Viability of a Serum Infliximab Concentration-Detecting Reagent as a Qualitative Assay for an Infliximab Biosimilar

Takanori Inagaki,*,a Tatsuya Isesaki,a Kumi Kawana,a and Ryohkan Funakoshi*a,b

Department of Pharmacy, Tesshokai Kameda Medical Center; 929 Higashi-cho, Kamogawa, Chiba 296–8602, Japan; and Drug Oversight Department, Medical Administration Headquarters, Medical Corporation Tesshokai; 929 Higashi-cho, Kamogawa, Chiba 296–8602, Japan.

Received February 15, 2021; accepted June 9, 2021

The efficacy of infliximab in treating rheumatoid arthritis depends on its serum trough concentration, which must be maintained at a minimum of 1 μg/mL to achieve the desired effects. However, Japan’s National Health Insurance system does not cover tests for rheumatoid arthritis patients undergoing treatment with biosimilar infliximab because its performance as a biosimilar remains unclear. This study aimed to investigate whether the Remi-check Q qualitative assay yields comparable results for biosimilar infliximab and the originator product. Infliximab BS 100 “NK” and Remicade 100® were separately diluted in pooled human serum to yield test samples at the following concentrations: 0.30, 0.70, 1.20, and 3.00 μg/mL. Prepared samples were quantitatively assessed using an enzyme-linked immunosorbent assay (ELISA) and qualitatively using Remi-check Q, and the results obtained for the originator and biosimilar product were compared. For both originator and biosimilar infliximab, Remi-check Q yielded a negative result for all 0.30 and 0.70 μg/mL samples and a positive result for all 3.00 μg/mL samples. However, negative results were obtained with a fraction of the 1.20 μg/mL samples (biosimilar, 4/15; originator, 3/15). Concordance rates between the results of quantitative ELISA and qualitative Remi-check Q analyses were comparable between originator and biosimilar infliximab at all tested concentrations. These results indicate that Remi-check Q yields comparable results for biosimilar infliximab and the originator product on being used as a qualitative assay for trough serum levels.

Key words infliximab; biosimilar; originator; trough serum level; rheumatoid arthritis; quick determining kit

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by joint degeneration, damage, and injury and is associated with a reduced QOL, fatigue, and other adverse health effects.1) Infliximab, a biopharmaceutical used to treat RA, first became eligible for reimbursement under Japan’s National Health Insurance (NHI) system in July 2003. The continued development and clinical application of a series of novel biologic medical products—including infliximab—has transformed treatment guidelines for RA, owing to their ability to effectively control disease activity.2) Today, Japanese patients and practitioners have a range of RA biologics from which to choose, including five types of tumor necrosis factor-alpha (TNF-α) inhibitors, two types of interleukin-6 (IL-6) blockers, and one T-cell activation inhibitor.3) Infliximab, an anti-TNF-α agent, is a “key drug” used to treat RA, considering its long track record of clinical success (over 17 years at the time of writing), its well-established usage protocols, and its safety profile.4) Indeed, the breadth of evidence supporting its efficacy renders infliximab a highly recommended treatment alternative in accordance with the clinical guidelines for RA treatment.5) However, infliximab and other biologics are costly, warranting the development and usage of biosimilar products to control healthcare costs, which continuously increase annually.6–8) The efficacy of infliximab depends on its trough serum concentration, which should be maintained at least 1 μg/mL.9) Kizaki et al.10) reported the dose of infliximab that must be administered to maintain a trough serum concentration above 1 μg/mL. Remi-check Q is a rapid screening kit for trough infliximab and has been approved for use in Japan since October 2016. The speed and objectivity of the test render it a valuable tool to design or adjust the treatment alternatives for RA.11) However, Remi-check Q is not covered by Japan’s NHI for RA patients on biosimilar infliximab because its performance has not been sufficiently validated for biosimilar products. To encourage the use of bio-generics, it is imperative that the performance of Remi-check Q as a serum infliximab assay be additionally validated for biosimilar products—to the same degree as the originator product—to allow practitioners to rapidly assess the status of patients taking biosimilar products and to adjust their treatment strategy accordingly.

No studies have assessed the potential of Remi-check Q to assess trough serum levels for biosimilar infliximab. This study aimed to investigate whether this qualitative assay yields comparable results for biosimilar infliximab and the originator product.

MATERIALS AND METHODS

Materials The biosimilar product under investigation was infliximab BS 100 mg for intravenous (i.v.) infusion “NK” (biosimilar); the reference product was Remicade® 100 mg for i.v. infusion (originator). Three lots of each product were tested; detailed information is provided in Table 1. For quantitative and qualitative testing, drugs were dissolved in pooled human serum prepared from multiple vials of single-donor serum (Clinical Trials Laboratory Services; KAC Corporation, Kyoto, Japan). The Remi-check Q kit, a rapid detection kit for

© 2021 The Pharmaceutical Society of Japan
serum infliximab, was used for the qualitative component of the testing (lot # B804: LSI Medience Corporation, Tokyo, Japan).

**Experimental Procedure** Infliximab BS 100 “NK” and Remicade 100® were separately diluted in pooled human serum to yield test samples at concentrations of 0.3, 0.7, 1.2, and 3.0 µg/mL. The diluted samples were evaluated quantitatively via an enzyme-linked immunosorbent assay (ELISA) and qualitatively using Remi-check Q. Qualitative assay results were compared between the biosimilar and originator products (Fig. 1). This study adhered to the *Ethical Guidelines for Medical and Health Research Involving Human Subjects* and was approved by the clinical research ethics committee of Kameda Medical Center (Approval No. 18-070-19111).

**Reagent Preparation**

**Pooled Human Serum**

Single-donor serum products were screened for infliximab content to ensure testing consistency, i.e., to ensure that the concentration of active substances in the test samples matches their designated value. Single-donor serum was prepared from blood obtained from donors providing informed consent. Infliximab levels in single-donor serum were measured using the Infliximab (Remicade®) ELISA SHIKARI® Q-INFLEXI kit (Matriks Biotek Laboratories, Ankara, Turkey #1903012). Only products with negligible hemolysis and lipemia, and infliximab <0.10 µg/mL, were selected. Pooled human serum was prepared by combining the selected products; this mixture was separated into 10 mL vials and stored at −60 °C. Vials were thawed immediately before use, either in a refrigerator (1.5–8.4 °C) or under ambient conditions at 14.5–30.4 °C.

**Buffer Solution for Test Samples**

Buffer solution was prepared by dissolving the following compounds in 500 mL H₂O: refined sucrose (25 g), sodium dihydrogen phosphate monohydrate (0.11 g), disodium hydrogen phosphate dihydrate (0.31 g), and polysorbate 80 (0.025 g). After confirming a pH of 7.2 ± 0.3, the solution was passed through a 0.22 µm filter before use.

**Stock Solutions (10 mg/mL) of Remicade and Infliximab BS**

Stock solutions were prepared by adding the respective biologics to vials of United States Pharmacopeia (USP) sterile water for injection (10 mL) and rotating the vials to gently dissolve them. The solution was allowed to stand for 5 min and then stored in a freezer at <−60 °C until use. Three lots were prepared for each product.

**Diluted Samples (infliximab in Pooled Human Serum)**

Matching cuvettes containing buffer solution were placed in the reference and measurement holders of a spectrophotometer (V-730: JASCO Corporation, Tokyo, Japan) to estab-
lish baseline absorbance. Diluted samples were prepared by adding 100 µL of each stock solution to 900 µL buffer. The absorbance of each test sample was measured at 280, 320, 325, 330, 335, 340, 345, and 350 nm. A curve was plotted through linear regression of a log-log plot of apparent absorbance (i.e., measurement data) versus wavelength. Absorbance from scattering at 280 nm was derived through extrapolation from this curve. Absorbance resulting from proteins in solution was determined by subtracting scattering absorbance from total absorbance at 280 nm. Protein concentrations were calculated using the method of Gill and von Hippel. Thereafter, each stock solution was diluted in physiological saline to a final protein concentration of 1 mg/mL. Furthermore, a diluted solution (10 µg/mL infliximab) was prepared and combined with pooled human serum. The final solutions for quantitative and qualitative analysis were prepared by separately mixing this solution to yield test samples with the following concentrations: 0.30, 0.70, 1.20, and 3.00 µg/mL. All test samples were stored at −80 °C until use.

**Quantitative Assay: ELISA** For ensuring integrity, we requested LSI Medience Corporation to carry out the ELISA quantitative test using the Infliximab (Remicade®) ELISA SHIKARI® Q-INFLIXI kit. This ELISA method has already been used and validated for determining infliximab concentrations in human serum.

First, 30 µL of the test sample was added to the assay buffer, and the mixture (110 µL) was transferred to a reaction plate well. Plates were incubated at 23 °C for 30 min, and then rinsed thrice with wash buffer (300 µL). Horseradish peroxidase (HRP)-conjugate probe (100 µL) was then added to the well. Again, the plate was incubated at 23 °C for 30 min and then triple-rinsed (300 µL wash buffer). Thereafter, 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (100 µL) was added to the well, and the mixture incubated without direct light for 10 min at 23 °C to facilitate the reaction. The reaction was terminated upon addition of TMB stop solution (100 µL). Finally, sample absorbance was measured using a microplate reader (excitation wavelength, 450 nm; emission wavelength, 650 nm). Infliximab concentration was determined from a (4-parameter) calibration curve. Absorbance resulting from proteins in solution was determined by subtracting scattering absorbance from total absorbance at 280 nm. Protein concentrations were calculated using the method of Gill and von Hippel. Thereafter, each stock solution was diluted in physiological saline to a final protein concentration of 1 mg/mL. Furthermore, a diluted solution (10 µg/mL infliximab) was prepared and combined with pooled human serum. The final solutions for quantitative and qualitative analysis were prepared by separately mixing this solution to yield test samples with the following concentrations: 0.30, 0.70, 1.20, and 3.00 µg/mL. All test samples were stored at −80 °C until use.

**Qualitative Assay: Remi-Check Q** Qualitative assays were performed using the Remi-check Q infliximab kit in accordance with the manufacturer’s instructions. Diluted test samples (“Diluted samples (inriximab in pooled human serum”) were allowed to thaw before use (in a refrigerator or under ambient conditions). Test samples (60 µL) were added to the diluent (240 µL) in a diluent tube provided in the kit. Some of this diluted sample (120 µL) was pipetted onto the application field of the Remi-check Q test plate, and the cut-off control (120 µL) onto its designated field immediately afterwards. The test plate was then allowed to stand on a level surface for 15 min. The outcome—positive or negative—was determined from a visual comparison of the coloration of the respective lines in the results field, which rapidly became visible in the course of the reaction. The outcomes were assessed in accordance with the manufacturer’s instructions: positive (≥1 µg/mL [+] if the diluted sample line was comparable or darker in hue than the cut-off control line; or, negative (<1 µg/mL [−]) if fainter. A single Remi-check Q was interpreted by 3 raters, and the final result adopted was the one

### Table 2. Infliximab Concentrations in Human Serum

| Batch number  | Serum infliximab concentration (µg/mL) | Description       |
|---------------|----------------------------------------|-------------------|
| SER018A020F013| 0.06                                   | slight emulsion   |
| SER018A020E007| 0.14                                   | slight emulsion   |
| SER018A020F023| 0.05                                   | slight emulsion   |
| SER020A020D024| 0.06                                   | slight emulsion   |
| SER018A020F014| 0.08                                   | slight emulsion   |
| SER018A020F025| 0.24                                   | slight emulsion   |
| SER018A020F029| 0.09                                   | slight emulsion   |
| SER018A020F026| 0.06                                   | slight emulsion   |
| CT-0375299-020| 0.07                                   | satisfactory      |
| CT-0375312-020| 0.03                                   | satisfactory      |
| CT-0367106-020| 0.12                                   | satisfactory      |
| CT-0375221-020| 0.08                                   | satisfactory      |
| CT-0375322-020| 0.09                                   | satisfactory      |
| CT-0375342-020| 0.07                                   | satisfactory      |
| CT-0375323-020| 0.30                                   | slight emulsion   |
| CT-0375296-020| 0.07                                   | satisfactory      |
| CT-0367447-020| 0.13                                   | satisfactory      |
| CT-0375298-020| 0.02                                   | satisfactory      |
| CT-0375297-020| 0.06                                   | satisfactory      |

### Table 3. ELISA Results for Infliximab Concentration

| Product name  | Lot No. | Preparation concentration (µg/mL) | Measurement (µg/mL) |
|---------------|---------|----------------------------------|---------------------|
| REMICADE®     | ICL87013| 3.00                             | 2.70                |
|               |         | 1.20                             | 1.13                |
|               |         | 0.70                             | 0.70                |
|               |         | 0.30                             | 0.39                |
| ICL90011      |         | 3.00                             | 2.90                |
|               |         | 1.20                             | 1.12                |
|               |         | 0.70                             | 0.74                |
|               |         | 0.30                             | 0.41                |
| ICL90012      |         | 3.00                             | 2.70                |
|               |         | 1.20                             | 1.08                |
|               |         | 0.70                             | 0.71                |
|               |         | 0.30                             | 0.40                |
| Infliximab BS | 250070  | 3.00                             | 2.54                |
|               |         | 1.20                             | 1.04                |
|               |         | 0.70                             | 0.69                |
|               |         | 0.30                             | 0.38                |
| 350080        |         | 3.00                             | 2.78                |
|               |         | 1.20                             | 1.07                |
|               |         | 0.70                             | 0.70                |
|               |         | 0.30                             | 0.38                |
| 350090        |         | 3.00                             | 2.60                |
|               |         | 1.20                             | 1.12                |
|               |         | 0.70                             | 0.71                |
|               |         | 0.30                             | 0.39                |
Table 4. Results of Measurement of Originator Infliximab by Remi-Check Q

| Lot No. ICL87013 | Preparation concentration | 0.30 µg/mL | 0.70 µg/mL |
|------------------|---------------------------|------------|------------|
|                  | Rater                     | Rating     | Rater      | Rating     |
|                  | Measurement number | A | B | C | A | B | C |
| 1                | − | − | − | − | − | − | − |
| 2                | − | − | − | − | − | − | − |
| 3                | − | − | − | − | − | − | − |
| 4                | − | − | − | − | − | − | − |
| 5                | − | − | − | − | − | − | − |

| Lot No. ICL90011 | Preparation concentration | 0.30 µg/mL | 0.70 µg/mL |
|------------------|---------------------------|------------|------------|
|                  | Rater                     | Rating     | Rater      | Rating     |
|                  | Measurement number | A | B | C | A | B | C |
| 1                | − | − | − | − | − | − | − |
| 2                | − | − | − | − | − | − | − |
| 3                | − | − | − | − | − | − | − |
| 4                | − | − | − | − | − | − | − |
| 5                | − | − | − | − | − | − | − |

| Lot No. ICL90012 | Preparation concentration | 0.30 µg/mL | 0.70 µg/mL |
|------------------|---------------------------|------------|------------|
|                  | Rater                     | Rating     | Rater      | Rating     |
|                  | Measurement number | A | B | C | A | B | C |
| 1                | − | − | − | − | − | − | − |
| 2                | − | − | − | − | − | − | − |
| 3                | − | − | − | − | − | − | − |
| 4                | − | − | − | − | − | − | − |
| 5                | − | − | − | − | − | − | − |
Table 5. Results of Measurement of Biosimilar Infliximab by Remi-Check Q

**Lot No. 250070**

| Preparation concentration | 0.30 µg/mL | 0.70 µg/mL |
|----------------------------|------------|------------|
| Measurement number         | A         | B         | C         | Rating | A         | B         | C         |
| 1                          | −         | −         | −         | −      | −         | −         | −         |
| 2                          | −         | −         | −         | −      | −         | −         | −         |
| 3                          | −         | −         | −         | −      | −         | −         | −         |
| 4                          | −         | −         | −         | −      | −         | −         | −         |
| 5                          | −         | −         | −         | −      | −         | −         | −         |
| Preparation concentration | 1.20 µg/mL | 3.00 µg/mL |
| Measurement number         | A         | B         | C         | Rating | A         | B         | C         |
| 1                          | −         | −         | −         | −      | −         | −         | −         |
| 2                          | −         | −         | −         | −      | −         | −         | −         |
| 3                          | −         | −         | −         | −      | −         | −         | −         |
| 4                          | −         | −         | −         | −      | −         | −         | −         |
| 5                          | −         | −         | −         | −      | −         | −         | −         |

**Lot No. 350080**

| Preparation concentration | 0.30 µg/mL | 0.70 µg/mL |
|----------------------------|------------|------------|
| Measurement number         | A         | B         | C         | Rating | A         | B         | C         |
| 1                          | −         | −         | −         | −      | −         | −         | −         |
| 2                          | −         | −         | −         | −      | −         | −         | −         |
| 3                          | −         | −         | −         | −      | −         | −         | −         |
| 4                          | −         | −         | −         | −      | −         | −         | −         |
| 5                          | −         | −         | −         | −      | −         | −         | −         |
| Preparation concentration | 1.20 µg/mL | 3.00 µg/mL |
| Measurement number         | A         | B         | C         | Rating | A         | B         | C         |
| 1                          | +         | +         | +         | +      | +         | +         | +         |
| 2                          | +         | +         | +         | +      | +         | +         | +         |
| 3                          | +         | −         | +         | +      | +         | +         | +         |
| 4                          | −         | +         | −         | +      | +         | +         | +         |
| 5                          | +         | +         | +         | +      | +         | +         | +         |

**Lot No. 350090**

| Preparation concentration | 0.30 µg/mL | 0.70 µg/mL |
|----------------------------|------------|------------|
| Measurement number         | A         | B         | C         | Rating | A         | B         | C         |
| 1                          | −         | −         | −         | −      | −         | −         | −         |
| 2                          | −         | −         | −         | −      | −         | −         | −         |
| 3                          | −         | −         | −         | −      | −         | −         | −         |
| 4                          | −         | −         | −         | −      | −         | −         | −         |
| 5                          | −         | −         | −         | −      | −         | −         | −         |
| Preparation concentration | 1.20 µg/mL | 3.00 µg/mL |
| Measurement number         | A         | B         | C         | Rating | A         | B         | C         |
| 1                          | +         | +         | +         | +      | +         | +         | +         |
| 2                          | +         | −         | +         | +      | +         | +         | +         |
| 3                          | +         | +         | +         | +      | +         | +         | +         |
| 4                          | −         | −         | −         | −      | −         | −         | −         |
| 5                          | +         | +         | +         | +      | +         | +         | +         |
where ≥2 raters agreed. The test was regarded as failed if a dark magenta line did not appear in the results field of the cut-off control strip. Each test sample was prepared independently, and each sample underwent qualitative testing by Remi-check Q five times in total.

Since the Remi-check Q band disappears immediately after the qualitative reaction and it is difficult to remove the plastic cover fixed to the Remi-check Q body, assessment by humans, rather than a machine, was performed.

**Statistical Analysis** Fisher’s exact test was performed to compare the concurrence rates between quantitative ELISA and qualitative Remi-check Q analyses for originator versus biosimilar infliximab. Statistical analysis was performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface developed for R software (The R Foundation for Statistical Computing, Vienna, Austria). Differences with \( p < 0.05 \) were considered statistically significant.

**RESULTS**

Pooled human serum samples were generated from six of the single-donor batches indicated in Table 2; all pooled batches had infliximab concentrations of <0.10 \( \mu \)g/mL and lacked lipemia and hemolysis. No marked discrepancies in the quantitative ELISA outcomes were observed, and all measured values were uniformly similar to the intended infliximab concentration for all lots and conditions (Table 3).

Tables 4 and 5 provide details regarding qualitative assay judgments (Remi-check Q) for originator and biosimilar infliximab, respectively. For reference, the photographs of some test plates used in the assays are provided in Fig. 2. For both originator and biosimilar infliximab, Remi-check Q yielded a negative result for all the samples at the lowest concentrations (0.30 and 0.70 \( \mu \)g/mL) and a positive result for all the samples at the highest concentration (3.00 \( \mu \)g/mL). However, only a fraction of samples of 1.20 \( \mu \)g/mL yielded negative results.
The raters disagreed in 4 out of 60 tests for both the originator and biosimilar samples, which were all at a concentration of 1.20 µg/mL (Fig. 3). No instances of testing failure were noted.

The concurrence between quantitative and qualitative assay results (i.e., ELISA vs. Remi-check Q) is summarized in Tables 6 and 7. Discrepancies were observed for 3/60 originator and 4/60 biosimilar samples. With 0.30, 0.70, and 3.00 µg/mL, no discrepancies between the quantitative ELISA and qualitative Remi-check Q outcomes were noted for both originator and biosimilar infliximab; however, the concurrence rate did not significantly differ between the two drugs (80% v. 73%, N.S.) (Fig. 4). The three divergent samples of Remicade were prepared from the same lot: these were ruled negative by Remi-check Q, yet classified as positive—i.e., trough serum infliximab ≥1 µg/mL—from quantitative ELISA (1.13 µg/mL). In contrast, the four divergent samples of infliximab BS were prepared from three different lots. They yielded negative results in the qualitative assay but positive results upon quantitative ELISA (1.04, 1.07, and 1.12 µg/mL).

**DISCUSSION**

This study aimed to investigate whether Remi-check Q is a feasible qualitative assay for biosimilar infliximab, comparable to its performance on the originator drug. Concurrence rates between the results of quantitative ELISA and qualitative Remi-check Q analyses were comparable between all tested concentrations of originator and biosimilar infliximab. Nonetheless, for samples prepared using 1.20 µg/mL, divergence was observed between the outcomes of qualitative and quantitative analyses for both the originator and biosimilar drugs.

The package insert for the Remi-check Q kit claims that the test results were different from those of quantitative ELISA at a rate of 5/150 samples, although we observed rates of 3/30 and 4/30 samples for the originator and biosimilar drugs, respectively. These discrepancies probably occurred because the designated concentrations of these samples were within the test’s “borderline” sensitivity range (0.70–1.20 µg/mL). Indeed, the package insert indicated that disagreements can occur at infliximab concentrations of 1.09–1.37 µg/mL, encompassing the range of sample concentrations at which disagreements were observed in the present study.

In Japan, healthcare costs among RA patients continuously increase annually, with greater expenditures associated with higher disease activity and disability levels. Furthermore, the use of biosimilar drugs is promoted as a strategy to limit medical costs. Inefficacy is one of the most common reasons for the discontinuation of biologic therapeutics among older RA patients. Regular measurement of blood infliximab concentrations among RA patients would facilitate the selection of optimum treatment strategies, potentially curtailing medi-

---

**Table 6. Correlation between the Results of Quantitative Measurement of Originator Infliximab by ELISA and the Results of Qualitative Reactions by Remi-Check Q**

| Remi-check Q | ELISA | Total |
|--------------|-------|-------|
| ≥1 µg/mL (+) | 27    | 27    |
| <1 µg/mL (−) | 0     | 0     |
| Total        | 27    | 0     |

**Table 7. Correlation between the Results of Quantitative Measurement of Biosimilar Infliximab by ELISA and the Results of Qualitative Reactions by Remi-Check Q**

| Remi-check Q | ELISA | Total |
|--------------|-------|-------|
| ≥1 µg/mL (+) | 26    | 26    |
| <1 µg/mL (−) | 4     | 4     |
| Total        | 30    | 30    |

---

Fig. 4. Rates of Agreement between the Quantitative (ELISA) and Qualitative (Remi-Check Q) Assay Results for Originator versus Biosimilar Infliximab

The rates are statistically comparable between the two drugs at each of the concentrations tested. p ≥ 0.05; N.S., not significant.
al costs. Certification of the use of Remi-check Q to monitor levels of biosimilar infliximab for NHI coverage could help practitioners determine optimum treatment strategies for patients not taking the originator drug, thus greatly reducing healthcare costs.

Because of ethical concerns, this was not an in vivo study involving human participants. All experiments were conducted in vitro using pooled serum combined from several single-donor samples. Nevertheless, an in vivo study involving human subjects is needed as the approval study for Remi-check Q involved patient samples. Before pooling for use in quantitative ELISA, serum products were first tested to confirm that they contained $\leq 0.1 \mu g/mL$ of infliximab-reactive protein. Unexpectedly, 6 of the 20 candidate batches assessed herein had higher measured concentrations of infliximab-reactive protein and could not be included. Infliximab can bind to various serum targets, their levels potentially varying among individuals. Therefore, although inter-lot variation in the ELISA kits used is a potential cause of this unusual finding, we cannot rule out the possibility that was a result of non-specific antigenic reactions. In either case, the issue demands further investigation. Future studies are required to measure baseline concentrations of infliximab-reactive protein before treatment initiation and investigate whether assessment for post-treatment differences in this value offers any clinical benefits.

A few instances of inter-rater disagreement were noted in the interpretation of the outcomes of the Remi-check Q assays, probably owing to difficulties comparing the colors of the cut-off control and diluted (test) sample, which are both rather faint. To prevent inter-rater disagreement arising from differences in interpretation, kits should be designed such that the coloration of the test line is clearly visible in the event of a positive result.

This study investigated only the specific infliximab biosimilar product currently used at Kameda Medical Center. Three preparations of biosimilar infliximab are available in Japan today, from five manufacturers. Further studies are required to determine whether Remi-check Q performs as well in assessing trough infliximab for these other biosimilar products as it does for the originator drug.

In summary, as a qualitative assay of trough serum infliximab, the Remi-check Q kit performs comparably on biosimilar infliximab and the originator product. Monitoring of blood levels is well positioned to enhance pharmacological outcomes. The Remi-check Q kit performs comparably on biosimilar infliximab and the originator product. Monitoring of blood levels is well positioned to enhance pharmacological outcomes.

**Acknowledgments** We would like to thank Nippon Kayaku Co., Ltd. for funding this research.

**Conflict of Interest** All authors received funding from Nippon Kayaku Co., Ltd.

**REFERENCES**

1. Russell AS. Quality-of-life assessment in rheumatoid arthritis. *Pharmacoeconomics*, 26, 831–846 (2008).

2. Japan College of Rheumatology. “Guidelines for the management of rheumatoid arthritis 2014.” <https://minds.jcqhc.or.jp/n/med4/med0064/G0000706/0007>, accessed 8 March, 2020.

3. Lau CS, Chia F, Dans L, Harrison A, Hsieh TY, Jain R, Jung SM, Kishimoto M, Kumar A, Leong KP, Li Z, Lichauroo JJ, Louthrenoo W, Luo SF, Mu R, Rash P, Ng CT, Suryana B, Wijaya LK, Yeap SS. 2018 update of the APLAR recommendations for treatment of rheumatoid arthritis. *Int. J. Rheum. Dis.*, 22, 357–375 (2019).

4. Kamata Y, Minota S. Wide difference in biologics usage and expenditure for the treatment of patients with rheumatoid arthritis in each prefecture in Japan analyzed using “National Database of Health Insurance Claims and Specific Health Checkups of Japan”. *Rheumatol. Int.*, 38, 663–668 (2018).

5. Lipsky PE, van der Heijde DM, St Clair EW, Farurst DE, Breedved FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN. Infliximab and methotrexate in the treatment of rheumatoid arthritis. *N. Engl. J. Med.*, 343, 1594–1602 (2000).

6. Jung YS, Park DI, Kim YH, Lee JH, Seo PJ, Cheon JH, Kang HW, Kim JW. Efficacy and safety of CT-P13, a biosimilar of infliximab, in patients with inflammatory bowel disease: a retrospective multi-center study. *J. Gastroenterol. Hepatol.*, 30, 1705–1712 (2015).

7. Safari S, Kolaee AA, Hoy D, Smith E, Bettampadi D, Mansouretia MA, Almasi-Hashami A, Ashrafi-Asgarabadi A, Moradi-Lakeh M, Qorbani M, Collins G, Wolf AD, March L, Cross M, Global, regional and national burden of rheumatoid arthritis 1990–2017: a systematic analysis for the Global Burden of Disease study 2017. *Ann. Rheum. Dis.*, 78, 1463–1479 (2019).

8. Zrubka Z, Gulácsy L, Brodzsky V, Remecz F, Alten R, Szekanecz Z, Péntek M. Long-term efficacy and cost-effectiveness of infliximab as first-line treatment in rheumatoid arthritis: systematic review and meta-analysis. Expert. *Rev. Pharmacoecon. Outcomes Res.*, 19, 537–549 (2019).

9. Takeuchi T, Miyasaka N, Inoue K, Abe T, Koike T. Impact of changes in interpretation of the outcomes of the Remi-check Q assay. *Bone Marrow Transplant.*, 48, 452–458 (2013).

10. Kizaki K, Yamashita F, Funakoshi N, Mori D, Otsuka K, Itoi M. Minimum infliximab dosage for keeping serum infliximab levels greater than 1 $\mu g/mL$ among patients with rheumatoid arthritis: results from the RISING study. *Mod. Rheumatol.*, 19, 478–487 (2009).

11. Remi-check Q [package insert]. LSI Medience Corporation, Tokyo, Japan, 2016.

12. Kanda Y. Investigation of the freely available easy-to-use software "EZR" for medical statistics. *Bone Marrow Transplant.*, 48, 452–458 (2013).

13. Burton W, Morrison A, Maclean R, Ruderman E. Systematic review of studies of productivity loss due to rheumatoid arthritis. *Occup. Med. (Lond.)*, 56, 18–27 (2006).

14. Ehina K, Hashimoto M, Yamamoto W, Hirano T, Hara R, Katayama M, Onishi A, Nagai K, Son Y, Amuro H, Yamamoto K, Maeda Y, Murata K, Jinno S, Takeuchi T, Hirao M, Kumanogoh A, Yoshikawa H. Drug tolerability and reasons for discontinuation of seven biologics in 4466 treatment courses of rheumatoid arthritis-the ANSWER cohort study. *Arthritis Res. Ther.*, 21, 91 (2019).

15. Mulleran D, Meric JC, Paintaud G, Ducourea E, Magdelaine-Beaizel C, Valat JP, Goupille P. Infliximab concentration monitoring improves the control of disease activity in rheumatoid arthritis. *Arthritis Res. Ther.*, 11, R178 (2009).

16. Laine J, Jokiranta TS, Eklund KK, Vakevainen M, Puolakka K. Cost-effectiveness of routine measuring of serum drug concentrations and anti-drug antibodies in treatment of rheumatoid arthritis patients with TNF-alpha blockers. *Biol. Pharm. Bull.*, 10, 67–73 (2016).