

## Letter to the Editor

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# Therapeutic drug monitoring of voriconazole: validation of a novel ARK<sup>TM</sup> immunoassay and comparison with ultra-high performance liquid chromatography

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To the Editor,

Voriconazole (VRC) is a second-generation triazole licensed to treat patients with invasive aspergillosis, invasive candidiasis caused by *Candida* species with reduced susceptibility to fluconazole, and serious infections caused by *Scedosporium* and *Fusarium* species [1, 2]. Recently, prophylaxis of invasive fungal infections (IFIs) in high risk allogeneic hematopoietic stem cell transplant recipients was added as a new indication for VRC [3]. According to the British Society for Medical Microbiology (BSMM), trough concentrations >1 mg/L and <4–6 mg/L are required to maximize efficacy and to minimize drug-related toxicity [4].

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In patients treated for IFIs with the recommended oral/intravenous VRC dosage regimen, large inter- and intra-individual variability has been found in VRC trough plasma concentrations, ranging from undetectable concentrations to 11 mg/L [5]. Oral bioavailability is a major determinant of variability of VRC plasma concentrations [6]. In healthy individuals, oral bioavailability is reported to be high, approximately 96%, when administered in a fasting state (1 h before/after meal) [2]. However, in patients, oral bioavailability might be much lower, as these patients are frequently suffering from gastro-intestinal complications [6, 7]. Other factors that contribute to the variability in VRC plasma concentrations are the Michaelis-Menten (non-linear) pharmacokinetics of VRC, polymorphisms of the gene encoding the CYP2C19 enzyme, drug-drug interactions, liver disease and age [4]. The large variability in VRC plasma concentrations together with the narrow therapeutic window for treating patients with IFIs, makes individualized dosing adjustments based on therapeutic drug monitoring (TDM) of VRC necessary to optimize therapeutic response and to minimize the probability of neurotoxicity [6]. According to the BSMM, VRC plasma concentrations should be measured in the first 5 days of therapy and regularly thereafter [4].

In Table 1, an overview is given of the analytical methods that are suitable for measuring VRC in plasma, including bioassays [8–11], HPLC [12–19] and LC-MSMS [20–29] methods. Currently, chromatographic methods are predominantly used, as can also be derived from proficiency testing results [30]. These techniques are accurate, precise and allow the simultaneous analysis of multiple anti-fungal drugs. However, these methods also include some disadvantages, such as the limited availability of chromatographic instruments in the core clinical laboratory, the need for skilled laboratory technicians, the use of (sometimes) time consuming sample preparation steps and the need

**Table 1** Characteristics of the analytical methods used for measurement of VRC plasma concentrations.

	Current methods			New method
	Bioassay	HPLC/UPLC-UV/ fluorescence detection	LC-MS(MS)	ARK™ immuno-assay
Sample volume (plasma)	10–25 µL	100–500 µL	5–200 µL	100 µL
Sensitivity/LOQ	0.25–0.5 mg/L	0.03–0.5 mg/L	0.002–0.1 mg/L	0.4 mg/L
TAT	20–24 h	1–3 h	<30 min	5 min
Use	Clinical practice (TDM)	Clinical practice (TDM)	Clinical practice (TDM) Pharmacokinetic studies	Clinical practice (TDM)
Advantages	<ul style="list-style-type: none"> <li>– Inexpensive</li> <li>– No expertise needed</li> </ul>	<ul style="list-style-type: none"> <li>– Less expensive equipment than LC-MS</li> <li>– Possibility of simultaneous analysis of multiple drugs</li> </ul>	<ul style="list-style-type: none"> <li>– Minimal sample preparation</li> <li>– Small sample volume</li> <li>– Very sensitive and specific</li> </ul>	<ul style="list-style-type: none"> <li>– No need for specific equipment/For use on random access chemistry analyzers</li> <li>– No need for analysis in batch</li> </ul>
Disadvantages	<ul style="list-style-type: none"> <li>– Unsuitable for patients receiving antifungal combination therapy</li> <li>– Lower precision and accuracy</li> <li>– Lack of standardization</li> </ul>	<ul style="list-style-type: none"> <li>– Large sample volume needed to ensure sensitivity</li> <li>– Subject to interference from multiple substances</li> <li>– Analysis in batch with calibration and controls in each batch</li> <li>– Expertise needed to perform analysis</li> </ul>	<ul style="list-style-type: none"> <li>– Expensive equipment</li> <li>– Not widely available</li> </ul>	<ul style="list-style-type: none"> <li>– Requirement of ±120 requests/month (kit stability)</li> </ul>
References	[8–11]	[12–19], this study	[20–29]	

LOQ, limit of quantification; TAT, turn-around-time (=plasma pretreatment+analysis time).

to work in batch with repetitive calibrations, resulting in a long turn-around-time. Consequently, TDM-assisted VRC dose adjustment with chromatographic techniques is very challenging. The implementation of a fast and reliable VRC immunoassay on an open-access chemistry analyzer could significantly improve VRC therapeutic target achievement, and minimize the drug-related adverse effects.

Recently, ARK™ Diagnostics, Inc. (Fremont, CA, USA) has introduced a VRC homogeneous enzyme immunoassay (Test principle: see Supplemental Material), for use

on random access chemistry analyzers. In this study, we evaluated an investigational reagent lot (not approved for routine clinical application) of this assay in an open channel on the c502 module of the Cobas 8000 analyzer (Roche Diagnostics, Mannheim, Germany) in the core laboratory of Ghent University Hospital. The application protocol was obtained from ARK™ Diagnostics (Supplemental Material, Table 1). One assay kit contains reagents for 210 tests and analysis time on the c502 instrument is 5 min. Full calibration and quality control evaluation were

**Table 2** Within-run imprecision, between-run imprecision and bias of the ARK™ VRC immunoassay on the c502 module of the Cobas 8000 analyzer.

Quality control	Target concentration, mg/L	Within-run imprecision (n=21)			Between-run imprecision (n=40, pool: n=22)			Bias (n=40)
		Mean	SD	CV	Mean	SD	CV	
Low	1.5	1.62	0.08	5.03%	1.58	0.11	6.77%	5.33%
Mid	5.0	4.93	0.19	3.88%	5.05	0.31	6.14%	1.00%
High	10.0	9.74	0.48	4.96%	10.17	0.68	6.72%	1.70%
Plasma pool		0.43	0.03	7.16%	3.11	0.16	5.06%	

CV, coefficient of variation.

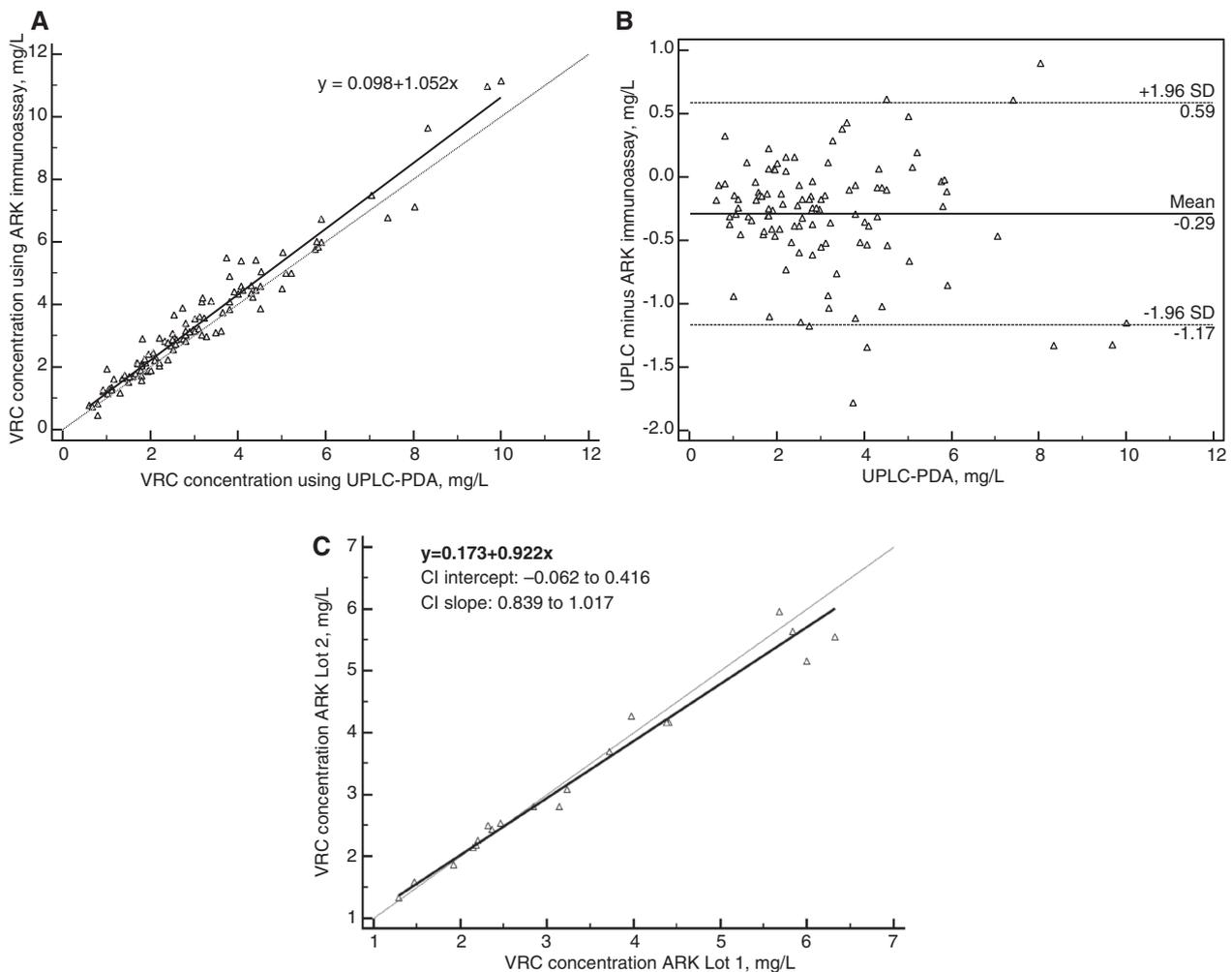
performed using the ready-to-use calibrators (6 points: 0.0, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/L) and quality controls (1.5, 5.0 and 10.0 mg/L).

Within-run ( $n=21$ ), between-run imprecision and accuracy ( $n=40$ , 25 days) were evaluated using quality control material and a plasma pool and were all found acceptable ( $<7\%$  at all tested concentrations, Table 2).

We evaluated the linearity of the VRC assay according to NCCLS guideline EP6-A. We prepared a dilution series covering the range 0.5–16.2 mg/L, as described in the Supplemental Material. As the deviation from linearity is considered not clinically important ( $<10\%$ ), linearity

is accepted within the tested range (Supplemental Material, Figure 1). Based on our experiments according to NCCLS EP17-A2 specifications, we obtained a functional sensitivity of 0.4 mg/L (CV 10%) (Supplemental Material, Figure 2). Recovery was tested by diluting (1:2, 1:4, 1:8 and 1:16) a native human plasma with a VRC concentration of 11.1 mg/L, where the result of the undiluted plasma was taken as 100% recovery. Mean recoveries of duplicate measurements of the diluted plasmas ranged between 99% and 107%.

Specificity was tested using samples provided by ARK™ Diagnostics containing 3 mg/L VRC in combination



**Figure 1** Passing-Bablok regression (A and C) and Bland-Altman plot (B) for comparison between ARK™ VRC immunoassay and UPLC-PDA ( $n=113$ , A and B) and between ARK™ Lot 1 (investigational lot) and Lot 2 (lot certified for clinical application) ( $n=20$ ).

Individual observations are represented as triangles. (A and B) Seven samples were excluded from the analysis because they had a VRC concentration below the LOQ. (A) The identity line is dashed. The continuous line shows the fitted regression line. Regression equation:  $y=0.098+1.052x$ . (B) Horizontal lines are drawn at the mean difference (continuous line), and at the limits of agreement (dashed lines), which are defined as the mean difference plus and minus 1.96 times the standard deviation (SD) of the differences. (C) The identity line is dashed. The continuous line shows the fitted regression line. Regression equation:  $y=0.173+0.922x$ . Confidence intervals (CI) for slope and intercept are indicated.

with the potential cross-reacting compounds fluconazole (30 mg/L), itraconazole (20 mg/L), posaconazole (20 mg/L) or the inactive N-oxide metabolite of VRC (10 mg/L). No interferences were found (Supplemental Material, Table 2).

During a period of 4 months, a method comparison was performed using 113 routine blood samples from patients receiving VRC. The VRC immunoassay was compared with the routinely used ultra-high performance liquid chromatography method with photodiode array detection (UPLC-PDA) carried out at the General Hospital St-Lucas, Ghent (Test principle: see Supplemental Material). Patient care relied solely on the UPLC-PDA method determination of VRC and the study was approved by the Ethics Committee of Ghent University Hospital (registration number B670201319303). Blood was drawn prior to administration of the next VRC dose and lithium heparin tubes without gel separator (Venosafe, Terumo Europe, Belgium) were used. As VRC is very stable in serum/plasma (at least 7 days at room temperature, and 1 month at  $-20^{\circ}\text{C}$ ), and is not affected by multiple freeze-thaw cycles (own unpublished data and [14]), fresh and frozen plasma samples were used. Results were analyzed using MedCalc Software (Mariakerke, Belgium). Seven samples, with results  $<0.5$  mg/L on both methods, were excluded from further analysis. Method comparison of the VRC immunoassay and UPLC-PDA showed a significant linear correlation (Pearson's  $r$ : 0.978,  $p < 0.0001$ ). Passing-Bablok regression showed an intercept of 0.098 (95% CI  $-0.025$  to 0.250) and slope of 1.052 (95% CI 0.996–1.105), with a substantial agreement between both methods, as indicated by a concordance correlation coefficient (CCC) of 0.96. Bland-Altman plot revealed a small systematic error of 0.29 mg/L between both methods (Figure 1A and B).

We also tested 12 control samples from United Kingdom National External Quality Assessment Service (UK NEQAS) with All Laboratory Trimmed Mean (ALTM) concentrations ranging from 0.37 to 9.03 mg/L (11/12 samples within measuring range). Median recovery of the UK NEQAS samples within the measuring range was 101% (recovery range 92%–117%). Our results did not significantly differ from the ALTM, as determined by the Wilcoxon paired-sample test ( $p = 0.42$ ). Lot-to-lot consistency was explored by re-measurement of plasma samples ( $n = 20$ ), using the first VRC reagent lot and calibrator lot, approved for routine clinical application. Passing-Bablok regression showed no significant systematic or proportional differences between both lots (Figure 1C). Analysis of three UK NEQAS ( $n = 3$ , ALTM concentrations 0.98, 1.59 and 8.16 mg/L) with the new reagent/calibrator lot resulted in a recovery of 99%–105%.

As the kit is containing 210 tests, reagent stability might be a point of concern for laboratories that have only a limited number of VRC requests. In our experience, the assay should be recalibrated on a weekly basis and kit stability on board of the analyzer is approximately 1 month.

This is the first study showing results of the first VRC immunoassay. An advantage of this fast and ready to use assay is the possibility to be used on random access analyzers in the core laboratory. Hence, this test can be performed on a 24/7 basis, allowing rapid adaptation of the dosing scheme of the patient under VRC therapy. Substantial agreement was found between the results of this new immunoassay and a validated UPLC-PDA assay, as well as an independent external assessment. In conclusion, the ARK™ VRC immunoassay proved to be acceptable for implementation in daily practice.

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