**Measuring Serum Vedolizumab and Antibodies to Vedolizumab: Comparison of Commercial Assays With the Vedolizumab Clinical Development Assay**

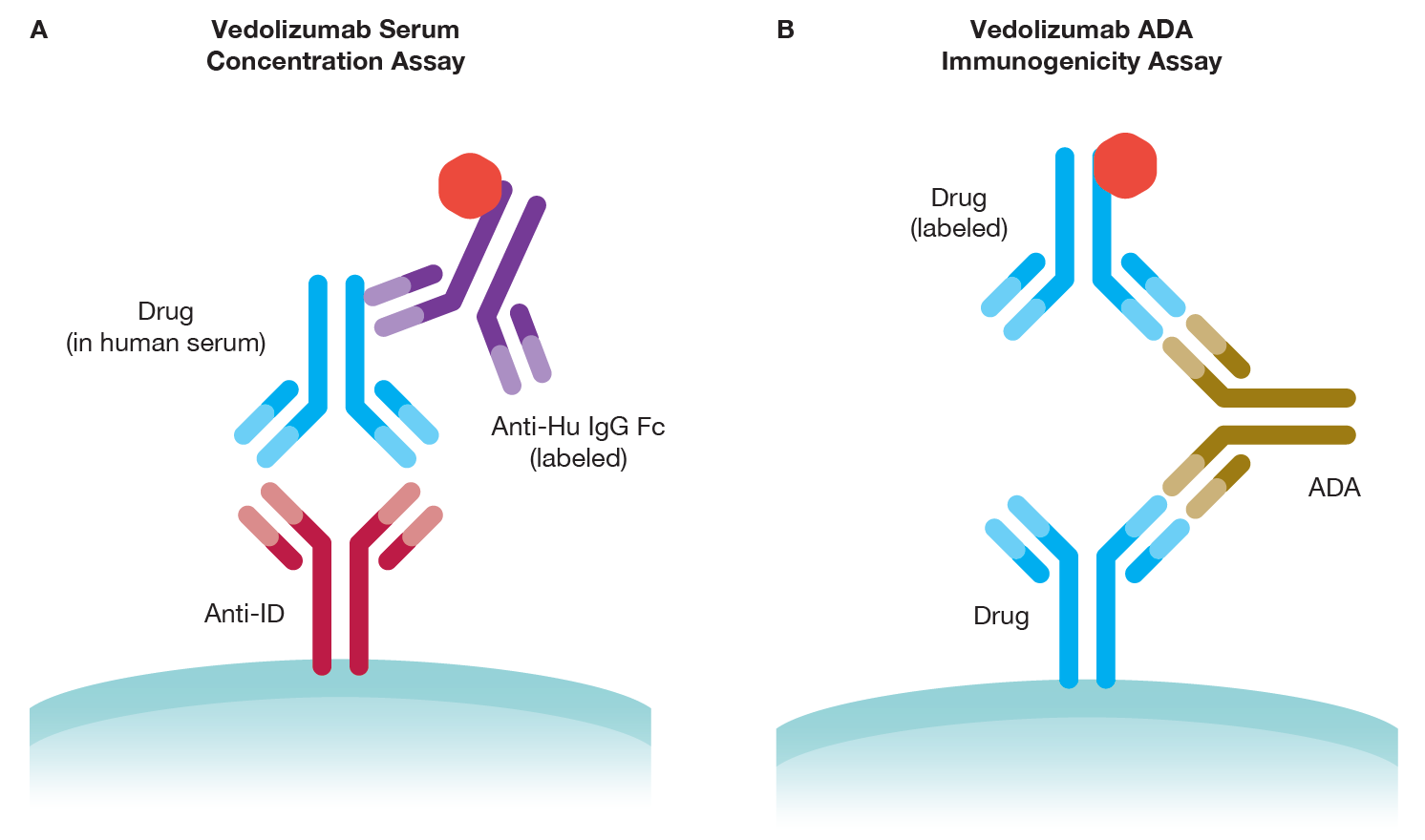
**Supplemental Digital Content**

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**FIGURE 1.** Reference assays



ADA, antidrug antibody; anti-Hu IgG Fc, anti-human immunoglobulin G antibody (Fc specific); anti-ID, anti-idiotype antibody.

**FIGURE 2.** Mean percentage differences in vedolizumab serum concentrations in 21 patient samples



Measured by the commercial assays compared with the reference assay. Bland-Altman analysis results of samples from vedolizumab-treated patients with inflammatory bowel disease. The red lines represent the mean percentage difference across the range. The blue lines indicate the limit of agreements. For more information on sample sizes see **Sample Sets** section of **Methods** and **Table 2**, **Supplemental Digital Content**.

**TABLE 1.** Comparison of Assay Attributes.

|  |  |  |  |
| --- | --- | --- | --- |
| Company (Assay Source) | Vedolizumab Serum Concentration Assay Format | Lower Limit of Quantification, µg/mL\* | Vedolizumab ADA Immunogenicity Assay Format |
| Takeda Pharmaceuticals | ELISA | 0.2 | ECL |
| Grifols Diagnostic Solutions (performed in TX) | ELISA,  PROMONITOR-VEDOLIZUMAB:ELISA | 1.4 | ELISA,  PROMONITOR-ANTI-VEDOLIZUMAB |
| Immundiagnostik AG | ELISA  (IDKmonitor® Vedolizumab drug concentration) | 1.7†,‡ | ELISA  (IDKmonitor® Vedolizumab free antidrug antibody) |
| Progenika Biopharma B  (performed in Spain) | ELISA,  PROMONITOR-VEDOLIZUMAB:ELISA | 1.4 | ELISA,  PROMONITOR-ANTI-VEDOLIZUMAB |
| Sanquin Diagnostic Services | ELISA  (Vedolizumab Spiegel ELISA) | 0.01 | RIA  (Vedolizumab-ABT) |
| Theradiag | ELISA  LISA Tracker® vedolizumab | 2 | ELISA  LISA Tracker® Anti-vedolizumab |

\*Samples with concentrations lower than the measurement range cannot be clearly quantified. The upper limit of the measurement range can be calculated as: highest concentration of the standard curve × sample dilution factor to be used.

†http://www.immundiagnostik.com/fileadmin/pdf/IDKmonitor\_Vedolizumab\_K9658.pdf.

‡Including a dilution factor of 200.

ADA, antidrug antibody; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay.

**TABLE 2.** Samples used for measuring vedolizumab and determining the presence of anti-vedolizumab antibodies.

|  |  |  |  |
| --- | --- | --- | --- |
| Samples used for measuring vedolizumab serum levels | | Total samples prepared and tested | Final sample numbers used in the statistical analysis |
| Accuracy: | QPS vs commercial lab concentration values | 10 pooled samples x triplicates = 30 samples  2 selective samples x duplicates = 4 samples  23 patient samples x singlet = 23  Total 30+4+23 = 57 samples tested | Sample values between 1–60 µg/mL are included:  Final sample numbers used: (30+4-6) + (23-2) = 49 |
| Precision: | % CV from replicates of vedolizumab concentration | 10 pooled samples x triplicates = 30 samples  2 selective samples x duplicates = 4 samples  Total 30+4 = 34 samples tested | Replicate sample values between 1–60 µg/mL are included:  Final sample numbers used: 30+4-6 = 28 |
| Precision Not applicable | 23 patient samples (singlet),  2 specificity samples (BQL) | Not applicable | Not applicable |
| Specificity: | IFX or ADA without presence of VDZ (BQL in VDZ assay) | IFX (Duplicates)  ADA (Duplicates)  Total 4 samples | Not applicable |
| Selectivity | IFX+VDZ combo,  ADA+VDZ combo | IFX + VDZ (Duplicatas)  ADA + VDZ (Duplicatas)  Total 4 samples | Total 4 samples |
| Samples used for determining presence of anti-vedolizumab antibodies | | | Total samples |
| Absence of VDZ | 4 different levels of positive control spiked with VDZ samples (singlet) | | Total 4 samples |
| Presence of VDZ | Samples above + 15µg/ml VDZ (singlet) | | Total 4 samples |

ADA, adalimumab; BQL, below limit of quantification; CV, coefficient of variation; IFX, infliximab; VDZ, vedolizumab.

**TABLE 3.** Mean for each QC and selectivity sample from each assay

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Grifols** | **Immunodiagnostik** | **Progenika** | **Sanquin** | **Theradiag** | **Takeda Reference Assay** |
| QC.1 | <1.00 µg/mL | <1.00 µg/mL | <1.00 µg/mL | <1.00 µg/mL | <1.00 µg/mL | <1.00 µg/mL |
| QC.2 | 2.83 | 2.36 | 2.82 | 2.28 | NA | 2.67 |
| QC.3 | 9.85 | 10.65 | 10.39 | 9.43 | 8.32 | 10.74 |
| QC.4 | 14.68 | 18.88 | 15.13 | 13.80 | 12.15 | 16.40 |
| QC.5 | 21.17 | 18.99 | 22.64 | 17.98 | 14.63 | 21.23 |
| QC.6 | 23.19 | 22.91 | 28.08 | 23.77 | 18.32 | 27.32 |
| QC.7 | 31.02 | 27.70 | 33.91 | 27.12 | 23.05 | 30.72 |
| QC.8 | 44.84 | 37.76 | 48.83 | 37.25 | 27.62 | 41.62 |
| QC.9 | 58.34 | 41.97 | 61.37 | 44.75 | 32.98 | 52.25 |
| QC.10 | >60.00 µg/mL | >60.00 µg/mL | >60.00 µg/mL | >60.00 µg/mL | >60.00 µg/mL | >60.00 µg/mL |
| SS.3 | 21.72 | 17.89 | 21.39 | 18.83 | 15.43 | 21.95 |
| SS.4 | 23.91 | 19.11 | 19.27 | 17.90 | 15.01 | 21.80 |

All values in µg/mL.

ID, identifier; QC, quality control; SS, selectivity sample

**TABLE 4.** Coefficient of variation for each QC and selectivity sample from each assay

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample ID | Grifols | Immunodiagnostik | Progenika | Sanquin | Theradiag | Takeda Reference Assay |
| QC.1 | Not calculated\* | Not calculated\* | Not calculated\* | Not calculated\* | Not calculated\* | Not calculated\* |
| QC.2 | 17.36 | 12.88 | 2.02 | 6.11 | NA | 3.59 |
| QC.3 | 10.03 | 20.07 | 9.48 | 6.29 | 2.66 | 0.69 |
| QC.4 | 10.95 | 41.59 | 10.15 | 6.03 | 2.97 | 3.18 |
| QC.5 | 12.44 | 7.67 | 15.42 | 5.40 | 5.30 | 6.05 |
| QC.6 | 10.54 | 13.73 | 11.63 | 1.27 | 9.08 | 4.57 |
| QC.7 | 5.32 | 3.61 | 17.48 | 14.81 | 6.64 | 4.94 |
| QC.8 | 13.32 | 8.77 | 7.33 | 3.17 | 7.35 | 7.75 |
| QC.9 | 13.59 | 15.68 | 11.66 | 9.24 | 2.31 | 9.41 |
| QC.10 | Not calculated\* | Not calculated\* | Not calculated\* | Not calculated\* | Not calculated\* | Not calculated\* |
| SS.3 | 0.68 | 6.78 | 2.20 | 6.57 | 12.53 | 6.57 |
| SS.4 | 8.56 | 16.90 | 9.09 | 8.69 | 7.16 | 6.09 |

All values are %

\*Not calculated since samples <1 µg/mL or >60 µg/mL were excluded from the analysis.

ID, identifier; QC, quality control; SS, selectivity sample

**TABLE 5.** Vedolizumab ADA Detection by Immunogenicity Assays

|  |  |  |
| --- | --- | --- |
| Company  (Assay Source) | Immunogenicity (Vedolizumab ADA Detection) [Yes/No] | Drug Tolerance (Immunogenicity Detected in Presence of 15 µg/mL Vedolizumab) [Yes/No] |
| Takeda Pharmaceuticals  (reference assay) | Yes | Yes |
| Grifols Diagnostic Solutions | Yes | No |
| Immundiagnostik AG | Yes | No |
| Progenika Biopharma B | Yes | No |
| Sanquin Diagnostic Services | Yes | No |
| Theradiag | Yes | No |

ADA, antidrug antibody.