

THE TIME COURSE OF WHITE CELL DESTRUCTION IN URINE SAMPLES AND THE NECESSITY OF IMMEDIATE ANALYSIS

Hypothesis / aims of study

Part of the diagnosis of overactive bladder (OAB) requires the exclusion of significant urine infection. It is now known that the routine culture of midstream urine (MSU) samples seeking isolation of $\geq 10^5$ colony forming units (cfu) ml⁻¹ is not sufficiently sensitive and results in 50% of genuine urine infections going undetected [1]. This implies that the screening for urinary infection in patients with OAB symptoms needs to be reassessed. There are significant difficulties with sampling and culture methods used in clinical practice, which mean that an improved culture method may be some time away. An alternative is to use a surrogate marker of infection. Since 1968 it has been known that the detection of ≥ 10 white blood cells (wbc) μL^{-1} of a fresh, unspun, urine sample, examined by light microscopy using a haemocytometer, is the best surrogate marker of urinary infection [2]. Despite this the literature is surprisingly uninformative about the sampling methods that should be achieved for optimum results. It is known that centrifuging urine causes white cells to lyse with resulting underestimate of the pyuria signal. There are no data to guide sampling method; MSU or catheter specimen (CSU) and method and duration of storage have not been studied. These are apposite matters since in the health services urine samples can spend many hours in the journey to the laboratory.

This study was designed to measure the timed decay of white cells in urine samples when stored at room temperature and when refrigerated at 4°C. The study hypothesis that was tested was that there would not be a significant decay in white cell counts in response to storage over a day.

Study design, materials and methods

Because there were no data to guide expectations a sample size calculation could not be effected.

Urine samples were obtained from women presenting with overactive bladder symptoms by the midstream clean-catch method.

A disposable pipette was used to load a clean haemocytometer chamber with a sample. This preparation was examined using a x20 objective with a x10 optical (magnification x200). The leucocyte count (wbc μL^{-1}) was enumerated by counting cells in five large squares and multiplying the result by 2.2, since the volume of the whole chamber was 0.9 μL (figure 1). If a cell overlapped a dividing line, it was counted if the line ran along the top or right side and ignored if the line ran along the bottom or left side.

The samples were divided into two aliquots of which one was stored at room temperature (18°C) and one refrigerated at 4°C. These samples were then examined repeatedly

in the same way every two hours for the duration of the working day (09:00 am to 17:00 pm) for the 2 days.

The samples were disposed of once the WBC count reached zero, or the two day time period had expired.

Results

90 female patients with OAB provided specimens that manifest pyuria >6 wbc μL^{-1} on sampling. Their mean age was 56 (sd=18).

In the samples stored at room temperature, WBC count decreased to about 60% of the original in the first two hours after collection (Figure 1).

In the samples that were refrigerated, the WBC count decreased to about 80% of the original during the first two hours after collection (Figure 2).

Figure 1 the proportion of the initial white cell count by time – Room temperature (18°C)

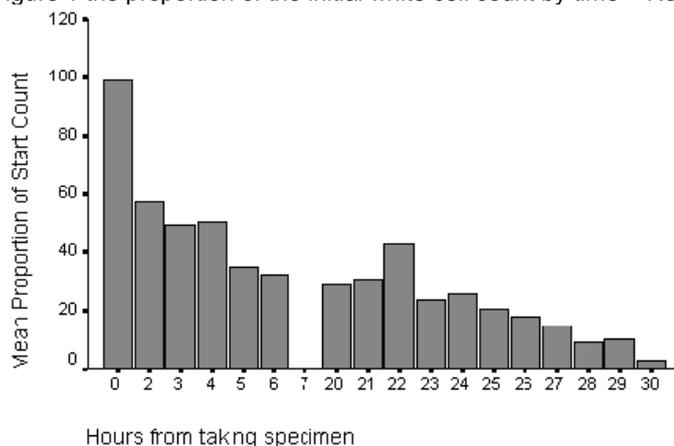
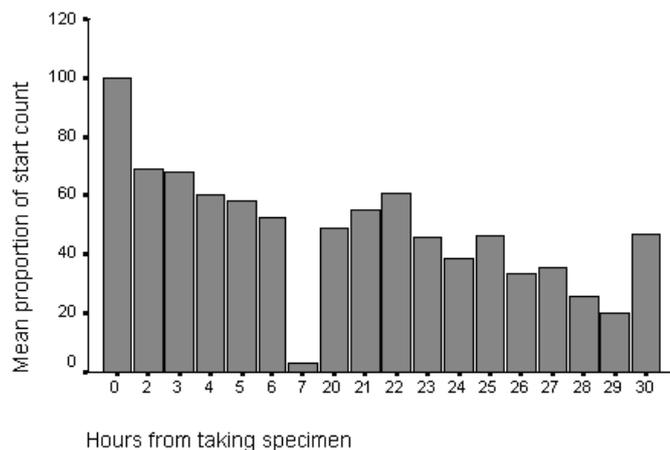


Figure 2 the proportion of the initial white cell count by time – Refrigeration storage (4°C)



Interpretation of results

Lysis of the white blood cells in urine appears to commence very shortly after sampling and is not significantly retarded by storage at 4°C.

Concluding message

It seems that delayed examination of the urine deposit for pyuria is associated with significant error that must lead to an underestimation of the inflammatory response. At this time the only solution seems to lie in immediate microscopic examination in the clinic at the time of collection.

References

1. Am.J.Med. 75(1B), 53-58. 28-7-1983
2. Ugeskr.Laeger 130(4), 136-139. 25-1-1968

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Is this a clinical trial?	No
What were the subjects in the study?	HUMAN
Was this study approved by an ethics committee?	Yes
Specify Name of Ethics Committee	The East London Ethics Committee
Was the Declaration of Helsinki followed?	Yes
Was informed consent obtained from the patients?	Yes