the shorter 4 h exposure to represent the duration after a straightforward excision transferred promptly to pathology, whereas the longer time was chosen to represent delayed fixation.

The concentration dynamics and the cell time exposures are likely to be highly complex. In cell line studies, direct and rapid exposure of the cells to the dye occurs. In the context of a complex tissue, even when the dye can be directly visualised pervading the tumour on gross analysis, the uniformity and certainty of cell exposure can only be hypothesised.

In summary, MB; which is one of the dyes used for SLNB worldwide, can reduce ER α and PR staining levels but not MNF116, *in vitro*. We suggest that if MB is used for SLNB, ER α should be checked on the core biopsy, if it was negative in the excised tumour.

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Comparison of estimated glomerular filtration rate with routine creatinine clearance using a kinetic alkaline picrate assay from Olympus Diagnostica

Established chronic kidney disease (CKD) requires treatment with dialysis or transplantation, both of which are expensive. In addition, the late referral of patients requiring renal replacement therapy can have negative effects.^{1–4} As a result, it is recommended that estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) equation be used to identify people with CKD and those at risk of end-stage CKD.^{5–7} However, MDRD-derived eGFRs are reportedly less precise above 60 ml/min/1.73 m² when a kinetic alkaline picrate assay is used.⁸

We derived eGFR using the four-variable MDRD equation in 100 patients undergoing the creatinine clearance test (CCT) as assessed by 24 h urine collections using a kinetic alkaline picrate assay (Olympus Life and Material Science Europe (Irish Branch) Lismeehan, O'Callaghan's Mills, Ireland).

$GFR (ml/min/1.73 m^2) = 186 \times ((\text{serum creatinine } (\mu\text{mol/l})/88.4)^{-1.154}) \times \text{age (years)} \times 0.742$ if female and $\times 1.21$ if African American.

Urine protein was requested for 85 patients and was measured using pyrogallol red-molybdate (Olympus Diagnostica). Absolute and relative difference plots were drawn (fig 1) and the median bias was calculated for all stages of CKD. For each level of proteinuria, the median percentage difference was calculated (table 1).

As neither the CCT nor the eGFR is a reference method, the mean of both these values was used for the classification of the 100 patients. The individual means ranged from 7 to 178 ml/min/1.73 m², with 22, 22, 39, 12 and 4 patients having values consistent with CKD stages 1, 2, 3, 4 and 5, respectively. The absolute difference plot showed that the difference between the CCT and eGFR increased as the average of the eGFR and CCT increased. This is not unexpected as the range

of the mean of the eGFR and CCT evaluated in numerical terms is relatively large; furthermore, these data are similar to those obtained when deriving the MDRD equation.⁹ However, the percentage difference plot showed no increase in the relative difference with increasing mean of the eGFR and CCT values (fig 1). Unfortunately, the authors of the MDRD equation did not provide any data on relative difference. Compared with the mean of the eGFR and CCT, the CCT had a median positive (95% CI) bias of 49.4% (31.0% to 60.8%), 43.3% (24.4% to 54.1%), 36.1% (31.3% to 41.8%), 34.5% (11.1% to 53.1%) and 60.4% (36.4% to 75.9%) in CKD stages 1, 2, 3, 4 and 5, respectively, with an overall median bias of 39.3% (35.3% to 47.3%). The Kruskal–Wallis one-way analysis of variance by ranks¹⁰ was used to see whether there was a difference between at least two of the medians of the five CKD stages. H was computed to be 5.75 for 4 degrees of freedom. As H was less than $\chi_{0.05} = 9.49$, there was no significant difference between any of the medians. The Passing Bablok linear regression equation¹¹ with creatinine clearance as x and eGFR as y was $y = 0.613x + 2.9$ with the 95% CIs for the slope and intercept being 0.55 to 0.682 and -0.7 to $+6.2$, respectively. Thus, the slope is significantly different from 1.0 and is consistent with a bias of approximately 38.7% between the two methods. The intercept is not significantly different from 0.0. The correlation coefficient r was calculated as 0.857 (0.869 if the data point with an absolute difference of 157 ml/min is excluded).

The relationship between proteinuria and the median percentage difference between creatinine clearance and eGFR was investigated. The protein concentration ranged from below the limit of detection (0.05 g/l) to 10.5 g/day (table 1). The Kruskal–Wallis one-way analysis of variance by ranks¹⁰ was used to see whether there was a difference between at least two of the medians of the five CKD stages. H was computed to be 3.38 for 6 degrees of freedom. As H was less than $\chi_{0.05} = 12.59$, there was no significant difference between any of the medians.

The CCT overestimates the true GFR because of renal secretion of creatinine. Ensuring accurately timed and complete urine collection is also notoriously difficult. Despite the labour-intensive CCT being far from ideal, it is routinely used in clinical practice. The four-variable MDRD equation has been criticised for its potential for patient misclassification due to analytical interference, imprecision or calibration issues.¹² However, these issues also affect the CCT—for example, the intraindividual coefficient of variation for the CCT and serum creatinine are 13.6% and 4.3%, respectively (<http://www.westgard.com/biodatabase1.htm>). As serum creatinine is the only one of the four variables that has imprecision, the eGFR should have a much smaller CI for individual patients than the CCT. Accordingly, the routine use of eGFR would result in fewer individual patients having a CI that crosses one of the CKD classification cut-offs compared with the CCT. The large intraindividual coefficient of variation for the CCT is probably the main reason for the poor correlation coefficient by method comparison standards ($r = 0.857$) found in this study. Added to this is the relatively large positive bias in the CCT owing to active secretion of creatinine. Compared with the CCT, the eGFR in our study is negatively biased (as expected) by about 39%, with no statistically significant difference

Figure 1 Absolute difference plot (A): absolute difference between creatinine clearance test (CCT) and estimated glomerular filtration rate (eGFR) plotted against the Mean of the creatinine clearance and eGFR. Percentage difference plot (B): percentage difference between creatinine clearance and eGFR plotted against the Mean of the creatinine clearance and eGFR. Difference in GFR with increasing proteinuria (C): percentage difference between creatinine clearance and eGFR plotted against the daily urinary protein.

Table 1 Relationship between proteinuria and the median percentage difference between creatinine clearance and estimated glomerular filtration rate

Proteinuria (g/day)	Number	Median % difference
<0.02	13	51.1
0.02–0.19	25	39.3
0.20–0.49	11	32.3
0.5–0.99	14	39.8
1.0–2.99	13	50.8
≥3.0	9	38.4
Unknown	15	41.8

between two or more of the medians of the five CKD stages. Also expected is the increased difference in the absolute difference with the increase in the GFR; however, the percentage difference plot shows no evidence of an increase in the range of individual values with increasing GFR, especially >60 ml/min/ 1.73 m².

Despite the problems with the CCT, it is still the method routinely used in the determination of GFR, with many patients being incorrectly classified as a result. Compared with the CCT, it is to be expected that eGFR will result

in fewer misclassifications despite issues such as analytical interference, imprecision and calibration. The user-friendly four-variable MDRD-derived eGFR overcomes the positive bias of the CCT across all stages of CKD and degrees of proteinuria when using the Olympus kinetic alkaline picrate assay. Accordingly, we believe that the four-variable MDRD-derived eGFR should be reported instead of the CCT across all stages of CKD.

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Over 18 h to positivity in the Bact/ALERT system with clustered Gram-positive cocci is highly predictive of coagulase-negative staphylococci

Coagulase-negative staphylococci (CoNS) are the most common bacteria isolated from blood cultures, but a great proportion of them are most likely contaminants.^{1,2} On the other hand, one single positive blood culture for *Staphylococcus aureus* is usually sufficient to diagnose bacteraemia. Therefore, a rapid method of differentiating CoNS from *S aureus* would assist in the clinical decision on starting antimicrobial treatment in a timely manner. As many laboratories routinely process blood culture bottles in automated systems that record time to positivity (TTP) and this information is readily available along with the Gram-smear results, we assessed the