



Spectrally multiplexed quantitative lateral flow immunoassay for *Bordetella pertussis* antibodies

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Introduction

Pertussis, also known as whooping cough, is a bacterial infection caused by *Bordetella pertussis*. Despite a high vaccination coverage, pertussis remains endemic worldwide, with an increasing reported incident rate. Serological testing for pertussis is based on measuring antibodies against pertussis toxin (PT) since it is solely produced by *Bordetella pertussis*. However, PT is also included in all acellular pertussis vaccines, which makes separating infection from vaccine induced responses challenging.

The aim of this research is a proof-of-concept for a spectrally multiplexed quantitative and standardized lateral flow immunoassay (LFIA) for anti-PT antibodies. The novel assay utilizes two upconverting labels with separate emissions, to measure anti-PT IgG and IgA from a single test line (figure 1). This could aid in identifying a pertussis infection, since childhood vaccinations are shown not to cause anti-PT IgA responses.¹

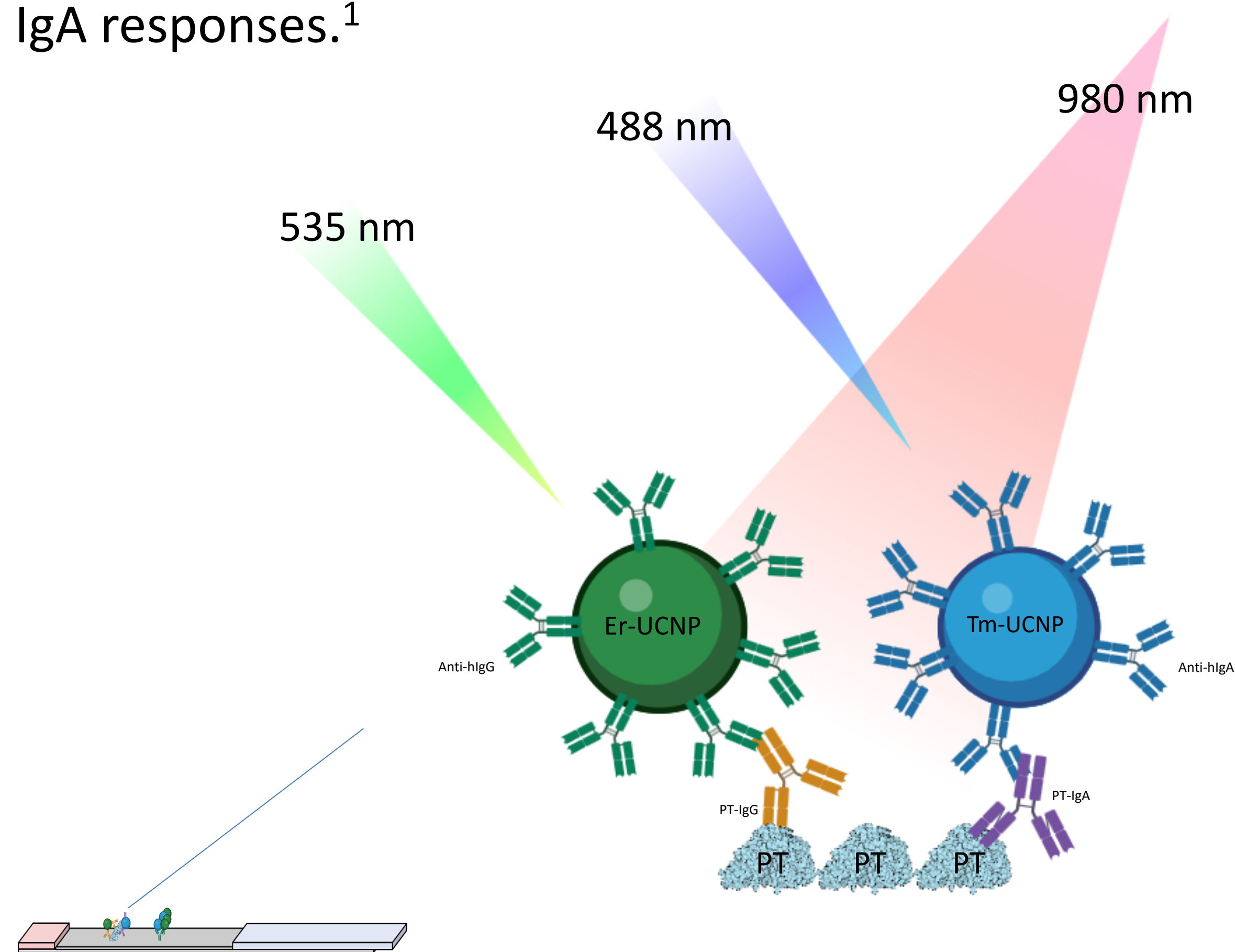


Figure 1. Multiplexed immunocomplex at LFIA test line. Bound anti-PT antibodies from patient serum (yellow and purple) at antigen coated test line (light blue), are measured using two different upconverting nanoparticles (green and blue) that have the same excitation wavelength, but separate emissions.

Methods

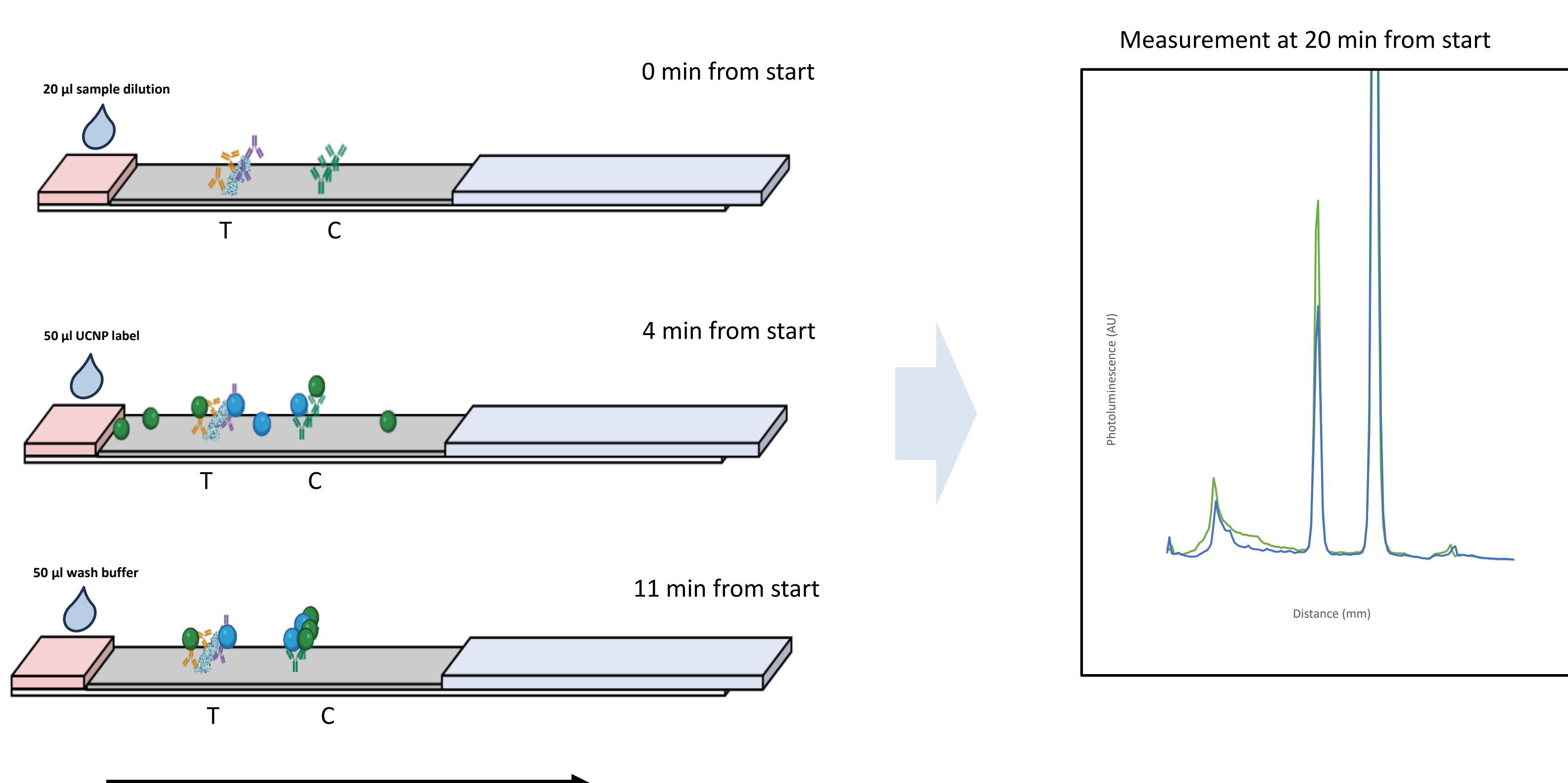


Figure 2. Assay protocol. On the lateral flow strip, Test line (T) and control line (C) are dispensed on a nitrocellulose membrane (grey). Sample, label and wash buffer are added in steps on the glass fiber sample bad (pink). Liquid flows towards the cellulose absorbent pad (lilac). Strips are line scanned with infrared laser excitation at 980 with Upcon reader (UnioGen, Finland).

Results

Spectral multiplexing was attained for both assays (figure 3), with minimal crosstalk (less than 1 %) and no steric hindrance.

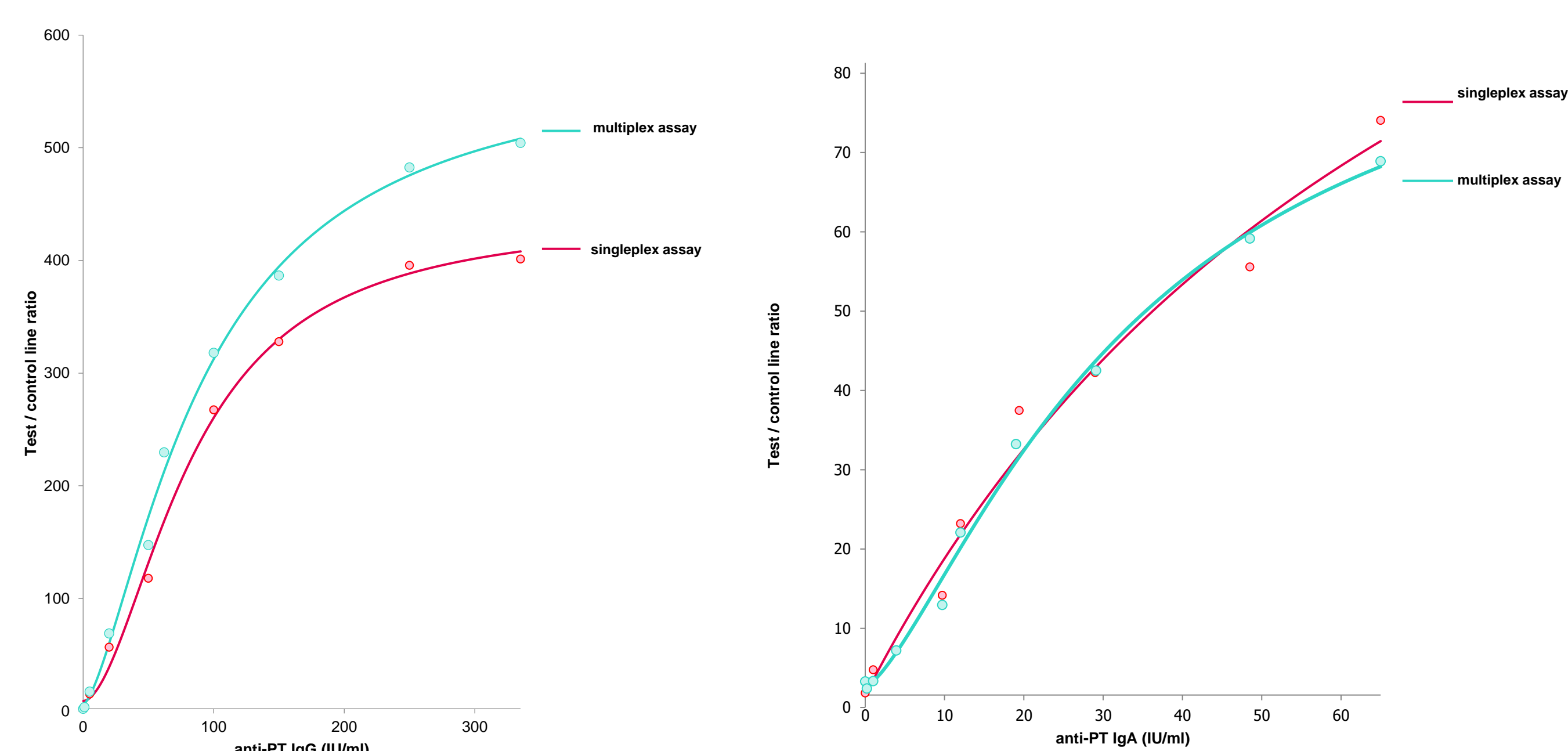


Figure 3. Spectral multiplexing. Both anti-PT IgG and IgA assays were carried out with either one UCNP label (singleplex) or both (monoplex) with the same protocol (figure 2). Singleplex and monoplex assays were compared using WHO International standard 06/140 dilutions.

The dynamic range for anti-PT IgG was from 4 to 335 IU/ml (figure 4) with the clinical cut-offs being between 40 and 100 IU/ml.

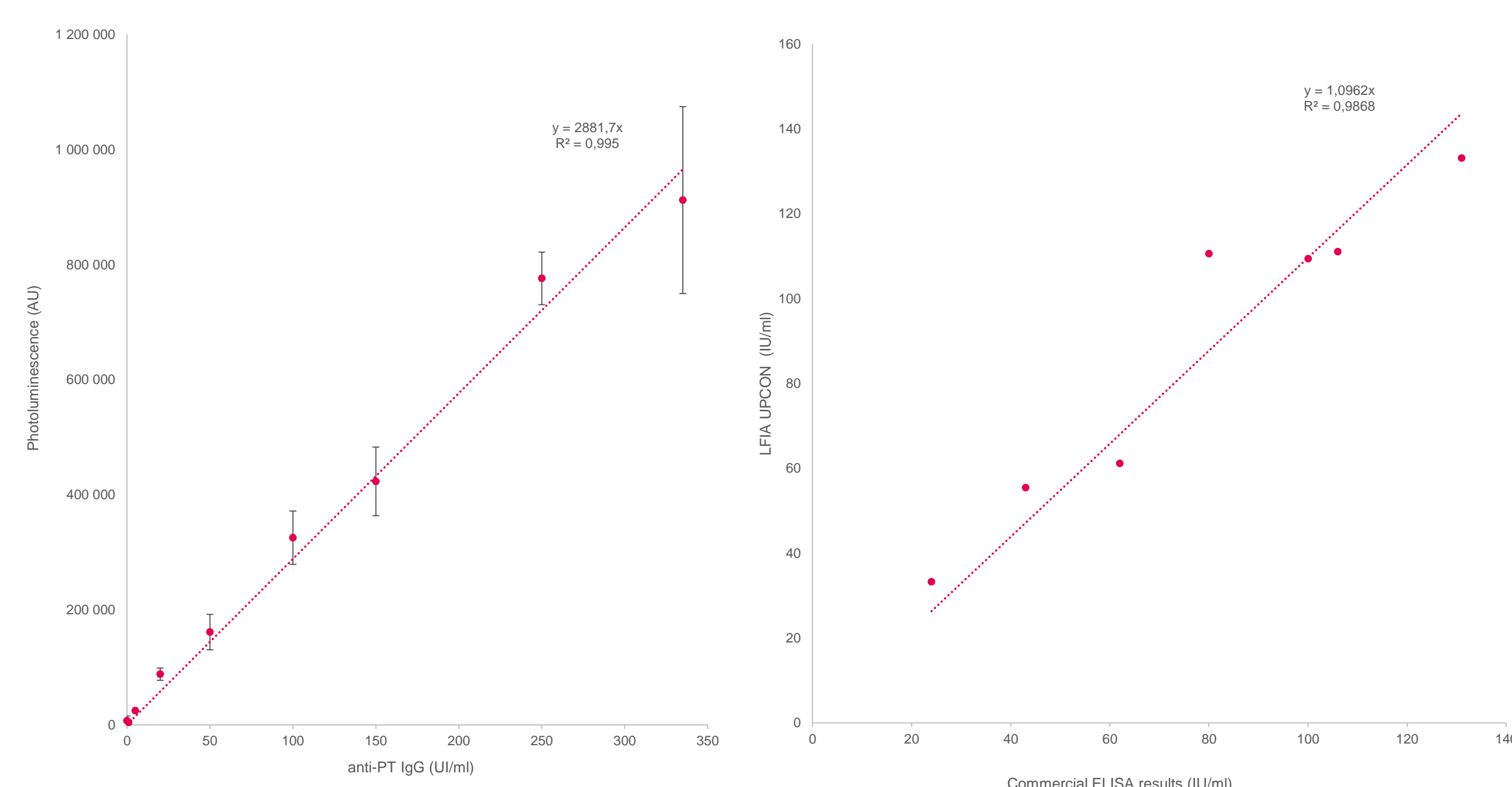


Figure 4. Anti-PT IgG quantification. Anti-PT IgG standard series was made using WHO international standard 06/140 (left). Patient sample panel (n=7) was measured using the novel LFIA and compared with reported standardized values (right).

Discussion

The developed LFIA enables measurement of two analytes from one test line with total assay time of only 20 minutes. Anti-PT IgG quantification shows great promise with an adequate dynamic range and a good correlation with commercial ELISAs. However, the correlation is shown with a very limited sample panel (n=7). In addition, anti-PT IgA quantification was unsuccessful due to variable effects of negative sera and needs further optimization.

¹Hendriks LH, et al. (2011) Serum IgA Responses against Pertussis Proteins in Infected and Dutch wP or aP Vaccinated Children: An Additional Role in Pertussis Diagnostics.