“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 Ca$^{2+}$ ions. The RBC front does not reach the wicking pad in any of the samples, even in the absence of added 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: \( \text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \).

\[
\text{Na}^+ \text{O} \quad \text{O} \\
\text{O} \quad \text{OH} \\
\text{O} \quad \text{O} \quad \text{Na}^+ \\
\text{O} \quad \text{O} \quad \text{Na}^+
\]

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 µL citrated rabbit blood,

- Volume of citrate solution in citrated rabbit blood: \( \frac{160}{5} = 32 \mu L \)
- 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\(^3^-\) concentration in citrated rabbit blood: \( \frac{0.005 \times 10^{-3}}{160 \times 10^{-6}} = 31.25 \times 10^{-3} M = 31.25 \text{ mM} \)

Average concentration of free Ca\(^{2+}\) ions is found to be 1.37 mM\(^{31}\) in rabbit whole blood. According to the chemical formula of trisodium citrate (two trisodium citrate molecules bind with three Ca\(^{2+}\) ions), only 0.92 mM of trisodium citrate is needed for Ca\(^{2+}\) immobilization. Therefore, the citrate amount is significantly beyond what is required to immobilize Ca\(^{2+}\) ions in rabbit whole blood.