Quantum Blue®
Infliximab

Quantitative
Lateral Flow Assay

For research use only.
Not for use in diagnostic procedures.

LF-TLIF25-U 25 tests

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ENGLISH

INTENDED USE

Quantum Blue® Infliximab is an *in vitro* lateral flow immunoassay for the quantitative determination of trough levels of infliximab in serum samples. The Quantum Blue® Infliximab is combined with the Quantum Blue® Reader.

For research use only.

PRINCIPLE OF THE ASSAY

The test is designed for the selective measurement of infliximab by a sandwich immunoassay. Recombinant tumor necrosis factor alpha (TNFα) is conjugated to gold colloids. On the test cassette (TC) the conjugate is released from a pad into the reaction system as the sample is applied. Infliximab present in the sample will bind to the conjugate. A monoclonal antibody, highly specific for the analyte, is immobilized on the test membrane and will capture the conjugate/analyte complex, resulting in a coloring of the Test Line (T). The remaining free TNFα/gold conjugate will bind to the Control Line (C). The signal intensities of the Test Line (T) and the Control Line (C) are measured quantitatively by the Quantum Blue® Reader.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Comments
Test Cassette	25 pieces	B-LFTLIF-TC	vacuum-sealed in a foil bag pouch
Chase Buffer	1 bottle 10 mL	B-LFTLIF-CB	Ready to use
Controls Low* / High*	2 vials, 0.5 mL	B-LFTLIF-CONSET	Ready to use
RFID Chip Card	1 piece	B-LFTLIF-RCC	**White** plastic card
RFID Chip Card	1 piece	B-LFTLIF-RCC15	**Green** plastic card

Table 1

* The controls contain lot specific amounts of infliximab. Refer to the additional QC data sheet for actual concentrations.

STORAGE AND SHELF LIFE OF REAGENTS

All kit components are stable at 2-8 °C until the expiration date printed on the labels.

PRECAUTIONS

Safety Precautions

- None of the reagents of this test contains components of human origin.
- Specimens should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practice (GLP) using appropriate precautions.
- Reagents: Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation can occur.
- Unused solution should be disposed according to local state and federal regulations.

Technical Precautions

Kit Components

- The test must be performed at room temperature (20-26 °C).
- All reagents and test samples must be equilibrated to room temperature (20-26 °C) before starting the assay.
- Before performing the test, remove the test cassette from the foil pouch and allow it to equilibrate at room temperature (20-26 °C).
- Mix well (e.g. vortex) the reagents before use.
- Components must not be used after the expiration date printed on the labels.
- Do not mix different lots of reagents.
- Do not disassemble the test cassettes.
- Test cassettes cannot be re-used.
- Handle the test cassettes with care. Do not contaminate the sample loading port or read-out window via skin contact, other liquids, etc. (Fig. 1D).
- Ensure a flat, horizontal position of the test cassette while performing the assay.

Test Procedure

- Read carefully the instructions prior to carrying out the test. Test performance will be adversely affected, if reagents are incorrectly diluted, handled or stored under conditions other than those as detailed in this instruction for use.
- The Quantum Blue® Reader must be switched on and programmed for the Quantum Blue® Infliximab assay: Load the assay method using the RFID Chip Card (B-LFTLIF-RCC or B-LFTLIF-RCC15), before starting the assay (see Quantum Blue® Reader Manual).
- Use the RFID Chip Card in order to change lot-specific test parameters.
- Samples that are not properly handled may cause inaccurate results.
- Diluted samples should be used within several hours (store at 2-8 °C) and cannot be stored for a longer period.

MATERIALS REQUIRED BUT NOT PROVIDED

- Vortex mixer
- Timer (optional)
- Precision pipettes with disposable tips: 10-100 µL and 100-1000 µL
- Eppendorf tubes (or equivalent) for dilution of serum samples
- Quantum Blue® Reader available from BÜHLMANN (order code: BI-POCTR-ABS)
- Gloves and laboratory coat

SPECIMEN COLLECTION AND STORAGE

Collect blood into plain venipuncture tubes without any additives avoiding hemolysis and let the serum clot at room temperature for at least 20 and for up to 60 minutes. Centrifuge at room temperature at ~2'000 x g for 15 minutes. Decant the serum.
Serum samples can be stored refrigerated at 2-8 °C for up to 10 days. For longer storage, keep serum samples at ≤-20 ºC. These samples are stable for at least 6 months at ≤-20 ºC.

ASSAY PROCEDURE

For the assay use only reagents equilibrated to room temperature (20-26 °C). The test cassette must be removed from the foil pouch prior to assay start.

The assay procedure consists of two steps:

1. Dilution of Serum Samples with Chase Buffer

Prior to measurement dilute the serum sample 1:20 with Chase Buffer (B-LFTLIF-CB) (e.g. mix 10 µL serum sample with 190 µL Chase Buffer) in a test tube and mix it by vortexing, pipetting or shaking.

2. Lateral Flow Assay Procedure and Readout

Two alternative methods can be loaded from the respective RFID Chip Card: TLIF_0 or TLIF_15. Select one of the RFID Chip Cards before starting the experiments. Load the test method from the RFID Chip Card.

2.1. Method <TLIF_15> with internal timer

- Use the green plastic card.
- Insert the test cassette into the test cassette holder of the Quantum Blue® Reader.
- Add 80 µL of the diluted serum sample onto the sample loading port of the test cassette (Fig. 1D).
- Close the test cassette holder and start the measurement by pressing the start button.
- The scan starts automatically after 15 minutes (900 seconds).
- For Low / High Controls: Repeat step 2.1 using 80 µL of Control instead of diluted serum.

2.2. Method <TLIF_0> without internal timer

- Use the white plastic card.
- Add 80 µL of the diluted serum sample onto the sample loading port of the test cassette (Fig. 1D).
- Incubate for 15 ± 1 minutes (set a timer manually).
- Insert the test cassette into the test cassette holder of the Quantum Blue® Reader.
- Scan the test cassette with the Quantum Blue® Reader by pressing the start (<ENTER>) button immediately.
- For Low / High Controls: Repeat step 2.2 using 80 µL of Control instead of diluted serum.

Remark: Please refer to the Quantum Blue® Reader Manual to learn about the basic functions and how to initialize and operate the Quantum Blue® Reader, especially how to select test methods, and how to load lot specific parameters from the RFID Chip Card in order to get the samples measured. Ensure the correct insertion of the test cassette into the Quantum Blue® Reader, with the read-out window first (Fig. 1 D).

QUALITY CONTROL

- If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: *i)* pipetting, temperature controlling and timing devices *ii)* expiration dates of reagents and *iii)* storage and incubation conditions.
- Result of the self-test of the Quantum Blue® Reader performed at the startup of the instrument has to be valid.

VALIDATION OF RESULTS

- For a valid test result, the Control Line (C) must be visible in any case (see Fig. 1A and Fig. 1B). It is used as a functional test control only and cannot be used for the interpretation of the Test Line (T). If the Test Line (T) is not detectable after 15 minutes of incubation time (Fig. 1A), the concentration of infliximab present in the serum sample is below the detection limit. If a Test Line (T) is detectable after 15 minutes of incubation time (Fig. 1B), the infliximab concentration present in the serum sample is calculated by the Quantum Blue® Reader.
- If only the Test Line (T) is detectable after 15 minutes of incubation time (Fig. 1C), the test result is invalid and the infliximab assay has to be repeated using another test cassette.
- If neither the Control Line (C) nor the Test Line (T) are detectable after 15 minutes of incubation time (Fig. 1D), the test result is invalid and the infliximab assay has to be repeated using another test cassette.
- As the Quantum Blue® Reader allows a quantitative evaluation of the Test (T) and Control (C) Line, an additional validity check of the Control Line (C) is undertaken. If the signal intensity of the Control Line (C) is below a threshold after 15 minutes of incubation time, the test result is also invalid and the infliximab assay has to be repeated using another test cassette.

STANDARDIZATION

- Calibrator values of the standard curve are assigned according to a value transfer protocol (ref. 1). The standard curve parameters are indicated in the enclosed QC data sheet. The calibrator material comprises infliximab (Remicade®) in a human serum matrix and is standardized against internal reference material.
- The Quantum Blue® Reader uses a lot-specific calibration curve to calculate the infliximab concentration. The measuring range is between 0.4 and 20.0 µg/mL.

LIMITATIONS

- The reagents supplied with this kit are optimized to measure trough levels of infliximab in diluted serum samples.

CHECK YOUR TEST KIT UPON ARRIVAL

BÜHLMANN products have been manufactured with the greatest of care and all possible efforts have been taken to ensure completeness of this test kit and its performance. Nevertheless, we advise you to verify your test kit for the condition of the test cassette and its pouch based on the following criteria:

- Expiration date
- The fault-free condition of the pouch (e.g. absence of any perforation that could be caused by improper handling).
- The fault-free condition of the test cassette (e.g. absence of scratches on the analytical membrane).

Should one of the test cassettes not fulfil the criteria mentioned above, please use another test cassette.

PERFORMANCE CHARACTERISTICS

Repeatability: 16.3 - 25.0 % CV

Within-Laboratory Precision: 18.5 - 25.3 % CV

Repeatability and within-laboratory precision were established according to CLSI guideline EP05-A3. Serum samples spiked with infliximab to cover the measuring range of the assay were tested over 20 days, in two independent runs with two replicates per run. The results are summarized in Table 2.

Limit of Blank (LoB): <0.10 µg/mL infliximab. The LoB was established according to the CLSI guideline EP17-A2 with four negative serum samples. The samples were measured over three days in five replicates each day to produce 60 blank values. The study was performed with two different test cassette lots. The LoB was calculated using non-parametric analysis.

Limit of Detection (LoD): <0.21 µg/mL infliximab. The LoD was established according to the CLSI guideline EP17-A2 with four pooled samples with concentrations of 0.63, 0.40, 0.26, and 0.15 µg/mL infliximab. The samples were measured over three days in five replicates each day to produce 60 values. The study was performed with two different test cassette lots. The LoD was calculated using parametric analysis.

Limit of Quantification (LoQ): ≤0.32 µg/mL infliximab. The LoQ was established according to the CLSI guideline EP17-A2. Calibrator material was diluted in negative serum to obtain samples with reference values of 0.50, 0.41, 0.30 and 0.20 µg/mL infliximab. The samples were measured over three days in five replicates each day to produce 60 values. The study was performed with two different test cassette lots. The LoQ was determined as the lowest reference value meeting the acceptance criterion of 30% relative total error, calculated using the RMS model from precision and bias estimates for each sample. Representative results obtained with one of the reagent lots are summarized in Table 3.

High Dose Hook Effect: A high dose hook effect was not observed for samples with infliximab concentrations of up to 1000 µg/mL.

Specificity (Cross-Reactivity): The Quantum Blue® Infliximab test specifically recognizes infliximab in serum. Spiked serum with TNFα blockers, such as adalimumab (Humira®), ethanercept (Enbrel®), golilumab (Simponi®), and certolizumab (Cimzia®) up to 100 µg/mL resulted in a read out below the limit of blank.

Linear Range: 0.14-20.37 µg/mL infliximab.

The linear range of the Quantum Blue® Infliximab test was determined according to the CLSI guideline EP06-A. Two sample pools, low and high, were blended to obtain at least 14 concentration levels covering and exceeding the expected measuring range. The blends were assayed in 10 replicates on two test cassette lots. The linear range was defined as the interval of concentration levels in which coefficients of the second and third order fits were determined as not significant. Results for one test cassette lot are shown in Fig. 2.

Method Comparison

Bias at 3 µg/mL: -2.4%

Bias at 7 µg/mL: -4.4%

The method comparison study was performed according to the CLSI guideline EP09-A3. One hundred and ten (110) samples were measured according to the instruction for use with the Quantum Blue® Infliximab assay and with a commercially available infliximab ELISA test (ref. 2). Measurements were performed over three days using two Quantum Blue® Infliximab test cassette lots. The correlation data is illustrated in Fig.3.

Recovery: 83 - 100 %.

To six samples 3.2 µg/mL infliximab in negative serum were added. “Base” samples were spiked with the corresponding amount of negative serum. “Base” and “base + spike” samples were measured in 10 replicates with one reagent lot. The results are summarized in Table 4.

INTERFERING SUBSTANCES

Within-Class Switch: No interference, tested for a minimum of two infliximab concentrations, was detected up to 10 µg/mL for adalimumab (Humira®), etanercept (Enbrel®), golilumab (Simponi®), and up to 1.7 µg/mL of certolizumab (Cimzia®).

Serum Indices: No interference was detected with the following substances up to the listed concentrations: Triglycerides (Intralipid® 1320 mg/dL; equivalent to 37 mmol/L triglyceride), conjugated bilirubin (342 µmol/L; 29 mg/dL), unconjugated bilirubin (342 µmol/L; 20 mg/dL), haemoglobin (2 g/L (= 200 mg/dL), TNFα (2.6 ng/mL) and rheumatoid factors (497.3 IU/mL).

Immunosuppressive Co-Medication: No interference was detected with immunsuppressive co-medication such as azathioprine (60 µmol/L), 6-mercaptopurine (37 µmol/L), and methotrexate (1363 µmol/L).

All performance characteristics were done with infliximab (Remicade®, MSD).

Test Results

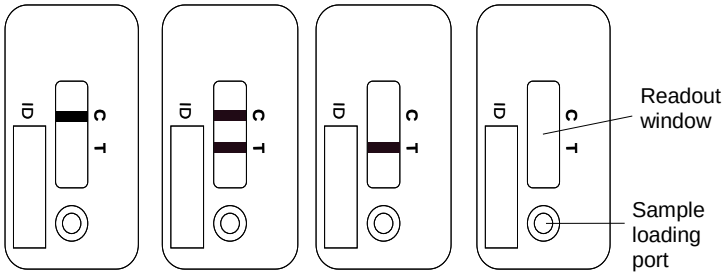

Fig. 1A Fig. 1B Fig. 1C Fig. 1D

Repeatability / Within-Laboratory Precision

Mean IFX Conc. [µg/mL]	Repeatability CV [%]	Between -run Precision CV [%]	Between -day Precision CV [%]	Within-lab Precision CV [%]
0.42	16.3	3.7	7.9	18.5
1.44	25.0	0.0	3.6	25.2
3.02	20.3	5.1	5.1	21.5
4.78	21.0	0.0	0.0	21.0
7.26	17.0	7.5	4.5	19.2
9.37	20.4	0.0	2.7	20.6
11.71	23.5	9.5	0.0	25.3

Table 2

Limit of Quantification

Sample	1	2	3	4
Reference value [µg/mL]	0.20	0.30	0.41	0.50
Bias [µg/mL] (Mean value obtained – reference value)	-0.01	-0.05	-0.05	-0.10
Precision [% CV]	24	20	18	16
% Total Error	**24**	**27**	**22**	**30**

Table 3

Recovery

Sample	Base [µg/mL]	Spike [µg/mL]	Expected Base + Spike [µg/mL]	Observed Base + Spike [µg/mL]	Reco-very [%]
S1	1.5	3.2	4.7	3.9	83
S2	2.0	3.2	5.3	5.1	98
S3	2.9	3.2	6.1	6.1	100
S4	4.3	3.2	7.6	7.2	95
S5	6.5	3.2	9.7	9.3	96
S6	9.9	3.2	13.2	11.8	89

Table 4table 7

Linearity Plot

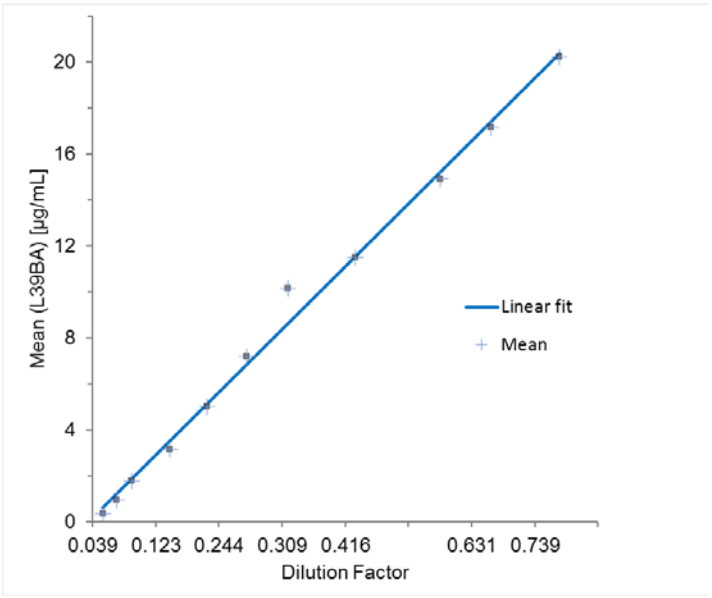

Fig. 2

Method Comparison

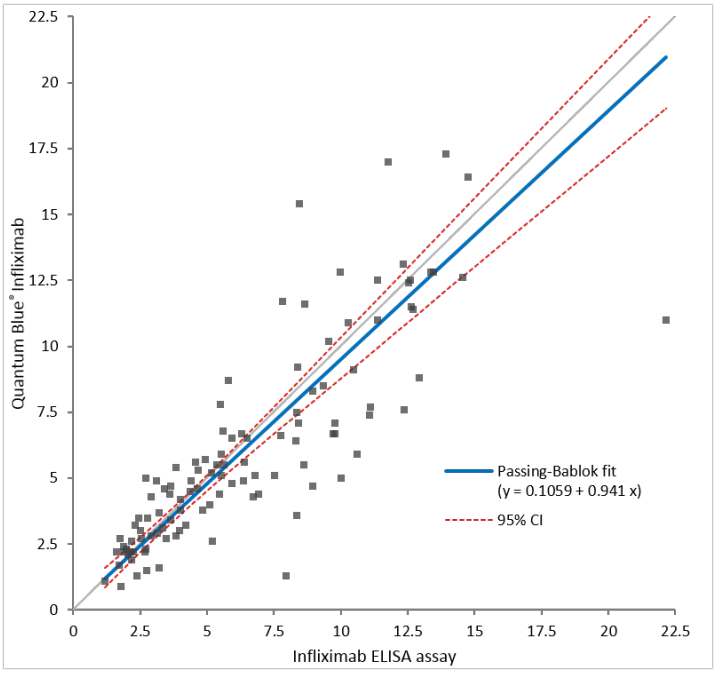

Fig. 3

APPENDIX II

REFERENCES

1. Blirup-Jensen et al.: *Protein standardization V: value transfer. A practical protocol for the assignment of serum protein values from a Reference Material to a Target Material.* Clin Chem Lab Med; **46**, 1470 – 9 (2008)

2. Vande Casteele, N. et al.: *Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays.* Aliment Pharmacol Ther, **36**, 765-771 (2012)

APPENDIX III

SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Contains sufficient for <n> tests
	Consult Instructions for Use

Symbol	Explanation
	Temperature limitation
TC	Test Cassette
BUFCHASE	Chase Buffer
CONTROL L	Control Low
CONTROL H	Control High
RCC	RFID Chip Card