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Measuring Serum Vedolizumab and Vedolizumab Antibodies: Comparison of Commercial Assays with the Vedolizumab Clinical Development Assay

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Background: Vedolizumab (VDZ) is an anti-$\alpha_4\beta_7$ integrin monoclonal antibody approved for inflammatory bowel disease treatment. VDZ serum and antidrug antibody (ADA) concentrations may be used for treatment optimization. In this article, the results of 5 commercial assays (Grifols, Immundiagnostik, Progenika, Sanquin, and Theradiag) measuring VDZ concentration and ADA were compared with those of the reference assays used in VDZ clinical studies. Our findings will assist clinicians in interpreting commercial assay results in the context of VDZ clinical trial data.

Methods: VDZ-treated patient samples were used to evaluate the agreement between commercial assays and the reference VDZ serum concentration assay, based on linear regression, Bland–Altman, and qualitative agreement analyses. VDZ ADAs were detected using qualitative assays. Specificity, selectivity, accuracy, and precision were assessed using serum samples from healthy donors or patients with IBD (VDZ serum concentration <0.5 mcg/mL) spiked with VDZ, with/without other biologics (identical sample sets per assay).

Results: All assays were specific and selective for VDZ. Overall, the commercial assay results for VDZ-spiked samples correlated well with those of the reference serum concentration assay ($R^2 \geq 0.98$). Compared with the Immundiagnostik and Theradiag assays, the Grifols, Sanquin, and Progenika assays had the best reference assay agreement (based on regression analysis, Bland–Altman plots, and qualitative agreement [Cohen's kappa $\geq$0.92]). All immunogenicity assays detected VDZ ADAs; only the reference assay detected VDZ ADAs in the presence of 15 mcg/mL VDZ, advising caution with commercial ADA assays if VDZ is present.

Conclusions: All 5 commercial assays are suitable for VDZ therapeutic monitoring and ADA testing. However, the absolute values from the reference assays and the different commercial assays were not comparable, indicating that the same assay must be used for repeated monitoring of VDZ serum concentrations.

Key Words: vedolizumab, assay, ulcerative colitis, Crohn's disease

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DATA AVAILABILITY

The data sets, including the redacted study protocol, redacted statistical analysis plan, and individual participants' data supporting the results reported in this paper, will be made available within 3 months from initial request to researchers who provide a methodologically sound proposal. The data will be provided after its deidentification in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization**.** Data are available on request by application at [https://search.vivli.org/].

INTRODUCTION

Therapeutic drug monitoring (TDM) of biologics by the use of assays to quantify drug concentrations and detect antidrug antibodies (ADAs) is increasingly being used to

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maintain drug concentrations within certain parameters in blood, thereby optimizing the therapeutic efficacy in patients with ulcerative colitis (UC) or Crohn disease (CD).[1,2] The goal of this strategy is to mitigate the loss of response or development of treatment-related complications by adjusting the dose and/or dosage interval. For TDM to be an effective strategy, a clear and measurable relationship between drug blood concentration and therapeutic response is required. This type of relationship has been demonstrated for antitumor necrosis factor alpha (anti-TNF-α) treatments, such as infliximab[2–7] and adalimumab,[8–10] which are used to treat patients with inflammatory bowel disease (IBD). Various commercial assays used to measure anti-TNF-α drug concentrations and immunogenicity offer good correlation, facilitating more uniform therapeutic decisions among clinicians.[2–12]

Vedolizumab, a humanized monoclonal immunoglobulin G1 antibody that targets a conformational epitope of the $\alpha_4\beta_7$ integrin heterodimer, is approved for the treatment of adult patients with moderately to severely active UC or CD.[13,14] Data on vedolizumab exposure–response highlight the potential benefit of TDM in patients with IBD.[15–17] The vedolizumab serum concentration and ADA immunogenicity assays (hereinafter referred to as reference assays) used in the phase 3 GEMINI 1, GEMINI 2, and VISIBLE 1 trials[18–20] are not commercially available. However, several commercial assays are available to measure vedolizumab serum concentration and ADAs; however, the relationships between the results obtained using these assays and the reference assays developed by Takeda Pharmaceuticals (Deerfield, IL) are unknown.

The comparison and harmonization of assays for TDM are important for cross-referencing the minimal effective drug concentration proposed in clinical research and guidelines. As a result, the data obtained using the reference assay for vedolizumab serum concentration and ADAs were compared with those from 5 commercial assays. These analyses were designed to determine whether the commercial assays cross-reacted with other antibodies (ie, specificity) and whether they selectively detected vedolizumab in the presence of other antibodies (ie, selectivity).

METHODS

Assays

The reference vedolizumab serum concentration assay is an enzyme-linked immunosorbent assay (ELISA) used to measure vedolizumab serum concentrations in the GEMINI and VISIBLE 1 clinical trials.[21,22] In brief, vedolizumab was bound by immobilized mouse antivedolizumab idiotypic antibodies in microtiter plates. The unbound mouse antivedolizumab idiotypic antibodies were then blocked, and serum samples were added to the wells. The captured vedolizumab was detected with F(ab')2 mouse antihuman immunoglobulin conjugated to horseradish peroxidase and visualized using a colorimetric substrate (**Fig. 1A, Supplemental Digital Content,** http://links.lww.com/TDM/A639). The lower limit of quantification (LLOQ) for this assay is 0.2 mcg/mL (**Table 1, Supplemental Digital Content,** http://links.lww.com/TDM/A639).

The vedolizumab ADA immunogenicity reference assay is a drug-tolerant bridging electrochemiluminescence assay. For this assay, serum samples were treated with acid to dissociate antibodies complexed to the drug; the acid was then neutralized using excess SULFO-TAG-labeled (Meso Scale Discovery, Rockville, MD) and biotinylated drugs. The captured ADA-drug complexes were detected using an MSD SECTOR instrument (Meso Scale Discovery) (**Fig. 1B, Supplemental Digital Content,** http://links.lww.com/TDM/A639). The reference ADA immunogenicity assay has a sensitivity of 3.9 ng/mL and detects 0.5 mcg/mL vedolizumab ADAs in the presence of vedolizumab at concentrations of ≥50 mcg/mL.

In this prospective study, reference assays developed at Takeda were compared with commercial assays by Grifols Diagnostic Solutions (Los Angeles, CA), Immundiagnostik (Bensheim, Germany), Progenika Biopharma (Bizkaia, Spain; called Progenika Biopharma B when the assay comparison was conducted), Sanquin (Amsterdam, The Netherlands), and Theradiag (Croissy-Beaubourg, France). The assay information is summarized in **Table 1, Supplemental Digital Content,** http://links.lww.com/TDM/A639. All assays for measuring vedolizumab concentration were ELISA-based, and their LLOQ ranged from 0.01 to 2 mcg/mL. These assays used antivedolizumab antibodies to capture and detect vedolizumab in samples, except for the Theradiag assay, which used the $\alpha_4\beta_7$ integrin for capture and antivedolizumab antibodies for detection. Most ADA assays are ELISA-based and use vedolizumab to capture and detect vedolizumab ADAs, except for the Sanquin assay, which detects vedolizumab ADAs bound to protein-A-sepharose beads by the biotin-labeled Fab portion of vedolizumab.

Infliximab, adalimumab, and vedolizumab were obtained from Fisher Clinical Service (Allentown, PA), Almac Clinical Services (Durham, NC), and Takeda (Deerfield, IL), respectively

Sample Sets

Human sera from healthy donors and patients with IBD with vedolizumab serum concentration <0.5 mcg/mL were purchased from Bioreclamation IVT (Westbury, NY) by QPS (Newark, DE) and used to prepare spiked samples for the assays described below. Serum from consenting vedolizumab-treated adult patients with UC or CD was purchased from Discovery Life Sciences (Huntsville, AL) or Sanguine BioSciences (Sherman Oaks, CA). These samples were collected in red-top or gray-top serum separator tubes and transported to QPS for further processing. These patients received their last dose of vedolizumab ≥2 weeks and ≤10 weeks before blood collection. The main exclusion criteria were UC or CD treatment with any other biologics within 12 weeks before blood collection, treatment with any investigational drug within 30 days before blood collection, pregnancy or nursing, and diagnosis of another autoimmune or inflammatory disease, HIV infection, or hepatitis.

Identical sets of randomized and blinded serum samples were sent to the respective commercial laboratories between January and March 2019 for analysis. The samples used in this study (including their definitions and replicates) are outlined in Figure 1. In the context of this comparative study, specificity was defined as the lack of assay cross-reactivity with other antibody therapies. The samples used to assess

assay specificity were prepared by spiking serum with infliximab (10 mcg/mL) and adalimumab (5 mcg/mL) (Fig. 1, box A). Selectivity was defined as the ability of each assay to detect vedolizumab in the presence of other antibodies. Test samples were prepared by spiking serum with infliximab (10 mcg/mL), adalimumab (5 mcg/mL), and vedolizumab (20 mcg/mL) (Fig. 1, box B).

In the comparison study, precision was evaluated using 10 pooled samples (triplicates; n = 30 samples tested) and samples for selectivity assessment (duplicates; n = 4 samples tested, resulting in a total of n = 34) (Fig. 1, box C), and accuracy was evaluated using 10 pooled samples (triplicates) and samples for selectivity assessment (duplicates), and 23 patient samples (singlets) (Fig. 1, box C and box D, n = 34 +

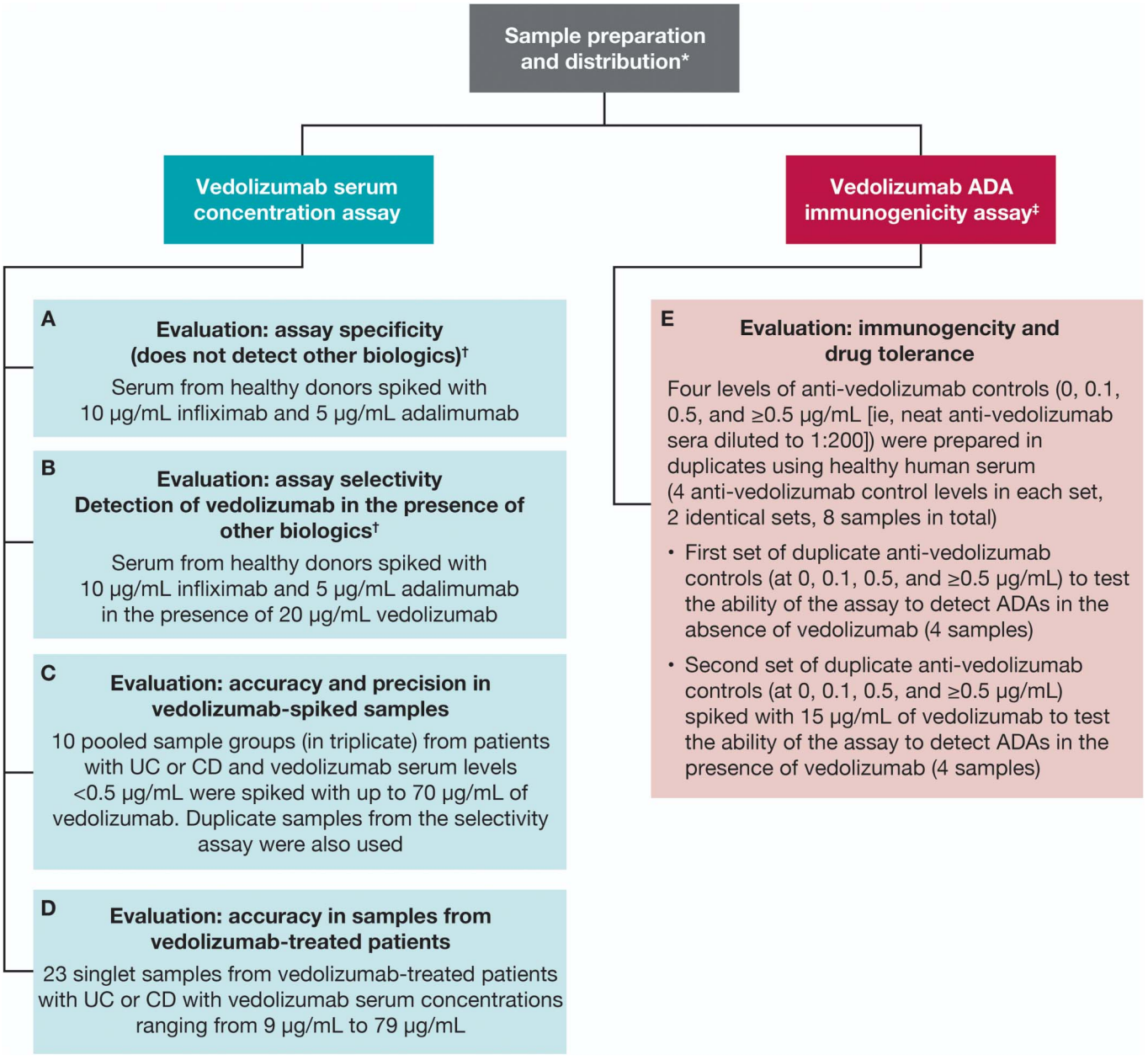


FIGURE 1. Study design. *Identical sets of serum samples were sent for analysis using each assay. †Two pooled sample groups from healthy donors were spiked with 10 mcg/mL infliximab and 5 mcg/mL adalimumab: a group without vedolizumab (specificity assay) and a second group with 20 mcg/mL vedolizumab (selectivity assay). Each sample group was provided in duplicates and the assays were performed in parallel; individual results were reported. ‡One set of 4 samples was also spiked with 15 mcg/mL vedolizumab to test the drug tolerance of the ADA assay. This vedolizumab concentration was selected as it is the approximate trough serum concentration observed when drug levels are at steady state during maintenance (dosing every 8 weeks).[14] The other set of 4 samples did not contain vedolizumab. ADA, antidrug antibody; CD, Crohn disease; UC, ulcerative colitis.

23 = 57). A concentration range of 1–60 mcg/mL (based on the Takeda reference assay) was used to include the greatest number of values over a similar range (most assays could not detect concentrations <1 mcg/mL and >60 mcg/mL). Six samples were excluded from the precision and accuracy sample set, reducing the sample number from 34 to 28. Furthermore, 2 samples were excluded from the IBD patient sample set, reducing the sample number to 21 (see **Table 2**, **Supplemental Digital Content,** http://links.lww.com/TDM/A639). For the immunogenicity comparison study, various levels of antivedolizumab–positive samples were spiked with vedolizumab (15 mcg/mL) or were not spiked (Fig. 1, box E). The results from each commercial assay were compared with those from the reference vedolizumab serum concentration and ADA immunogenicity assays.

Statistical Analyses

To evaluate assay accuracy and precision, the mean serum vedolizumab concentrations were calculated for triplicate samples from patients with IBD with vedolizumab serum levels <0.5 mcg/mL, and duplicate samples from vedolizumab-spiked samples from healthy donors. The experiments included different runs performed on separate days. A weighted least-squares regression model with inverted standard deviations across replicates as weights was fitted to the mean serum vedolizumab concentrations. The equation for the line fitting the model is $y = mx + b$, where m is the slope and b is the shift. Values below the LLOQ, values above the upper limit of quantification, and concentrations outside the detection interval (1–60 mcg/mL) were excluded from the analyses, as noted above. For the samples from vedolizumab-treated patients, a linear regression model was fitted to vedolizumab serum concentrations measured using commercial assays and the reference serum concentration assay. The analysis was performed using samples provided for each assay within the 1–60 mcg/mL range, according to the reference serum concentration assay. Vedolizumab serum concentrations were predicted using the weighted least-squares regression model obtained from the vedolizumab-spiked samples and compared with the actual observed values. Thus, the concordance between the predicted and actual values from the reference serum concentration assay and commercial assays was used to determine the performance of each commercial assay.

Modified Bland–Altman plots were produced to evaluate and illustrate the agreement between each commercial assay and the reference serum concentration assay. These plots demonstrated the difference between the corresponding values of both assays (commercial assay—reference assay) and the corresponding values of the reference assay. The mean (or percentage) difference and limits of agreement (defined as the range in which 95% of the observed differences between the 2 compared assays were found) are presented.

A qualitative agreement analysis was performed to assess the agreement of each assay with the reference serum concentration assay in the detection of vedolizumab concentrations that fell into 3 clinically meaningful intervals: <15, 15–30, and >30 mcg/mL. Cohen kappa statistic (χ), a measure of agreement, was calculated for each commercial assay. The value of χ is interpreted as follows: <0, no agreement; 0–0.2, no to slight agreement; 0.2–0.4, fair agreement; 0.4–0.6, moderate agreement; 0.6–0.8, substantial agreement; and 0.8–1, almost perfect agreement.

The overall agreement of the commercial assay with the reference serum concentration assay was assessed considering all 3 statistical analyses described above, instead of individual interpretation of the analyses.

Vedolizumab ADA assay results were qualitative and were reported as ADA positive or ADA negative. Statistical analyses were performed using R software, version 4.0.4.

Ethical Considerations

The serum samples used for this study were obtained from commercial suppliers under institutional review board–approved protocols with informed consent.

RESULTS

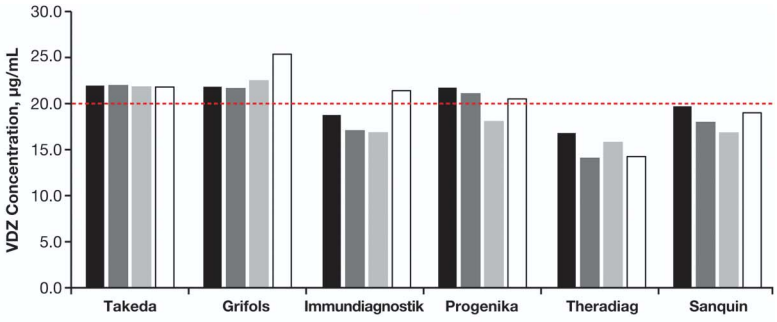
Specificity and Selectivity of the Vedolizumab Serum Concentration Assay

Consistent with other comparative studies, such as that of Marini et al,[4] specificity was defined as the lack of assay cross-reactivity with other antibody therapies. Selectivity was defined as the ability of each assay to detect vedolizumab in the presence of other antibodies. To demonstrate the specificity of the assays, healthy donor samples without vedolizumab were spiked with infliximab or adalimumab. The reference serum concentration assay and all commercial assay measurements were below the LLOQ for these serum samples (data not shown). To demonstrate the selectivity of the assays, healthy donor samples were spiked with infliximab or adalimumab in the presence of 20 mcg/mL vedolizumab (Fig. 2). Only slight differences were observed in the detection of vedolizumab in the presence of these biologics, suggesting that the overall impact was negligible.

Accuracy and Precision of the Vedolizumab Serum Concentration Assay

Pooled serum samples from patients with IBD (vedolizumab serum concentration <0.5 mcg/mL) were spiked with increasing concentrations of vedolizumab. Data from each commercial assay were compared with those from the reference serum concentration assay (red symbols and equations, Fig. 3) by a regression model. Based on the defined range of the sample results, 28 of the 34 samples for the commercial assays were included in the analysis. A weighted regression model was used to account for triplicates and duplicates. Overall, the commercial assays correlated well with the reference assay ($R^2 \geq 0.98$) (Fig. 3); however, there were differences in the absolute measured values and the slopes (range, 0.60–1.05).

Similar regression models for each commercial assay were generated using 21 samples obtained from vedolizumab-treated patients that had concentrations within the 1–60 mcg/mL range based on the reference serum concentration assay (black symbols and equations, Fig. 3). Among these samples, one had a concentration >60 mcg/mL based on the Progenika assay and was included in the analysis as the Takeda



	Takeda	Grifols	Immundiagnostik	Progenika	Theradiag	Sanquin
IFX + VDZ (replicate 1)	22.0	21.8	18.8	21.7	16.8	19.7
IFX + VDZ (replicate 2)	22.0	21.6	17.0	21.1	14.1	18.0
ADA + VDZ (replicate 1)	21.8	22.5	16.8	18.0	15.8	16.8
ADA + VDZ (replicate 2)	21.8	25.4	21.4	20.5	14.2	19.0

FIGURE 2. Assay selectivity. Data from duplicates are presented as independent replicates. Red line indicates the concentration of VDZ (20 mcg/mL) spiked into samples. ADA, adalimumab; IFX, infliximab; VDZ, vedolizumab.

reference assay result for this sample was <60 mcg/mL. In general, all 5 commercial assays correlated well with the reference serum concentration assay ($R^2 \geq 0.91$). The slopes were found to differ between the commercial assay regression models (range 0.59–1.19), indicating differences in the absolute measured vedolizumab concentrations between assays.

The performance (predictive value) of the derived regression models built on vedolizumab-spiked samples was determined using vedolizumab-treated patient samples (red symbols, Fig. 3). Generally, for all 5 commercial assays, the models built based on vedolizumab-spiked serum samples from patients with IBD predicted the concentrations in

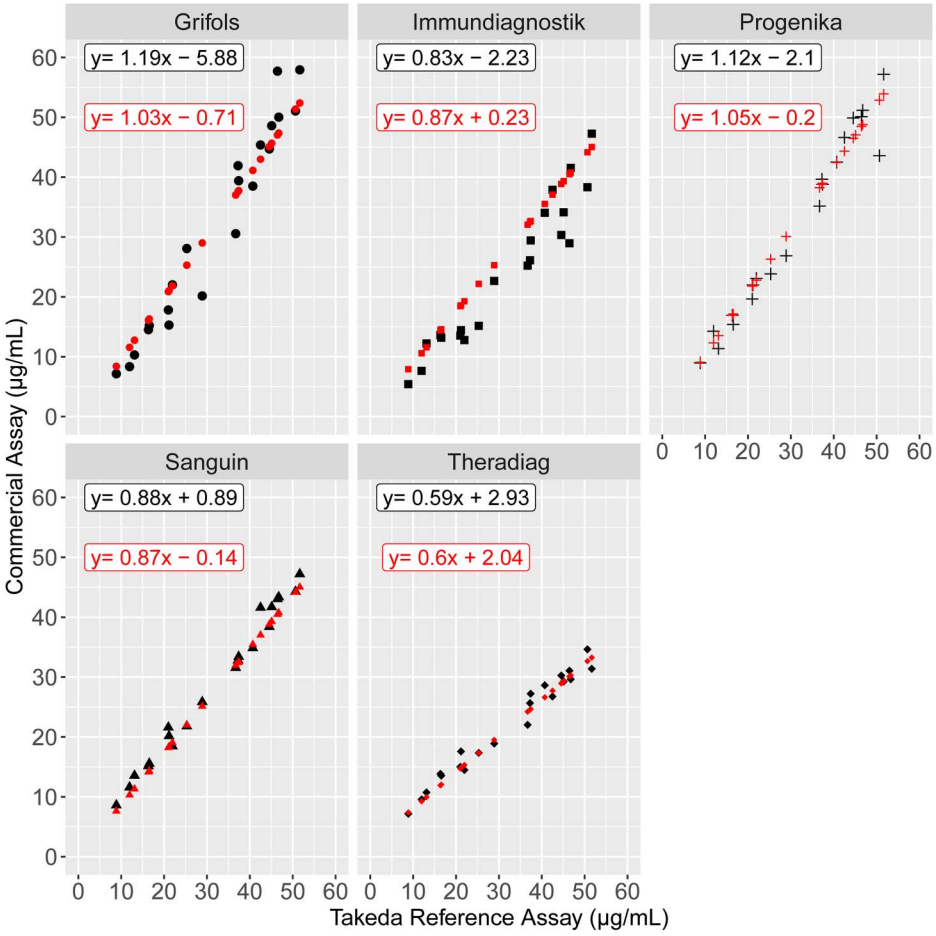


FIGURE 3. Consistency of the predicted and measured concentrations in patient samples. Black symbols represent the actual values of samples from vedolizumab-treated patients with IBD (n = 21). Black equations represent the regression fit of samples from vedolizumab-treated patients with IBD. Red symbols and red equations represent predicted values and predicted regression fit for the 21 patient samples based on regression fit from 28 vedolizumab-spiked serum samples (see Sample Sets section of Methods and **Table 2, supplemental digital content,** http://links.lww.com/TDM/A639). The adjusted R^2 values for the correlation with the reference assay were as follows: Theradiag 0.97, Immunodiagnostik 0.91, Progenika 0.93, Grifols 0.95, and Sanquin 0.99. The coefficients of variation for the assays (calculated based on 23 patient samples) were as follows: Theradiag 38.8%, Immunodiagnostik 50.5%, Progenika 48.6%, Grifols 53.8%, Sanquin 43.2%, and reference 44.2%. IBD, inflammatory bowel disease.

samples from patients treated with vedolizumab. The mean concentrations and precision for each assay based on replicated samples are presented in **Tables 3 and 4, Supplemental Digital Content,** http://links.lww.com/TDM/A639.

Agreement Between Each Commercial Assay and the Reference Assay for the Assessment of Vedolizumab Serum Concentration

Based on the reference serum concentration assay of the 21 analyzed patient samples (see Sample Sets section of Methods and **Table 2**, **Supplemental Digital Content,** http://links.lww.com/TDM/A639), 3 samples had vedolizumab concentrations <15 mcg/mL, 7 samples had concentrations of 15–30 mcg/mL, and 11 samples had concentrations >30 mcg/mL. The Grifols assay had a good linear relationship with the reference assay based on spiked samples from patients with IBD (m = 1.03; b = −0.71) (Fig. 3). However, for vedolizumab-treated patients, the assay tended to agree less with the reference assay based on the slope (m = 1.19); the shift suggests underestimation and overestimation of concentrations compared with the reference assay for concentrations <20 or >40 mcg/mL, respectively. This level of agreement was confirmed by the Bland–Altman analysis (mean difference −0.01 mcg/mL; half-width of the limits of agreement, ∼9 mcg/mL) (Fig. 4). The pattern of data distribution on the Bland–Altman plots indicated that this assay tended to underestimate concentrations <30 mcg/mL and overestimated higher concentrations of vedolizumab (Fig. 4). The results with concentrations >45 mcg/mL must be interpreted with caution, as not all data points fell within the limits of agreement. The qualitative agreement analysis revealed nearly ideal agreement (χ = 0.92), with only one sample placed in a different value range compared with its placement with the reference assay (Table 1).

The Immundiagnostik assay generally underestimated reference assay concentrations based on spiked samples from patients with IBD (m = 0.87, b = 0.23) and vedolizumab-treated patients (m = 0.83, b = −2.23) (Fig. 3). The mean (percentage) difference between the 2 assays was approximately −7.7 mcg/mL (∼25.6%), whereas differences from the reference assay appeared to increase for larger concentrations (Fig. 4 and **Fig. 2, Supplemental Digital Content,** http://links.lww.com/TDM/A639). The limits of agreement were relatively narrow (half-width of ∼8 mcg/mL) (Fig. 4). The qualitative agreement analysis revealed fair agreement (χ = 0.37) between this assay and the reference assay; 9 samples were placed in a different value range (Table 1).

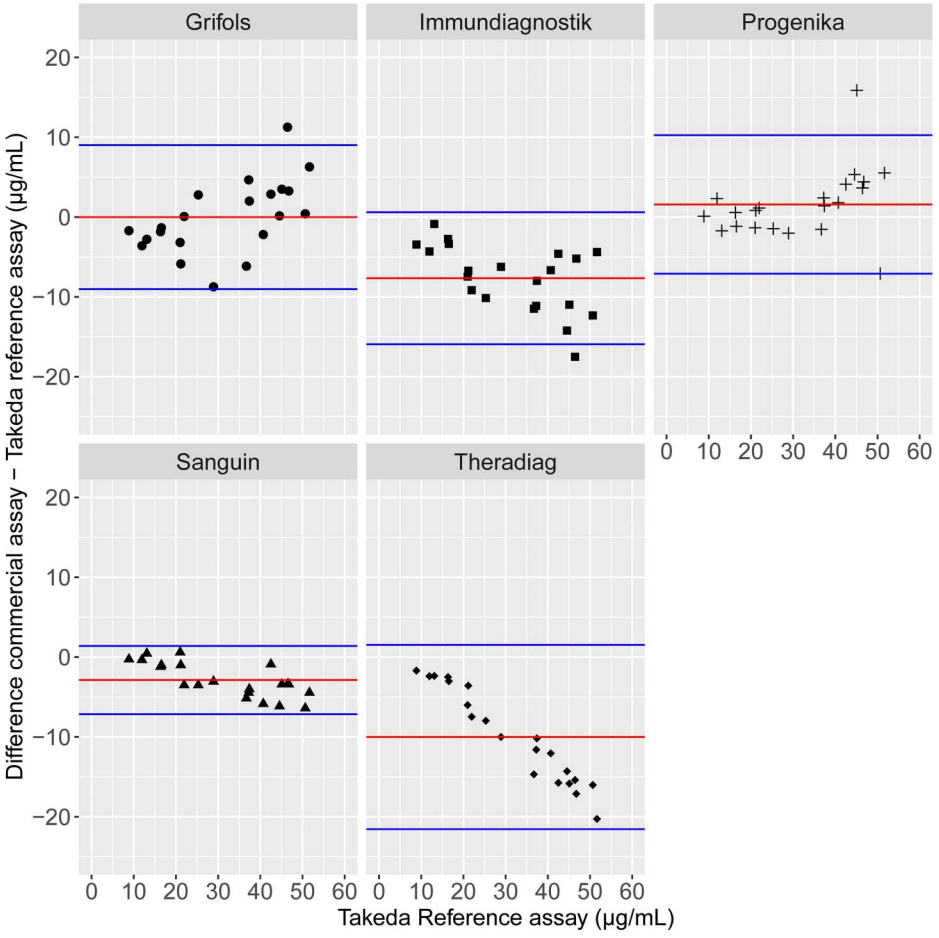


FIGURE 4. Mean differences in vedolizumab serum concentrations measured using the commercial assays compared with the reference assay. Bland–Altman analysis results of 21 singlet samples from vedolizumab-treated patients with inflammatory bowel disease (IBD) illustrating the comparability of the commercial assays to the Takeda reference assay. The red lines represent the mean difference of the commercial and reference assays across the investigated range. The blue lines indicate the limits of agreement.

TABLE 1. Qualitative Agreement Analysis on Vedolizumab Serum Concentration

Assay	Commercial Assay Results, mcg/mL	Takeda Reference Assay Results, mcg/mL <15	15–30	>30	Cohen's Kappa (95% CI)
Grifols	<15	3	1*	—	0.92 (0.77–1.0)
	15–30	—	6	—	
	>30	—	—	11	
Immundiagnostik	<15	3	5*	—	0.37 (0.09–0.64)
	15–30	—	2	4*	
	>30	—	—	7	
Progenika	<15	3	—	—	1.0 (1.0–1.0)
	15–30	—	7	—	
	>30	—	—	11	
Sanquin	<15	3	—	—	1.0 (1.0–1.0)
	15–30	—	7	—	
	>30	—	—	11	
Theradiag	<15	3	4*	—	0.25 (−0.04 to 0.53)
	15–30	—	3	7*	
	>30	—	—	4	

*Samples with discrepancies in results between the commercial and Takeda reference assays.

N = 21 singlets were used for analysis, as described in the Methods and **Table 2, Supplemental Digital Content,** http://links.lww.com/TDM/A639.

CI, confidence interval.

The Progenika assay had a good linear relationship with the reference assay based on spiked samples from patients with IBD (m = 1.05, b = −0.20) (Fig. 3), with a risk of underestimation or overestimation in vedolizumab-treated patients (m = 1.12, b = −2.10) (Fig. 3). Based on the Bland–Altman plots (Fig. 4), a very good general agreement existed for values < 45 mcg/mL. The results of concentrations >45 mcg/mL must be interpreted with caution, as not all data points fell within the limits of agreement. The mean difference was 1.58 mcg/mL and the limits of agreement were reasonably narrow (half-width ~8.7 mcg/mL) (Fig. 4). Qualitative agreement analysis revealed ideal agreement (χ = 1) between this assay and the reference assay (Table 1).

The Sanquin assay slightly underestimated the reference assay concentrations based on spiked samples from patients with IBD (m = 0.87, b = −0.14) (Fig. 3) and vedolizumab-treated patients (m = 0.88, b = 0.89) (Fig. 3). Based on the Bland–Altman analysis (Fig. 4), this assay slightly overestimated the values for concentrations <30 mcg/mL based on the reference assay and slightly underestimated the values for concentrations >35 mcg/mL based on the reference assay. The mean (percentage) difference between the 2 assays was −2.9 mcg/mL (~8%), and the limits of agreement were very narrow (half-width ~4 mcg/mL) (Fig. 4 and **Fig. 2, Supplemental Digital Content,** http://links.lww.com/TDM/A639). Qualitative agreement analysis revealed ideal agreement (χ = 1) between this assay and the reference assay.

The Theradiag assay generally underestimated the reference assay results based on spiked samples from patients with IBD (m = 0.60, b = 2.04) (Fig. 3) and vedolizumab-treated patients (m = 0.59, b = 2.93) (Fig. 3). The mean (percentage) difference between the 2 assays was −10.0 mcg/mL (~29%). For concentrations <30 mcg/mL, the absolute difference from the reference assay was moderate (<10 mcg/mL); for larger concentrations, differences >10 mcg/mL (up to 20 mcg/mL) were observed (Fig. 4 and **Fig. 2, Supplemental Digital Content,** http://links.lww.com/TDM/A639). The limits of agreement were the largest of the 5 commercial assays (half-width of ~11.5 mcg/mL) (Fig. 4). Qualitative agreement analysis indicated fair agreement (χ = 0.25) between this assay and the reference assay; 11 samples were placed in a different value range (Table 1).

ADA Assays

The reference vedolizumab ADA assay and all 5 commercial ADA assays detected vedolizumab ADAs without vedolizumab present (**Table 5, Supplemental Digital Content,** http://links.lww.com/TDM/A639). The reference ADA assay was the only assay that detected vedolizumab ADAs in the presence of 15 mcg/mL vedolizumab, thereby confirming drug tolerance. Adalimumab (100 mcg/mL) was tested using the vedolizumab ADA assay, which revealed no interference (data not shown). Infliximab interference was not determined in this study.

DISCUSSION

All 5 commercial assays (Grifols, Immundiagnostik, Progenika, Sanquin, and Theradiag) could measure vedolizumab concentrations in vedolizumab-treated patients and could thus be used to monitor treatment. However, all commercial assays differed from the reference assay for their ability to quantify vedolizumab concentrations. The 5 assays were compared with the reference serum concentration assay using 3 statistical analyses (regression, Bland–Altman, and qualitative agreement), which were interpreted together to formulate an overall conclusion on correlation and agreement.

Regression analysis was used to investigate the linear relationship between each commercial assay and the reference serum concentration assay across the full concentration range, resulting in an equation for the regression line (y = mx + b). Assays were indicated to agree when the equation is, or is close to, y = x, where the slope is 1 and the shift is 0.[23] The slope and shift should be considered together when interpreting the agreement between the 2 assays. The Bland–Altman analysis was used to illustrate the agreement between each commercial assay and the reference serum concentration assay by estimating the distance between the values of the 2 compared assays; on average, a mean difference close to 0 indicates high agreement. The interval around the mean difference (limits of agreement) indicates the consistency of the difference between the 2 assay methods across a range of values. Values plotted outside the limits of agreement should be considered with caution. Of note, what is regarded as "close" is a clinical decision and not a statistical decision.[23–26] The qualitative agreement analysis between the commercial assays and the reference serum concentration

assays was performed by categorizing vedolizumab concentrations into 3 clinically meaningful intervals (<15, 15–30, and >30 mcg/mL according to the trough serum concentrations observed at steady state during maintenance dosing every 8 weeks, maintenance dosing every 4 weeks, and induction),[14] without providing any quantitative information. These results inform physicians of the probability that a commercial assay result would fall into the same interval if tested using the reference serum concentration assay.[26–28]

Based on these analyses, the Grifols, Progenika, and Sanquin assays yielded results similar to the reference serum concentration assay. However, owing to the interassay differences and intra-assay variations over time, a direct serum concentration comparison and, thus, a simple conversion factor from these commercial assays to the reference serum concentration assay cannot be provided. The Immundiagnostik and Theradiag assays generally tended to underestimate serum vedolizumab concentration compared with the reference serum concentration assay. Thus, any interpretation of serum concentrations should be based solely on the assay used. The differences in assay procedures and critical reagents used in the capture and detection steps might explain the differences in the serum concentrations of vedolizumab. Generally, if vedolizumab serum concentrations are monitored over time in a patient, the same vendor assay should be consistently used to enable accurate comparisons between time points. Furthermore, drifts in assay performance are expected and further limit the ability to calculate conversions between results obtained using different assays.

Only the reference ADA assay was drug tolerant. The commercial ADA assays did not detect vedolizumab ADAs in the presence of 15 mcg/mL vedolizumab. Thus, if vedolizumab is present in a patient sample, drug-sensitive commercial assays may lead to false-negative results. In practice, vedolizumab ADA data should be interpreted while considering the vedolizumab concentrations obtained from the same sample.

Our study had strengths and limitations. To the best of our knowledge, this is the first study to compare commercially available assays for the measurement of vedolizumab and ADA concentrations against the assay used in vedolizumab registration trials. Our findings highlight the importance of conducting rigorous assay comparison studies owing to potential differences between assays. In this article, infliximab interference with the vedolizumab ADA assay was not tested; however, adalimumab 100 mcg/mL was tested and was not found to interfere with the detection of antivedolizumab antibodies. In addition, the clinical relevance of the measurement of vedolizumab concentrations or ADA levels could not be assessed. Although this relevance was not within the scope of the current article, it has been addressed in other studies.[29]

CONCLUSION

By comparing commercial assays to the reference assays used in vedolizumab clinical studies, the 5 commercial assays were found to be suitable for monitoring serum vedolizumab and ADA concentrations. However, longitudinal monitoring and interpretation of vedolizumab concentrations in patients should be performed using the same commercial assay. Furthermore, caution should be exercised when interpreting ADA assay data in the presence of vedolizumab. The differences between assays for detecting vedolizumab serum concentrations and ADAs should be considered during the comparison of commercial assay results with those from clinical studies.

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