System for Automated Detection of 4-Part Complete Blood Count

Rutgers Aresty Research Center for Undergraduates

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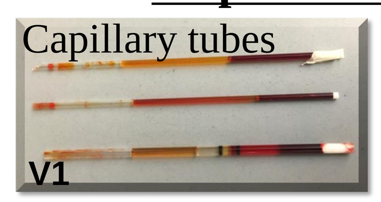
Background

- Blood testing is the most commonly performed clinical procedure in the U.S. [1].
- In the U.S. the most routine type of blood test is the complete blood count (CBC) [2], which provides critical information about overall patient health that may often be time-sensitive.
- Traditionally, a trained lab technician draws a blood sample from a peripheral vein and sends it to a centralized lab for analysis. This is a highly segmented process requiring separate, specialized facilities with complex equipment and trained technicians that provide possibility for human error [3].
- **Our device aims to provide the clinician with a CBC at the point of care (POC) within 5 min and to do so completely autonomously to remove possible human error.**

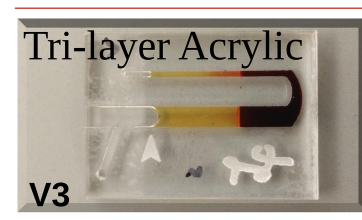
Device Evolution

Disposable Blood Chip Evolution



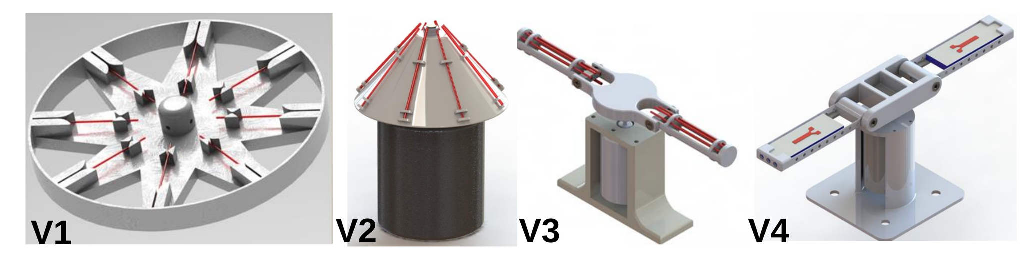
- \+ Commercially available
- \+ Consistent dimensions
- \- Imaging difficulty
- \- Fluid handling issues

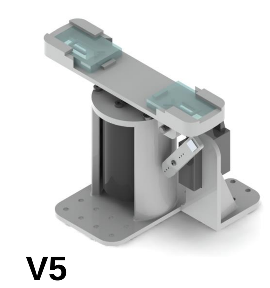
- \+ Ease of imaging
- \+ Consistent sizing
- \- Labor & time-intensive production
- \- Damaged by centrifugation



- \+ Mass Producible
- \+ Precision Laser Cut
- \+ Optically Ideal
- \+ Viable at speeds >12,000 RPM

Centrifuge evolution

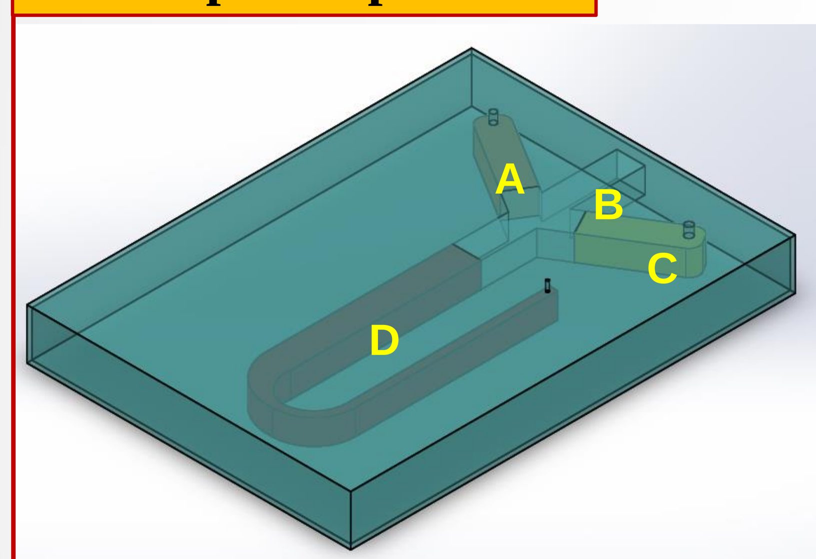




The first generation centrifuges were capable of housing capillary tubes. Later designs were edited to house our custom chip design. The final centrifuge includes position locking and is more compact

Materials and Methods

1. Sample Preparation



Custom-designed, precision laser cut, tri-layer acrylic chip

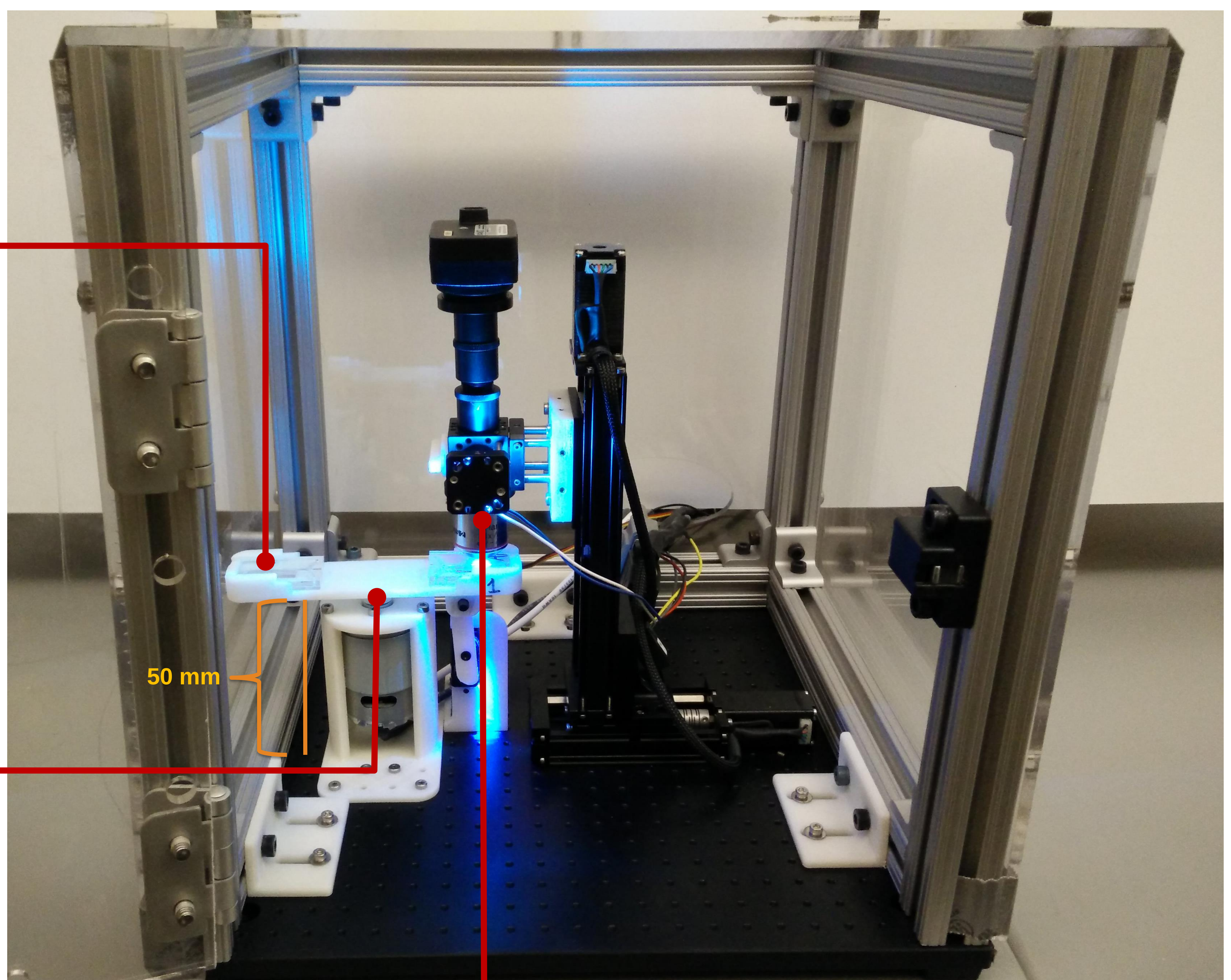
Disposable Blood Chip

A. Acridine orange (7 µL), a nucleic stain used to identify white blood cells (WBCs) under fluorescence microscopy

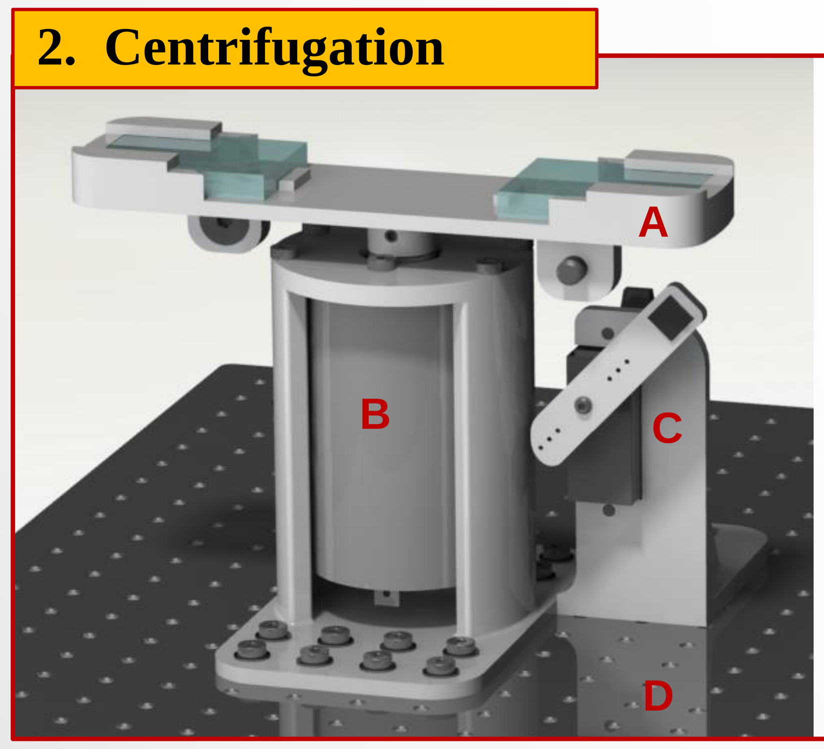
B. Air outlet to allow air displaced by blood to escape

C. Ficoll-Paque 1.077 g/mL (5 µL) separation media to differentiate WBC cell subtypes by density

D. Whole blood sample (70 µL)



2. Centrifugation



Centrifuge

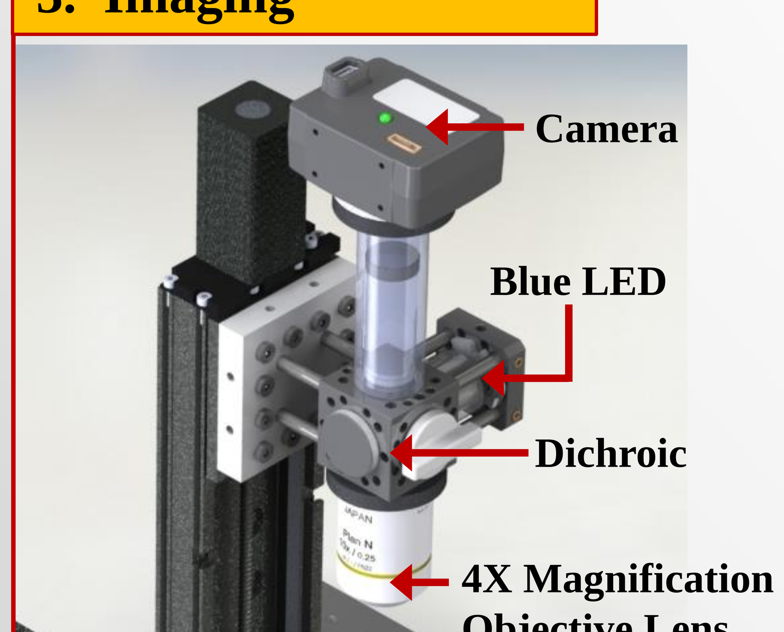
A. Custom 3-D printed blood chip housing

B. DC brushed motor capable of 3000 - 5000G (9000 - 13000 RPM). Spin time of 5 min

C. RC servo alignment locking mechanism to secure sample for imaging

D. Vibration-dampening optical breadboard

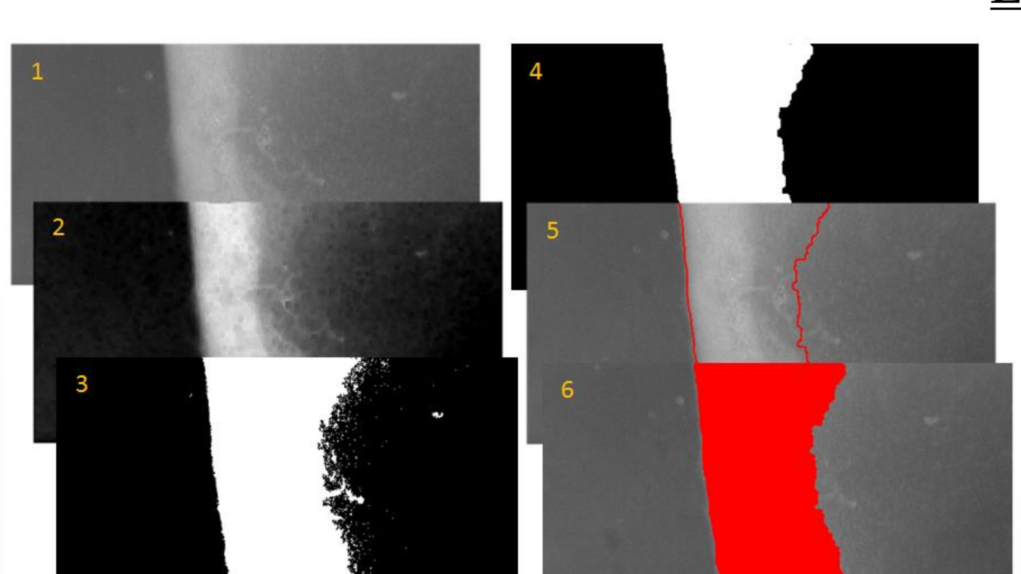
3. Imaging



Optical detection System

- A blue 3.5 V (470 nm) LED transmits light to dichroic filter
- The filter only reflects blue light downward
- The blue light causes the sample to fluoresce green (570 nm)
- The dichroic filter only allows green light to pass through to the camera
- The camera feeds real-time images to computer software

4. Processing



LabView image processing software

- Contrast enhancement and Gaussian blur are added to the original image
- Thresholding then converts it into a binary image
- Morphological closing operation fills in gaps and gets rid of false objects
- Perimeter of segmented image is overlaid onto the original image
- Finally, segmentation pixel area is correlated to white blood cell count

Results

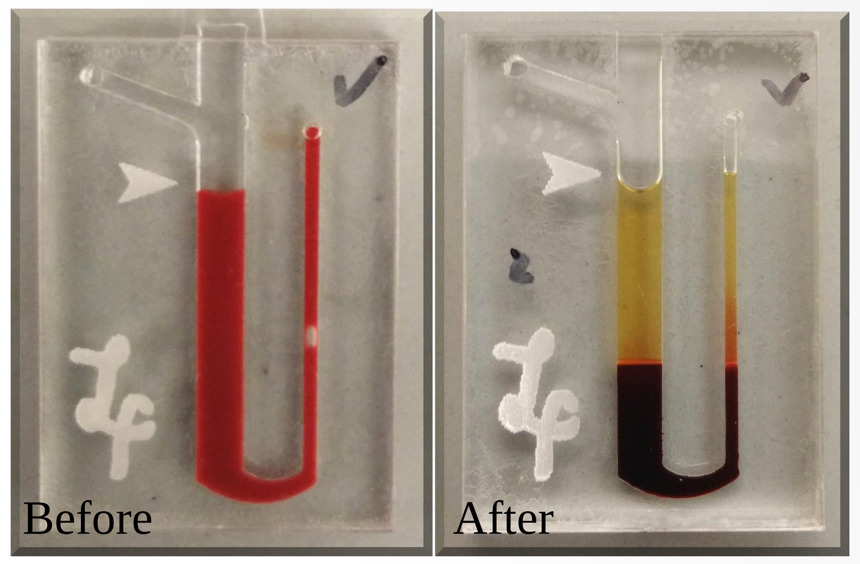


Figure 1. Before and after photos of the disposable blood chip after 5 minutes of centrifugation at 4000 RFC. The boundary between fractionated layers is clearly visible.

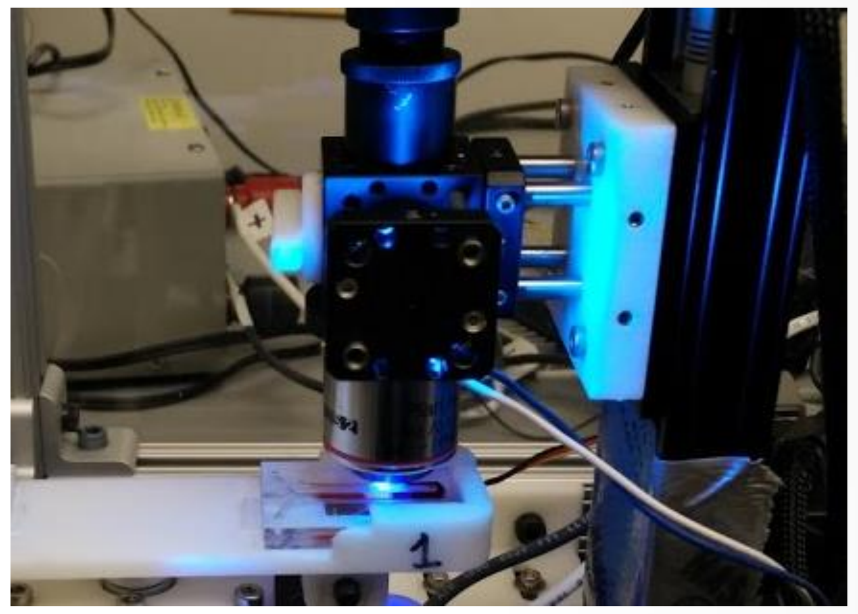


Figure 2. Close-up of completed system. The optics move to image the sample immediately after centrifugation. All data is sent and analyzed in real time by a PC. The entire process is automated

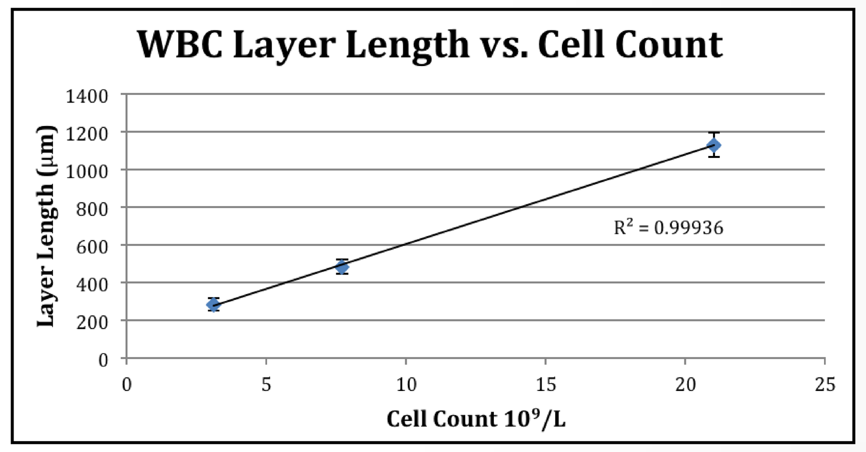


Figure 3. Standard curve generated for proof of concept using white blood cell controls, (N = 3 for each data point).

Future Directions

- Increase the validation sample study size to generate a robust standard curve for white cell counts.
- Incorporate the detection of other critical blood parameters (e.g., cardiac markers, blood chemistry, and metabolic analytes) in our POC device.
- Integrate the POC system with a robotic venipuncture device currently being developed in the Yarmush lab to enable fully automated blood diagnostics at the point of the venipuncture.

References

1. G. Walsh, “Difficult peripheral venous access: Recognizing and managing the patient at risk,” *Journal of the Association for Vascular Access*, vol. 13, no. 4, pp. 198-203, 2008.
2. C. Hsiao, *et al.* “National ambulatory medical care survey” *National Health Statistics Reports*, 2010.
3. G. Fermann, *et al.* “Point of care testing in the emergency department,” *The Journal of Emergency Medicine*, vol. 22, pp. 393-404, 2002.

Acknowledgments

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