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Urea: another analyte recommended for harmonised reference intervals

Sir,

The Australasia Association of Clinical Biochemists (AACB) has used an evidence-based checklist approach to assess the feasibility of developing and recommending common reference intervals (RI).[1]

After reviewing and considering studies related to bias as well as both *a priori* and *a posteriori* RI studies nationally and internationally and the consideration of flagging rates, partitioning, units of measurement, and clinical relevance, the AACB has recommended and received endorsement by the Royal College of Pathologists of Australasia (RCPA) for 18 harmonised RIs for commonly performed clinical chemistry analytes. Using these same criteria, age and gender based concentrations for urea estimation in serum are now also to be recommended as harmonised RIs seeking endorsement by the RCPA. The analysis of bias utilising commutable samples undertaken by the AACB[2] as well as analysis of the liquid serum chemistry program provided by the RCPA Quality Assurance Program (QAP) showed that bias would not prevent the implementation of harmonised RIs for urea in serum. This assessment was based on a traffic light system[2] that incorporated the RCPAQAP allowable limits of performance (±12%) from the target or median value as its guide.

In determining the concentrations for the upper reference limit (URL) and lower reference limit (LRL), studies reviewed included the Aussie Normals,[3] data from the Sonic Health laboratory group and Dorovitch Pathology, a multicentre study undertaken by Ozarda *et al.* in Turkey,[4] the NORIP study[5] and the NHANES 111 study.[6] All of these studies have shown gender differences and that there is a progressive increase in concentration and range of concentrations for urea in healthy individuals as they age. These increases in concentration are at both the URL and the LRL and the age related concentrations obtained by the Aussie Normals study are shown in Fig. 1.

Analysis to assess if the recommended harmonised reference intervals meet the principles associated with flagging rates showed that if a uniform URL of 8.5 mmol/L was used, 1–2% of young adults (<40 years) gave flagging rates of 4–6%. However, using the same URL resulted in a steady increase in flagging rates with 40–60% of patients over 70 years of age having flagging rates of 20–30%. Conversely if flagging rates were age specific these flagging rates in the elderly were significantly reduced. If a uniform LRL of 2.5 mmol/L was used, very few flags resulted over the age

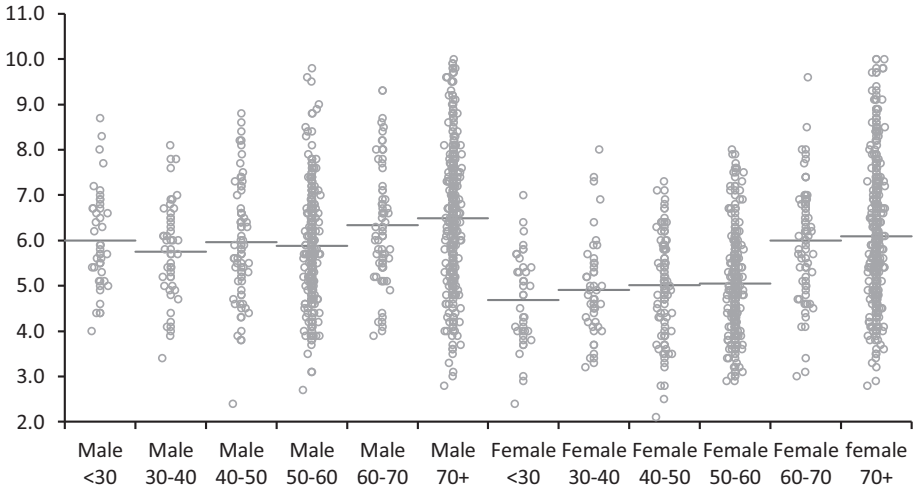

Fig. 1 Urea concentrations by age and gender derived from the Aussie Normals study.[3] The median urea concentrations (mmol/L) are shown by the horizontal bars. The x-axis shows the gender and age ranges in years while the y-axis shows the urea concentration in mmol/L.

Table 1 Proposed harmonised age and gender specific reference intervals for urea in serum

	<50 years	50−69 years	70+ years
Women (mmol/L)	3.0−7.0	3.5−8.0	4.0−9.0
Men (mmol/L)	3.5−8.0	4.0−9.0	4.5−10.0

range; however, if age related cut-offs were used, then the flagging rate was around 2.5% across all ages. The age and gender specific reference intervals meet the flagging rate criteria minimal standard of about 5.7% at the URL and the expected 2.5% at the LRL.[7]

Table 1 shows the recommended age and gender related reference intervals for urea proposed by the AACB.

One of the strategic priorities of the AACB is to achieve harmonisation of reference intervals where sound calibration and traceability are in place. The RIs recommended for harmonisation are based on professional opinion and consensus. These opinions are based on review of appropriate *a priori* and *a posteriori* analytical programs and studies, clinical relevance and flagging rates. The AACB has planned future workshops where discussions relating to other analytes as candidates for harmonisation will be discussed.

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Routine susceptibility testing for *Candida albicans* isolated from blood stream infections

Sir,

Blood stream infection (BSI) with *Candida* species is associated with significant morbidity and mortality.[1] Traditionally species level identification has been utilised to predict antifungal susceptibility patterns in *Candida* infections. Antifungal susceptibility (AFS) testing instead is now being increasingly employed to guide the management of *Candida* BSI. At our 1500 bed tertiary care hospital in Singapore, AFS has been conventionally performed by the Sensititre YeastOne (TREK Diagnostic Systems, USA) on index isolates of non-*albicans* species from positive blood culture broths. With the growing demand for comprehensive testing, routine susceptibilities were extended to *Candida albicans* with effect from February 2014.

According to previous Infectious Disease Society of America (IDSA) guidelines,[2] routine susceptibility testing was not required for *C. albicans*, a pathogen deemed typically sensitive to the azoles, unlike other *Candida* species. An update[3] by the IDSA now recommends susceptibility testing to azoles for all *Candida* species from bloodstream infections. These latest guidelines could potentially translate into additional workload and increased cost considerations for the clinical laboratory.

In this retrospective study we addressed the above recommendation by analysing the susceptibility profiles of all *Candida* species isolated from BSIs over a period of 3 years (February 2014 to December 2016) at Tan Tock Seng Hospital, Singapore. As identification and susceptibility testing are part of routine clinical care, we extracted the susceptibility profiles of all *Candida* species BSI using the laboratory information system. Isolates from expired and discharged patients were not subjected to susceptibility testing as per laboratory policy and were thus excluded from this analysis.

Briefly, blood cultures with smears containing budding yeast cells were subcultured on blood agar, chocolate agar, MacConkey agar and Sabouraud's dextrose agar (BBL; Biomedia, Singapore). These were incubated aerobically at 37°C with 5% CO_2. Visible colonies were analysed on the MALDI-TOF Biotyper system. The assay was performed on the Microflex mass spectrometer using the FlexControl software (version 3.3.108.0; Bruker, Germany). Next, the obtained spectra were imported into the Biotyper software (version 3.0; Bruker) and were compared to the reference spectra in the Bruker library to carry out a species level identification using a previously established score threshold $\geq$1.8.[4]

In this study, the Sensititre YeastOne (SYO)-YO10 version, a commercially available colorimetric microdilution panel was employed for susceptibility testing. This panel has been previously validated for AFS testing on *Candida* species and *Cryptococcus* species.[5] The susceptibilities were set up as per the manufacturer's recommendations.

Endpoints were determined as the lowest antifungal concentration to yield a substantial growth inhibition as visually confirmed by comparing the colour change to the drug-free growth control well. The antifungal minimum inhibitory concentrations (MICs) were interpreted as per the recently revised CLSI species-specific clinical breakpoints for azoles