Low-cost semi-quantitative lateral flow assay format

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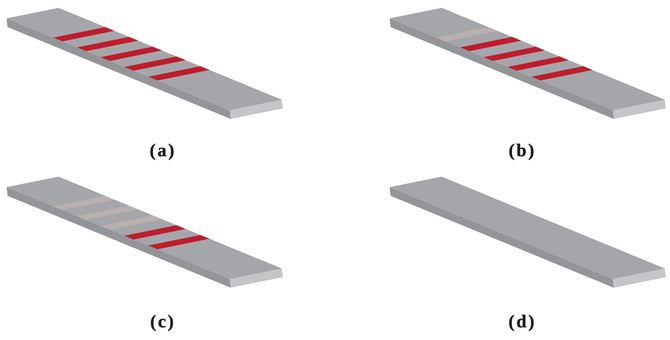
Abstract

The purpose of this research is to improve the performance of lateral flow assays. The majority of lateral flow tests are non-quantitative assays which function by measuring the presence (or absence) of a colored spot in order to determine the presence (or absence) of the analyte of interest. Alternatively, quantitative conventional lateral flow tests require additional instrumentation to measure the intensity of the spot as a reading of the concentration of the analyte of interest, adding significant cost. The ladder bar assay[1] is a promising assay format that can reduce cost and enable use in low-resource settings by producing a semi-quantitative readout by eye without requiring additional instrumentation. The ladder bar design was simplified to reduce manufacturing cost and a model sandwich assay was used to demonstrate the proof of concept for the assay.

Striped assay design

Concept: pattern stripes of capture molecules onto paper, as the analyte is applied it will bind sequentially to the stripes, the colored label will stick to the sections where analyte has bound and will cause a striped pattern to appear. The stripes can then be counted to determine the concentration of analyte.



Reading the results of a striped semi-quantitative assay is as easy as counting the dark stripes that appear:
(a) shows a full response, (b) and (c) show partial responses, and (d) shows a negative response.

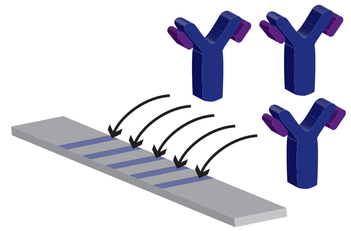
Methods

The specific reagents used were as follows: anti-biotin IgG was used as a capture molecule, BSA-biotin was used as the model analyte, and streptavidin-gold was used as a visual label.

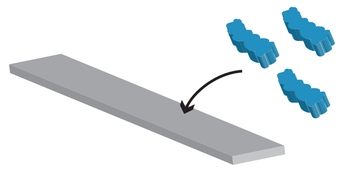
The capture molecules were patterned using an industrial spotter or a home-made striper. Videos of the flow were taken using an inexpensive webcam in conjunction with HandyAvi software.

System Overview

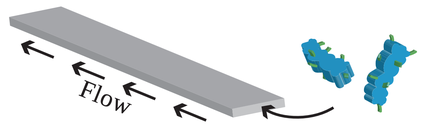
The strip is coated with a striped pattern of capture molecules which nonspecifically bind to the nitrocellulose membrane.



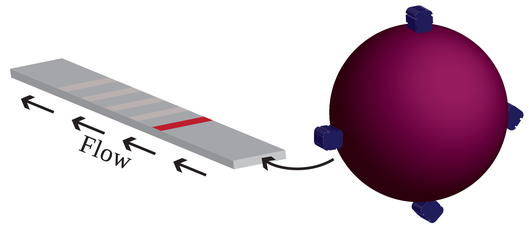
The strip is then put through a blocking procedure that prevents future proteins from sticking to the membrane.



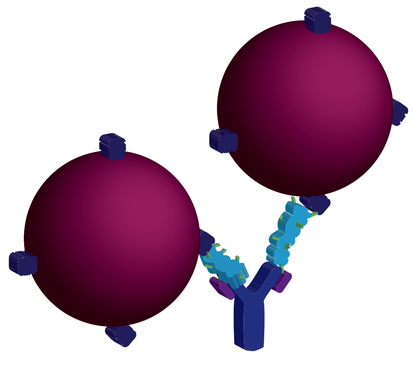
To run the test,wash fluid is applied first to wet-out the strip, then the analyte is applied. Analyte, as available, will bind to capture molecules in the successive stripes



Without letting the strip dry, more wash fluid is applied. The colored label is then applied. The label then binds to the analyte.

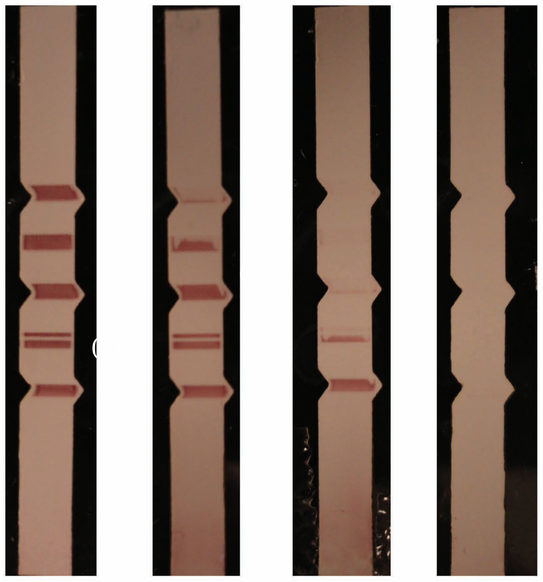


After the assay has run, the proteins are attached like this:



Results

Below is an image of several responses obtained from the assays which were run: (a) shows a full response, (b) and (c) show partial responses, and (d) shows a negative response. The assay can be modified by changing the number of stripes or the density of capture molecules.



Future development

The ladder bar assay format is planned to be implemented in a two-dimensional paper network[2] format for inexpensive developing world diagnostics.

The assay format must be further characterized before it can be used. An accurate relationship between the concentration of analyte and the signal generated in the assay must be generated. Furthermore, design parameters such as the number of stripes and capture density must be optimized to improve the dynamic range and sensitivity of the assay.

Acknowledgments

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References

1. C. Lou, C. Patel, S. F. Ching and J. Gordon, *Clin. Chem.*, 1993
2. E. Fu, B. Lutz, P. Kauffman, P. Yager, *Lab On a Chip*, 2010