Instrumentation Science and Technology, 42:635–645, 2014

ISSN: 1073-9149 print/1525-6030 online
DOI: 10.1080/10739149.2014.930877

Taylor & Francis
Taylor & Francis Group

POINT-OF-CARE TEST FOR C-REACTIVE PROTEIN BY A FLUORESCENCE-BASED LATERAL FLOW IMMUNOASSAY

Yongbin Gu,[1] Yongliang Yang,[1] Jing Zhang,[2] ShengXiang Ge,[2] Zhanghong Tang,[3] and Xianbo Qiu[1]

[1] *College of Information Science and Technology, Beijing University of Chemical Technology, Beijing, P. R. China*
[2] *National Institute of Diagnostics and Vaccine Development in Infectious Diseases, Xiamen University, Xiamen, P. R. China*
[3] *College of Material Science and Engineering, Beijing University of Technology, Beijing, P. R. China*

□ *An innovative, portable fluorescence reader was developed for the determination of C-reactive protein based on a lateral flow immunoassay. The C-reactive protein concentration was proportional to the intensity of the test line which was calibrated relative to the control line. To quantify the fluorescence intensity of the lateral flow strip, a custom illumination module, which concentrated the excitation beam from an ultraviolet light-emitting diode, was developed for strip scanning. Accordingly, a high sensitive photodiode with a preamplifier was chosen as the detector for fluorescence. For good repeatability, the strip scanning resolution was set to 5 μm between data points by controlling a linear stage actuated by a stepper motor. Four double-logistic calibration models were compared. The sensitivity for C-reactive protein was 0.1 mg/L and the linear dynamic range extended to 400 mg/L. The optical reader provides a new and simple approach for the determination of C-reactive protein and may be modified for other similar biomarkers.*

Keywords C-reactive protein, fluorescence, optical reader, point-of-care test

INTRODUCTION

Point-of-care-tests, that are conducted at or near the site of patient,[1] have become increasingly popular in diagnostics[2,3] to provide faster results, decreased cost, diagnosis in remote areas, enhanced privacy, and improved

Yongbing Gu and Yongliang Yang contributed equally to this study.

Address correspondence to Xianbo Qiu, College of Information Science and Technology, Beijing University of Chemical Technology, No. 15 Bei San Huan Dong Lu, Beijing 100029, P. R. China. E-mail: xbqiu@mail.buct.edu.cn

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/list.

public health. In recent years, there is a growing interest in the determination of C-reactive protein, a critical biomarker for inflammation, colorectal cancer, and heart disease.[4–11] Several studies to determine C-reactive protein concentrations in blood for diagnostics have been reported.[12–15] As a biomarker of inflammation, C-reactive protein provides clinical information to physicians, especially in the emergency room. However, traditional C-reactive protein measurement is performed on automated instruments in central labs with complicated procedures, which is inconvenient in terms of time, cost, and location. Therefore, it is necessary to employ a portable, simple, low-cost and faster method for quantitative C-reactive protein to develop a point-of-care test with good accuracy and specificity.

Immunochromatographic assays, also known as lateral flow immunoassays, offer low-cost, ease-of-use, speed, and high sensitivity, have been widely used in point-of-care tests.[16] Lateral flow strips are commonly used to determine antigens and antibodies. The users only need to bring the strips in contact with the raw sample or a mixture of sample and buffer (diluent), and then the sample or mixture is wicked along the lateral flow strips by capillary force, and results are obtained within a few minutes. The lateral strips are functionalized with immobilized proteins capture the analytes; and a C line is used to guarantee that the sample has, indeed, travelled up the strips and the reporters (label) sample have not decayed. Either a sandwich assay[17] or a competitive assay[18] is employed in the lateral flow test. In some cases, gold conjugate labels are used for qualitative or semi-quantitative detection, and the test results can be directly read visually or with a reader.[19,20] In contrast, fluorophores, which require excitation at specific wavelengths and whose signal is detected with optical components, are often used for quantitative lateral flow tests[21] with an optical reader.

Several quantitative immunoassays based on lateral flow strips with fluorescence labels have been reported.[16,22–30] For example, Yan et al. developed a biosensor used for the determination of *Yersinia pestis* based on a lateral flow immunoassay with upconverting phosphors.[29] A complicated, expensive and bulky optical module, which included an infrared (IR) laser for excitation and a photomultiplier (PMT) for detection, was employed. Song et al. developed a quantitative C-reactive protein measurement system using time-resolved luminescence fluorescence with a sensitivity of 0.2 ng/mL.[30] Time-resolved fluorescence was employed to enhance the signal-to-noise ratio. However, this detection system is more complicated than a traditional fluorescence reader. Li et al. described a quantitative system for cytokine interferon-γ with upconverting phosphor labels. A two-dimensional optical scanner with high resolution was developed to measure across the reaction area. However, 8 min were required to scan the entire area, which is significantly longer than reported in other studies.[31] Previously, a portable analyzer was developed to detect

from a lateral flow strip in a microfluidic cassette by our group. Because a single excitation point was applied to scan the strip in one direction, only qualitative results may be obtained with that analyzer.[16]

Therefore, for quantitative C-reactive protein determination on lateral flow strips, a new compact, low-cost optical reader was developed. To facilitate quantitative strip scanning, a custom illumination module was built to focus the light emitting diode beam into a line-shape. The detection module was designed to collect fluorescence into a high sensitivity photodiode with a preamplifier. Previously, an optical reader with a laser and a photomultiplier tube were employed.[30] Compared to the previous design, the developed optical reader is more compact and inexpensive, which is more suitable for point-of-care tests. Strip scanning was done by controlling a linear stage actuated by a high-resolution stepper motor. To improve the accuracy, four calibration models were compared to determine the most suitable. The sensitivity of the optical reader was shown to be appropriate for the determination of C-reactive protein.

MATERIALS AND METHODS

Three antibodies made in house were involved in the lateral flow immunoassay. Mouse against human monoclonal antibodies (cell line: 7D9) were immobilized on the test line of the nitrocellulose membrane of the lateral flow strip, and goat against mouse polyclonal antibodies on the control line. Mouse against human monoclonal antibodies (cell line: 10C5) were conjugated with fluorescence particles for labeling. The fluorescence particles (fluoro-max-fluorescent-carboxylate-modified particles, Thermal Scientific, Texas, USA) with diameters of 0.322 μm were purchased from Thermal China. The excitation wavelength was 365 nm and the emission wavelength was 610 nm. Samples were prepared by the addition of standardized C-reactive protein (GK-039, GuiKang Biotech, Shanghai, China) to phosphate buffer (pH: 7.3, 20 mM).

The monoclonal antibodies (cell line: 7D9) in buffer were added to the sample area of the nitrocellulose membrane (HF135, EMD Millipore, Massachusetts, USA) at a concentration of 2 mg/mL for immobilization using a spotter (BioDot XYZ3050, BioDot, Inc. California, USA), while polyclonal antibodies in buffer with the concentration of 1 mg/mL were introduced on control area of the membrane. The fluorescence particles (volume concentration: 1%) were conjugated with monoclonal antibodies (cell line: 10C5) with the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Thermal Scientific, Inc. Texas, USA) reagent within 2 hr at room temperature using in house modification of the standard protocol.[32] The conjugates were freeze-dried on the sample pad (8 × 4 mm) at an antibody concentration of 0.1 μg/mL.

The lateral flow cassette was obtained from Beijing Wantai Biological Pharmacy Enterprise (Beijing, China). The lateral flow strip (55 mm × 4 mm) included a sample pad, a conjugate pad, a nitrocellulose membrane, an absorbent pad, and a lamination layer.

Once the sample was in contact with the sample pad, it was wicked along the lateral flow strip by capillary force and readings were made a few minutes later. First, the target proteins in the test sample were bound to the fluorescence particles stored on the conjugate pad, and they moved together along the strip until captured by specific proteins immobilized on the test line area. The free fluorescence particles were then captured in the control line area for assay verification.

A fluorescence label with wide stock shift (excitation wavelength: 365 nm, emission wavelength: 610 nm) was selected to achieve the desired signal-to-noise ratio with low system complexity.

Instrumentation

To read the fluorescence, a portable, compact, easy-to-use reader with low cost was developed, which incorporated an optical module, a linear stage, and an electrical module with two microcontrollers, as shown in Figure 1. The optical module included an illumination module and a detection module. For illumination, a 365 nm ultraviolet light emitting diode (Sensor Electronic Technology, Inc., USA) was used for excitation with a band-pass filter (320–390 nm, Jingyibodian Ltd, Beijing, China) focused into a single point with two collimating lenses (Changchun Fortune Optronics, Changchun, China) and transformed into a line-shape beam (length: 4 mm, width: 0.8 mm) by two cylindrical lenses (Changchun Fortune Optronics, Changchun, China), whose length is close to the width

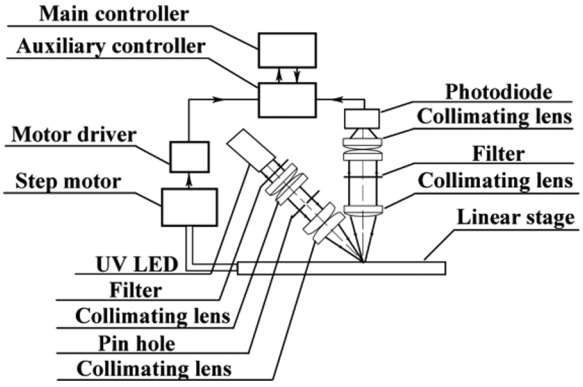

FIGURE 1 Schematic of the optical reader.

of the flow strip. The custom line-shaped excitation beam was able capable of quantitative fluorescence. To scan the strip, the illumination module was located at an angle of 45° relative to the strip surface. For fluorescence signal detection, a photodiode (Opto Diode, USA) with a preamplifier was used for photoelectrical conversion. The emission from the strip was collected by two collimating lens (Changchun Fortune Optronics, Changchun, China) through a high-pass filter (580 nm, Jingyibodian, Beijing, China) before reaching the photodiode. The detection system is located above the strip vertically for a wide detection angle. Flexible adjustment was employed to ensure that the illumination module and the detection module met at the same point on the lateral flow strip. To ensure the effectiveness of the optical module, the above design was evaluated with optical simulation software (Zemax, Radiant Zemax, USA).

A compact linear stage, including a stepper motor, a gear belt, and a cassette tray, was constructed to facilitate smooth cassette movement with high resolution for strip scanning. The scanning resolution was modified by control of the speed of the cassette.

An electrical module with two microcontrollers (main and auxiliary) was constructed. The auxiliary controller, AT89C51ED2 (Atmel, USA) was designed to control the linear stage and collect the photoelectric signal with a 16 bit analog-to-digital convertor. The main controller, S3C2440AL-40 (Samsung, Korea) analyzed the scanning measurements, identified the peak and boundary with a custom algorithm, and calculated the sample concentration. Serial communication was incorporated between the two microcontrollers. The optical reader was accessed with a touch-screen interface, and the test results may be saved and printed.

CALIBRATION MODEL FOR QUANTITATIVE TEST

In principle, the concentration of the sample is proportional to the signal intensity of the test line. However, to compensate for error from the optical reader, the lateral flow strip, and lateral flow immunoassay, the signal intensity of the test line was calibrated with the intensity of the control line. Instead of using four-parameter logistic model, a double-logistic model with low complexity was chosen to calculate the concentration of the sample:

$$\lg c = \alpha \lg(T/C) + \beta, \tag{1}$$

where c is the concentration of the test sample, T and C are the intensities of test and control lines, and α and β are modeling parameters.

Figure 2 shows the principle of lateral flow strip scanning with the line-shape excitation beam. The lateral flow strip moves smoothly on the

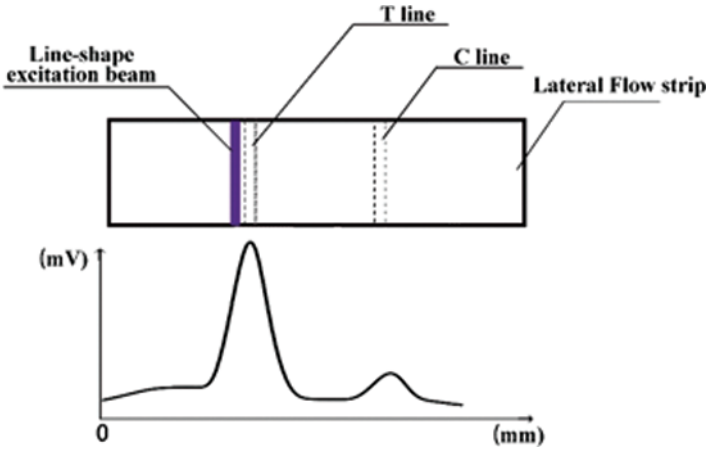

FIGURE 2 Strip scanning with a line-shape excitation beam.

linear stage toward the line-shape illumination beam which is fixed in position, and the fluorescence from the excitation area is collected and recorded to generate a curve with two peaks corresponding to the test (T line) and control lines (C line), respectively. To obtain the best accuracy, the lateral flow strip should be scanned with an extremely thin line, which, however, is difficult to achieve experimentally.

Because of the limited thickness of the excitation beam, there are two designs. In one design, the thickness of the line-shape beam is much smaller than that of the test line, which allows high resolution scanning. In the other approach, the two dimensions are comparable, which allows low resolution scanning. For high resolution scanning, the area of the test line of the scanning curve may be regarded as the fluorescence intensity. The fluorescence intensity of the test line area was used to determine the sample concentration. Because a sandwich immunoassay was adopted, free fluorescence labels escaping from the test line were captured by the control line area. An empirical model was therefore developed to calibrate the test line signal with the control line in the ratio between test line and control line signals.[28] Therefore, Eq. (1) can be rewritten as:

$$\lg c = \alpha \lg(S_T/S_C) + \beta, \tag{2}$$

where c is the concentration of the test sample, S_T and S_C correspond to the area of the test and control lines, and α and β are modeling parameters.

However, for low resolution scanning, it is more reasonable to use the peak value of the test line to approximate the fluorescence intensity because the boundary between the background and the physical domain

of the test line cannot be identified accurately with the thick excitation beam. Therefore, Eq. (1) is rewritten as:

$$\lg c = \alpha \lg(V_T/V_C) + \beta, \tag{3}$$

where c is the concentration of the test sample, V_T and V_C correspond to the peak value of the test and control lines separately, and α and β are modeling parameters.

C-reactive protein concentrations in healthy people are normally less than 10 mg/L, which may increase to more than 350 mg/L due to disease.[33] For clinical analysis, quantitative determination of C-reactive protein sample is not significant at concentrations exceeding 400 mg/L. Therefore, 9 standards with C-reactive protein concentrations between 0.1 mg/L and 400 mg/L were used to develop model parameters using least squares.[34] Different models were compared to evaluate their compatibility to the developed optical reader.

RESULTS AND DISCUSSION

To determine the performance of the optical reader, a phosphate buffer control, 1 mg/L C-reactive protein in phosphate buffer, and 400 mg/L C-reactive protein in phosphate buffer were analyzed (Figure 3). 70 µL of sample were applied into the sample inlet of the strip cassette, and the

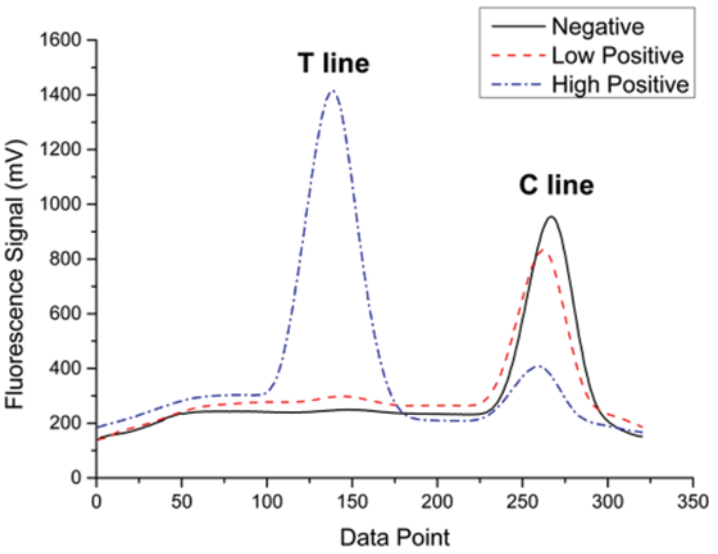

FIGURE 3 Fluorescence scans of control phosphate buffer (negative), 1 mg/L C-reactive protein in phosphate buffer (low positive), and 400 mg/L C-reactive protein in phosphate buffer (high positive).

lateral flow strip was incubated for 15 min before reading. The control phosphate buffer yielded no test peak and a high control peak. The 1 mg/L C-reactive protein in phosphate buffer yielded a low test and a moderately high control peak. The 400 mg/L C-reactive protein in phosphate buffer yielded a high test peak and a low control peak. This experiment indicated that the optical reader is able to distinguish different concentrations of analyte.

In principle, there are two calibration models which may be employed. Because the background needs to be subtracted from the signal measurement, single and variable baselines were considered. For a single baseline, a common background was subtracted from the scanning measurement. For a variable baseline, more than one background was employed if the baseline changed around the test or control peaks. Consequently, four calibration models were considered.

Using the least squares method, nine standards between 0.1 mg/L and 400 mg/L (0.1, 1, 5, 10, 50, 100, 200, 300, and 400 mg/L) were investigated for linear fitting of the four models as shown in Table 1. The models include the ratio of area (S_T/S_C) with a single baseline, the ratio of area (S_T/S_C) with variable baseline, the ratio of peak (V_T/V_C) with a single baseline, and the ratio of peak (V_T/V_C) with a variable baseline. The correlation coefficients of four calibration models were all larger than 0.99 and the model with the ratio of peaks with a variable baseline, V_T/V_C was the most suitable ($R^2 = 0.9988$). The model for concentration estimation is described as:

$$C = 10^{\lg(V_T/V_C)*0.94036-1.4084}. \quad (4)$$

It can be concluded that it is more reasonable to use a variable baseline, instead of single baseline to extract the most accurate fluorescence signal because of background shifting on the lateral flow strip due to the relatively large size of the beam from the light emitting diode.

The accuracy and repeatability of the optical reader were evaluated with samples of different concentrations. For repeatability verification, each test was repeated 20 times. It is found that the coefficient of variation (CV) in the entire quantitative detection range (0.1–400 mg/L) was less than 5%.

TABLE 1 Comparison of four calibration models

Model type	Slope	Intercept	R^2
V_T/V_C (Variable Baseline)	0.9404	−1.4084	0.9988
S_T/S_C (Variable Baseline)	1.0274	−1.5284	0.9953
V_T/V_C (Single Baseline)	0.8804	−1.2473	0.9937
S_T/S_C (Single Baseline)	0.9390	−1.2560	0.9938

The detection limit was defined as the sample concentration corresponding to the test signal at blank ± three times the standard deviation, which was 0.1 mg/L. The blank was measured 20 times.

Parallel tests were performed with a standard enzyme-linked immunosorbent assay (ELISA) (RT-6100, Rayto Shenzhen, China). The ELISA requires a higher sample volume and a longer reaction time with more complicated detection procedure than the fluorescence reader method. However, using a high sensitivity C-reactive protein kit (HuiJia Biotechbology, China), the ELISA sensitivity was 0.625 ng/mL with 100% specificity, while the sensitivity of the developed reader was 0.1 mg/L with 100% specificity. Although ELISA provided much better sensitivity, for clinical detection, the sensitivity and specificity of the developed method is satisfactory.

Compared with the existed optical readers in point-of-care tests, such as the immunoassay fluorescence reader Finecare (Guangzhou Wondfo Biotech, Guangzhou, China), the developed optical reader has better sensitivity (0.1 mg/L vs. 0.5 mg/L), a wider detection range (0.1–400 mg/L) vs. (0.5–200 mg/L), a shorter read time (5 s vs. 10 s), and a larger sample size (70 μL vs. 10 μL). The performance of the developed reader is superior to that of the commercial system, except it requires a higher sample volume.

CONCLUSIONS

A portable, compact optical reader for quantitative C-reactive protein determination was developed and shown to be suitable for point-of-care tests. The limit of detection limit for C-reactive protein was 0.1 mg/L. Four calibration models were compared to determine the most compatible one with the optical reader; the model with ratio of peaks with variable baseline was shown to be the most appropriate. The coefficient of variation of the optical reader was less than 5% across the linear dynamic range from 0.1–400 mg/L, and less than 16 min for the optical reader is required for the analysis. The optical reader is easily operated with a touch-screen, and the data may be saved and printed. A similar device is in preparation by our group for procalcitonin, which is another important biomarker. We anticipate that the developed optical reader may be easily modified for diagnostics of other biomarkers for point-of-care tests.

FUNDING

This work was partly supported by the National Nature Science Foundation of China under Grant 81371711, Fundamental Research Funds for the Central Universities of China under Grant ZZ1329 and YS1404, and the Fujian Study-Production Cooperation Foundation Key Project under Grant 2012Y4011.

REFERENCES

1. Kost, G.J. *Goals, Principles and Practice of Point-of-Care Testing*; Lippincott Williams and Wilkins: Philadelphia, 2002.
2. St-Louis, P. Status of Point-of-Care Testing: Promise, Realities, and Possibilities. *Clin. Biochem.* **2000**, *33*(6), 427–440.
3. Price, C.P.; John, A. St.; Hicks, J.M. *Point-of-Care Testing*; AACC Press: Washington DC, 2004.
4. Gabay, C.; Kushner, I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. *N. Engl. J. Med.* **1999**, *340*(6), 448–454.
5. Du Clos, T.W. Function of C-Reactive Protein. *Ann. Med.* **2000**, *32*(4), 274–278.
6. Sin, K.K.; Chan, C.Y.; Leung, W.M.; Seydack, M.; Renneberg, R. Fluorogenic Nanocrystals for Highly Sensitive Detection of C-Reactive Protein. *Proc. IEE Nanobiotechnol.* **2006**, *153*(3), 54–58.
7. Erlinger, T.P.; Platz, E.A.; Rifai, N.; Helzlsouer, K.J. C-Reactive Protein and the Risk of Incident Colorectal Cancer. *JAMA.* **2004**, *291*(5), 585–590.
8. Danesh, J.J.; Wheeler, G.; Hirschfield, G.M.; Eda, S.; Eiriksdottir, G.; Rumley, A.; Lowe, G.D.; Pepys, M.B.; Gudnason, V. C-Reactive Protein and Other Circulating Markers of Inflammation in the Prediction of Coronary Heart Disease. *New. Engl. J. Med.* **2004**, *350*(14), 1387–1397.
9. Latina, J.M.; Mark Estes III, N.A.; Garlitski, A.C. The Relationship between Obstructive Sleep Apnea and Atrial Fibrillation: A Complex Interplay. *Pulm. Med.* **2013**, 2013.
10. McDonnell, B.; Hearty, S. Leonard, P.; O'Kennedy, R. Cardiac Biomarkers and the Case for Point-of-Care Testing. *Clin. Biochem.* **2009**, *42*(7), 549–561.
11. Melin, J.; Rundström, G.; Peterson, C.; Bakker, J.; MacCraith, B.D.; Read, M.; Öhman, O.; Jönsson, C. A Multiplexed Point-of-Care Assay for C-Reactive Protein and N-Terminal Pro-Brain Natriuretic Peptide. *Anal. Biochem.* **2011**, *409*(1), 7–13.
12. Roberts, W.L.; Moulton, L.; Law, T.C.; Farrow, G.; Cooper-Anderson, M.; Savory, J. Rifai, N. Evaluation of Nine Automated High-Sensitivity C-Reactive Protein Methods: Implications for Clinical and Epidemiological Applications. Part 2. *Clin. Chem.* **2001**, *47*(3), 418–425.
13. Roberts, W.L.; Sedrick, R.; Moulton, L.; Spencer, A.; Rifai, N. Evaluation of Four Automated High-Sensitivity C-Reactive Protein Methods: Implications for Clinical and Epidemiological Applications. *Clin. Chem.* **2000**, *46*(4), 461–468.
14. Rothkrantz-Kos, S.; Schmitz, M.P.; Bekers, O. Menheere, P.P.C.A.; van Dieijen-Visser, M.P. High-Sensitivity C-Reactive Protein Methods Examined. *Clin. Chem.* **2002**, *48*(2), 359–362.
15. Ledue, T.B.; Rifai, N. Preanalytic and Analytic Sources of Variations in C-Reactive Protein Measurement: Implications for Cardiovascular Disease Risk Assessment. *Clin. Chem.* **2003**, *49*(8), 1258–1271.
16. Qiu, X.; Liu, C.; Mauk, M.G.; Hart, R.W.; Chen, D.; Qiu, J.; Kientz, T.; Fiene, J.; Bau, H.H. A Portable Reader for Pouch-Actuated, Immunoassay Cassettes. *Sens. Actuators, B.* **2011**, *160*(1), 1529–1535.
17. Qian, S.; Bau, H.H. A Mathematical Model of Lateral Flow Bioreactions Applied to Sandwich Assays. *Anal. Biochem.* **2003**, *322*(1), 89–98.
18. Qian, S.; Bau, H.H. Analysis of Lateral Flow Biodetectors: Competitive Format. *Anal. Biochem.* **2004**, *326*(2), 211–224.
19. Leung, W.; Chan, C.P.; Rainer, T.H.; Ip, M.; Cautherley, G.W.H.; Renneberg, R. InfectCheck C-Reactive Protein Barcode-Style Lateral Flow Assay for Semi-Quantitative Detection of C-Reactive Protein in Distinguishing between Bacterial and Viral Infections. *J. Immunol. Methods.* **2008**, *336*(1), 30–36.
20. Wang, S.; Zhang, C.; Wang, J.; Zhang, Y. Development of Colloidal Gold-Based Flow-Through and Lateral-Flow Immunoassays for the Rapid Detection of the Insecticide Carbaryl. *Anal. Chim. Acta.* **2005**, *546*(2), 161–166.
21. Li, Z.; Wang, Y.; Wang, J.; Tang, Z.; Pounds, J.G.; Lin, Y. Rapid and Sensitive Detection of Protein Biomarker Using a Portable Fluorescence Biosensor Based on Quantum Dots and a Lateral Flow Test Strip. *Anal. Chem.* **2010**, *82*(16), 7008–7014.
22. Niedbala, R.S.; Vail, T.L.; Feindt, H.; Li, S.; Burton, J.L. Multiphoton Up-Converting Phosphors for Use in Rapid Immunoassays, *Proc. of BiOS 2000 The International Symposium on Biomedical Optics*, San Jose, California, Jan. 22, 2000.
23. Corstjens, P.L.A.M.; Li, S.; Zuiderwijk, M.; Kardos, K.; Abrams, W.R.; Niedbala, R.S.; Tanke, H.J. Infrared Up-Converting Phosphors for Bioassays. *Proc. IEE –Nanobiotechnol.* **2005**, *152*(2), 64–72.

24. Huang, L.; Zhou, L.; Zhang, Y.; Xie, C.; Qu, J.; Zeng, A.; Huang, H.; Yang, R.; Wang, X. A Simple Optical Reader for Upconverting Phosphor Particles Captured on Lateral Flow Strip. *IEEE Sens. J.* **2009**, *9*(10), 1185–1191.
25. Corstjens, P.L.A.M.; Zuiderwijk, M.; Nilsson, M.; Feindt, H.; Niedbala, R.S.; Tanke, H.J. Lateral-Flow and Up-Converting Phosphor Reporters to Detect Single-Stranded Nucleic Acids in a Sandwich-Hybridization Assay. *Anal. Biochem.* **2003**, *312*(2), 191–200.
26. Niedbala, R.S.; Feindt, H.; Kardos, K.; Vail, T.; Burton, J.; Bielska, B.; Li, S.; Milunic, D.; Bourdelle, P.; Vallejo, R. Detection of Analytes by Immunoassay Using Up-Converting Phosphor Technology. *Anal. Biochem.* **2001**, *293*(1), 22–30.
27. Malamud, D.; Bau, H.; Niedbala, S.; Corstjens, P. Point Detection of Pathogens in Oral Samples. *Adv. Dent. Res.* **2005**, *18*(1), 12–16.
28. Corstjens, P.; Zuiderwijk, M.; Brink, A.; Li, S.; Feindt, H.; Niedbala, R.S. Tanke, H. Use of Up-Converting Phosphor Reporters in Lateral-Flow Assays to Detect Specific Nucleic Acid Sequences: A Rapid, Sensitive DNA Test to Identify Human Papillomavirus Type 16 Infection. *Clin. Chem.* **2001**, *47*(10), 1885–1893.
29. Yan, Z.; Zhou, L.; Zhao, Y.; Wang, J.; Huang, L.; Hu, K.; Li, H.; Wang, H.; Guo, Z.; Song, Y.; Huang, H.; Yang, R. Rapid Quantitative Detection of Yersinia Pestis by Lateral-Flow Immunoassay and Up-Converting Phosphor Technology-Based Biosensor. *Sens. Actuators B.* **2006**, *119*(2), 656–663.
30. Song, X.; Knotts, M. Time-Resolved Luminescent Lateral Flow Assay Technology. *Anal. Chim. Acta.* **2008**, *626*(2), 186–192.
31. Li, J.J.; Ouellette, A.L.; Giovangrandi, L.; Cooper, D.E.; Ricco, A.J.; Kovacs, G.T.A. Optical Scanner for Immunoassays with Up-Converting Phosphorescent Labels. *IEEE Biomed. Eng.* **2008**, *55*(5), 1560–1571.
32. Hermanson, G.T. *Bioconjugate Techniques*; Academic Press: Salt Lake City, UT, 2013.
33. Clyne, B.; Olshaker, J.S. The C-Reactive Protein. *J. Emerg. Med.* **1999**, *17*(6), 1019–1025.
34. Xu, T.; Bashford, G.R. Lateral Blood Flow Velocity Estimation Based on Ultrasound Speckle Size Change with Scan Velocity. *IEEE Ultrason. Ferroelectr. Freq. Control*, **2010**, *57*(12), 2695–2703.