitivity rates when both are executed (Table 2).

228 In accordance with other reports [3, 5-7] this study also confirms that the UV test can be  
229 performed on urine specimens of follow-up patients, who received intravesical therapy.

230 Sensitivity scores in our study are even higher in this population than in those without a  
231 tumour history.

232 There are some hypotheses for the false negative UV cases we encountered. Firstly, UCs in  
233 these cases may have other chromosomal alterations than those detectable by UV. Secondly,  
234 it is also known that low grade UCs are mostly diploid tumours [22, 23] and this may explain  
235 the false negative results, since most of our false negative UV cases were low grade tumours.

236 What to do with a positive UV test without detectable UC? In this regard, a positive UV test  
237 has been shown to be predictive of a future recurrence [8, 24-25] as it may be an indicator of  
238 unstable urothelium capable of or primed for malignant transformation, thus detecting patients  
239 at significant risk [26]. However, tetrasomic cells, mostly umbrella cells or dividing cells, can  
240 be present in urine samples and should not be interpreted as FISH-positive, malignant cells [8,  
241 18].

242 In summary, our results suggest that a modification of the standard UV evaluation criteria on  
243 pre-screened preparations can result in a higher detection rate of UCs in patients with  
244 equivocal urinary cytology. Future prospective, blinded studies may help to validate our  
245 results in a higher number of patients and on a diversity of specimens (voided urine  
246 specimens, bladder washings, bladder and ureter catheterizations).

## 247 REFERENCES

248 [1]Eble J, Sauter G, Epstein J and Sesterhenn I. WHO Classification of Tumors. Pathology  
249 and genetics of tumors of the urinary system and male genital organs. Lyon, France: IARC  
250 Press; 2004. p. 93.

251 [2]Schmidbauer J, Witjes F, Schmeller N, Donat R, Susani M, Merberger M. Improved  
252 detection of urothelial carcinoma in situ with hexaminolevulinate fluorescence cystoscopy. J  
253 Urol 2004;171:135-8.

254 [3] Têtu B. Diagnosis of urothelial carcinoma from urine. Mod Pathol 2009;22:S53-9.

255 [4] Hajdinjak T. Urovysion FISH test for detecting urothelial cancers: meta-analysis of  
256 diagnostic accuracy and comparison with urinary cytology testing. Urol Oncol 2008;26:646-  
257 51.

258 [5] Kipp B, Karnes R, Brankley S, et al. Monitoring intravesical therapy for superficial  
259 bladder cancer using fluorescence in situ hybridization. J Urol 2005;173:401-4.

260 [6] Mian C, Lodde M, Comploj E, et al. The value of the ImmunoCyt/uCyt+ test in the  
261 detection and follow-up of carcinoma in situ of the urinary bladder. Anticancer Res  
262 2005;25:3641-4.

263 [7] Savic S, Zlobec I, Thalmann G, et al. The prognostic value of cytology and fluorescence  
264 in situ hybridization in the follow-up of nonmuscle – invasive bladder cancer after  
265 intravesical Bacillus Calmette-Guérin therapy. Int J Cancer 2009;124:2899-904.

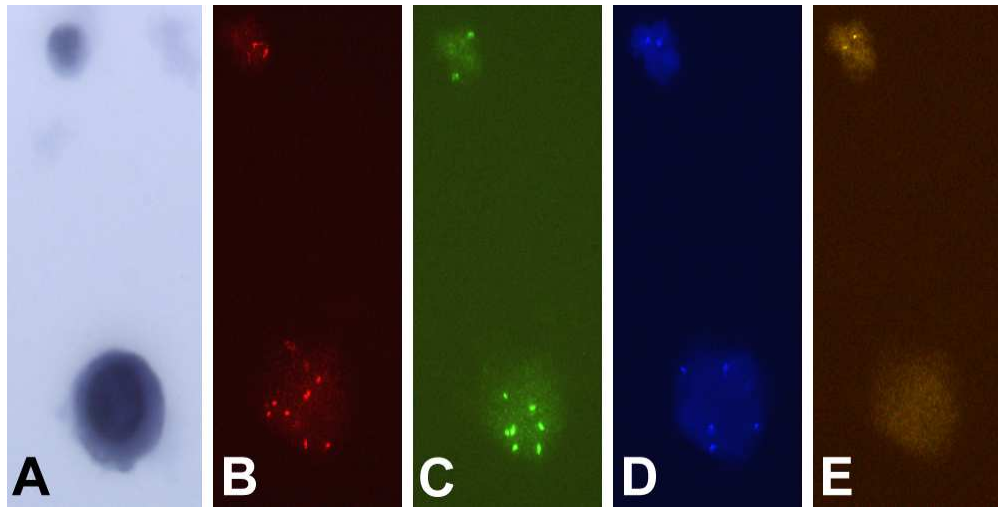
- 266 [8] Bubendorf L, Grilli B, Sauter G, Mihatsch M, Gasser T, Dalquen P. Multiprobe FISH for  
267 enhanced detection of bladder cancer in voided urine specimens and bladder washings. *Am J*  
268 *Clin Pathol* 2001;116:79-86.
- 269 [9] Ferra S, Denley R, Herr H, Dalbagni G, Jhanwar S, Lin O. Reflex UroVysion testing in  
270 suspicious urine cytology cases. *Cancer Cytopathol* 2009;117:7-14.
- 271 [10] Desphande V, McKee GT. Analysis of atypical urine cytology in a tertiary care center.  
272 *Cancer Cytopathol* 2005;105:468-75.
- 273 [11] Garbar C, Mascaux C, Wespes E. Is urinary tract cytology still useful for diagnosis of  
274 bladder carcinomas? A large series of 592 bladder washings using a 5-category classification  
275 of different cytological diagnoses. *Cytopathology* 2007;18:79-83.
- 276 [12] Highman W, Wison E. Urine cytology in patients with calculi. *J Clin Pathol*  
277 1982;35:350-6.
- 278 [13] Kannan V, Gupta D. Calculus artifact. A challenge in urinary cytology. *Acta Cytol*  
279 1999;43:794-800.
- 280 [14] McVey RJ, Persad RV, Brown CWS, Robinson E, Payne SR. How long is urinary  
281 cytology abnormal after flexible cystoscopy. *BJU Int* 2004;94:548-51.
- 282 [15] Nabi G, Greene DR, O'Donnell M. How important is urinary cytology in the diagnosis of  
283 urological malignancies? *Eur Urol* 2003;43:632.
- 284 [16] Kipp B, Halling K, Champion B. Assessing the value of reflex fluorescence in situ  
285 hybridization testing in the diagnosis of bladder cancer when routine cytological examination  
286 is equivocal. *J Urol* 2008;179:1296-1301.

- 287 [17] Lotan Y, Bensalah K, Ruddell T, Shariat S, Sagalowsky A, Ashfaq R. Prospective  
288 evaluation of the clinical usefulness of reflex fluorescence in situ hybridization assay in  
289 patients with atypical cytology for the detection of urothelial carcinoma of the bladder. *J Urol*  
290 2008;179:2164-9.
- 291 [18] Halling K, Kipp B. Bladder cancer detection using FISH (UroVysion assay). *Adv Anat*  
292 *Pathol* 2008;15:279-86.
- 293 [19] Akkad T, Brunner A, Pallwein L, et al. Fluorescence in situ hybridization for detecting  
294 upper urinary tract tumors – a preliminary report. *Urology* 2007;70:753-7.
- 295 [20] Luo B, Li W, Deng CH, et al. Utility of fluorescence in situ hybridization in the  
296 diagnosis of upper urinary tract urothelial carcinoma. *Cancer Genet Cytogenet* 2009;189:93-7.
- 297 [21] Marin-Aguilera M, Mengual L, Ribal MJ, et al. Utility of fluorescence in situ  
298 hybridization as a non-invasive technique in the diagnosis of upper urinary tract urothelial  
299 carcinoma. *Eur Urol* 2007;51:409-15.
- 300 [22] Kipp B, Tanasescu M, Else T, et al. Quantitative fluorescence in situ hybridization and  
301 its ability to predict bladder cancer recurrence and progression to muscle-invasive bladder  
302 cancer. *J Mol Diagn* 2009;11:148-54.
- 303 [23] Richter J, Jiang F, Gorog JP, et al. Marked genetic differences between stage pTa and  
304 stage pT1 papillary bladder cancer detected by comparative genomic hybridization. *Cancer*  
305 *Res* 1997;57:2860-64.
- 306 [24] Skacel M, Fahmy M, Brainard J, et al. Multitarget fluorescence in situ hybridization  
307 assay detects transitional cell carcinoma in the majority of patients with bladder cancer and  
308 atypical or negative urine cytology. *J Urol* 2003;169:2101-5.



- 309 [25] Yoder B, Skacel M, Hedgepeth R, et al. Reflex UroVysion testing of bladder cancer  
310 surveillance patients with equivocal or negative urine cytology: a prospective study with  
311 focus on the natural history of anticipatory positive findings. *Am J Clin Pathol* 2007;127:295-  
312 301.
- 313 [26] Veeramachaneni R, Nordberg M, Shi R, Herrera G, Turbat-Herrera E. Evaluation of  
314 fluorescence in situ hybridization as an ancillary tool to urine cytology in diagnosing  
315 urothelial carcinoma. *Diagn Cytopathol* 2003;28:301-7.

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Figure 1. The coordinates of the atypical urothelial cell (A, under) on a haematoxylin pre-stained preparation are annotated during prescreening. Note the neutrophilic granulocyte (A, above). After fluorescence in situ hybridization the same atypical urothelial cell shows 7 copies of the centromeric region of chromosome 3 labeled with the fluorophore SpectrumRed (B), 7 copies of the centromeric region of chromosome 7 labeled with SpectrumGreen (C), 5 copies of the centromeric region of chromosome 17 labeled with SpectrumAqua (D) and homozygous loss of 9p21 labeled with SpectrumGold (E), while the neutrophilic granulocyte keeps 2 copies of each locus.

## 1 Table 1: Patient characteristics

Number of patients	82
<ul style="list-style-type: none"> <li>• No history of UC</li> <li>• History of UC <ul style="list-style-type: none"> <li>○ Bladder UC</li> <li>○ Ureter UC</li> <li>○ Bladder + ureter UC</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• 34</li> <li>• 48 <ul style="list-style-type: none"> <li>○ 45</li> <li>○ 1</li> <li>○ 2</li> </ul> </li> </ul>
Patients with cystoscopic evaluation	82
Patients with histologic evaluation	36
Male/female	4.1/1
Median $\pm$ SD patient age (years)	70 $\pm$ 11.4

## 2 UC: urothelial carcinoma

Table 2: Cystoscopy and UV test performance characteristics for UC detection in patients with equivocal cytology specimens (n = 82 patients)

Examination	Sens	Spec	PPV	NPV	p
Cystoscopy	65% (20/31)	NA	NA	82% (51/62)	p<0.0001
UV1	68% (21/31)	84% (43/51)	72% (21/29)	81% (43/53)	p<0.0001
UV2	81% (25/31)	82% (42/51)	74% (25/34)	88% (42/48)	p<0.0001
UV1/cysto+	90% (28/31)	84% (43/51)	78% (28/36)	94% (43/46)	p<0.0001
UV2/cysto+	100% (31/31)	82% (42/51)	78% (31/40)	100% (42/42)	p<0.0001

UV: UroVysion® / UC: urothelial carcinoma

Sens: sensitivity / Spec: specificity

PPV: positive predictive value / NPV: negative predictive value

UV1: UV test positive according to the strict manufacturer's criteria

UV2: UV test positive when polysomy in at least one chromosome (3, 7 or 17) and/or heterozygous or homozygous deletion of 9p21

UV1/cysto+: UV test positive according to the strict manufacturer's criteria and/or cystoscopy positive

UV2/cysto+: UV test positive when polysomy in at least one chromosome (3, 7 or 17) and/or heterozygous or homozygous deletion of 9p21 and/or cystoscopy positive

NA: not applicable, since cystoscopy was used as gold standard in case of no histology control

1 Table 3: Relationship between tumour grade, stage, cystoscopy and UV test

Patients with equivocal cytology and histology-proven UC (n = 24 patients)								
	G1 (n=3)	G2 (n=5)	G3 (n=12)	CIS (n=4)	Ta (n=15)	T1 (n=3)	T2 (n=1)	T3 (n=5)
Cysto-	0	2 <sup>a</sup>	6 <sup>b</sup>	3	5 <sup>c</sup>	1 <sup>d</sup>	0	5 <sup>e</sup>
Cysto+	3	3	6	1	10	2	1	0
UV-	3	1	1	0	5	0	0	0
UV1	0	1	10 <sup>b</sup>	4	8 <sup>c</sup>	3 <sup>d</sup>	1	3 <sup>f</sup>
UV2	0	4 <sup>a</sup>	11 <sup>b</sup>	4	10 <sup>c</sup>	3 <sup>d</sup>	1	5 <sup>e</sup>
UV1/cysto+	3	3	11 <sup>b</sup>	4	14 <sup>c</sup>	3 <sup>d</sup>	1	3 <sup>f</sup>
UV2/cysto+	3	5 <sup>a</sup>	12 <sup>b</sup>	4	15 <sup>c</sup>	3 <sup>d</sup>	1	5 <sup>e</sup>

2 **UC**: urothelial cell carcinoma / CIS: carcinoma in situ

3 <sup>a</sup>: incl 2 pyelum **UCs** / <sup>b</sup>: incl 2 ureter **UCs** and 3 pyelum **UCs** / <sup>c</sup>: incl 1 pyelum **UC** / <sup>d</sup>: incl 1

4 ureter **UC** / <sup>e</sup>: incl 1 ureter **UC** and 4 pyelum **UCs** / <sup>f</sup>: incl 1 ureter **UC** and 2 pyelum **UCs**

5 Cysto-: cystoscopy negative / Cysto+: cystoscopy positive

6 UV-: UV test without chromosomal alterations

7 UV1: UV test positive according to the strict manufacturer's criteria

8 UV2: UV test positive when polysomy in at least one chromosome (3, 7 or 17) and/or

9 heterozygous or homozygous deletion of 9p21

10 UV1/cysto+: UV test positive according to the strict manufacturer's criteria and/or cystoscopy  
11 positive

12 UV2/cysto+: UV test positive when polysomy in at least one chromosome (3, 7 or 17) and/or  
13 heterozygous or homozygous deletion of 9p21 and/or cystoscopy positive

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 Contributor address: Kwademeerslaan 19 8531 Hulste BELGIUM  
 Manuscript number (if known): HISTOP-08-10-0444  
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