# Capillary Agglutination Technology

**Problem presented by**

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## Problem statement

In medical diagnostic tests, including pregnancy testing and tests for typed red blood cells, a small fluid sample is placed at one end of a capillary channel, which has been lined with a dried reagent. If the sample contains the analyte (the substance being tested for) then an agglutination reaction occurs between it and the reagent in the channel, and the agglutinated complexes progressively slow the flow and may even block the channel, partially or completely, so that the flow only reaches the far end very slowly, or not at all. The aim is that this should give a reliable detection of quite low concentrations of analyte in the sample. Platform Diagnostics would like a mathematical model of the process, so that, for known binding forces in the agglutination complexes, the channel size and shape, and the fluid viscosity, can be designed to maximize the reliable detection of low concentrations. A key question is how the flow time depends on channel size, fluid surface tension and viscosity, (a) in the absence of agglutination, and (b) in the presence of agglutination.

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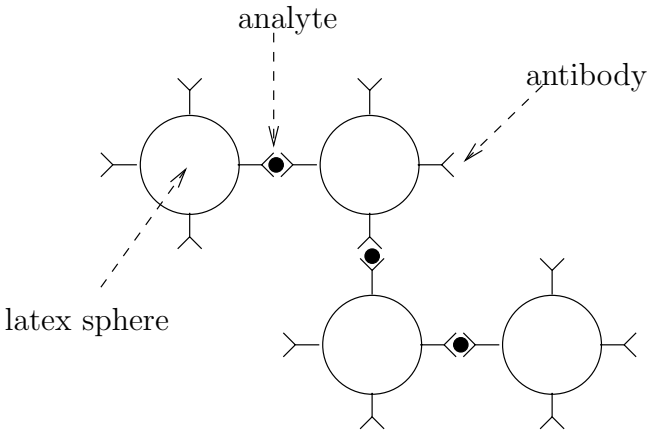


Figure 1: Figure showing the method of aggregation .

# 1 Introduction

The aim of the Platform Diagnostics device is to reliably test for a very low concentration of a specific analyte. The principle on which this device works is based on a relatively old technology termed *latex agglutination* which has been in use in clinical laboratories since the 1950s. The latex agglutination procedure, as it was originally devised, is performed by coating many latex microparticles with an antibody to the analyte. These are then mixed with a sample on a glass slide and the slide examined under a microscope. If latex particle aggregates are detected in this mixture it demonstrates the presence of analyte in the sample, the aggregates being formed by the analyte binding to antibodies on different microspheres (see figure 1).

Platform Diagnostics' device consists of two identical capillary tubes, with triangular cross section, formed by impressing grooves into a plastic sheet. These grooves are covered with an adhesive tape, which forms the upper surface of the tubes (see figure 2). Both tubes start from a reservoir of fluid and make their way, after several turns, to two separate small chambers (see figure 3). Each chamber is hooked up to a pair of electrodes which can be used to detect when fluid reaches the end of its capillary. The operation of the device requires that fluid is introduced into the reservoir; capillary forces then pull the fluid from the reservoir up the capillaries and into the chambers. In practice this requires that the surface of the plastic sheet be treated with a reagent to make it perfectly wetting; the adhesive tape (which forms the top of the capillary) is non-wetting.

Latex microspheres are placed into the bottom of each of the capillaries. In one, a control experiment is carried out using microspheres with no attached antibodies while in the other the full experiment is carried out using microspheres with antibodies attached. Thus, in the presence of the analyte, the microspheres are able to bind together to form aggregates whereas in the control they are not. The formation of aggregates, in the presence of the analyte, increases the viscosity in the full experiment, causing the time for the fluid to reach the chamber at the end of the capillary to increase in comparison

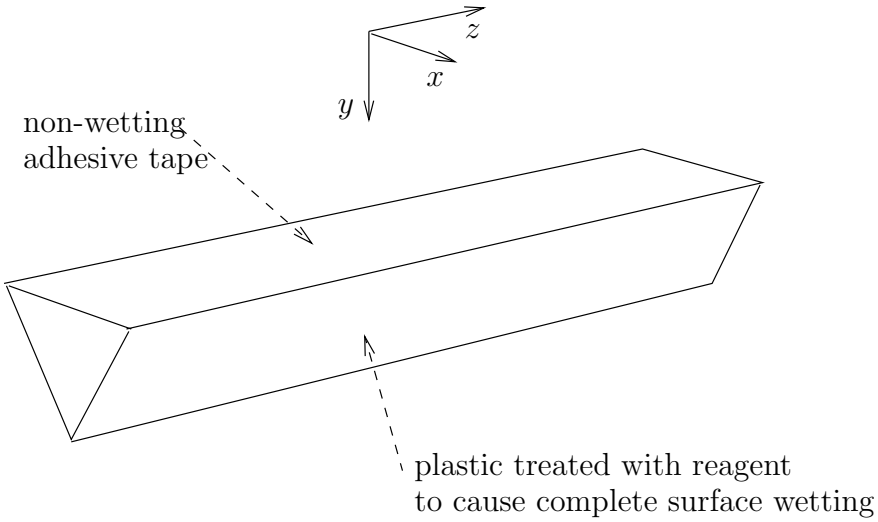


Figure 2: The capillary shape.

with that of the control. The difference between these two times indicates the presence of the analyte.

## 1.1 Application

A similar device has been successfully used to test for blood type in a scenario in which a reaction between the blood and a reagent causes clotting and leads to a detectable slowing of the flow in comparison to a control in which no reaction (and hence clotting) occurs. Platform Diagnostics are currently trying to develop the technology in order to detect the hormone hCG in the urine of pregnant women. The level of accuracy required in this device is that it is able to reliably detect 25 mIU per millilitre of urine (1mIU= 54.1 ng) which corresponds to the level present in a pregnant woman 2 days after a missed period. Current pregnancy tests can be completed within 3 minutes, so they need this new device to work at least as fast as that.

## 1.2 Experimental parameters

Laboratory experiments have been carried out using the following protocol. A solution containing a certain concentration of hCG is prepared. Latex beads (5% by volume) with the antibodies attached are introduced, and the mixture is well stirred. The solution is then put into the reservoir of the apparatus and is allowed to flow along the tubes. The experimental data is shown in figure 4.

We can see that the time taken for the liquid to flow through the apparatus increases with concentration, as expected. Note that 25 mIU/ml level lies at the left hand side of the graph, and that the error bars here indicate that reliable detection of a difference

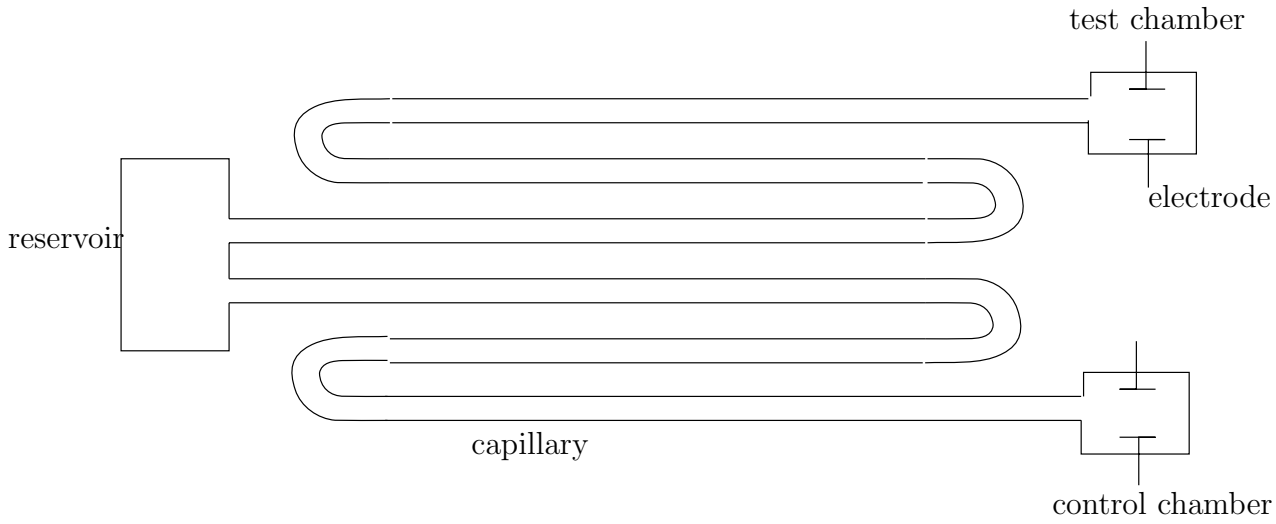


Figure 3: Schematic of the device.

between the control track and active track is impossible. In the experiments, a "tongue" of fluid is seen to travel down the capillary ahead of the main body of fluid.

There are a number of physical parameters in the system, and a number of design parameters. The physical parameters are

$$\begin{aligned} \text{Density, } \rho &\approx 10^3 \text{ kg m}^{-3}, \\ \text{Surface tension, } \gamma &\approx 70 \times 10^{-3} \text{ kg s}^{-2}, \\ \text{Viscosity, } \mu &\approx 10^{-3} \text{ kg m}^{-1}\text{s}^{-1}, \\ \text{Diffusivity of hCG in water, } D &\approx 2 \times 10^{-10} \text{ m}^2\text{s}^{-1}, \end{aligned}$$

and the design parameters are

$$\begin{aligned} \text{Length of capillary tube, } L_{\text{tot}} &\approx 0.3 \text{ m}, \\ \text{Radius of microspheres, } r_B &\approx 200 \text{ nm}, \\ \text{Width of channel top, 2d} &\approx 5 \times 10^{-4} \text{ m}. \end{aligned}$$

We note that the easiest thing to change in the design of the experiment is the radius of the latex microspheres.

# 2 Flow in the capillary

### The flow equations and boundary conditions

We assume the fluid in the capillary is Newtonian (albeit that its viscosity is affected by the size of latex bead complexes). The flow thus satisfies the Navier-Stokes equations (see [2])

$$\rho\left(\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u}\cdot\nabla)\mathbf{u}\right) = -\nabla p + \rho g \boldsymbol{e}_y + \mu\nabla^2\mathbf{u}\,, \tag{1}$$

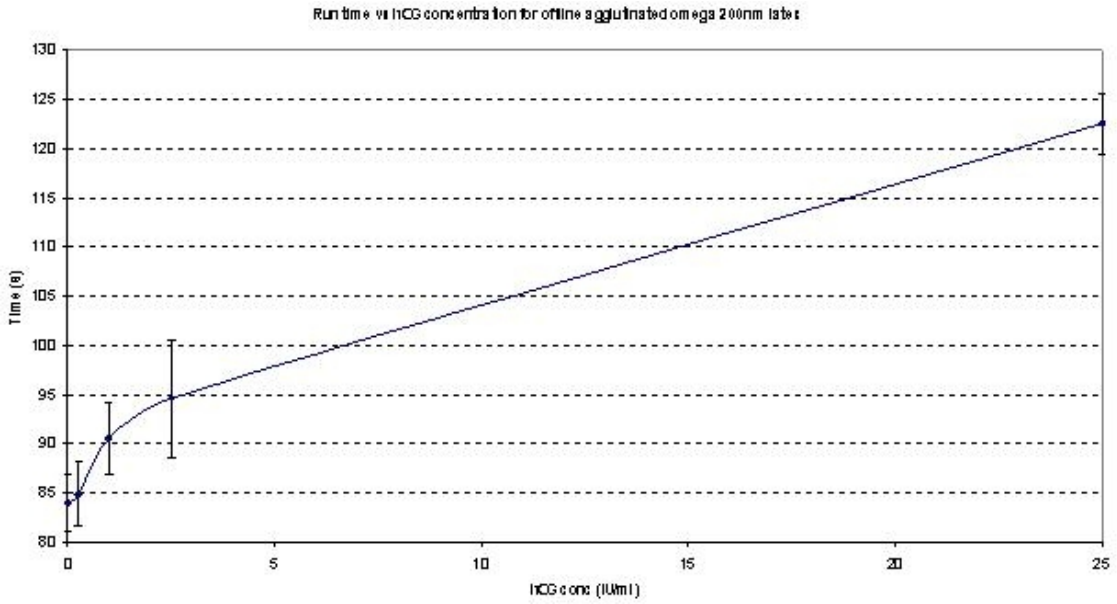


Figure 4: Graph showing how the time taken for the liquid to reach the end of the tube varied with concentration.

where $g$ is the acceleration due to gravity, $y$ measures the distance below the adhesive tape and $\boldsymbol{e}_y$ is the unit vector in this direction, $p$ is the pressure in the fluid, $\mathbf{u}$ is the velocity, $\rho$ is the density and $\mu$ is the viscosity. On the edge of the capillary $\partial\Omega_e$ the no-slip boundary condition,

$$\mathbf{u}|_{\partial\Omega_e} = \mathbf{0}, \tag{2}$$

is satisfied. At the free surface formed by the advancing edge of the fluid in the capillary, $\partial\Omega_{fs}$, the normal and tangential force balances and kinematic conditions

$$\mathbf{n}.\sigma.\mathbf{n} = -2\gamma\kappa, \qquad \mathbf{t}.\sigma.\mathbf{n} = 0, \qquad v_n = \mathbf{u}\cdot\boldsymbol{n}|_{\partial\Omega_{fs}}, \tag{3}$$

are satisfied. Here $\sigma$ is the stress tensor, $\gamma$ is the surface tension, $v_n$ is the normal velocity of the free boundary, $\mathbf{n}$ is the normal to the free surface, $\mathbf{t}$ is a tangent to the surface and $\kappa$ is the mean curvature. Finally, the pressure in the reservoir is equal to atmospheric pressure, which gives us the initial condition $p|_{z=0} = 0$. We note that the length of the liquid, $L(t)$, in the capillary is not known *a priori* and we must specify a law of motion in order to determine the position of the contact line.

**An ad-hoc approximation to the flow problem**

Rather than solve the full problem comprised by equations (1)-(3) we look for an approximate solution which will enable us to find an expression for the length of fluid, $L(t)$, in the capillary as a function of time $t$ and for now we neglect gravitational effects assuming that the device lies on a level surface.

For a capillary cross-section formed by an equilateral triangle (as used in practice)[1]

[1]providing the tube remains completely flooded

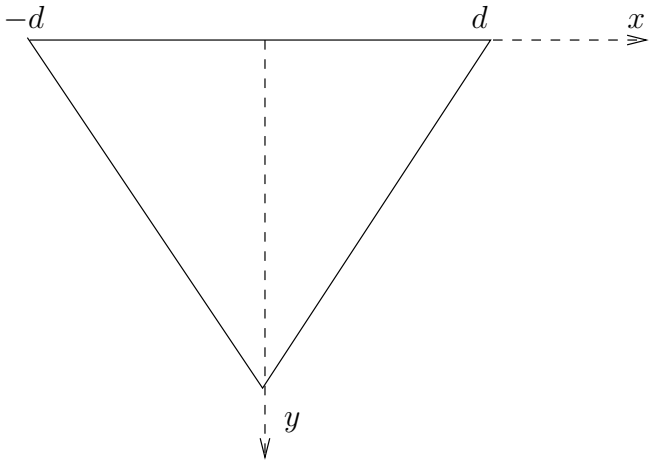


Figure 5: The coordinates in the plane of the capillary cross-section .

there is an exact Poiseuille solution[2] to the Navier-Stokes equations (1) with no-slip boundary conditions (2). This takes the form

$$\mathbf{u} = \lambda y(y + \sqrt{3}x - \sqrt{3}d)(y - \sqrt{3}x - \sqrt{3}d)\boldsymbol{e}_z, \qquad p = -\frac{\Delta p_1}{L} z\,, \tag{4}$$

where $\boldsymbol{e}_z$ is the vector pointing down the capillary, $x$ and $y$ are coordinates in the plane of the capillary cross-section (see figure 5) and $2d$ is the length of the sides of the triangle. The parameter $\lambda$ can be evaluated in terms of the pressure drop along the capillary, $\Delta p_1$, the length of the fluid in the capillary, $L$, and the viscosity, $\mu$, and is given by

$$\lambda = \frac{\Delta p_1}{4\sqrt{3}Ld\mu}. \tag{5}$$

Note that the assumption that the flow has the Poiseuille profile (4) all the way down the capillary is unrealistic; there is an adjustment region in the vicinity of the front. The reduced Reynolds number $Re$ for the flow along the capillary ($Re = \rho d^2/\mu T$, where $T$ is the time taken for the fluid to reach the end of the tube) is approximately $1.4 \times 10^{-3}$. In turn the small size of $Re$ implies that the adjustment region is of length $O(d)$. When $L \gg d$ the effects of the adjustment region on the Poiseuille flow taking place in the majority of the capillary is negligible.

Since the major pressure drop in the capillary occurs over the Poiseuille flow region (and not in the adjustment region) the pressure at the capillary front is constant to leading order. This means that the mean curvature of the free boundary $\kappa$ is constant (to a first approximation) and given by

$$2\kappa \approx -\frac{p|_{\partial\Omega_{fs}}}{\gamma} \approx \frac{\Delta p_1}{\gamma}. \tag{6}$$

Here we have made use of boundary condition (3a) and the fact that $p = 0$ at the capillary entrance ($z = 0$). Furthermore we know that the contact angle that the fluid makes with

[2]see the appendix for a proof

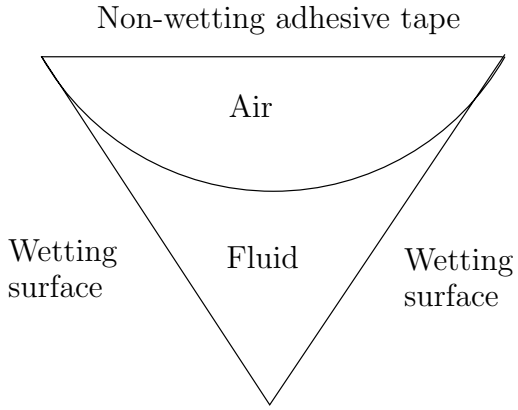


Figure 6: The fluid profile in the capillary at a point near the front.

the completely wetting surface is zero while that on the non-wetting surface is given by some constant $\theta_c$. Given that the mean curvature of the surface is constant we could, in theory, solve a PDE for the free surface shape using the contact angle conditions as boundary conditions on the PDE. The result of this calculation would determine the constant mean curvature of the free boundary and hence the pressure at the interface. In practice though all we require is a fairly rough estimate of the mean curvature and pressure drop. We thus consider the cross-section of the interface (in the $x$-$y$ plane) at the value of $z$ at which the fluid first completely uncovers the adhesive tape covering the top surface. It will look somewhat like that presented in figure 6. Assuming that the mean curvature is dominated by curvature of the interface in the $x$-$y$ plane, the shape of the interface in this cross-section is approximately circular with radius $R = 2d/\sqrt{3}$. Hence the pressure drop along the capillary is given by

$$\Delta p_1 \approx \frac{\sqrt{3}\gamma}{2d} = 240 \text{ Pa}, \tag{7}$$

which is the equivalent to several centimetres of water. The average fluid velocity $u_{\text{av}}$ along the capillary can be calculated by integrating the Poiseuille profile (4) across the cross section of the capillary and dividing by its area. On substitution for $\lambda$ from (5), this gives

$$u_{\text{av}} = \frac{\sqrt{3}\lambda d^3}{5} = \frac{d^2 \Delta p_1}{20 L \mu} \tag{8}$$

Substituting for $\Delta p_1$ from (7) gives

$$u_{\text{av}} = \frac{\sqrt{3} d \gamma}{40 \mu L}. \tag{9}$$

It follows that the length $L$ of fluid in the capillary satisfies the ordinary differential equation

$$\frac{dL}{dt} = \frac{\sqrt{3} d \gamma}{40 \mu} \frac{1}{L}, \tag{10}$$

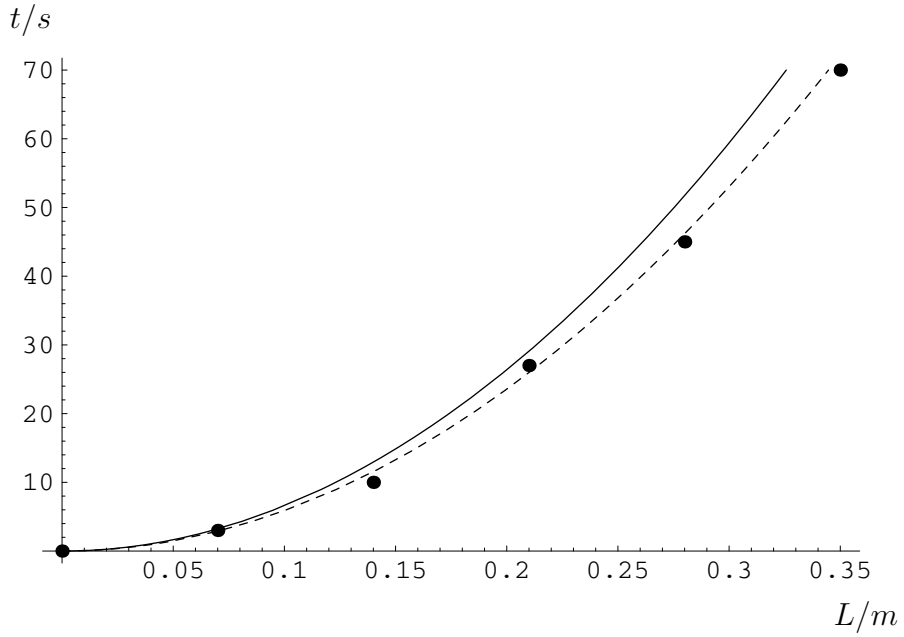


Figure 7: Graph showing the experimental results (dots) and the results given by (11) for pure water (solid line). The dashed line provides a better fit to the data.

and hence

$$L = \frac{3^{1/4}}{\sqrt{20}} \left( \frac{d\gamma t}{\mu} \right)^{1/2} . \tag{11}$$

Thus, the width of the channel, the surface tension of the interface and the viscosity of the liquid all play a role in determining the speed with which the front moves. In particular, increasing the viscosity is not the only way to slow down the front; it can also be achieved by reducing the width of the channel or reducing the surface tension.

We carried out a table-top experiment to see whether the evolution of the length of the fluid was captured by the solution given in (11). We put some water (dyed blue) into the well at the end of the device and recorded the time that it took for the liquid to progress to the end of each of the lanes. The experiment was repeated. The experimental results are shown as dots in figure 7, and the prediction from (11) using parameters for pure water is shown by the solid line. We see that there is remarkably good agreement between the two (especially given the table-top nature of our experiment!). We note that a better fit to the data can be obtained (dashed line) by slightly altering the geometric factor.

**The effect of gravity on the experiment**

If the device is placed on a non-level surface (as might happen in practice) gravitational effects may be important. In order to estimate whether this is the case we compare the size of the gravitational term in (1) with the typical pressure gradient

induced by capillary forces (in the first section of the capillary tube). The key parameter is the ratio of the hydrostatic pressure to the capillary pressure, the Bond number $Bo$, given by

$$Bo = \frac{\rho g H}{\Delta p_1} = \frac{\rho g H}{\sqrt{3}\gamma/2d}, \tag{12}$$

where $H$ is the difference in height between the ends of the device. Provided $Bo \ll 1$ gravitational effects are negligible and thus (in keeping with the size of the capillary pressure found earlier) we find that

$$H \ll \frac{1}{8.25 \times 10^4 d}. \tag{13}$$

The current device has $d = 2.5\times10^{-4}$ m which implies that $H \ll 5$ cm. This suggests that the test may be insufficiently robust, as it stands, to be used at home rather than in the laboratory, since the presence of any significant incline will always result in a retardation of the flow in part of the capillary which may prevent the front from progressing. It may therefore be worth considering using a chemical reaction to lower the pressure in the chamber at the end of the capillary. This would enhance the pressure difference between the ends of the capillary and correspondingly reduce the Bond number for any given $H$.

Reducing the width of the channel would also make the experiment more robust. Reducing $d$ by a factor of 10 would mean that the difference in height between the two ends could increase by a factor of 10. However, this would increase the time of the test by a factor of 10, all other things being equal, which is undesirable.

# 3 The agglutination process

For the device to be successful as a pregnancy test kit, it must be able to detect concentrations of hCG as low as 25 mIU per millilitre. This corresponds to approximately $2.1 \times 10^{10}$ molecules per millilitre of fluid. In contrast to this a 1% solution of 200 nm diameter beads gives approximately $2.5 \times 10^{12}$ beads per millilitre. There are therefore many more beads than molecules. If the beads and hCG are stirred together only a very small percentage of beads will find an hCG molecule to bind to. In a homogeneous mixture this, in turn, means that only a tiny percentage of these beads will bond to form anything other than a dimer. There is clearly an advantage, therefore, in ensuring that the mixing is slow enough to form a non-homogeneous mix.

**Diffusion timescales for hCG**

The timescale $\tau$ for a substance to diffuse a distance $L$ is given by

$$\tau = \frac{L^2}{D}, \tag{14}$$

where $D$ is the diffusivity of the substance in a particular solvent. We suspect that $D$ for hCG in water is about $D \approx 2 \times 10^{-10}$ m$^2$ s$^{-1}$ (this is based on the assumption that

the Stokes radius for hCG is approximately 1 nm). The timescale for diffusion of hCG across the capillary (a distance of 0.5 mm) is thus approximately 1250 seconds (much longer than the time of the experiment). The diffusion timescale for hCG over the typical distance between 200nm particles at 1 percent (*i.e.* approx $10^{-6}$ m) is about $5 \times 10^{-3}$ s, whereas the diffusion timescale over the typical distances between 5 $\mu$m beads at 1 percent (*i.e.* $2.5 \times 10^{-5}$ m) is about 2.5 s.

**Bond strength and shear forces**

Typical bond strengths between an antibody and a hCG molecule are of the order of $5 \times 10^{-11}$ N. A typical shear rate in the device is of the order of $10\text{s}^{-1}$. The force $F$ on the bond between two latex particles, radius $r_B$, travelling in this flow is then given very approximately by

$$F \sim 6\pi\mu s r_B^2, \tag{15}$$

where $s$ is the shear rate of the flow. If the two particles are held together by a single bond then it is a requirement, in order for the bond to hold, that $F < 5 \times 10^{-11}$ N. This gives a critical radius $r_B$ (in order for a dimer to hold together) of order of $10^{-5}$ m. In the case of a chain of $m$ particles $F \sim 6m\pi\mu s r_B^2$.

**The Einstein formula for the viscosity of dilute suspensions**

This formula relates the volume fraction $\phi$ of spheres in a dilute suspension to the viscosity of the suspension $\eta$ and can be simply stated as

$$\eta = \mu(1 + 2.5\phi), \tag{16}$$

where $\mu$ is the viscosity of the fluid. A consequence of this formula is that dense aggregations of particles will hardly change the viscosity of the suspension, since they do not significantly change the underlying volume fraction. However, sparse (or fractal) aggregations can be expected to significantly change the effective volume fraction and hence the viscosity (see figure 8). In particular, dimers can be thought of as a type of dense cluster and are therefore not desirable since they will not significantly affect the viscosity of the solution.

## Conclusion

We are left with the problems that (i) it takes a long time for hCG to diffuse across the capillary, (ii) there are vastly more beads than hCG molecules and so we are most likely to form dimers, (iii) dimers won't change the viscosity of the solution enough to provide a detectable change in the duration of the flow.

It is conceivable that extremely heterogeneous mixing in which a fluid layer, containing hCG and no beads, is sheared over a second fluid layer, containing beads and no hCG, will lead to significantly larger numbers of clusters being formed than the homogeneous mixing we have been discussing above. However the treatment of this subject is considerably beyond the scope of this report.

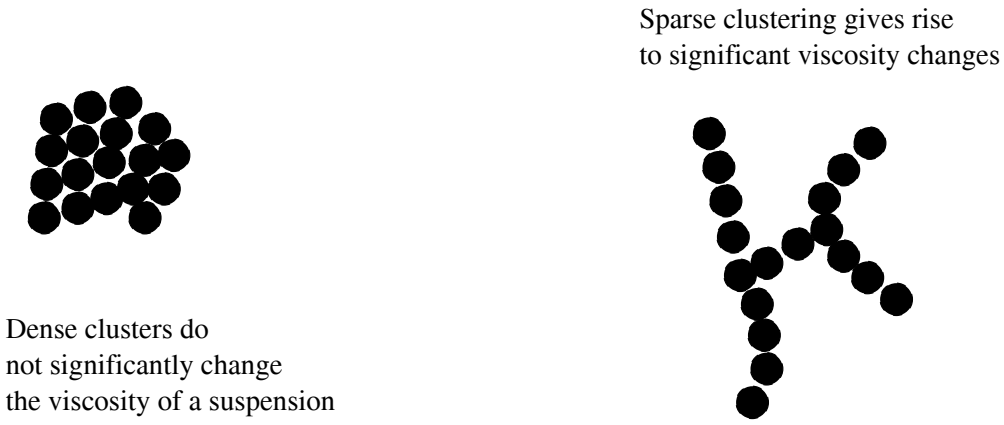


Figure 8: Sketches showing different types of clustering of latex microspheres.

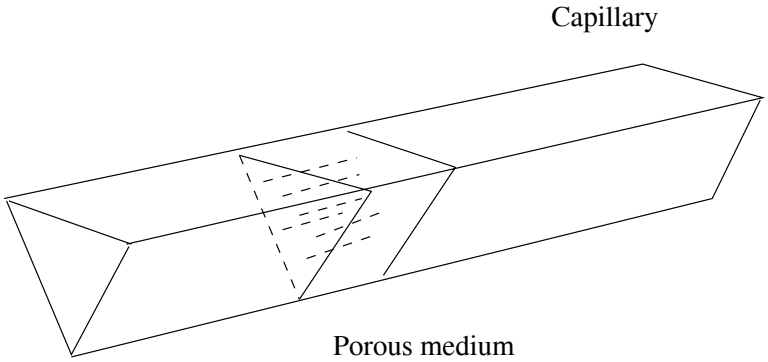


Figure 9: Redesign of the device with an inserted porous medium.

# 4 Process redesign to overcome the difficulties with the low concentration of hCG

The main difficulty to be overcome when designing a device to detect small concentrations of hCG is to ensure that the hCG molecules can form bonds in a manner which gives rise to a significant change in the flow time in the capillary device. One way of doing this is to introduce a porous medium previously coated in antibodies, then flush through a quantity of the sample (in the absence of latex beads) before introducing latex beads, coated in antibodies, into the flow. If designed properly this will cause (i) most of the hCG to bind to the walls of the porous medium before (ii) latex beads flow through the medium binding to sites previously activated by the hCG; in turn this will (iii) lead to constriction of the pores (in the medium) and a consequent retardation of the flow.

For this device to work the following requirements must be met:

1. The bond must be sufficiently strong to hold a latex bead to the porous medium walls against the action of fluid shear.

2. The bead radius must be large enough to cause a significant narrowing of the pores it adheres to.

3. There must be sufficient hCG to bind beads over a surface area comparable to the surface area of the porous medium.

4. The diffusion timescale of the hCG across a pore must be less than (or equal to) the typical time for flow through the porous medium.

5. The pressure drop across the porous medium must be of the same order (or greater) than the maximum pressure drop along the rest of the capillary.

6. The time required to perform the test must be less than three minutes.

7. The porous plug must fit inside the capillary.

As a first step to evaluating these criteria we assume that the porous medium is formed by $N$ identical cylindrical pores of length $\Lambda$ with radius $a$.

Furthermore we denote the average fluid velocity in a pore by $v_{\text{av}}$ and the pressure drop across the pore by $\Delta p_2$. Note that the average velocity is related to the pressure drop across the porous medium by the relation

$$v_{\text{av}} = \frac{a^2 \Delta p_2}{8\Lambda\mu}, \tag{17}$$

and thus conservation of fluid dictates that

$$N\pi a^2 v_{\text{av}} = \sqrt{3} d^2 u_{\text{av}}. \tag{18}$$

With these definitions the criteria outlined above can be rewritten as

1. $$\left( \frac{\text{Bond strength}}{6\pi\mu r_B^2 (-\,\partial u/\partial r|_{r=a})} \right) > 1 \Longrightarrow r_B < \left( \frac{Ba}{24\pi\mu v_{\text{av}}} \right)^{1/2},$$

   where $B$ = bond strength.

2. Given some $O(1)$ constant $k$,
   $$r_B = \frac{a}{k}.$$

3. $$\frac{\text{Number of molecules hCG} \times \pi r_B^2}{\text{Surface area of porous medium}} \geq O(1).$$

   Assuming that a volume $V_0$ millilitres of sample, carrying hCG at concentration $C$ molecules per millilitre, passes through the porous medium before the beads start passing (*i.e.* $M$ molecules bind) then
   $$\frac{V_0 C r_B^2}{2N\Lambda a} \geq O(1).$$

4.

$$\frac{\text{Diffusion timescale across pore}}{\text{Flow timescale through medium}} \geq O(1) \Longrightarrow \frac{a^2/D}{8\Lambda^2\mu/(a^2\Delta p_2)} \geq O(1). \qquad (19)$$

5.

$$\frac{\text{Pressure drop across porous medium}}{\text{Pressure drop across rest of capillary}} = \frac{\Delta p_2}{\Delta p_1} \geq O(1).$$

This ratio can be calculated by substituting (8) and (17) (the expressions for $u_{\text{av}}$ and $v_{\text{av}}$) into (18), noting that the maximum drop is when $L = L_{\text{tot}}$, the total length of capillary, to obtain

$$\frac{\Delta p_2}{\Delta p_1} = \frac{2\sqrt{3}d^4\Lambda}{5\pi a^4 N L_{\text{tot}}} \geq O(1).$$

6. Once the front of the flow has passed through the porous medium it is driven by the pressure difference generated by surface tension in the capillary, $\sqrt{3}\gamma/(2d)$. In line with assumption 5 we assume that the major pressure drop occurs over the porous medium so that $\Delta p_2$ is approximated by the surface-tension-generated pressure drop leading to the relation

$$\Delta p_2 \approx \sqrt{3}\gamma/(2d). \qquad (20)$$

We then use (17) and the continuity relation (18) to derive expressions for $v_{\text{av}}$ and $u_{\text{av}}$:

$$v_{\text{av}} \approx \frac{\sqrt{3}\gamma a^2}{16 d\Lambda\mu}, \qquad u_{\text{av}} \approx \frac{N\pi a^4\gamma}{16 d^3\Lambda\mu}. \qquad (21)$$

Furthermore, since in all cases of practical interest $L_{\text{tot}} \gg \Lambda$, this implies that for the flow to take place over a timescale less than $T_{\text{req}}$ (= 180 seconds) we require

$$\frac{16 d^3\Lambda L_{\text{tot}}\mu}{N\pi a^4\gamma T_{\text{req}}} < 1.$$

7. The porous plug must fit inside the capillary. The cross sectional area of the plug is $\sqrt{3}d^2$ and, assuming hexagonal packing of the pores, the area associated with the porous medium is approximately $2\sqrt{3}Na^2$. Thus we must choose $N$ such that

$$2Na^2 \leq d^2.$$

In order to examine these conditions, we first choose the relationship between the bead radius and the pore radius, and set the number of pores. We set $k = 4$ and $N = d^2/2a^2$ thus obviating relations 2 and 7. The other relations, taken in numerical order, can be simplified by eliminating $\Delta p_2$, $u_{\text{av}}$ and $v_{\text{av}}$ using (21)-(20) to give

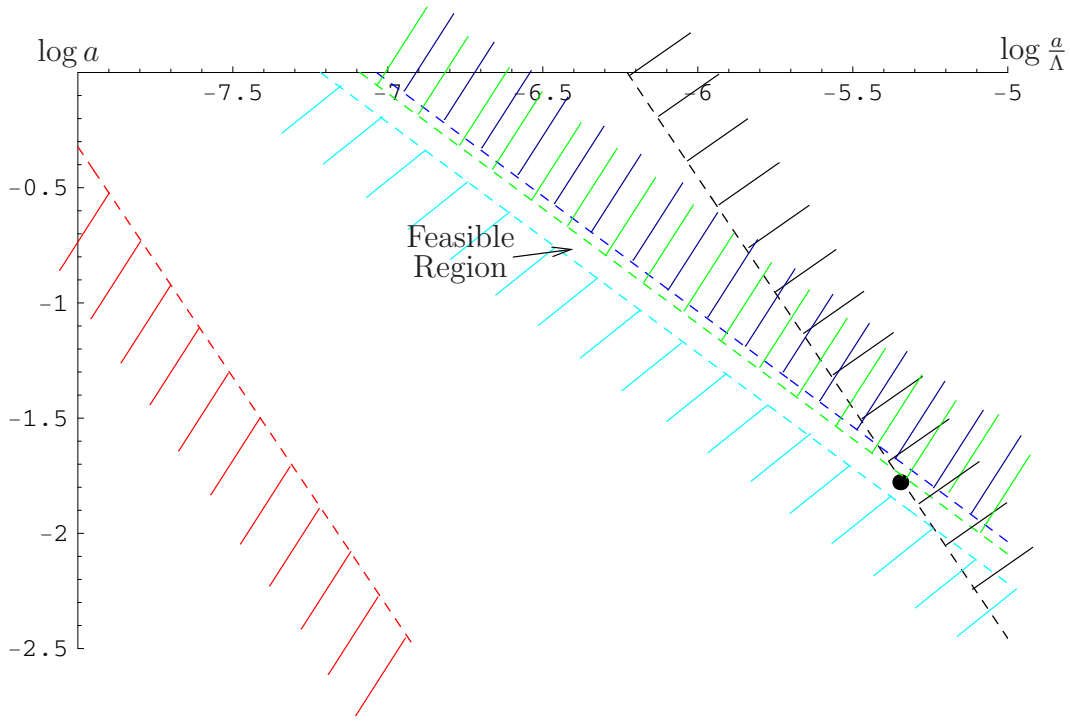


Figure 10: Graph showing the feasible region for the solution to (22). The black line represents the first condition, the red line, the second condition, the green line, the third condition, the blue line, the fourth condition and the cyan line, the fifth condition. Shaded areas are excluded from solution space. The dot indicates a possible solution.

$$\begin{aligned} &\frac{a^3}{\Lambda} < \frac{32Bd}{3\sqrt{3}\pi\gamma}, \qquad \frac{a^3}{\Lambda} \geq O\left(\frac{16d^2}{V_0 C}\right), \quad \frac{a^2}{\Lambda} \geq O\left(\sqrt{\frac{16\mu D d}{\sqrt{3}\gamma}}\right), \\ &\frac{a^2}{\Lambda} \leq O\left(\frac{4\sqrt{3}d^2}{5\pi L_{\text{tot}}}\right), \quad \frac{a^2}{\Lambda} > \frac{32dL_{\text{tot}}\mu}{\pi T_{\text{req}}\gamma}. \end{aligned} \tag{22}$$

To determine if there are possible solutions to this system, we suppose that, in addition to the parameter values stated earlier, we have

$$B = 5 \times 10^{-11} \text{ N}, \quad C = 2.1 \times 10^{10} \text{ molecules ml}^{-1}, \quad V_0 = 1 \text{ ml}, \quad T_{\text{req}} = 180 \text{ s}. \tag{23}$$

In figure 10 we show the range of solutions to (22) in $\log a$—$\log(a/\Lambda)$ space. We can see that there is a small feasible region for picking $a$ and $\Lambda$. We indicate one such solution by the dot in figure 10. This solution corresponds to the following set up. The porous plug is 0.27 mm long, contains $1.5 \times 10^3$ pores, each with radius 4.5 $\mu$m. The beads required are of 1.1 $\mu$m radius, and the timescale associated with the experiment

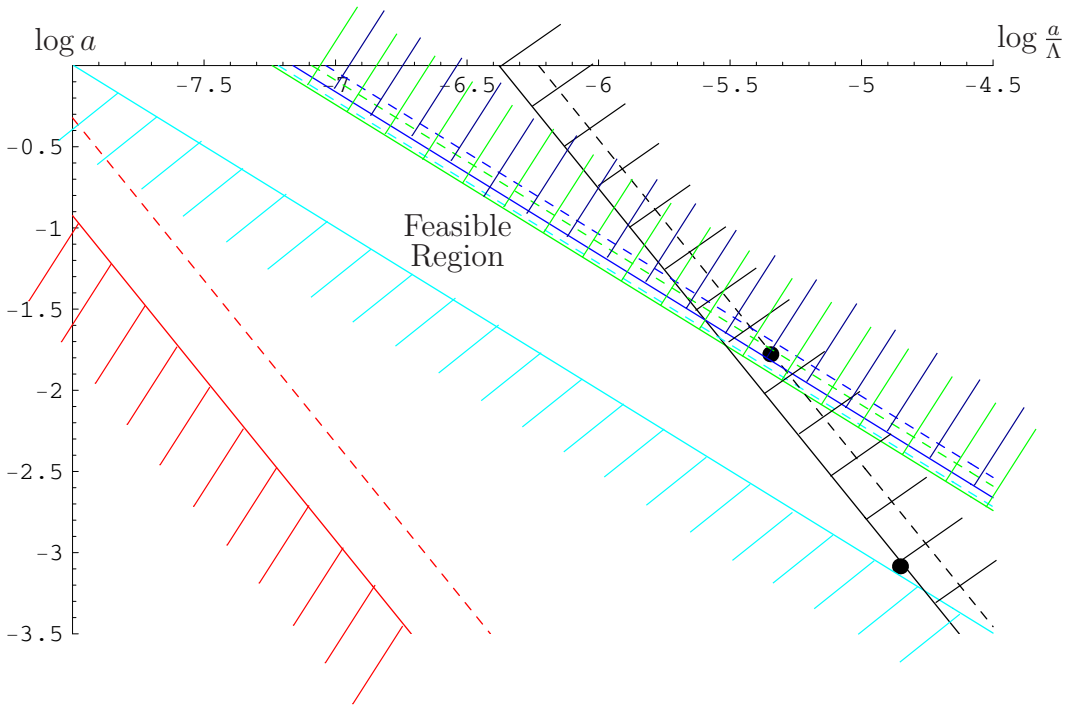


Figure 11: Graph showing the feasible region for the solution to (22). The black line represents the first condition, the red line, the second condition, the green line, the third condition, the blue line, the fourth condition and the cyan line, the fifth condition. The dashed lines correspond to the feasible region shown in figure 10. Shaded areas are excluded from solution space. The dots indicate the original solution and solution consistent with the shifted constraints.

is 172 seconds. Note that, to use beads of radius 200 nm, we would beed to reduce the length of the porous block, and increase the number of pores.

Of course, there are many other possible choices for the parameters which will give different porous media. For example, if we reduce $d$ by a factor of 2 and reduce $L_{\text{tot}}$ to 0.1m, we shift the feasible region to that shown by the solid lines in figure 11. We see that the solution given above does not reside in the new feasible region, but one in which the porous plug is 1.7 cm long, contains 40 pores, each with radius 14 $\mu$m does. The beads required are of 3.5 $\mu$m radius, and the timescale associated with the experiment would be 162 seconds.

Of course, it would be straightforward to investigate the effects of changing each of the design parameters, but we do not do that here.

# 5 Other possible ways to redesign the apparatus

In the previous section we looked at including a porous medium in the flow so that we could *concentrate* the hCG and get it closer to the surfaces and hence clog the system. Another way to slow up the capillary would be to clog, and thus retard, the air/liquid interface. One way of doing that would be to make the beads surface active (*i.e.* hydrophobic) so that they accumulate at the surface. In the control track, these would be swept away by the stagnation point flow to the edge of the capillary (where they would clump). However, in the reacting track (where the beads are coated with hCG antibody) the hCG near the surface will move to the surface and bind the beads together. There will then be replenishment of the subsurface hCG by diffusion from the bulk. A better solution might be to add surfactant to the solution. The hCG-surfactant complexes that result would be surface active (even if the hCG is not) and reside preferentially at the air-liquid interface [1, 3]. In the reacting capillary, the beads would then see a much-increased concentration of hCG near the surface and so the possibility of binding and forming chains would be much higher. In both cases, the process would rely on transporting the hCG near to the surface by convection, and then diffusion taking over and being the rate-limiting step.

# 6 Discussion

We decomposed the workings of the proposed test for hCG into several parts. First we looked at the flow down a capillary with triangular cross-section, and obtained a crude (but accurate) model for capillary length as a function of time. This showed that the controlling parameter was the ratio of the product of the surface tension of the air-liquid interface and the width of the channel to the viscosity, and that the capillary length is proportional to the square root of time.

We also looked at the effect of gravity on the operation of the device and showed that a 5 cm height difference between the ends was all that is needed to retard the flow significantly. This implies that the design may not be robust enough to work in the home.

We considered the mass transfer and agglutination processes and noted that there are considerably many more latex microspheres in solution than hCG molecules. A consequence of this is that it is unlikely that anything other than dimers will form and, in turn, no significant viscosity change can be observed.

We looked at how the apparatus could be redesigned to induce significant clogging. One way to do this is to place a plug of porous medium into the capillary tube. We wrote down a number of design specifications that need to be satisfied in order for this method to work based on a porous medium with straight channels. We used these criteria to demonstrate the feasibility of this approach and note that the same method could be generalised to porous media with non-straight, tortous, pores.

Finally we looked at other mechanisms for retarding the flow and a totally new approach for measuring hCG concentrations.

# Appendix: Polynomial viscous flow in a polyhedral pipe

It is well-known that an equilateral triangle is the *only* polygonal pipe cross-section for which there is a *polynomial* solution of the viscous flow equation. However, we do not know of a proof of this fact in the literature, so we give one here for interest.

If the axial flow velocity is $w(x, y)$, then after rescaling it obeys $\nabla^2 w = 1$ in a polygon $P$ in the $(x, y)$-plane, with $w = 0$ on the boundary. We shall assume that $w$ is a polynomial and show that $P$ must be an equilateral triangle. Each edge of the triangle is a line segment on which $w = 0$. However since $w$ is a polynomial it must vanish on the whole continuation of that line segment, and must have the equation of that line as a factor. Let $l$ be the number of such factors. Also $w - \frac{1}{2}x^2$ is a *harmonic* polynomial, and is therefore the real part of a complex polynomial when expressed in terms of $z = x + iy$, so we have

$$w = \tfrac{1}{2}x^2 + \mathrm{Re}(a_n z^n + a_{n-1} z^{n-1} + \ldots + a_0) \tag{24}$$

with $a_n \neq 0$ and $n \geq l \geq 3$. Let $c$ be a corner of $P$ and $\zeta_1, \zeta_2$ be unit complex numbers in the directions of the two edges at $c$, so $w$ vanishes at $z = c + r\zeta_i$ with $r$ real. Considering the coefficients of $r^n$ and $r^{n-1}$ in $w(c + r\zeta_i)$, we have

$$\mathrm{Re}(a_n \zeta_i^n) = 0 \; ; \quad \text{and, if } n \geq 4, \quad \mathrm{Re}((a_n n c + a_{n-1})\zeta_i^{n-1}) = 0. \tag{25}$$

Since $a_n \neq 0$ the first of these implies that $\zeta_1^n / \zeta_2^n$ is real. If $n \geq 4$ then choose a corner $c$ such that $a_n n c + a_{n-1} \neq 0$ and the second would imply that $\zeta_1^{n-1} / \zeta_2^{n-1}$ is also real. However, these cannot both hold, as then $\zeta_1 / \zeta_2$ would be real, contradicting the choice of $c$ as a corner. Hence in fact $n = 3$ and $(\zeta_1 / \zeta_2)^3$ is real. Hence $l = 3$ and at each corner the edges meet at $\pi/3$, so $P$ is an equilateral triangle.

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