

Analytical Evaluation of a Vancomycin Immunoassay in Synovial Fluid

Francesca Luceri¹ Fiamma Balboni² Giovanni Balato³ Paola Pezzati¹ Nicoletta Cini¹ Gianni Virgili⁴
Andrea Baldini³

¹Laboratorio Generale, Dipartimento dei Servizi, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy

²Laboratorio Analisi, Istituto Fiorentino Cura E Assistenza, Firenze, Italy

³UO Ortopedia, Istituto Fiorentino Cura E Assistenza, Firenze, Italy

⁴Dipartimento di Chirurgia e Medicina Translazionale, Università di Firenze, Oculistica, Italy

Address for correspondence Paola Pezzati, MD, Laboratorio Generale, Dipartimento dei Servizi, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy (e-mail: p.pezzati@live.it).

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Abstract

In clinical laboratories performing routine activities, the need to answer the burning clinical question in emerging field may be limited by lack of technology support or assays accessibility. Commercially available methods, although originally validated for specific biological matrices, may be employed for other matrices, following appropriate guidelines such as Clinical and Laboratory Standards Institute (CLSI) EP 19. We investigated the use of a vancomycin assay with synovial fluid samples, in view of a possible employment in vancomycin release study. The standard of care of periprosthetic joint infection is a two-stage revision surgery with antibiotic-loaded bone cement implantation. Vancomycin, for its activity against gram-positive bacteria even multidrug-resistant staphylococci, is the most widely used antibiotic. Despite the widespread use of such devices, little is known about the *in vivo* elution in the joint space. Clinical laboratories equipped with a validated, affordable method to quantify vancomycin in synovial fluid, may support clinical research, and give an important contribution to the study of the pharmacokinetics of antibiotic release from bone cement matrix.

Keywords

- ▶ vancomycin
- ▶ clinical laboratory services
- ▶ immunoassay synovial fluid
- ▶ antibacterial agents

Introduction

Clinical laboratories are required to answer several emerging clinical questions; however, any request for implementation, in terms of a new biomarker, innovative assay, or unconventional samples, and alternative matrices, needs to be evaluated according to best practice and to certification rules.

When instruments or reagents, intended for a specific sample matrix, are used to analyze a different matrix, laboratory personnel must validate the procedure, according to the international guideline such as Clinical and Laboratory Standards Institute (CLSI) EP19.

Clinical laboratories routinely perform serum vancomycin assay for therapeutic drug monitoring (TDM). Recently, assessment of vancomycin levels in synovial fluid from patient affected by periprosthetic joint infection (PJI) and undergoing revision surgery has been suggested.¹

PJI is a rare yet devastating complication after total joint replacement, ranging between 0.5 and 3%, with total knee arthroplasty (TKA) having a higher prevalence than total hip arthroplasty.² Two-stage revision surgery with antibiotic-loaded spacer implantation is now considered the standard of care.^{3–5} Vancomycin is widely used and, although the pharmacokinetic properties of aminoglycosides have been thoroughly investigated *in vitro* and *in vivo* studies,^{5–8} only limited data are available on the vancomycin release *in vivo* from prosthetic devices after implantation.¹ Accordingly, it appears to be of paramount importance the availability of an affordable and reliable analytical method to study vancomycin *in vivo*.

The aim of this study was to verify if vancomycin assay validated for TDM in human plasma and serum can be used to measure vancomycin in drainage fluid.



Methods

K₂EDTA blank drainage fluid samples were obtained from patients ($n = 3$) who underwent primary TKA. No systemic vancomycin was given before or after surgery in any patient. The samples collected 24 hours after surgery were pooled, centrifuged, transferred to Microcon YM10 filters (Merck Millipore, Billerica, Massachusetts, United States), designed to remove proteins from viscous biological samples. According to manufacturer instructions, filters were loaded and centrifuged at 15,000 g for 20 minutes, and the ultrafiltrates were then collected (Filtered Synovial Fluid [FSF]).

Vancomycin hydrochloride for injection USP 500 mg was purchased from Mylan N.V. Pharmaceutical (Amsterdam, The Netherlands) and a stock solution of vancomycin (1 mg/mL) was prepared in blank FSF. Calibration standards at vancomycin concentrations of 5, 10, 25, 50, and 100 µg/mL were obtained from appropriate additions of stock solution to blank FSF.

Three levels of quality control (2.5, 25.0, and 50.0 µg/mL) were analyzed during each routine analysis, and these were prepared by the appropriate addition of another (independently prepared and weighed) stock solution of vancomycin (1 mg/mL).

All samples were analyzed on CDx90 (Thermo Scientific, Waltham, Massachusetts, United States) instrument by Quantitative Microsphere System (QMS) vancomycin assay (Thermo Scientific); the vancomycin assay is a homogeneous particle-enhanced turbidimetric immunoassay, and it is based on competition between drug in the sample and drug coated onto a microparticle for antibody-binding sites of the vancomycin antibody reagent.

The vancomycin-coated microparticle reagent is rapidly agglutinated in the presence of the antivancomycin antibody reagent and the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically.

When a sample containing vancomycin is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained with a maximum rate of agglutination at the lowest vancomycin concentration and the lowest agglutination rate at the highest vancomycin concentration.

Results

The method was linear from 0 to 100 µg/mL ($r_2 = 0.987$), selectivity was checked by analyzing 25 blank drainage fluid samples, the limit of quantification was 2.5 µg/mL and the accuracy ranged from 82 to 93% at three concentration levels (2.5, 25, and 50 µg/mL).

The precision of the method was determined by calculating the coefficient of variability at three concentration levels (2.5, 25, and 50 µg/mL) for 10 times in the same day (intra-assay precision) and once for 10 days (inter-assay precision). Intra-assay precision ranged from 1.8 to 4.9% at each level and the inter-assay precision from 4.0 to 10.4%.

Discussion

Since the introduction of antibiotic-loaded acrylic bone cement, antibiotic-impregnated bone cement have been widely used as a delivery vehicle for the local administration of antibiotics in joint-infected sites, reaching higher concentrations compared with systemic administration.⁵

The most commonly used antibiotics include tobramycin, gentamicin, vancomycin, and cephalosporins that show a wide spectrum activity even against the multidrug-resistant bacteria. Strikingly, a recent systematic review of the use of antibacterial cement spacers reports that a significantly high infection eradication rate, ranging from 73 to 100%, can be achieved even when the antibacterials placed into the spacers are not active against the infecting organisms.⁵

Despite these appealing results, several questions still remain to be addressed to optimize the use of these devices and to define the ideal moment for prosthetic reimplantation. Indeed, the therapeutic concentration levels of antibiotics present at the implantation site and the actual inhibitory activity of antibiotics released at the infection site, in the first postoperative period, are still open questions, along with the duration of the antimicrobial effect.

The possibility to measure vancomycin levels in drainage fluid is the basis for studying the antibiotic release in vivo from prosthetic devices after implantation and might represent a step ahead toward treatment optimization. Nevertheless, significant heterogeneity caused by differences in methods and experimental conditions have complicated the attempts to draw definite conclusions from available studies. The QMS method guarantees enough precision and accuracy at low costs to answer, at least, some of the clinical questions related to PJI.

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