The Diagnostic Reliability of Urinary Cytology:
A Retrospective Study

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The aim of this study was twofold. The first aim was to estimate the diagnostic reliability of urinary cytology for detection and management of urothelial neoplasms by using a specific preserving fluid for sample collection, and the liquid-based thin layer method for specimen preparation, the estimate was based on the correlation between the cytological findings of 10,000 non-hospitalized patients, and their histological diagnoses. A second aim was to compare the reliability of two instruments for thin-layer preparation, i.e., TP2000, TP3000, capable of processing the specimens at very different rates. The preservation of cell structure is ameliorated by the procedure of sample collection and treatment here described. This allows a more accurate reading of LBC slides as shown by: (a) the significant concordance between cytological and histological diagnosis (92%); (b) the significant number of low-grade urothelial carcinomas (20.5%) revealed by urinary cytology and validated by histologic diagnosis; (c) the low rate (8%) of misjudgement of cytological diagnosis reached in this study. The quality of performances of the two instruments tested for thin-layer preparation, i.e., TP2000 and TP3000, is statistically comparable. We recommend the procedure that makes use of preserving fluid for sample collection (cytolyt®) and treatment (preservcyt ™) as here described. We also recommend the use of thin-layer method for specimen preparation since it allows a more uniform distribution of the cells on the support with reduction of overlapping phenomena. Finally, economic considerations suggest the preferential use of Thin Prep 3000. Diagn. Cytopathol. 2012;40:608–614.

Key Words: urine cytology; collection fluids; thin-layer technique; ThinPrep2000; ThinPrep3000

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Since many years, urinary cytology is largely used in daily practice of pathology as a method of choice for non-invasive detection and management of neoplastic lesions of urothelial tract.[1–5] The method is diagnostically accurate for high-grade transitional cell neoplasms, but it is much less specific and reliable in the case of low grade urothelial neoplasms.[4–7] This relevant limitation is the result of adverse factors including the cell morphology of low grade urothelial neoplasms, the poor preservation of cell integrity by the use of conventional procedure for urine sample collection and delivery and the artifacts due to specimen preparation methods. In fact, disaggregated cells from low-grade urothelial neoplasms lack easily recognizable morphological features indicative of neoplastic transformation.[8] On the other hand, the possibility of an accurate cytological diagnosis depends chiefly on the preservation of morphological characteristics of the cells, that in turn depends largely on the procedures for collection and treatment of urine samples. As a matter of fact, a good preservation of cell integrity is not guarantied by conventional procedure for collection of urine samples since the samples are collected and transported to the laboratory without any treatment, and thus the cell preservation depends largely on the mode and time of transport. It is recently reported that this problem could be removed by mixing the urine samples with preserving fluids immediately after collection, and by keeping them at $\sim 3°C$.[7,8] The composition and the chemical activity of the preserving fluid used may have some relevance as to the quality of the results. Substantial limitations of the diagnostic accuracy may also derive from artifacts resulting from the specimen preparation methods that may greatly hinder the resolution of reading and implicitly the quality of observations. Reports from literature suggest that these difficulties could be surmounted by a thin layer preparation procedure. This method denoted "liquid-based cytology thin prep method"[8–10] allows the formation of a well dispersed thin layer of cells on the support. The more uniform and

random distribution of the cell thus obtained may reduce or even eliminate the artifacts due to cell overlapping. Since a number of years, this laboratory has applied systematically these improved technique and method in urinary cytology: here we report a detailed analysis of the results obtained.

The aim of this study was twofold. The first aim was to establish the diagnostic accuracy of urine cytology when performed under the conditions here described, i.e., the use of a specific preserving fluid for sample collection and delivery (Cytolyt solution Hologic™) and the application of Liquid-based Cytology Thin Layer technique (Preservcyt solution Hologic™) for specimen preparation. The diagnostic accuracy was established by correlating the cytological results to histological diagnoses. The second aim was to compare the reliability of two instruments capable of processing the specimens for LBC Thin Prep Layer preparation at very different rates. In fact the processing rate of an instrument, Thin Prep 2000, is one specimen per cycle, whereas that of Thin Prep 3000 is of the order of 70 specimens per cycle.

Materials and Methods

Material

Data from a set of more than 10,000 non-hospitalized patients who underwent cytological examination because of symptoms suggesting neoplastic lesions in bladder and upper urinary tract or under follow-up, were reviewed. Of the 10,017 total urinary cytological tests that were reviewed, 8,855 test results were read as benign (88.3%), one test result were read inadequate, 443 test results were read as malignant (4.4%), and 718 test results were read as suspicious (7.2%). The over-all group distribution resulting from this review agrees with data from literature when urine cytology is routinely used in the initial evaluation of patients with micro- or macrohaematuria.[11,12]

To evaluate the cytological accuracy, data were extracted from the charts of a subset of patients with malignant or suspicious test results who underwent immediately cystoscopic evaluation subsequent biopsy to provide a histological correlate to cytology. Only this subset of patients was included in this study.

To compare the reliability and the efficiency of TP3000 versus TP2000, 2,997 specimens from 999 patients randomly extracted from the set of patients who underwent urinary cytology examination, were processed in parallel with the two instruments using the blue filter 5 μ, 2 Flu/Fna programme in the case of TP2000, and the white filter 7 μ, Gyn programme in the case of TP3000. The agreement between the two instruments was assessed by using the Cohen's K statistics.[13] This statistics is frequently used in the literature to measure the inter-rater agreement for qualitative (categorical) items. Kappa statistics measures the percentage of data values in the main diagonal of the 2 × 2 table and adjusts these values for the amount of agreement that could be expected by chance alone. Kappa statistics is always less than or equal to 1. A value of Kappa equal to 1 implies perfect agreement; values between 0.80 and 1.0 imply a very good agreement.

Urine Sample Collection and Delivery Procedure

The patients were provided with an adequate amount of Cytolytic solution in closed beakers. The Cytolytic solution, purchased from Hologic™, is an isotonic saline solution containing 20% methyl alcohol. At this concentration the methyl alcohol is inhibitor of the microbial growth, but cannot act as a fixative although it may help to stabilize the cell morphology. The patients were invited to collect urine samples of three successive days. Immediately after collection, they have to mix aliquots of 70 ml of urine with 30 ml of Cytolytic solution and keep the samples in closed beakers at ~ 3°C. The third day, the patients were recommended to deliver immediately the three samples to local centers specifically dedicated to the delivery of samples to laboratory. The samples were then processed separately.

Specimen Preparation Method

Aliquots of 50 ml of each sample were centrifuged at 1,900 r.p.m. for 10 minutes, and the sediment, resuspended in Preservcyt solution, was carefully dispersed by mild shacking. 30,051 samples were then processed by Thin Prep 2000 using the blue filter 5 μ, 2 Flu/Fna programme. The thin layer thus formed was immediately fixed for 15 minutes with absolute ethyl alcohol and stained according to the modified Papanicolau method by an automatic multi-stainer robotic cover-slipper ST 5020 CV 5030 (Leica). To obtain a satisfactory thin layer with the cells well dispersed, it is essential to avoid any possible air drying of the specimen and to fix it with absolute ethyl alcohol immediately after formation of the thin layer.

Cytological Classification

The cytological observations were grouped according to WHO/ISUP (2004) in four diagnostic categories:

1. Inadequate (when the number of transitional cells observed was minor than three in the whole sediment);
2. Benign (inflammation-related lesions);
3. Suspicious;
4. Malignant.

Histological Classification

The histological observations were grouped according to WHO/ISUP (2004-1997) in three diagnostic categories[14–17]:

1. Negative: Benign papillary hyperplasia; urothelial papilloma

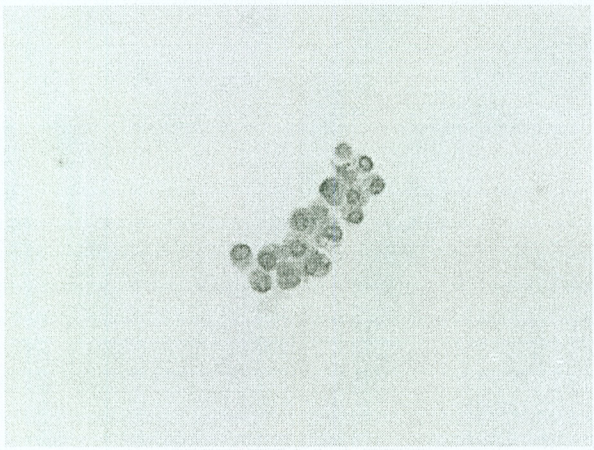

Fig. C-1

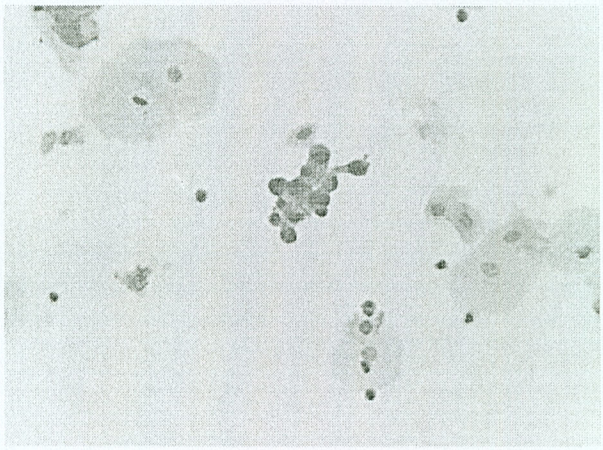

Fig. C-2

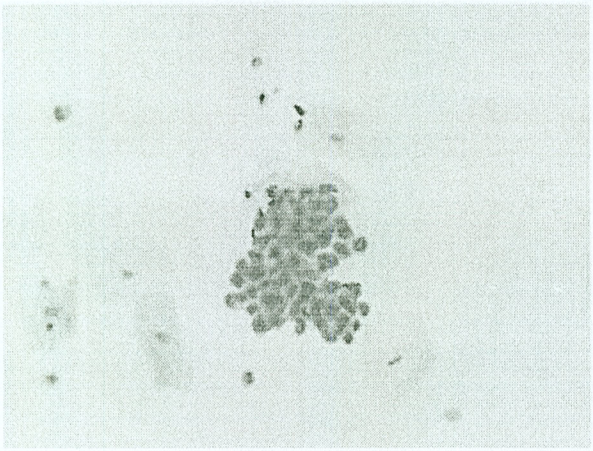

Fig. C-3

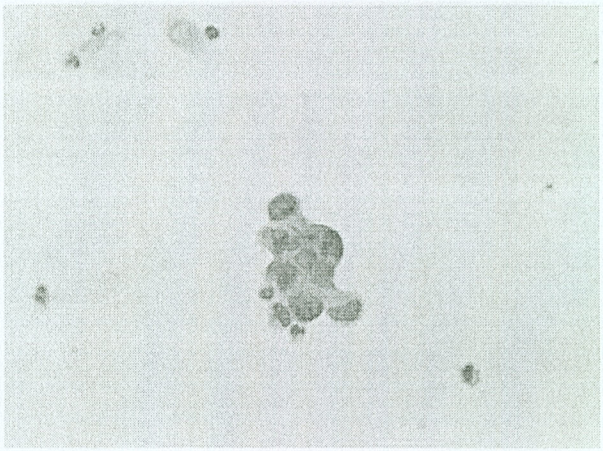

Fig. C-4

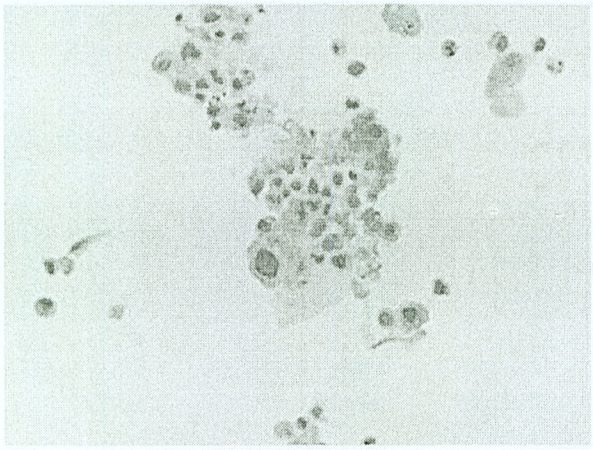

Fig. C-5

Figs. C-1–C-5

2. Low grade urothelial lesions: papillary urothelial carcinoma Grade I, papillary urothelial carcinoma Grade II
3. High Grade urothelial lesions: Papillary urothelial carcinoma Grade III, urothelial carcinoma in situ, invasive carcinoma.

Results

Figures C-1–C-5 are micrographs from different urine samples to illustrate the morphological criteria used to achieve the cytological diagnosis of benign, suspicious, and malignant lesions. Preliminary observations revealed that the use of collection solution (Cytolyt^{TM}) preserves the morphological features of the cells for at least one week and allows a better standardization of cytological diagnosis.

Figure 1 illustrates the concordance or not-concordance between cytological and histological diagnoses in the 318 patients that underwent biopsy for histological correlation. It appears that 218 cytological diagnoses over 234 cases judged malignant with urinary cytology, equal to 93%, were confirmed by the histological diagnosis, and that 74 cytological diagnoses over 84 judged suspicious, i.e., 88%, were validated as malignant by histological diagnosis. This means that the false-positive cytological diagnoses amount to 7% of all malignant cytological diagnoses, 12% of the suspicious ones and 8% of the total.

Tables I and II illustrate the diagnostic accuracy of urinary cytology with respect to different grade, age and gender of urothelial neoplasms, as validated by histological correlates. Table I illustrates the group distribution in the case of tumors differing per grade of malignancy in relation to age and gender. The data show that of 218 cytological tests read as malignant, 38 were validated as low grade neoplasm by histological diagnosis (17.5%), and of 74 tests categorized as suspicious by cytology, 22 were confirmed as low grade neoplasms by the histological diagnosis (29.7%) with suspicious cytological diagnosis. Moreover, 26 cases with malignant cytological diagnosis and 3 cases with suspicious cytological diagnosis were validated as high-grade in situ carcinoma by histological examination. This observation is noteworthy since the in situ carcinoma, accounting for 9.1% of all malignant tumors in this study, is difficult to be detected by cystoscopy. Table II illustrates the group distribution of cytological diagnoses correlated to histological diagnoses of tumors differing per site, age, and gender. It appears that the pool of tumors examined in this study is representative of all tumors of the urinary tract. The overall frequency distribution resulting from the Table II is in good agreement with data reported in the literature as to the incidence of the different neoplastic lesions in human urinary apparatus.[18,19] As expected, bladder carcinoma is the most frequent neoplastic lesion of urinary apparatus. In our study, it amounts to 94% of all malignant cytological diagnoses validated histologically, and to 86.5% of suspicious cytological diagnoses validated as malignant by histological diagnosis. According to our observations, it is seven times more frequent in men than in women with a very high peak of incidence between 60 and 90 year. Also the frequency distribution of prostate, kidney, and ureter carcinomas is in agreement with data from the literature.[18,19]

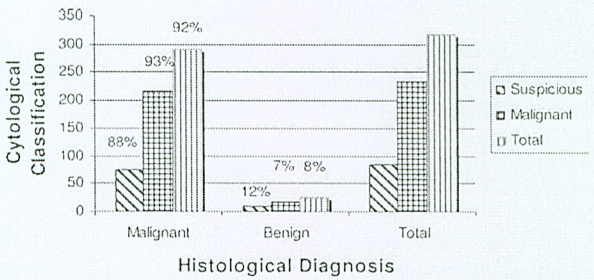

Fig. 1. Validation of cytological classification by histological diagnosis.

Table III illustrates the performance of TP3000 compared with that of TP2000. To this aim, 2,997 specimens from 999 patients were processed by both TP2000 and TP3000. The Table III reports the diagnostic groups resulting by the use of the two instruments. It appears that, by examining the urine specimens of 999 patients by Thin-

Figs. C-1–C-5. Fig. C-1. Example of benign lesion. The micrograph shows pseudopapillary cluster of urothelial cells. Voided urine of a 29-year-old man suffering from lithiasis. The specimen was processed by TP3000 and stained with Papanicolaou, ×40. **Fig. C-2.** Example of suspicious lesion. The micrograph illustrates the presence of cluster of urothelial cells with slight nuclear enlargement and slight hypercromasia, scanty isolated urothelial atypical cells on a clean background. The histological diagnosis was: Papillary Urothelial Carcinoma Low Grade of the bladder. Voided urine of a 74-year-old man. The specimen was processed by TP2000 and stained with Papanicolaou, ×40. **Fig. C-3.** Example of malignant lesion of low grade. A papillary cluster of overlapping urothelial cells with slight nuclear enlargement and hypercromasia is seen on a clean background. The histological diagnosis was: Papillary Urothelial Carcinoma Low Grade of the bladder. Voided urine of a 80-year-old man. Specimen processed by TP2000 and stained with Papanicolaou, ×40. **Fig. C-4.** Example of malignant lesion of high-grade. The micrograph illustrates the presence of papillary cluster of overlapping urothelial cells with nuclei of variable size, marked hypercromasia and irregular configuration on a clean background. The histological diagnosis was: Urothelial Carcinoma in situ of the bladder. Voided urine of a 75-year-old man. Specimen processed by TP 2000 and stained with Papanicolaou, ×40. **Fig. C-5.** Example of malignant lesion of high-grade. The micrograph shows a population of pleomorphic frankly malignant cancer cells with overlapping nuclei of irregular shape, variable size and marked hypercromasia. The background was dirty with the presence of blood cells and necrotic cells. The histological diagnosis was: Invasive Papillary Carcinoma of the bladder. Voided urine of a 84-year-old man. Specimen processed by TP 2000 and stained with Papanicolaou, ×40.

Table I. Frequency Group Distribution of Tumours Differing Per Grade of Malignancy in Relation to Gender and Age

Grade of neoplastic lesion			*Malignant cytological and histological diagnosis*		*Suspicious cytological and histological diagnosis*	
			No.	*%*	*No.*	*%*
UCG1-2 Low Grade	Overall		38	17.5	22	29.7
	Gender	Male	31	81.6	20	90.9
		Female	7	18.4	2	9.1
	Age	<59	1	2.6	2	9.1
		60–90	37	97.4	20	90.9
UC in situ UCG3 High Grade	Overall		26	11.9	3	4.1
	Gender	Male	24	92.3	3	100
		Female	2	7.7	0	0
	Age	<59	1	3.8	0	0
		60–90	25	96.2	3	100
Invasive lesion	Overall		154	70.6	49	66.2
	Gender	Male	132	85.7	47	95.9
		Female	22	14.3	2	4.1
	Age	<59	6	3.9	4	8.2
		60–90	148	96.1	45	91.8
Total			218	100	74	100

Table II. Frequency Group Distribution of Tumours Differing Per Site in Relation to Gender and Age

Site of neoplastic lesion			*Malignant cytological and histological diagnosis*		*Suspicious cytological and histological diagnosis*	
			No.	*%*	*No.*	*%*
Bladder Carcinoma	Overall		205	94	64	86.5
	Gender	Male	178	86.8	60	93.7
		Female	27	13.2	4	6.3
	Age	<59	7	3.4	6	9.4
		60–90	198	96.5	58	90.6
Prostate Carcinoma	Overall		5	2.2	4	5.4
	Gender	Male	5	100	4	100
		Female	0	0	0	0
	Age	<59	1	20	0	0
		60–90	4	80	4	100
Kidney Carcinoma	Overall		3	1.4	4	5.4
	Gender	Male	2	66.7	4	100
		Female	1	33.3	0	0
	Age	<59	0	0	0	0
		60–90	3	100	4	100
Ureters Carcinoma	Overall		2	0.9	1	1.4
	Gender	Male	2	100	1	100
		Female	0	0	0	0
	Age	<59	0	0	0	0
		60–90	2	100	1	100
Metastatic Carcinoma	Overall		3	1.4	1	1.4
	Gender	Male	0	0	1	100
		Female	3	100	0	0
	Age	<59	0	0	0	0
		60–90	3	100	1	100
Total			218		74	

Prep2000, 884 patients were diagnosed to have benign lesions, 62 suspicious lesions, and 52 malignant lesions, whereas the urine specimens of one patient were judged inadequate for diagnosis. The results obtained by examining the same specimens by ThinPrep3000 were 871 benign, 73 suspicious, and 50 malignant, respectively, with five cases judged inadequate. By crossing the data obtained by the two instruments, it appears that there is an excellent agreement between the performance of ThinPrep3000 and that of ThinPrep2000: the value of this agreement is in fact equal to 0.96 with a Cohen K of 0.84.

Discussion

Several studies suggest that the diagnostic value of urinary cytology is indisputable for diagnosis and management of high-grade neoplasms such as the in situ carcinoma or the occult invasive carcinoma, but it is questionable for the diagnosis of low-grade neoplastic lesions.[2–5] This conclu-

Table III. Statistics and Comparison of Thin Prep 2000 and Thin Prep 3000 Output

	ThinPrep 3000				
ThinPrep 2000	*Inadequate*	*Negative*	*Suspicious*	*Positive*	*Total*
Inadequate	**1**	0	0	0	1
Negative	4	**861**	18	1	884
Suspicious	0	7	**54**	1	62
Positive	0	3	1	**48**	52
Total	5	871	73	50	**999**

2,997 specimens of 999 patients were examined by both ThinPrep 2000 and ThinPrep 3000.
Kappa statistics measures the percentage of data values in the main diagonal of the 2 × 2 table and adjusts these values for the amount of agreement that could be expected by chance alone. Kappa statistics is always less than or equal to 1: a value of 1 implies perfect agreement; values between 0.80 and 1.0 imply a very good agreement.
In the Table, the agreement is equal to (1 + 861 + 54 + 48)/999 = 964/999 = 0.96.
$K = 0.84$.

sion, widely accepted, is drawn from the rather high number of false-positive and false-negative cytological diagnoses and the frequent diagnosis of non-conclusive atypia[5] in the case of low grade urothelial neoplasms. The limited diagnostic utility of urinary cytology is assumed to depend on the low sensitivity of this technique, particularly inadequate when used to distinguishing, in disaggregated cells, the initial neoplastic cell transformations from alterations that may be due to infections, inflammations, stones, instrumental treatment.[2,20] The data reported in this study indicate that, in spite of lack of highly specific features of initial neoplastic transformation, the use of more adequate techniques for sample collection and delivery and for specimen preparation, may help to overcome the above difficulties. This is proved by the sensitivity of the cytological method performed under the conditions described, as well as the remarkably lower rate of misjudgement of cytology test results, compared with data of literature.[5,21]

As to the procedure for sample collection, the present data confirm the findings by Raistrick et al.[7] that the immediate mixing of urine samples with preserving fluids ameliorates the preservation of cell integrity in voided urine cytology. The preferential use of Cytolyt and Preservcyt solution as a preserving fluid is suggested by a series of considerations regarding the chemical composition of these fluids. These include the isotonicity of the saline solution, which prevents osmotic alterations; the presence of methanol that inhibits the microbial growth; the absence of immediate fixation that impedes the formation of cell aggregates. In fact, at the concentrations used, it is absolutely improbable that methanol may act as a fixative, although a positive effect on cell structure stabilization cannot be excluded, especially as to the integrity of RNA.[22] In conclusion, the use of this fluid as a collection fluid undoubtedly impedes the loss of relevant morphological features of the cells.

An additional advance in the diagnostic accuracy of urine cytology certainly results from the use of Preservcyt liquid-based cytology thin prep method since it facilitates the formation of a well dispersed thin layer of cells on the support. A uniform and random distribution of the cells is in fact a prerequisite for an accurate reading of the specimen, as it has been stressed also by Davey et al. in a study of cytological diagnoses of cervical pathology.[23] Clearly, methods for revealing specific tumor markers such as multi-fluorescence in situ hybridization, may further ameliorate the diagnostic reliability of urinary cytology.[24] Molecular data will be useful to identification of aggressive genetically unstable neoplasms and also for further refinements of the classification system of non invasive papillary and nonpapillary urothelial neoplasms.[16]

An interesting fact resulting from the present analysis is the significant percentage of low-grade transitional cell neoplasms that can be detected by urinary cytology and subsequently validated by histological diagnosis. In fact, of 292 test results read as malignant or suspicious by cytology, 60 (20.5%) were validated as low-grade neoplastic lesions by histological diagnosis. This finding suggests the possibility that low grade urothelial carcinomas can be followed by routine urine cytology. However, before establishing this conclusion with reasonable confidence, it is necessary to know what percent of cytologically negative cases were defined as low grade urothelial carcinoma by histology. This topic is at the present under analysis by our group.

The data reported in this study also illustrate a detailed comparison between the outcome of two different instruments characterized by very different rates of sample processing. A statistical analysis demonstrated that there is an excellent agreement between the results obtained by the two instruments. Since, however, the number of samples processed per unit of time by Thin Prep 3000 is much higher than that obtainable by Thin Prep 2000, economic considerations suggest the preferential use of Thin Prep 3000 to process urine samples.

In summary, this data show that the procedure for sample collection, delivery and treatment for thin-layer liquid-based cytology may play a role in improving the diagnostic reliability of urine cytology and, as a consequence, its utility in the clinical practice. In addition it may be presumed that the procedure for sample collection and delivery here described, is more convenient for the patient since he/she is not obliged to deliver the urine samples every day for three consecutive days immediately after collection, with the risk of damages due to an accidental delay.

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