

CAN WE DISTINGUISH BETWEEN CELL TYPES PRESENT IN MIDSTREAM AND CATHETER URINE SAMPLES FROM WOMEN WITH INCONTINENCE?



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HYPOTHESIS AND AIMS

Urine samples may be obtained from patients with Lower Urinary Tract Symptoms (LUTS) by either midstream urine (MSU) or catheter specimens of urine (CSU). Most commonly urine microbiology and microscopy is performed on MSU specimens, so-called 'clean catch' urine specimens. These samples potentially contain both urothelial cells and squamous epithelial cells of vaginal origin.

In contrast, CSU are more difficult to obtain but should only contain cells from a single source, that is urothelial cells from the bladder.

- The aim of this study was to identify variations in cell types present in MSU and CSU specimens.
- We have employed 3 staining methods in order to compare the differences in morphology and staining characteristics between MSU and CSU.
- We aim to establish a protocol to identify the origin of cells in MSU (vaginal and bladder) and CSU (bladder only) specimens.

These findings would be important in future efforts to examine the prevalence of occult bacterial cystitis in women with refractory detrusor overactivity, which needs to be distinguished from the report of "mixed growth due to vaginal contamination".

STUDY DESIGN, MATERIALS AND METHODS

Paired MSU and CSU specimens were obtained from 10 women (age range 32 to 79 years) who presented to our centre for investigation of incontinence.

Patients were asked to arrive with a comfortably full bladder and then to provide a **MSU specimen** on arrival.

All patients were then catheterised by the continence nurse 15-20 minutes later and a **CSU specimen** was collected just prior to urodynamics testing.

Aliquots of paired MSU and CSU specimens were preserved for urine cytology. Samples were centrifuged to concentrate the urothelial or vaginal epithelial cells; the cells were fixed with formalin.

Fixed cells were then centrifuged onto microscope slides by cytospin.

Cells preparations were subjected to two different staining techniques:

A) Wright's Giemsa stain:

usually used for identification of white blood cells

B) Papanicolaou stain:

usually used for cervical cytology, differentiates keratinised and non-keratinised epithelial cells [1]

The proportion of keratinised (orange-stained) and non-keratinised (blue-stained) cells in Papanicolaou stained paired MSU and CSU specimens was estimated in four low-power (10x magnification) fields by two investigators independently.

REFERENCES

1. Stremler KM et al., J Urology 2013; 189 (1), 343-351
2. De May R, The Art and Science of Cystopathology
3. Wiener DP et al., J Urology 1979; 122, 317-321

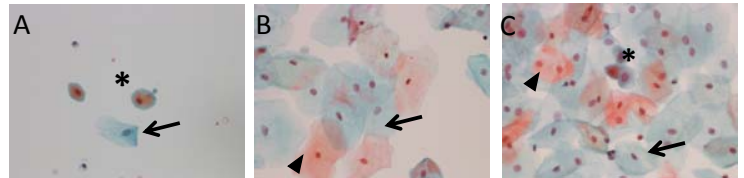
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RESULTS

Figure 1. Papanicolaou staining of MSU specimens (40 x magnification)



- In **MSU specimens** a number of individual cell types were identified using Papanicolaou stain:-
 1. large, polygonal superficial squames with pyknotic nuclei and variable staining of their cytoplasm (Figure 1, arrows)
 - A. Some cells demonstrated pale blue cytoplasm: indicating metabolically active cells that are possibly non-keratinised [2] (Figure 1, arrows)
 - B. Others with orange-stained cytoplasm: indicating possibly keratinised cells or cellular degeneration [2] (Figure 1B and C, arrowhead)
 2. small, round ovoid transitional cell (Figure 1A and C, *)
- In MSU specimens, there was great variability in the proportion of the cell population that were orange-staining (10 to 80%).
- On Wright's stain, cells of similar shape and size could be seen, although these were unable to be distinguished by their staining.
- In **CSU specimens** the most prominent cell type was the cells with a blue-stained cytoplasm (similar to those shown by the arrows in Figure 1) indicating the presence of metabolically active, non-keratinised cells.
- In approximately 50% of CSU specimens, cells with orange-stained cytoplasm (similar to those shown by the arrowhead in Figure 1B and C) were still present.
- These cells represented approximately 0 to 30% of the cells stained in CSU specimens.

SUMMARY

- **MSU specimens** contain: both orange-stained, keratinised, and blue-stained, non-keratinised cells, that is cells presumed to be of both bladder and vaginal origins.
- In **CSU specimens** there was a significant reduction in the presence of orange-stained (keratinised) cells however, it was interesting that these keratinised epithelial cells were still present in approximately 30% of CSU specimens.
- The presence of these orange-stained epithelial cells in CSU specimens could be explained by the presence of trigonal urothelial cells which are known to be squamous keratinised epithelium in up to 50% of women [3].
- Wright's staining was unable to distinguish between keratinised and non-keratinised cells although cellular morphology was similar in both Papanicolaou and Wright's staining.
- Preliminary studies using CK20 immunocytochemistry in five paired samples demonstrated the presence of urothelial umbrella cells in specimens from both MSU and CSU.

CONCLUSION

This study indicates that Papanicolaou stain appears to be the best protocol to identify the origins of cells in MSU and CSU specimens, although this is complicated by the presence of keratinised trigonal cells in some patients.

These findings could have implications for the manner in which specimens from women with LUTS are analysed. Due to the difficulty of distinguishing cells on a purely morphological basis, establishment of a protocol combining a number of stains and a definitive urothelial cell marker would be valuable in studies where the urothelial origin of the cells being examined is important.