

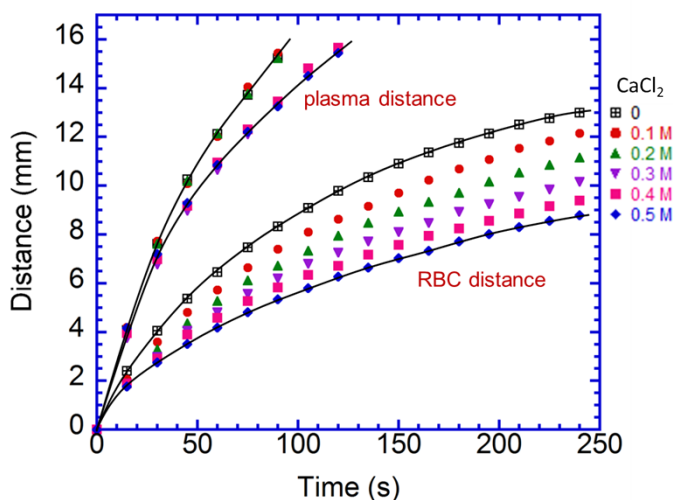
“Blood coagulation screening using a paper-based microfluidic lateral flow device”

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Supporting Information

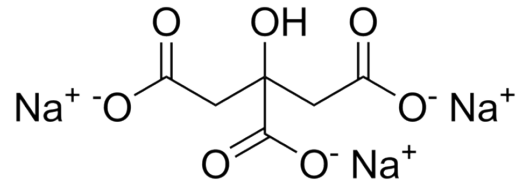
1. RBCs and plasma transport in LFA devices: distance vs time.



In this figure, the travel distance vs. time for the plasma and RBC fronts are plotted on a linear scale. The plasma component travels much faster than the RBCs, reaching the end of the observation window (coincident with the edge of the wicking pad) in 90 s (for low or no added Ca concentrations) to 120 s (for high added Ca concentration). As expected, the RBC flow rate is significantly slower and more strongly affected by the addition of  $\text{Ca}^{2+}$  ions. The RBC front does not reach the wicking pad in any of the samples, even in the absence of added  $\text{Ca}^{2+}$  ions. The saturation of travel distance with time for both the plasma and RBC components seen in this figure indicates that the flow rate is a function of flow time, which is typical fluid flow in capillary systems.

## 2. Calculation of citrate concentration in citrated rabbit blood.

Chemical formula of trisodium citrate:  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ .



According to the information provided by manufacturer (HemoStat Laboratories), citrated rabbit blood is made by draining fresh rabbit blood directly into citrate, keeping a volume ratio of fresh blood : citrate = 4 : 1, which means one fifth of the blood product volume is citrate solution. 4% (w/v) trisodium citrate solution from Sigma-Aldrich is used as the standard citrate, which has a molecular weight of 258.06 g/mol and a concentration of 4 g/100 mL.

Calculation on citrate concentration in citrated rabbit blood is listed as followed.

In 160  $\mu\text{L}$  citrated rabbit blood,

- Volume of citrate solution in citrated rabbit blood:  $\frac{160}{5} = 32 \mu\text{L}$
- Mass of trisodium citrate in citrated rabbit blood:  $\frac{4}{100} \times 32 = 1.28 \text{ mg}$
- Mole of trisodium citrate in citrated rabbit blood:  $\frac{1.28}{258.06} = 0.005 \text{ mmol}$
- Citrate<sup>3-</sup> concentration in citrated rabbit blood:  $\frac{0.005 \times 10^{-3}}{160 \times 10^{-6}} = 31.25 \times 10^{-3} \text{ M} = 31.25 \text{ mM}$

Average concentration of free  $\text{Ca}^{2+}$  ions is found to be 1.37 mM<sup>31</sup> in rabbit whole blood.

According to the chemical formula of trisodium citrate (two trisodium citrate molecules bind with three  $\text{Ca}^{2+}$  ions), only 0.92 mM of trisodium citrate is needed for  $\text{Ca}^{2+}$  immobilization. Therefore, the citrate amount is significantly beyond what is required to immobilize  $\text{Ca}^{2+}$  ions in rabbit whole blood.