

PRESERVATION OF URINARY WHITE CELLS TO ENABLE ADOPTION OF MICROSCOPY OF UNSPUN URINE FOR PYURIA INTO ORDINARY CLINICAL ASSESSMENT PROTOCOLS OF LOWER URINARY TRACT SYMPTOMS.

Hypothesis / aims of study

The International Consultation on Incontinence states that “*Urinanalysis is a fundamental test that should be performed in all urological patients although in many instances a simple dipstick urinalysis provides the necessary information; a complete urinalysis includes both chemical and microscopic analysis*”. Two meta-analyses of urinary dipsticks, used to assess acute frequency & dysuria, in adults(1;2) and one in children(3) have criticised the sensitivity and specificity of urinary dipsticks in diagnosing acute urinary tract infection (UTI). There are no validation data relevant to other lower urinary tract symptoms (LUTS). Similar criticisms have been voiced against the laboratory culture of midstream urine specimens, using a diagnostic threshold of 10^5 colony forming units (cfu) ml⁻¹, when assessing acute symptoms. Again there are no informative data to reassure us when assessing patients with LUTS. There are grounds for great concern about the accuracy of the exclusion of UTI when evaluating LUTS. Numerous studies have shown that the best surrogate method of diagnosing urine infection, in symptomatic patients, is the identification of pyuria of ≥ 10 white blood cells (wbc) μL^{-1} , counted by microscopic examination of fresh unspun urine in a haemocytometer. Catheter specimen (CSU) studies have shown that this is superior to MSU culture at 10^5 cfu ml⁻¹. It may be that, given evidence, practice guidelines should stipulate that UTI be screened-out for patients with LUTS by measuring microscopic pyuria. This technique is not usually available in the ordinary clinics; the test is often performed in a central laboratory. The urine sample must be transported to the laboratory site, which takes time. Regrettably it has been shown that at room temperature the pyuria count decrease to about 60% of the original in the first two hours after collection and to a higher 80% if the samples are refrigerated. There is an evident need for a method of preserving the white cells in the urine during the period of transport to the central laboratory. Surprisingly there are no published data on the action of preservatives for this purpose. Therefore this study was designed to identify and then test the performance of a putative agent for preserving white blood cells in urine samples.

Study design, materials and methods

This study was a blinded, observational cohort study with randomisation of samples. A pilot study screened a series of candidate chemicals for preservative properties: Hydrochloric acid, ethanol, acetic acid showed no action but boric acid, previously used to preserve bacteria in urine, did show some promise. The experimental procedure used to assess the function of boric acid was divided into two parts. The basis of the first experiment was to determine whether boric acid had a significant effect with regards to the preservation of pyuria in urine samples. The eventual outcome of this experiment led to the logical follow-up investigation of verifying whether the preservation of pyuria in urine due to boric acid was dose-dependent.

Experiment 1: Fresh urine samples with ≥ 10 wbc/ μL obtained from patients presenting with LUTS, were divided into 2ml aliquots and kept in three conditions: (i) Bench storage at room temperature (RT) (20°C), (ii) Bench storage at RT 20°C with 2% boric acid concentration, and (iii) With 2% boric acid concentration and refrigerated at 4°C. Microscopic haemocytometer wbc counts were performed at 2hours, 4hours, 6hours, 24hours and 48hours after collection.

Experiment 2: microscopic counts were performed as in experiment 1 but the samples were stored on the bench at 20°C in different concentrations of boric acid: 0%, 1%, 2%, 4% and 8%.

The data were collated and Prism-3 software was used to fit decay curves to the mean white blood cell counts plotted against time. The Wilcoxon paired tests for non-parametric data were performed to determine the statistical significance of the results. The study had 80% power to detect a 30% between group difference ($\alpha=0.05$).

Results

Experiment 1: wbc survival (%) decreased with time for all samples before a plateau developed at about six hours. The plateau for boric acid (4°C) was at 59.5% (95% CI= 53% to 65%) of initial count; significantly higher than boric acid at 20°C (55.8% - 95% CI = 49% to 61%), which was significantly higher than that for the bench urine sample (29.6% - 95% CI = 22% to 33% - $p<0.001$): See figure 1. Experiment 2: All boric acid concentrations significantly retarded wbc lysis but no difference between the concentrations was identified ($p<0.001$): See figure 2

Figure 1

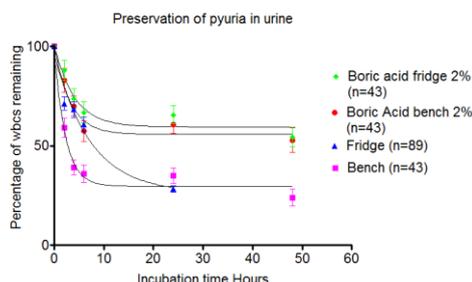
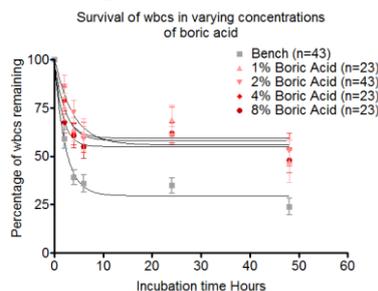


Figure 2



Interpretation of results

Retarding white blood cell lysis with 2% boric acid appears to be a clinically useful method that could help towards the adoption of microscopy for pyuria more widely in the assessment of LUTS. The plateau that develops from about six hours suggests selection of certain cells with specific morphological features that now need to be explored. The preparations studied in this experiment were not stained. A Giemsa stain of fixed preparations would differentiate neutrophils from lymphocytes and that should precede more

sophisticated analysis. If the decay curve proves as reproducible as in these studies it should be possible to fit a reliable time-function to the results of counting that should provide an accurate estimate of the original urinary white cell numbers.

Concluding message

Current evidence implies that microscopy of urine to count white blood cells should be a part of the assessment of LUTS. This study, on preserving the white cells during specimen transport, brings the technique an important step closer to adoption in ordinary clinical practice.

References

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