Quantum Blue®
Anti-Adalimumab

Qualitative
Lateral Flow Assay

For *In Vitro* Diagnostic Use

LF-ADAD25 25 tests
LF-ADAD10 10 tests

Release date: 2023-04-27
Version A3

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ENGLISH

INTENDED USE

The Quantum Blue® Anti-Adalimumab test is a qualitative *in vitro* diagnostic immunoassay for the detection of anti-adalimumab antibodies in human serum samples. The test serves as an aid to therapeutic drug monitoring in patients with inflammatory bowel disease (IBD) and rheumatoid arthritis (RA) under adalimumab therapy. The test is used in conjunction with other clinical and laboratory findings. Quantum Blue® Anti-Adalimumab is combined with the Quantum Blue® Reader.

For laboratory use.

PRINCIPLE OF THE ASSAY

The test is designed for the selective measurement of anti-adalimumab antibodies by a sandwich immunoassay. The signal intensities of the test line (T) and the control line (C) are measured by the Quantum Blue® Reader. The results are reported as negative <neg(-)> or positive <pos(+)>.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity		Code	Comments
	LF-ADAD25	LF-ADAD10		
Test Cassette	25 pieces	10 pieces	B-LFADAD-TC	Vacuum-sealed in a foil bag pouch
Chase Buffer	1 bottle 10 mL	1 bottle 10 mL	B-LFADAD-CB	Ready to use
Controls Low* / High*	2 vials 0.1 mL	2 vials 0.1 mL	B-LFADAD-CONSET	Dilute 1:10 in chase buffer before use
RFID Chip Card	1 piece	1 piece	B-LFADAD-RCC	**White** plastic card
RFID Chip Card	1 piece	1 piece	B-LFADAD-RCC15	**Green** plastic card
Barcode Card	1 piece	1 piece	B-LFADAD-BCC	2D Barcode plastic card

Table 1

* Controls are lot-specific. The controls low and high should be reported as <neg(-)> and <pos(+)>, respectively.

CHECK YOUR TEST KIT

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.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened reagents	
Store at 2-8 °C. Do not use the reagents beyond the expiration date printed on the labels.	
Opened reagents	
Test Cassette	Test cassettes removed from the foil pouch must be used within 4 hours.
Chase Buffer	Store for up to 6 months at 2-8 °C after opening.
Controls Low / High	Store for up to 6 months at 2-8 °C after opening.

Table 2

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

WARNINGS AND PRECAUTIONS

Safety precautions

- Specimens should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practice (GLP) using appropriate precautions.
- The controls and chase buffer of this kit contain components classified in accordance with the Regulation (EC) No. 1272/2008: 2-methyl-4-isothiazolin-3-one hydrochloride (conc. ≥ 0.0015%), thus the reagents may cause allergic skin reactions (H317).
- 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 before starting the assay.
- Once equilibrated to room temperature remove the test cassette from the foil pouch. Allow the test cassette to equilibrate in the laboratory environment for at least 2 minutes before starting the assay.
- 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. (figure 1D).
- Ensure a flat, horizontal position of the test cassette while performing the assay.

Test procedure

- Read the instructions carefully 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 detailed in this instruction for use.
- Please note that there are two generations of readers: The Quantum Blue® Reader 2nd Generation with serial numbers between 1000 and 3000 (QB2) and Quantum Blue® Reader 3rd Generation with serial numbers above 3000 (QB3G).
- The QB2 must be switched on and programmed for the Quantum Blue® Anti-Adalimumab assay: Load the assay method using the RFID chip card (B-LFADAD-RCC or B-LFADAD-RCC15), before starting the assay (see Quantum Blue® Reader manual).
- The QB3G must be switched on and programmed for the Quantum Blue® Anti-Adalimumab assay either by using the barcode card (B-LFADAD-BCC) or by selecting from the test menu (Fast Track Mode only). For more information please refer to the Quantum Blue® Reader manual.
- Use the RFID chip card (QB2) / barcode card (QB3G) in order to change lot-specific test parameters.
- Samples that are not properly handled may cause inaccurate results.
- Diluted samples should be measured promptly and cannot be stored longer than 15 hours at room temperature (≤23 °C).

SPECIMEN COLLECTION AND STORAGE

Collect blood into plain venipuncture tubes without any additives and avoid hemolysis. Perform serum preparation according to manufacturer's instructions. Decant the serum.
Undiluted serum samples can be stored unrefrigerated (temperatures up to 28 °C) for up to 9 days or stored cooled (2-20 °C) for 15 days. For longer storage, keep undiluted serum samples at ≤-20 °C. The samples are stable for at least 13 months at ≤-20 °C. More than six freeze-thaw cycles should be avoided.

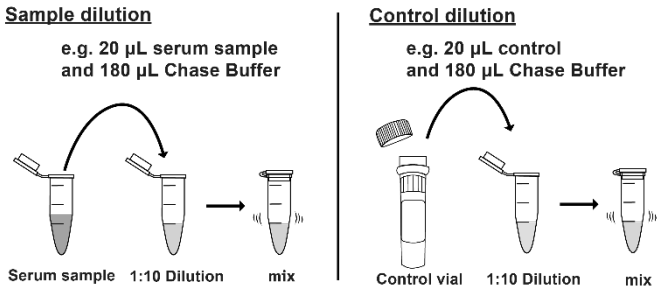
ASSAY PROCEDURE

For the assay use only reagents equilibrated to room temperature. 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 and controls with chase buffer

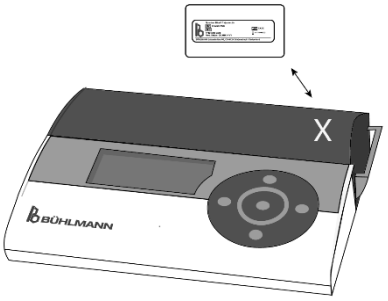
Prior to measurement dilute the serum sample and controls 1:10 with chase buffer (B-LFADAD-CB) (e.g. mix 20 µL serum sample or control with 180 µL chase buffer in a test tube) and mix by vortexing, pipetting or shaking.



2. Lateral flow assay procedure and readout

QB2

Two alternative methods can be loaded from the respective RFID chip card: B-LFADAD-RCC15 (with internal timer) or B-LFADAD-RCC (without internal timer). Select one of the RFID chip cards before starting the experiments. Load the test method from the RFID chip card on the Quantum Blue® Reader.



QB3G

Two different modes of operation are available to measure samples with the QB3G: Fast Track Mode or Fail Safe Mode. Before starting the assay, please inform yourself in which operation mode your reader is working.
The test method can be loaded from the barcode card (Fast Track and Fail Safe Mode) or, if previously used, selected from the test menu (Fast Track Mode only). Measurements can be performed with or without an internal timer in the Fast Track Mode. Measurements in the Fail Safe Mode can be performed with internal timer only.
Follow the instructions provided on the screen of the QB3G. You may also refer to the QB3G Quick Guides for the Fast Track and Fail Safe Mode.

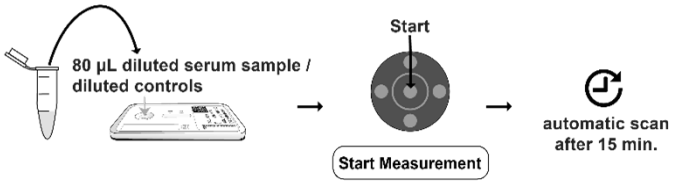
2.1. Method with internal timer

QB2: Use the green RFID chip card B-LFADAD-RCC15

QB3G (Fast Track Mode): when prompted by the QB3G to skip the incubation time, select “NO”

QB3G (Fail Safe Mode): default setting

- Unpack the test cassette. Allow the test cassette to equilibrate in the laboratory environment for at least 2 minutes.
- Add 80 µL of the diluted serum sample onto the sample loading port of the test cassette (figure 1D).
- Insert the test cassette into the test cassette holder of the Quantum Blue® Reader.
- Close the test cassette holder and start the measurement by pressing the start button on the QB2 or the “Start Measurement” option on the QB3G.
- The scan starts automatically after 15 minutes.
- For low / high controls: Repeat step 2.1 using 80 µL of diluted controls instead of diluted serum.



2.2. Method without internal timer

QB2: Use the white RFID chip card B-LFADAD-RCC

QB3G (Fast Track Mode): when prompted by the QB3G to skip the incubation time, select “YES”

QB3G (Fail Safe Mode): option not available

- Unpack the test cassette. Allow the test cassette to equilibrate in the laboratory environment for at least 2 minutes
- Add 80 µL of the diluted serum sample onto the sample loading port of the test cassette (figure 1D).
- Incubate for 15 ± 1 minute (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 button on the QB2 or the “Start Measurement” option on the QB3G.
- For low / high controls: Repeat step 2.2 using 80 µL of diluted controls 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® Readers, especially how to select test methods and how to load lot-specific parameters from the RFID chip card (QB2) / barcode card (QB3G) on the Quantum Blue® Reader. Ensure the correct insertion of the test cassette into the Quantum Blue® Reader, with the read-out window first (figure 1D).

QUALITY CONTROL

- If the performance 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 *ii)* expiration dates of reagents and *iii)* storage and incubation conditions.
- The result of the self-test of the Quantum Blue® Reader performed at the startup of the instrument has to be valid.

STANDARDIZATION

- The calibrator material is a monoclonal anti-adalimumab human IgG antibody in a human serum matrix.
- The Quantum Blue® Reader uses a lot-specific calibration curve to calculate the anti-adalimumab concentration in equivalents (μg_{eq}/mL) to the monoclonal anti-adalimumab IgG calibrator. The 95% confidence interval of the combined uncertainty of the product calibrator is lower than 25.0%, the combined uncertainty of the controls lower than 30.0%.
- The Quantum Blue® Reader indicates the result as negative <neg(-)>, if the sample concentration is below 0.2 μg_{eq}/mL. Results equal to and above 0.2 μg_{eq}/mL are indicated as positive <pos(+)>.

VALIDATION OF RESULTS

- For a valid test result, the control line (C) must be visible in any case (see figure 1A and figure 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 (figure 1A), the concentration of anti-adalimumab antibodies present in the serum sample is below the detection limit. If a test line (T) is detectable after 15 minutes of incubation time (figure 1B), the anti-adalimumab antibody 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 (figure 1C), the test result is invalid and the Quantum Blue® Anti-Adalimumab 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 (figure 1D), the test result is invalid and the Quantum Blue® Anti-Adalimumab assay has to be repeated using another test cassette.
- As the Quantum Blue® Reader allows an 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 specific preconfigured threshold after 15 minutes of incubation time, the test result is also invalid and the Quantum Blue® Anti-Adalimumab assay has to be repeated using another test cassette.

LIMITATIONS

- The Quantum Blue® Anti-Adalimumab test is a drug-sensitive assay and therefore can only be used on samples with undetectable adalimumab concentrations.

- The reagents supplied with this kit are optimized to measure levels of anti-adalimumab antibodies in serum samples.
- Quantum Blue® Anti-Adalimumab test results should be interpreted in conjunction with other clinical and laboratory findings. These may include the determination of disease activity, determination of adalimumab trough levels, as well as information on patient's adherence to therapy.

INTERPRETATION OF RESULTS

The Quantum Blue® Reader displays the following result categories for the Quantum Blue® Anti-Adalimumab assay:

Display	Interpretation
neg(-)	negative result
pos(+)	positive result

Table 3

CUT-OFF ESTABLISHMENT

The technical cut-off was established as 0.2 μg_{eq}/mL, based on results obtained in a reference interval study and the limit of detection (LoD) of the assay (please refer also refer to section Performance Characteristics).

Reference Interval

The reference interval of the Quantum Blue® Anti-Adalimumab test was established according to CLSI C28-A3 with 120 serum samples from healthy individuals, with equal numbers of women and men. Measurements were performed over 3 days by 2 operators using two Quantum Blue® Anti-Adalimumab lots, resulting in 240 values (ref. 1).

Reference Interval [μg_{eq}/mL]	
2.5th percentile (90% CI)	**97.5th percentile (90% CI)**
0.0 (0.0 - 0.0)	0.1 (0.0 - 0.2)

Table 4

PERFORMANCE CHARACTERISTICS

The following performance characteristics have been established with the Quantum Blue® Reader 2nd and 3rd generation.
Indicated performance characteristics apply for both reader generations.

Method comparison

Assay agreement: 91.3%

One hundred and twenty (120) clinical samples were measured with 2 lots of the Quantum Blue® Anti-Adalimumab assay over 4 days, resulting in 240 values, out of which 42 were negative and 198 were positive. The same samples were measured in duplicate using a commercially available ELISA assay. Mean ELISA values were classified as positive or negative according to the indicated cut-off and the total agreement between both assays was calculated. The results are summarized in table 5.

Within-laboratory precision: 100% within-category

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3 using the standard 20 days x 2 runs x 2 replicates study design (ref. 2). Three (3) pooled serum samples: negative, low positive and high positive, were assayed. The results are summarized in table 6.

Reproducibility: 100% within-category

Reproducibility was established according to the CLSI guideline EP05-A3 using a 3 instruments / lots / operators x 5 days x 5 replicates study design (ref. 2). Three (3) pooled serum samples: negative, low positive and high positive, were assayed. The results are summarized in table 7.

Limit of Detection (LoD): 0.1 μg_{eq}/mL

The LoD was established according to the CLSI guideline EP17-A2 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5% based on 120 determinations, with 60 blank and 60 low level replicates; and a LoB of 0.0 μg_{eq}/mL (ref. 3).

High dose hook effect

No negative results for contrived samples with theoretical anti-adalimumab concentrations of up to 592.5 μg_{eq}/mL were observed. The study was performed with two Quantum Blue® Anti-Adalimumab lots.

INTERFERING SUBSTANCES

The susceptibility of the Quantum Blue® Anti-Adalimumab test to interfering substances was assessed according to the CLSI-approved guideline EP07-A2. Interfering substances were tested at concentrations three-fold higher than those reported or expected in clinical samples or at concentration levels recommended by the CLSI guideline EP07-A2 (ref. 4). At least 7 replicates per interferent were tested. An out of category result for a single replicate was considered interference.

TNFα blocker

TNFα blockers were tested at concentrations exceeding lowest recommended drug trough levels by three-fold. No interference was detected with the following substance at the listed concentration: infliximab (Remicade®, 10 µg/mL). Interferences were detected with the substance adalimumab (Humira®, 15 µg/mL). The interference effect was further characterized by a dose response test. Results indicate that anti-adalimumab presence can only be assessed in samples with undetectable adalimumab levels.

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), Hemoglobin (200 g/L) and rheumatoid factors (up to 600 IU/mL).

Immunosuppressive and other medications

No interference was detected with the following substances, up to the listed concentrations: azathioprine (10.8 µmol/L, 3.0 µg/mL), 6-mercaptopurine (13.1 µmol/L, 2.0 µg/mL), and methotrexate (149.6 µmol/L, 68.0 µg/mL).

TABLES AND FIGURES

Test results

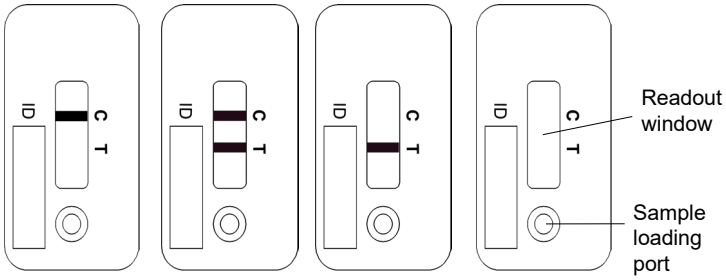


Figure 1A Figure 1B Figure 1C Figure 1D

Method Comparison

		Comparator		
		Negative	**Positive**	**Total**
Quantum Blue® Anti-Adalimumab	**Negative**	**19.2%**	6.3%	25.4%
	Positive	2.5%	**72.1%**	74.6%
	Total	21.7%	78.3%	100%

91.3%

Table 5

Repeatability / Within-Laboratory Precision

Sample	Description	n	Mean conc. [μg_{eq}/mL]	% Within category
S1	Negative	80	0.0	100
S2	Low Positive	80	0.3	100
S3	High Positive	80	1.4	100

Table 6

Reproducibility

Sample	Description	n	Mean conc. [μg_{eq}/mL]	% Within category
S1	Negative	75	0.0	100
S2	Low Positive	75	0.4	100
S3	High Positive	75	2.0	100

Table 7

REFERENCES

1. CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition.* CLSI document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

2. CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

3. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition.* CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

4. CLSI. *Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.* CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

CHANGELOG

Date	Version	Change
2023-04-27	A3	Update to chapter *Warnings and precautions* Inclusion of uncertainty values for product calibrator and controls in chapter *Standardization* Addition of chapter *References* Revision of chapter *Symbols* Inclusion of notified body number to CE-mark – conformity assessment procedure according to IVDR 2017/746

INCIDENT REPORTING IN EU MEMBER STATES

If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

SHIPPING DAMAGE

Please notify your distributor, if this product was received damaged.

SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1. In addition the following symbols and signs are used:

Symbol	Explanation
TC	Test Cassette
BUF CHASE	Chase Buffer
CONTROL L	Control Low
CONTROL H	Control High
RCC	RFID Chip Card
BCC	Barcode Card
	**EN**: electronic instruction for use available in different languages at:/ **BG**: електронни инструкции за употреба на различни езици на адрес:/ **CS**: elektronický návod k použití dostupný v různých jazycích na adrese:/ **DA**: elektronisk brugsanvisning på forskellige sprog på:/ **DE**: elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ **EL**: ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ **ES**: instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ **ET**: elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ **FR**: un mode d'emploi électronique disponible en différentes langues à l'adresse:/ **HU**: különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ **IT**: istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ **LT**: elektroninės naudojimo instrukcijos įvairiomis kalbomis:/ **LV**: dažādās valodās pieejama elektroniska lietošanas instrukcija:/ **NO**: elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ **PL**: elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ **PT**: instrução electrónica para utilização disponível em diferentes línguas em:/ **RO**: instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ **SK**: elektronický návod na použitie dostupný v rôznych jazykoch na:/ **SL**: elektronska navodila za uporabo so na voljo v različnih jezikih na:/ **SR**: elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:/ **SV**: elektronisk bruksanvisning på olika språk på följande adress: **www.buhlmannlabs.ch/support/downloads/**

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