Regular Article

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

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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). Concurrence 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-α (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

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Table 1. Information on Each Infliximab

Category	Product name	Lot No.	Expiration date	Manufacturer
Original	REMICADE® for I.V. Infusion100	ICL87013	2021.2	Mitsubishi Tanabe Pharma, Osaka
		ICL90011	2021.2	
		ICL90012	2021.2	
Biosimilar	Infliximab BS for I.V. Infusion 100 mg “NK”	250070	2020.1	Nippon Kayaku, Tokyo
		350080	2020.2	
		350090	2020.2

Fig. 1. Overview of the Qualitative Reaction Test Workflow Using Remi-Check Q for Originator and Biosimilar Infliximab

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. 18-070-191111).

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-INFLIXI 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 (infiximab 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 μ L of each stock solution to 900 μ L 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 μ g/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 μ g/mL. All test samples were stored at -60°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 μ L of the test sample was added to the assay buffer, and the mixture (110 μ L) was transferred to a reaction plate well. Plates were incubated at 23°C for 30 min, and then rinsed thrice with wash buffer (300 μ L). Horseradish peroxidase (HRP)-conjugate probe (100 μ L) was then added to the well. Again, the plate was incubated at 23°C for 30 min and then triple-rinsed (300 μ L wash buffer). Thereafter, 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (100 μ L) 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 μ L). 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 plotted using the reference solution (Std A: 3.0 μ g/mL, Std B: 1.0 μ g/mL, Std C: 0.3 μ g/mL, Std D: 0.1 μ g/mL, Std E: 0 μ g/mL) provided in the kit; specifically, the absorbance of a blank (Std E: 0 μ g/mL) was subtracted from values measured for respective samples.

Qualitative Assay: Remi-Check Q Qualitative assays were performed using the Remi-check Q infliximab kit in accordance with the manufacturer's instructions.¹¹⁾ Diluted test samples ("Diluted samples (infliximab in pooled human serum)") were allowed to thaw before use (in a refrigerator or under ambient conditions). Test samples (60 μ L) were added to the diluent (240 μ L) in a diluent tube provided in the kit. Some of this diluted sample (120 μ L) was pipetted onto the application field of the Remi-check Q test plate, and the cut-off control (120 μ L) 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 instructions¹¹⁾: *positive*

(≥ 1 μ g/mL [+]) if the diluted sample line was comparable or darker in hue than the cut-off control line; or, *negative* (<1 μ g/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 (μ g/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	emulsion
SER018A020F029	0.09	slight emulsion
SER018A020F026	0.06	emulsion
SER018A020F028	0.13	slight emulsion
CT-0375299-020	0.07	satisfactory
CT-0375312-020	0.03	satisfactory
CT-0367106-020	0.12	slight emulsion
CT-0375321-020	0.08	satisfactory
CT-0375322-020	0.09	slight emulsion
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 (μ g/mL)	Measurement (μ g/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 $\mu\text{g/mL}$				0.70 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
Preparation concentration	1.20 $\mu\text{g/mL}$				3.00 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	+	—	—	+	+	+	+
2	+	+	+	+	+	+	+	+
3	—	—	—	—	+	+	+	+
4	+	+	+	+	+	+	+	+
5	—	—	—	—	+	+	+	+
Lot No. ICL90011								
Preparation concentration	0.30 $\mu\text{g/mL}$				0.70 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
Preparation concentration	1.20 $\mu\text{g/mL}$				3.00 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	+	+	+	+	+	+	+	+
2	—	+	+	+	+	+	+	+
3	+	—	+	+	+	+	+	+
4	+	+	—	+	+	+	+	+
5	+	+	+	+	+	+	+	+
Lot No. ICL90012								
Preparation concentration	0.30 $\mu\text{g/mL}$				0.70 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
Preparation concentration	1.20 $\mu\text{g/mL}$				3.00 $\mu\text{g/mL}$			
	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 $\mu\text{g/mL}$				0.70 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
Preparation concentration	1.20 $\mu\text{g/mL}$				3.00 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	—	—	—	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	—	—	—	+	+	+	+
5	+	+	+	+	+	+	+	+
Lot No. 350080								
Preparation concentration	0.30 $\mu\text{g/mL}$				0.70 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
Preparation concentration	1.20 $\mu\text{g/mL}$				3.00 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	—	+	+	+	+	+	+
4	—	+	—	—	+	+	+	+
5	+	+	+	+	+	+	+	+
Lot No. 350090								
Preparation concentration	0.30 $\mu\text{g/mL}$				0.70 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		A	B	C	
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
Preparation concentration	1.20 $\mu\text{g/mL}$				3.00 $\mu\text{g/mL}$			
	Rater			Rating	Rater			Rating
Measurement number	A	B	C		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).¹²⁾ 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 $< 0.10 \mu\text{g/mL}$ 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 $0.70 \mu\text{g/mL}$) and a positive result for all the samples at the highest concentration ($3.00 \mu\text{g/mL}$). However, only a fraction of samples of $1.20 \mu\text{g/mL}$ yielded negative results

Fig. 2. Representative Photographs of the Infliximab Remi-Check Q Qualitative Test

(a, b) Originator infliximab, 0.3 (a) and $3 \mu\text{g/mL}$ (b). (c, d) Biosimilar infliximab, 0.3 (c) and $3 \mu\text{g/mL}$ (d).

Fig. 3. Representative Remi-Check Q Tests Where There Was Disagreement on the Assessment by the Raters

(a) Originator infliximab, $1.2 \mu\text{g/mL}$; (b) biosimilar infliximab, $1.2 \mu\text{g/mL}$.

(biosimilar: 4/15, originator: 3/15). The raters disagreed in 4 out of 60 tests for both the originator and biosimilar samples, which were all at a concentration of $1.20\mu\text{g/mL}$ (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 $3.00\mu\text{g/mL}$, no discrepancies between the quantitative ELISA and qualitative Remi-check Q outcomes were noted for both originator and biosimilar infliximab (concurrence: 100%). With $1.20\mu\text{g/mL}$, the quantitative and qualitative outcomes diverged for 3/15 originator and 4/15 biosimilar samples; 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 $\geq 1\mu\text{g/mL}$ —from quantitative ELISA ($1.13\mu\text{g/mL}$). 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 $1.12\mu\text{g/mL}$).

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 $1.20\mu\text{g/mL}$, 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⁽¹⁾ 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 ($0.70\text{--}1.20\mu\text{g/mL}$). Indeed, the package insert indicated that disagreements can occur at infliximab concentrations of $1.09\text{--}1.37\mu\text{g/mL}$,⁽¹⁾ 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.⁽³⁾ 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.⁽⁴⁾ Regular measurement of blood infliximab concentrations among RA patients would facilitate the selection of optimum treatment strategies,⁽⁵⁾ 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

		ELISA		Total
		$\geq 1\mu\text{g/mL}$	$< 1\mu\text{g/mL}$	
Remi-check Q	$\geq 1\mu\text{g/mL}$ (+)	27	0	27
	$< 1\mu\text{g/mL}$ (–)	3	30	33
Total		30	30	60

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

		ELISA		Total
		$\geq 1\mu\text{g/mL}$	$< 1\mu\text{g/mL}$	
Remi-check Q	$\geq 1\mu\text{g/mL}$ (+)	26	0	26
	$< 1\mu\text{g/mL}$ (–)	4	30	34
Total		30	30	60

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. $p \geq 0.05$; N.S., not significant.

cal costs.¹⁶⁾ 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 $\leq 0.1 \mu\text{g/mL}$ 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 concentrations is well positioned to enhance pharmacological therapies in future, with potential applications extending to other biologics and biosimilars other than infliximab and to diseases other than RA.

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

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