Screening urine samples for the absence of urinary tract infection using the sediMAX automated microscopy analyser

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Urinalysis culminates in a workload skew within the clinical microbiology laboratory. Routine processing involves screening via manual microscopy or biochemical dipstick measurement, followed by culture for each sample. Despite this, as many as 80% of specimens are reported as negative; thus, there is vast wastage of resources and time, as well as delayed turnaround time of results as numerous negative cultures fulfil their required incubation time. Automation provides the potential for streamlining sample screening by efficiently (>30% sample exclusion) and reliably [negative predictive value (NPV) ≥ 95%] ruling out those likely to be negative, whilst also reducing resource usage and hands-on time. The present study explored this idea by using the sediMAX automated microscopy urinalysis platform. We prospectively collected and processed 1411 non-selected samples directly after routine laboratory processing. The results from this study showed multiple optimum cut-off values for microscopy. However, although optimum cut-off values permitted rule-out of 40.1% of specimens, an associated 87.5% NPV was lower than the acceptable limit of 95%. Sensitivity and specificity of leukocytes and bacteria in determining urinary tract infection was assessed by receiver operator characteristic curves with area under the curve values found to be 0.697 [95% confidence interval (CI): 0.665–0.729] and 0.587 (95% CI: 0.551–0.623), respectively. We suggested that the sediMAX was not suitable for use as a rule-out screen prior to culture and further validation work must be carried out before routine use of the analyser.

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common indications for prescribing antibiotics. Urine samples form a significant proportion of routine laboratory workload, but up to 80% of urine cultures are negative (Okada et al., 2000). Therefore, a rapid and reliable method for identifying those samples with significant bacteriuria or pyuria, and thus requiring further culture, and those likely to be negative for UTI by culture would substantially reduce labour costs and unnecessary antimicrobial prescribing.

We sought to establish if an automated microscopy method for urinalysis that provides bright-field pictures of an aliquot (sediMAX; A. Menarini Diagnostics) could be used routinely to rapidly exclude negative samples that do not require further culture. The outcome was to attain an acceptable negative predictive value (NPV) of ≥ 95%, as recommended by the supplying company and cited by Kellogg et al. (1987), and reliably exclude a proportion (>30%, determined locally for financial viability of analyser implementation) of samples from culture techniques in order to reduce turnaround times of results. This study aimed to formulate optimum cut-off values for microscopy using the sediMAX automated analyser, to produce a high NPV for UTI using the Uri System (Mast Group) culture method, whilst maintaining adequate standards of sensitivity and specificity.

METHODS

Setting. Peterborough and Stamford Hospitals NHS Foundation Trust is a district general hospital with 612 beds covering a population of 450 000 people in Cambridgeshire, UK with ~75 000 admissions per year. The microbiology laboratory processed 460 502 samples in 2012, of which 78 980 (17.2%) were urine samples.
**Data collection.** Data were collected for all urine specimens submitted prospectively over a 2 week period. The sample population incorporated general practice, inpatient, outpatient and day clinics to ensure a representative dataset.

**Specimen collection.** Samples were collected in non-preservative sterile universal containers and tested within 4 h of arrival in the laboratory or refrigerated until processing was commenced – in accordance with the local laboratory protocol for the processing of urine specimens.

**Specimen processing.** Specimens were routinely processed to standard protocol, consisting of a dipstick technique (Clinitek; Siemens Diagnostics) semi-automated reader and sterile aliquoting for culture onto chromogenic, Gram-positive selective agar (Mast Group) and positive control medium using the Uri System (Mast Group) method. Screening was then performed with the sediMAX system for automated microscopy analysis within 15 min following inoculation for culture. On-screen review and necessary editing was only performed for samples which were flagged on the system as ‘crowded’, in line with how the analyser would be used if implemented routinely. After 15–21 h of incubation at 37 °C, plates were read by a specialist biomedical scientist who was denied access to the results of the sediMAX. Culture results were deemed positive if ≥10^4 c.f.u. ml⁻¹ pure growth of an organism was observed. For the purposes of this study, a sample was considered negative for UTI if there was no growth, no significant growth (≤10^3 c.f.u. ml⁻¹ growth) or mixed growth detected (two or more predominating organisms – on the basis that this indicated contamination of the sample).

**Statistical analysis.** SediMAX and culture results were collated in Microsoft Office Excel 2007 and divided into two groups determined by the gold standard ‘culture-positive’ or ‘culture-negative’. Receiver operating characteristic (ROC) curves were constructed for analysis of sensitivity and specificity for leukocyte and bacterial cell components. Curves were constructed using statistical package SPSS (IBM) in order to determine the accuracy of the investigated parameters in predicting UTI-positive samples.

**Ethical approval.** Ethical approval was not sought as this study was deemed a service improvement. Patient management was not affected and no patient-identifiable data were kept.

**RESULTS**

We processed 1411 samples over a 2 week period. The ages of patients ranged from <1 to 99 years (median 58 years) with 987 (70.0%) samples from females, 406 (28.8%) from males and ‘unclassified gender’ in 18 (1.2%) cases. A total of 387 (27.4%) urine samples tested were culture-positive for single isolates, 22.0% of samples were classified as ‘mixed growth’ (n=311), 7.7% ‘no significant growth’ (n=109) and 42.8% ‘no growth’ (n=604). The urine culture positivity rate was 31.3, 18.2 and 22.2% in female, male and unclassified gender groups, respectively.

**Escherichia coli** was the most frequent isolate (n=261; 77.4%) followed by **Enterococcus faecalis** (n=49; 12.7%), **Klebsiella** spp. (n=34; 8.8%), **Proteus** spp. (n=11; 2.8%), **Candida** spp. (n=10; 2.6%), **Pseudomonas aeruginosa** (n=9; 2.3%), coagulase-negative staphylococci (n=4; 1%), group G β-haemolytic streptococci (n=3; 0.8%), **Enterobacter** spp. (n=4; 1%) and **Staphylococcus saprophyticus** (n=2; 0.5%).

**Fig. 1.** ROC curves for WBC and bacterial cell (BAC) detection of the sediMAX analyser demonstrating sensitivity and specificity in prediction of UTI based upon culture result (positive or negative). Generation of WBC and bacterial cell performance characteristics: AUC=0.697 (95% CI: 0.665–0.729) and AUC=0.587 (95% CI: 0.551–0.623), respectively.

The ROC curves (Fig. 1) indicated the test performance for predicting UTI based on culture result. The area under the curve (AUC) for white blood cells (WBCs) and bacteria was 0.697 [95% confidence interval (CI): 0.665–0.729] and 0.587 (95% CI: 0.551–0.623), respectively.

Extensive functions composed in Microsoft Excel 2007 determined parameters of NPV, positive predictive value, sensitivity, specificity and percentage of samples potentially ruled out prior to culture from input combined cut-off values for leukocyte and bacterial cell counts. Table 1 illustrates the determination of combined cut-off values for the generation of optimum test parameters. Multiple cut-off points generated the same level of NPV. Upon slight leukocyte adjustment (e), it was shown that subsequent parameters were the same across all optimum bacterial counts highlighted in (d). Thus, there were multiple optimum cut-off values for the sediMAX as a screen based upon the present data. The optimum cut-off values permitted 40.1% of specimens to be excluded from culture; however, the associated NPV of 87.5% fell short of the acceptable limit of 95%.

Calculation of cut-off values based upon a favourable NPV produced no single optimal threshold criteria. Bacterial counts of 167, 168, 169, 170, 171 and 172 bacteria μl⁻¹ gave the same optimal NPV of 87.5% when combined with leukocyte counts of 8 and 11 leukocytes μl⁻¹. At a threshold of 11 leukocytes μl⁻¹, percentage rule-out
increased by 2.6% compared with 8 leukocytes $\mu l^{-1}$; thus we determined the optimum combined cut-off values to be 11 leukocytes $\mu l^{-1}$ and 167–172 bacteria $\mu l^{-1}$, which generated a NPV of 87.5% whilst excluding 40.1% of samples. Although this cut-off exceeded the 30% minimal target for sample exclusion, the NPV was <95%. The manufacturer’s recommended cut-offs to achieve a NPV of >95% were 150 bacteria $\mu l^{-1}$ and 35 leukocytes $\mu l^{-1}$. When these parameters were applied to our data, 50.8% of samples could be excluded, but the NPV we achieved was only 85.6%.

**DISCUSSION**

Our analysis suggests the platform failed the minimal targets of 95% NPV and 30% exclusion for the sediMAX to be reliably and efficiently installed as a urinalysis screen for negative samples. The highest NPV we achieved was 89.1%, based on a cut-off of 1 leukocyte $\mu l^{-1}$, which excluded just 12.4% of samples. Microscopy did not distinguish between positive and negative samples to an acceptable degree for its intended use.

Our study contrasts with another study using the sediMAX system (Falbo et al., 2012). Falbo et al. (2012) achieved a NPV of 99.4%, but their positive culture rate was only 18%; this may have been due to their definition of UTI ($>10^5$ c.f.u. $ml^{-1}$). Karakukcu et al. (2012) achieved a NPV of 100% using another automated urinalysis system when 85 bacteria $\mu l^{-1}$ and 13 WBCs $\mu l^{-1}$ counts were chosen as the cut-offs. They used the same definition of UTI as our study.

Whilst the detection of bacilli using the sediMAX was found to be almost always correct, that of cocci presents problems when excessive amounts of cellular or...
amorphous debris are present (Zaman et al., 2010). This may account for poor correlation between positive microscopy and positive cultures of Enterococcus faecalis of this study; 59.2% of Enterococcus faecalis cultures were detected compared with 86.7% for E. coli using the established optimum cut-off values. Automated detection of Gram-positive bacteria using microscopy may be further compromised due to the aggregation of cells and their small size relative to that of the more common Gram-negative uropathogens, thus increasing the false-negative margin. This may help explain the discrepancy between our study and others (Falbo et al., 2012; Karakukcu et al., 2012); whilst the proportion of patients with E. coli was similar (~60–66%), our study had a higher proportion of Enterococcus faecalis (12 versus 7%). Whilst correction of automated particle detection can be performed on screen, it was only performed on those flagged as ‘crowded’. If applied for every sample, false-negative and false-positive rates may improve. However, it should be noted that it is felt that the resolution of the images needs to improve.

We did not feel confidence could be placed in the detection of certain components using image review. This is described in other studies (Zaman et al., 2010) that found intra-batch precision (coefficient of variation) for erythrocytes and leukocytes to vary between 5 and 19% over a large range of counts. Furthermore, Zaman et al. (2010) found leukocyte and bacterial cell NPV to be 78 and 73%, respectively. Whilst it has been proposed that sediment analysis is equivalent to strip analysis (Lammers et al., 2001), Zaman et al. (2010) suggested that the sediMAX may only be used as a screen for negative samples in conjunction with the dipstick method. However, such a combination of methods defeats the purpose of installing an automated screening method on a local level as it would counteract a potential reduction in workload and cost. Despite this, it supports the prediction from the present study that the sediMAX as a stand-alone screen is not a viable replacement for the current routine method.

Inter-laboratory disagreement exists as to what constitutes the ‘gold standard’ culture result. Some authors believe a culture to be negative until a count of \( \geq 10^5 \) c.f.u. ml\(^{-1} \) is reached (Manoni et al., 2002; Falbo et al., 2012), whilst others deem samples positive at \( 10^5 \) c.f.u.ml\(^{-1} \) (Brihla et al., 2010; Kim et al., 2007). Culture classification has previously been suggested to have a profound effect on the calculated performance of microscopy analysers. This was demonstrated by Broeren et al. (2011), who investigated automated urinalysis screening using the UF-1000i against different gold standard criteria for defining negative cultures: no growth, \(< 10^4 \) c.f.u.ml\(^{-1} \) and \(< 10^5 \) c.f.u.ml\(^{-1} \). They found optimum performance in terms of specificity and sensitivity of the analyser was for those cultures deemed positive at a minimum of \( 10^5 \) c.f.u.ml\(^{-1} \), with leukocytes being least indicative of culture. Such criteria are locally complicated further by separate criteria for Gram-positive and Gram-negative bacteria (Koken et al., 2002), mixed growth being deemed at a minimum of three organisms (Parta et al., 2013), as well as influence from clinical input (Sultana et al., 2001). Indeed, mixed culture prevalence of this study was recorded relatively high at 22% of total samples. It would be useful to review the origin of these specimens as this might indicate an issue with the quality control of sample collection. Variation in protocols for antimicrobial prescription prior to laboratory testing (Broeren et al., 2011) may further act to distinguish negative from positive samples and it would be useful to know the origins of those samples recorded as ‘no growth’ on culture. Thus, these factors may in part account for the variation in evaluations for such analysers.

AUC values (Fig. 1) for both leukocyte (0.687; 95% CI: 0.665–0.729) and bacterial count (0.587; 95% CI: 0.551–0.623) showed leukocytes were a better predictor of culture by sensitivity and specificity than bacterial cell count. However, neither parameter generated sensitivity or specificity within acceptable limits for a diagnostically useful test (AUC≥0.9). Leukocyte specificity is expected to be low due to the range of factors capable of inducing pyuria other than UTI. However, a high bacterial count would also be picked up for mixed growth cultures (22.0%, \( n=311 \)) which are subsequently classified as ‘non-indicative’ of UTI, thus generating low specificity for bacterial microscopy. The same applies for patients who have received prior antibiotic therapy, potentially rendering bacteria non-viable. These factors may account for the finding that bacterial testing becomes a negative predictor of UTI towards the top right area of the curve as the ROC falls below the line of identity (AUC=0.5), representing a non-discriminatory test. Sensitivity may be low for both parameters due to crowding of samples (e.g. in high levels of amorphous debris) or due to inherent reading errors of the detection system. Our results contrast with those of other studies (Broeren et al., 2011; De Rosa et al., 2010), where the authors demonstrated that at any specificity, the corresponding sensitivity was higher for bacteria than for leukocytes. Where the ROC curve for bacterial cells falls below the line of identification in Fig. 1, culture is predicted incorrectly more often than infection is distinguished, potentially suggesting that ‘over-reporting’ of results may have occurred.

Limitations of the study include the single-centre nature of the study, the short time frame (2 weeks), and the fact that aspects such as cost and process integration were not specifically considered. In addition, the pre-analytical phase (i.e. prior to laboratory receipt) was not monitored or controlled as this is outside the realms of routine urinalysis at this district general hospital. The relatively high proportion of mixed cultures (22%) may have been due to poor specimen collection (which front-line staff are expected to advise patients upon), or time delays prior to receipt in the laboratory or processing. It may be useful for the laboratory to provide more detailed instructions regarding appropriate pre-analytical handling. Carry-over of specimens was not considered in this study; however, Zaman et al. (2010)
concluded leukocyte and bacteria cross-contamination to be absent in their study. All samples from patients were included, which contrasts with some other studies (Karakukcu et al., 2012), but is similar to others (Falbo et al., 2012). Exclusion of certain patient populations (e.g. paediatric patients, immunosuppressed, antenatal patients, the elderly and renal patients, as well as division of symptomatic from non-symptomatic individuals), whose samples would likely be cultured regardless of microscopy findings, would also affect the performance of the test. In the future, a more focussed approach to specific patient groups could be taken. Indeed, it may be believed that screening applied in such an exclusionary manner may only be suitable for non-symptomatic, otherwise healthy patients. It may be worthwhile investigating any benefit of a cross-check rule between sediMAX and dipstick methods as a sample screen prior to culture. Strengths of our study include size [our study was as large as some other published studies (Broeren et al., 2011; Falbo et al., 2012) and larger than others (Karakukcu et al., 2012)], time between culture and processing was minimal, and operators were controlled, competent and blinded to the results.

In summary, we found that the analyser cannot be reliably used as a stand-alone negative screen for the exclusion of samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population. We recommend that laboratories consider samples prior to culture due to a NPV of only 89.1% in our population.

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