

P202 Quantum Blue® Anti-Infliximab: Development and evaluation of a point of care rapid test for measuring anti-infliximab antibodies in human serum

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BACKGROUND

The treatment of patients suffering from an inflammatory disease, like inflammatory bowel disease (IBD) may involve biologicals like infliximab. However, infliximab is a chimeric human/murine monoclonal antibody and can induce a significant immune response. Within this immune response different anti-infliximab antibodies are formed, belonging to different isotypes (e.g. IgM, IgG₁, IgG₄ and IgE) with different specificities and affinities. The detection of these anti-infliximab antibodies is crucial to adjust the therapy with infliximab or to justify a switch of the used drug. For the fast and easy detection of the anti-infliximab response a lateral flow test was developed and preliminary evaluated.

METHODS

A drug-sensitive bridging lateral flow test was developed using infliximab-fragment coated gold nanoparticles and membrane immobilized infliximab to detect polyclonal anti-infliximab antibodies in a diluted human serum sample. Standardization is based on a specific monoclonal anti-infliximab antibody. Using this approach Limit of Detection (LoD) and Limit of Quantification (LoQ) were determined according to CLSI EP17-A2 guideline. The influence of rheumatoid factors as well as various blood conditions was evaluated. Patient samples were used to compare the Quantum Blue® Anti-Infliximab rapid test with a commercially available ELISA test. These results were used to establish a ROC curve analysis and to identify a clinical relevant cut-off value.

RESULTS

The current Quantum Blue® Anti-Infliximab test allows the analysis of diluted human serum samples within 15 minutes. The samples are diluted in chase buffer (1:10) before application on the test cassette. The readout is performed with a Quantum Blue® Reader resulting in a measuring range of 0.5 to 12 µg/mL (Table 1). Due to missing international standard material and the polyclonal immune response in patients, the Quantum Blue® Anti-Infliximab was classified as semi-quantitative. However, the Quantum Blue® Anti-Infliximab shows outstanding concentration CVs within the measuring range (Fig. 1). The test exhibits a LoD of 0.31 µg/mL and a LLoQ of 0.5 µg/mL (Fig. 2). Rheumatoid factors as well as various blood conditions showed no interference to test results. A clinical cut-off value of 1.44 µg/mL results in a sensitivity of 0.86 and a specificity of 0.94 obtained by ROC curve analysis with 78 patient samples (Fig. 3).

Table 1: Quantum Blue® Anti-Infliximab Test Specifications

Quantum Blue® Anti-Infliximab	
Measuring Range	0.5 - 12 µg/mL
Sample Specimen	Serum
Application Volume	80 µL
Sample Dilution	1:10
Time to Result	15 minutes
Assay Read-out	Quantum Blue® Reader

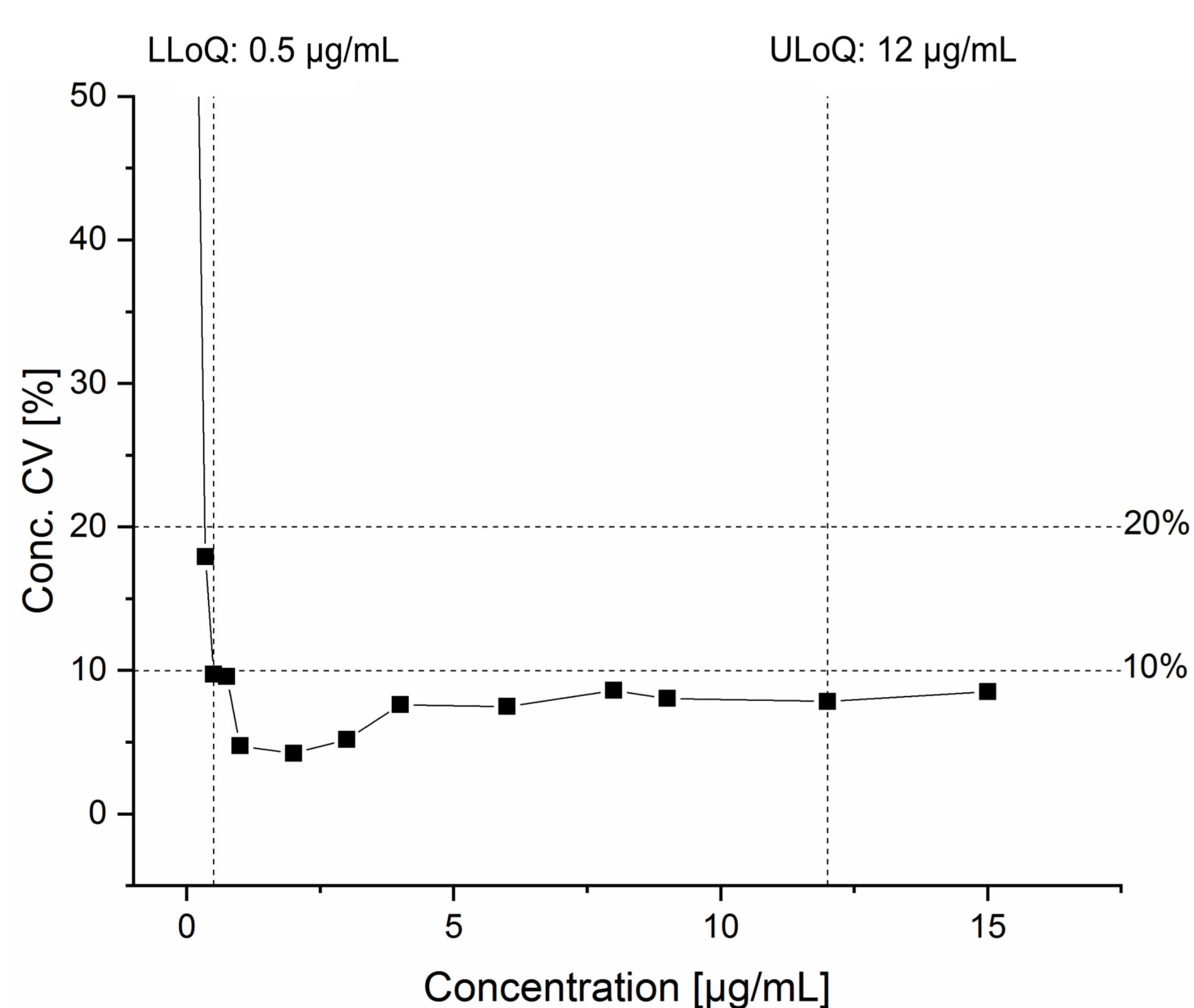


Figure 2: Precision Evaluation of Quantum Blue® Anti-Infliximab

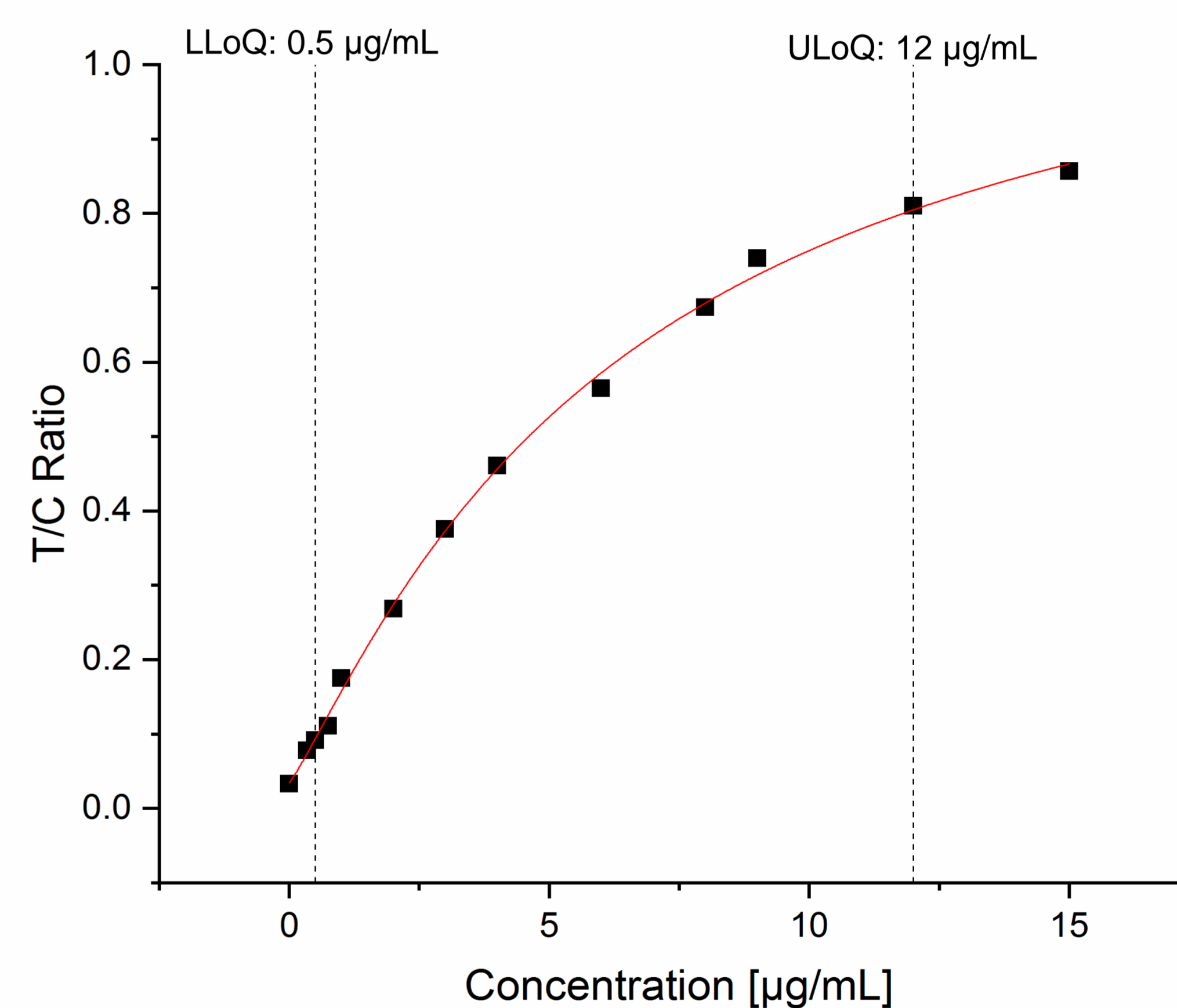


Figure 1: Sensitivity Evaluation of Quantum Blue® Anti-Infliximab

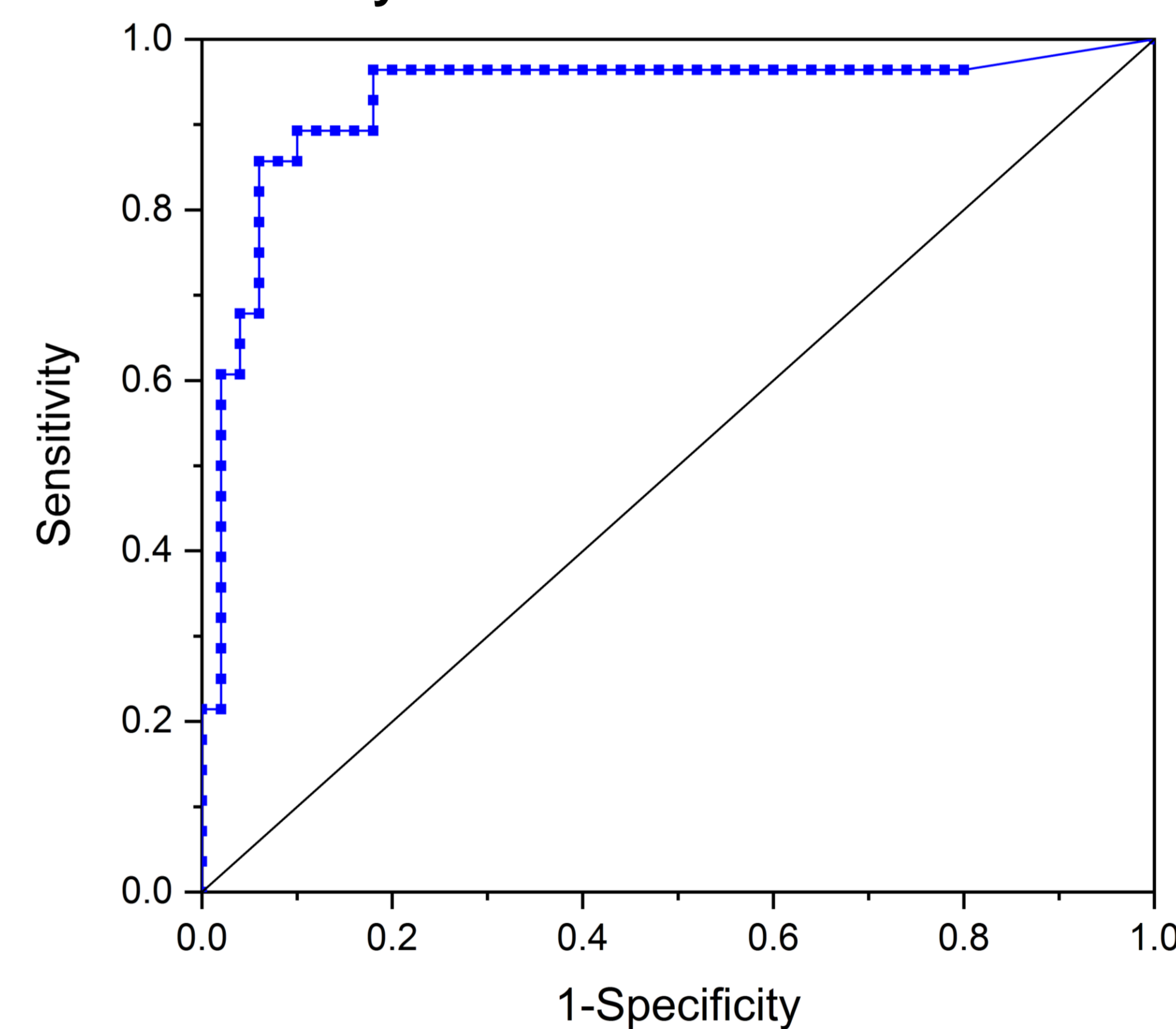


Figure 3: ROC Curve Evaluation of Quantum Blue® Anti-Infliximab

CONCLUSIONS

The here presented Quantum Blue® Anti-Infliximab test allows the fast and easy detection of anti-infliximab antibodies in human serum within 15 minutes. The assay can be carried out with a minimum of external equipment and may therefore support a fast adaption of the treatment regime, providing a valuable tool for pro-active therapeutic drug monitoring.