Immunoturbidimetric assay for in-process determination of polyclonal antibody functionality

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Introduction

Polyclonal antibodies are produced by host animals immunity against the antigen used in immunization. Polyclonal antibodies have several advantages due to their versatile binding properties but the production method causes variation between batches.

Haemoglobin is an oxygen-carrying protein found in blood. Its functional forms are oxygen-bound oxyhaemoglobin and oxygen-free deoxyhaemoglobin. Oxyhaemoglobin can also auto-oxydize into methaemoglobin, which is not able to bind oxygen.

In this thesis, the aim was to improve an immunoturbidimetric assay for antibody titre determination.

Methods

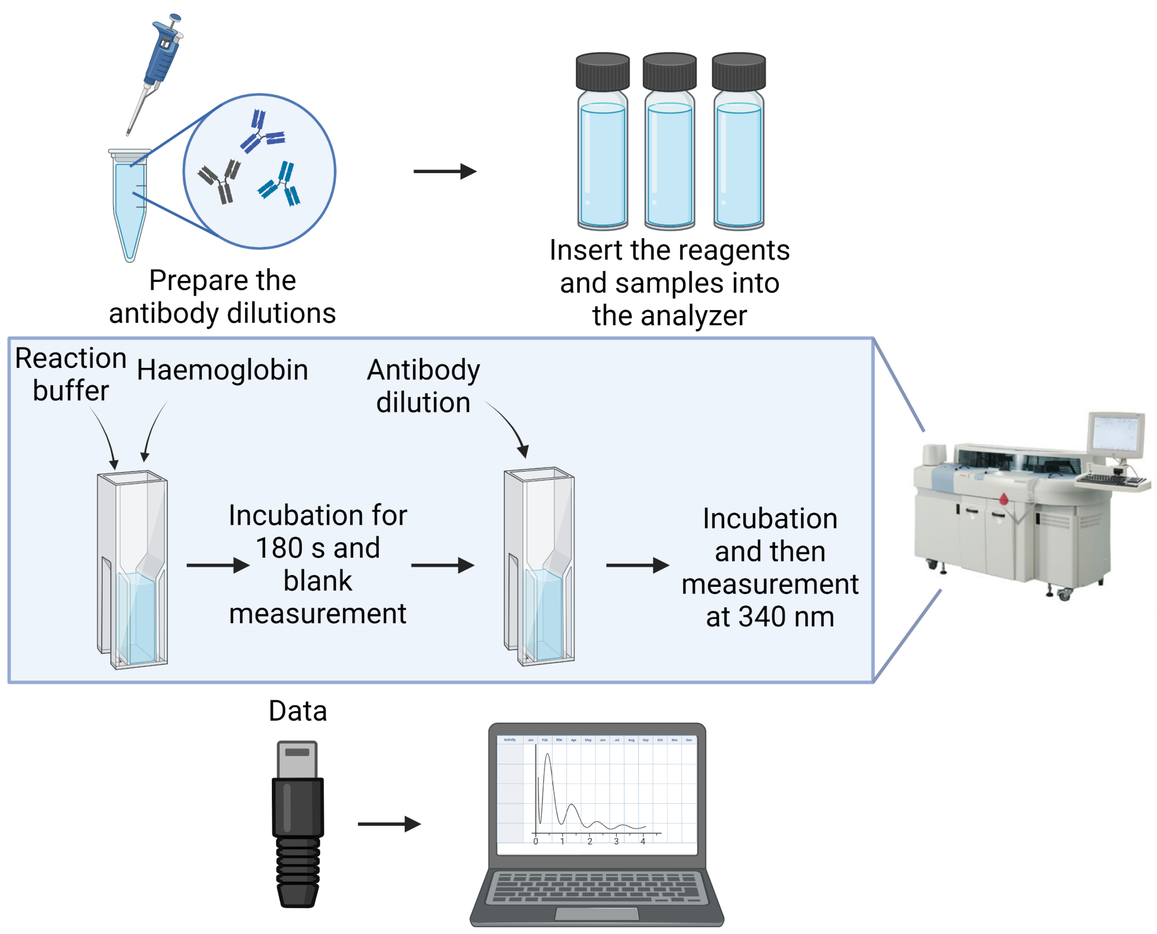

Figure 1. The assay protocol for titre determination assay. Reagents, haemoglobin and dilutions of antibodies were added to the measuring tube. The analyzer diluted and dispensed the reagents into the cuvettes and measured the blank and final absorbances. Created with BioRender.com

Conclusions

The method that was decided to be implemented included buffers containing azide, methaemoglobin as an antigen, pseudo end-point at 1000 seconds and 20 measuring points from haemoglobin range 7-250 µg/ml.

Results

Azide is a common preservative in buffers and reacts with methaemoglobin. Azide did not affect the titres, however, it increased the measurement signals.

Incubation time was studied with kinetic measurement. At equivalence zone, the absorbance responses increased strongly as a function of reaction time, but the increase clearly claimed down at 2000 seconds. The apparent titre showed an increase with longer reaction times.

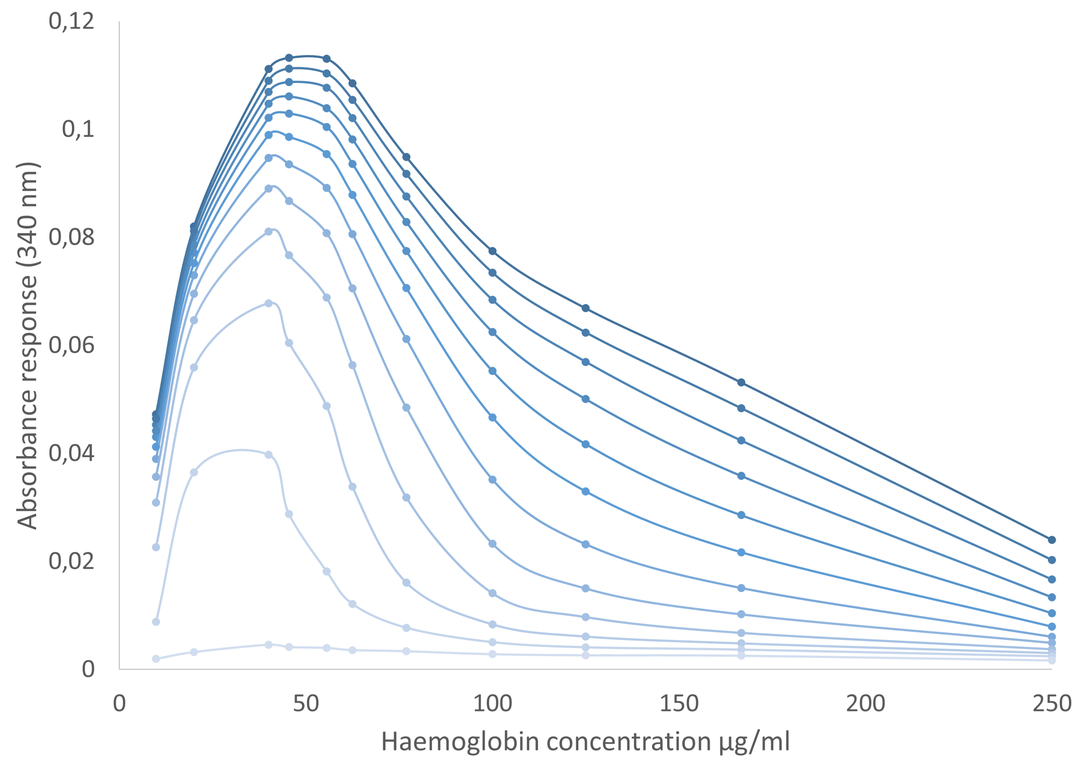

Figure 2. Data from the incubation measurements. The darkening color describes the increase in reaction time, which is between 0-2000 seconds.

Methaemoglobin and oxyhaemoglobin forms were compared and methaemoglobin increased the titres by 80 % and overall signals by 30 %.

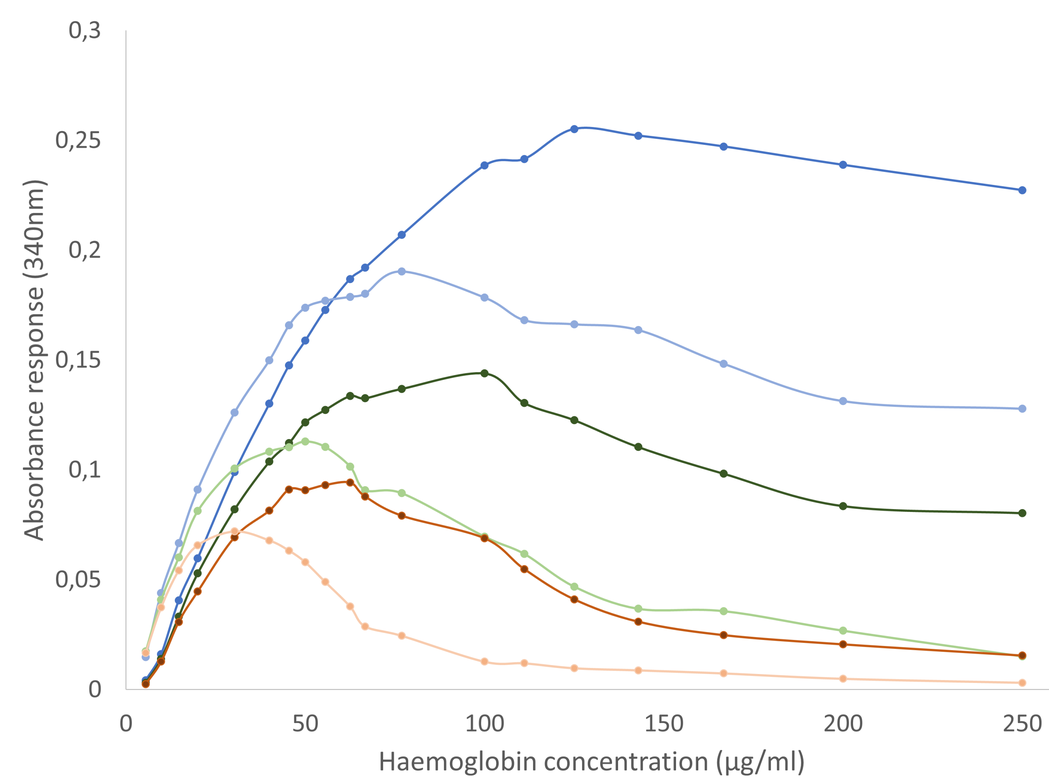

Figure 3. Comparison of the methaemoglobin and oxyhaemoglobin in titre determination assay. Three dilutions of antibodies 1/4 (blue), 1/6 (green) and 1/8 (red) were measured with methaemoglobin (darker colour) and oxyhaemoglobin (lighter colour).