Electronic Supplementary Material (ESI) for Lab on a Chip.


**SUPPLEMENTARY DATA**

# Lab on a Stick: Multi-Analyte Cellular Assays in a Microfluidic Dipstick

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## Supplementary Methods

*Scanning Electron Microscopy (SEM)*

A Carl Zeiss (Leo / Cambridge) 360 SEM was used for scanning the characteristics of inner surfaces of the PVOH-coated microcapillaries. A scanning electron microscope works by focusing a beam of high energy electrons to produce a variety of signals at the surface of the sample, and the signals generated from the electron-sample interactions provide information about the sample including morphology, chemical composition, and crystalline structure. MCF samples were prepared by gently drying the capillaries with nitrogen and slicing the strips along and through the middle of the capillaries in order to reveal the inner surface of the microcapillaries. The length of the samples scanned were about 4 mm, which was fixed to a pin stub mount with double-side tape. Samples where coated with a thin 20nm layer of gold using an Edwards S150 bench-top sputter coater for 60 seconds.

*Atomic Force Microscopy (AFM)*

AFM is a powerful technique for imaging surfaces at micrometre or nanometre resolution. In contrast to SEM, it does not require samples to be conductive or metal-coated. A Veeco atomic

force microscope (Model Explorer) with both non-contact and pulsed force imaging modes was used to obtain topography images of the uncoated and coated MCF microcapillaries. Strips were cut along the capillaries at an angle (starting from the middle part of the microcapillary) to allow the AFM probe to reach the inner surface of the microcapillaries.

*Confocal fluorescence imaging of reagents release*

Dried PVOH-coated strips were loaded with 1 mg/mL of mouse anti-human IgM or IgG fluorescently labelled with FITC (Sigma-Aldrich, Dorset, UK) and incubated for at least 2 hours, after which the excess of reagent was removed by flushing the capillaries with air. The MCF strips were then examined using a Nikon TE300 inverted microscope equipped with a Bio-Rad Radiance 2000 confocal laser scanning head. A Nikon 10x/0.25 Ph1 DL objective lens was used for imaging. The confocal microscope was computer controlled using the software LaserSharp 2000 (Bio-Rad), equipped with argon laser with peak excitation 488 nm and long pass 500 nm filter. The laser intensity, scan speed (500 lines per secod), iris, gain, offset and resolution (512 pixels * 512 pixels) were all kept constant, meaning results from multiple replicas and runs are directly comparable quantitatively.

*Microtiter plate MIC assays*

Samples of *E. coli* from a reference strain (ATCC ) and *E. coli* clinical isolate from a UTI patient that were tested with MCF Lab-on-a-Stick test strips (Fig. 5c) were also tested in parallel following the standardized protocol fully described in literature.[1]

## Supplementary Results

### Analysis of concentration gradient along the microcapillaries

Detailed confocal fluorescence imaging of *in-situ* release of IgM and IgG in the PVOH-coated strips revealed a gradient of concentration along the strip which varies with the length of the strip but also with the reagent type. The strips where scanned along the entire length once the meniscus reached the equilibrium height. In the case of Fig. S1 the release of IgM and IgG was directly compared and showed a sharper concentration gradient for the smaller molecule (i.e. IgG).

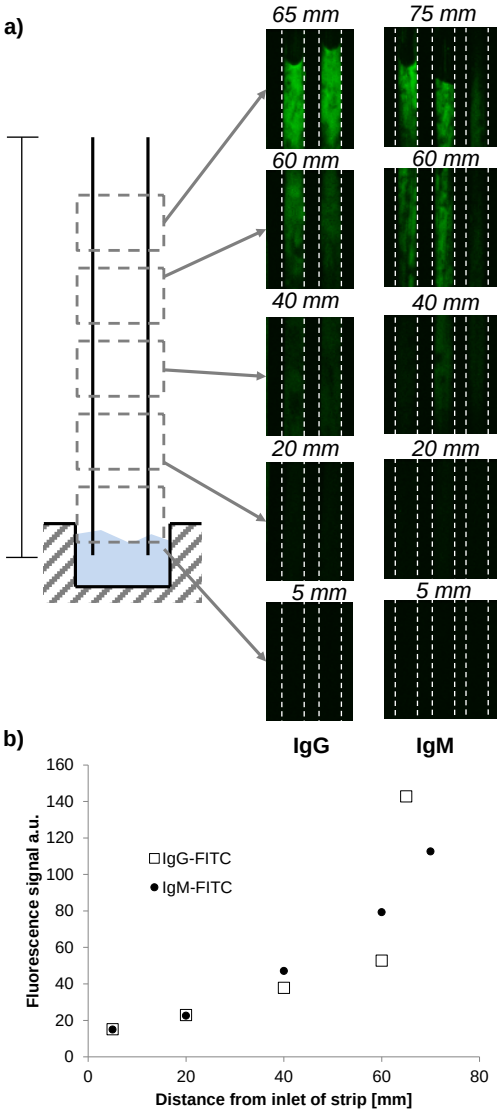


**Fig. S1** Confocal imaging of reagents release in PVOH-coated microcapillaries. All capillaries in the same strip were loaded with same reagent (IgM-FITC or IgG-FITC at 1 mg/ml), and the strips imaged once the meniscus reached the equilibrium height. Images show the capillaries in the centre of the strip at middle height of the capillary, at the axial distance shown in the labelling.

A similar study examined the concentration gradient when colored reagents were loaded and released from hydrophilic-coated MCF test strips. As an example, we selected an important colorimetric enzyme for detecting antibiotic resistance. Beta-lactamase enzymes can degrade antibiotics of the beta-lactam class (e.g. penicillins, crbapenems) and these resistance enzymes are a major global threat. Some beta-lactamase enzymes can be rapidly detected using yellow colorimetric substrate nitrocefin, which is turned red in the presence of beta-lactamase enzymes. Hydrophilic coated MCF was loaded with 10mg/mL nitrocefin dissolved in DMF, and the loading solution gently removed with air using a syringe. As expected, when long (6cm) test strips were dipped into water, a yellow colour was seen (Fig. S2) whereas when dipped into a beta-lactamase enzyme solution, a strong red colour was observed indicating rapid enzymatic conversion of nitrocefin. The reagent gradient was clearly visible, with the top (i.e. near meniscus) showing most intense colour indicating highest reagent concentration, as seen with antibody reagent in Fig S1. This gradient could be quantified by plotting an intensity plot for the blue channel (Fig. S2b). In contrast, a uniform colour was observed when 1mg/mL solutions of nitrocefin or enzymatically converted red product were loaded into MCF strips (Fig. S2c). In spite of the gradient of reagent release, the colour change was evident along the length of the strip, and as evident from the profile plots the dye reagent could be quantified at both the inlet and the meniscus.

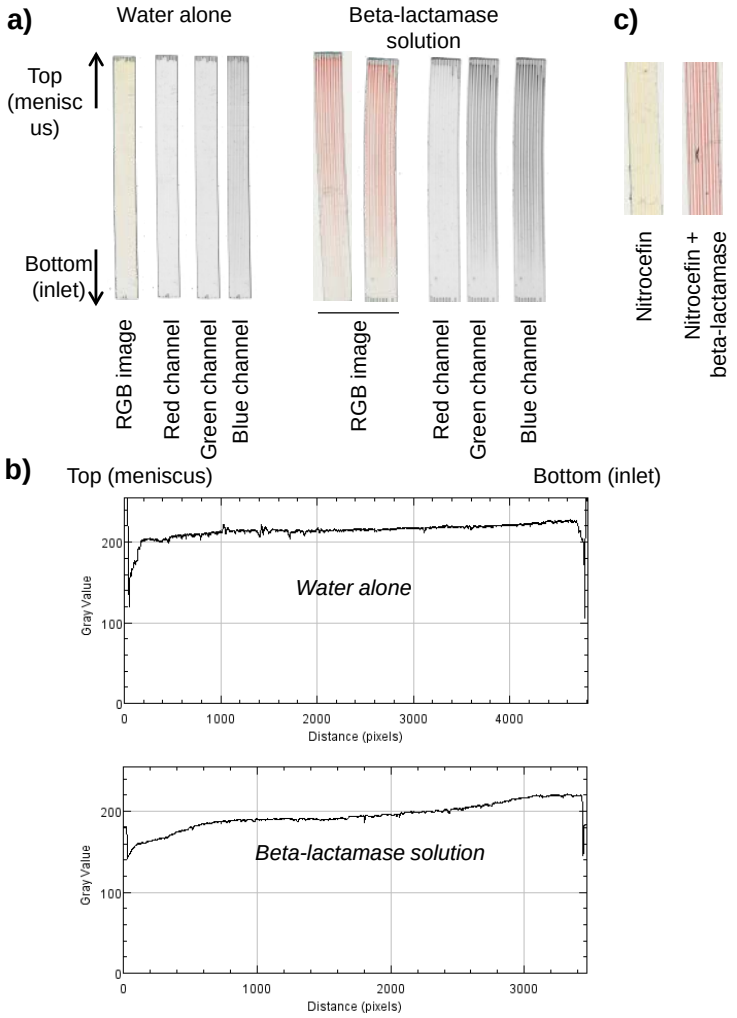


**Fig. S2** Demonstration of gradient of antibiotic resistance enzyme reagent released from Lab-on-a-Stick test strips. a) hydrophilic coated MCF was loaded with 10 mg/mL nitrocefin in DMF, loading solution removed using air, and short test strips cut. These were dipped either into water alone or a solution of beta-lactamase enzyme. Images illustrate RGB image, and greyscale images of the three colour channels split to see the increase in green absorbance on enzymatic conversion, leading to the colour change from yellow to red. b) The blue channel intensity was plotted along the length of a single capillary to illustrate the variation in reagent concentration from the top (meniscus) to the bottom (inlet) of the test strip. c) Solutions of 1mg/mL nitrocefin with or without beta-lactamase enzyme were prepared in microtubes, then filled into MCF strips to illustrate the uniform colour when reagent was directly filled into microcapillaries.

**Analysis of reagents release from the thin film**

A further set of confocal imaging experiments aimed studying the dynamic release of reagents from the thin film as shown in Fig. S3 and further understand the concentration gradients observed e.g. in Fig. 4, Fig. 5 or Fig. S2. All capillaries were loaded with 1 mg/ml of mouse anti-human IgG or IgM fluorescently labelled with fluorescence FITC similar to data in Fig. S1. Capillaries where observed to flow at different time points, this is linked to some variability in the contact angle in the capillaries in this particular experimental setup, which required taping a section of the strip on a glass slide for imaging in the microscope and carefully adding a small volume of PBS buffer in the area around the inlet of the strip.

Fig. S3 shows confocal images of a set of microcapillaries during capillary rise of PBS buffer in strips pre-loaded with IgM-FITC or IgM-FITC. Initially, only a thin layer of FITC labelled antibody is visible in the image on the internal wall of the microcapillary at time 0; note the *z* height of the stack selected was halfway through the height of the microcapillary, therefore two side walls are visible in the image in Fig. S3a. During capillary rise of liquid in the capillaries (it took few seconds for liquid to be detected in the first capillary) it is well visible the higher concentration of reagent dragged by the meniscus, which is further highlighted in Fig. S3b. Once liquid has reached equilibrium height in the capillary, the FITC labelled antibody could be detected everywhere across the capillary (Figure S3c), especially near the walls and quickly diffused towards the centre of the capillary. It was noticed a major difference in diffusion rates of IgM and IgG molecules from the thin film as shown in Fig. S3d, which is linked to very distinct molecular weight of these two antibody molecules. It is believed the concentration gradient generated along the microcapillaries result from the combined effect of the meniscus and passive diffusion of molecules from the thin film, and can be controlled by manipulating diffusion rate of reagents and/or the length of the microcapillary strip.

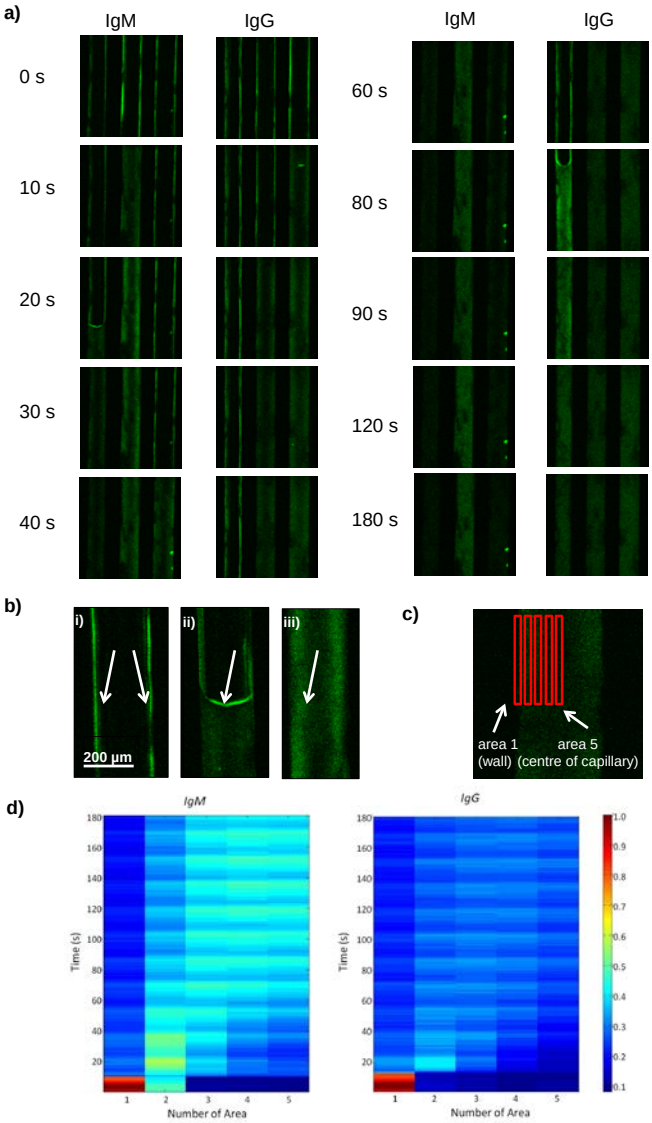


**Fig. S3** Kinetic analysis of reagents release in FEP microcapillaries coated with crosslinked PVOH with confocal microscopy. a) Release of IgM-FITC or IgG-FITC imaged at 60 mm from the inlet of the strip (stacks show 3 capillaries in the centre of the strip at middle height in the capillaries). b) Key features of *in-situ* reagents release, showing in (i) a thin film of FITC-IgG deposited onto the hydrophilic capillary - the arrows point to the position of the walls, (ii) PBS buffer rising into capillary – the arrow points to the position of the rising meniscus and in (iii) release of FITC-IgG by diffusion– the arrow points to the area with the highest antibody concentration. c) Areas of the capillaries where fluorescence signal was interrogated with time allowing to study reagents release from the thin film and diffusion across the microcapillaries. d) Normalised mean fluorescence intensity for the capillaries in the centre of the images in a) for the areas of identified in c).

**Surface characterization of the inner surface of POVH-coated microcapillaries**

SEM and AFM analysis (Figs. S4 and S5, respectively) to the inner surfaces of the microcapillaries revealed the PVOH-coating is homogeneous along and across the microcapillaries. In particular, AFM images show the surface topography of PVOH-coated capillaries is very distinct from that of uncoated microcapillaries.

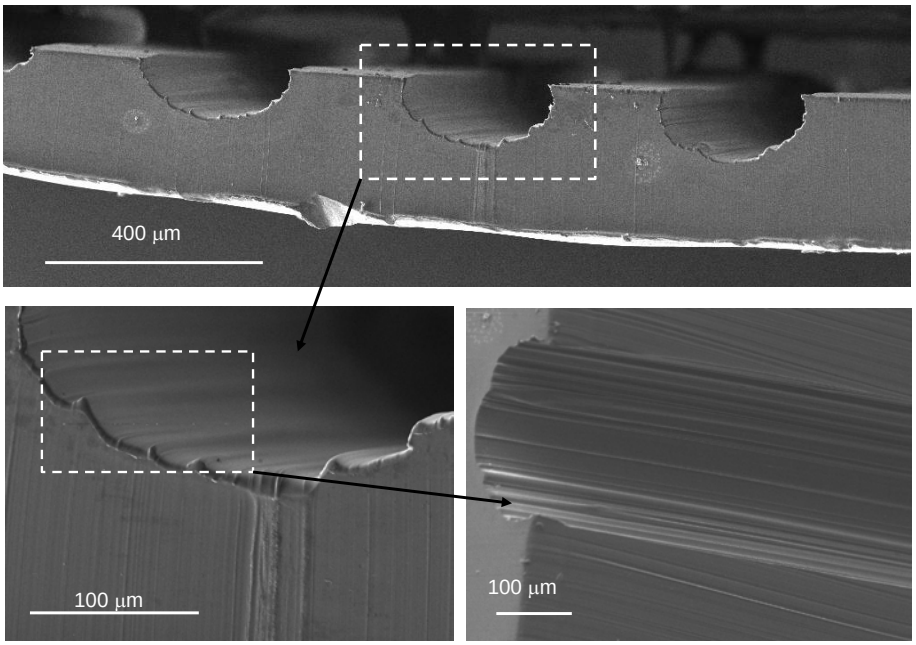


**Fig. S4** Scanning Electron Microscopy image of MCF coated with PVOH, showing a smooth and evenly coated surface. The ridges along the capillaries are due to the cooling during the melt-extrusion process, being also observed in uncoated MCF material (image not shown).

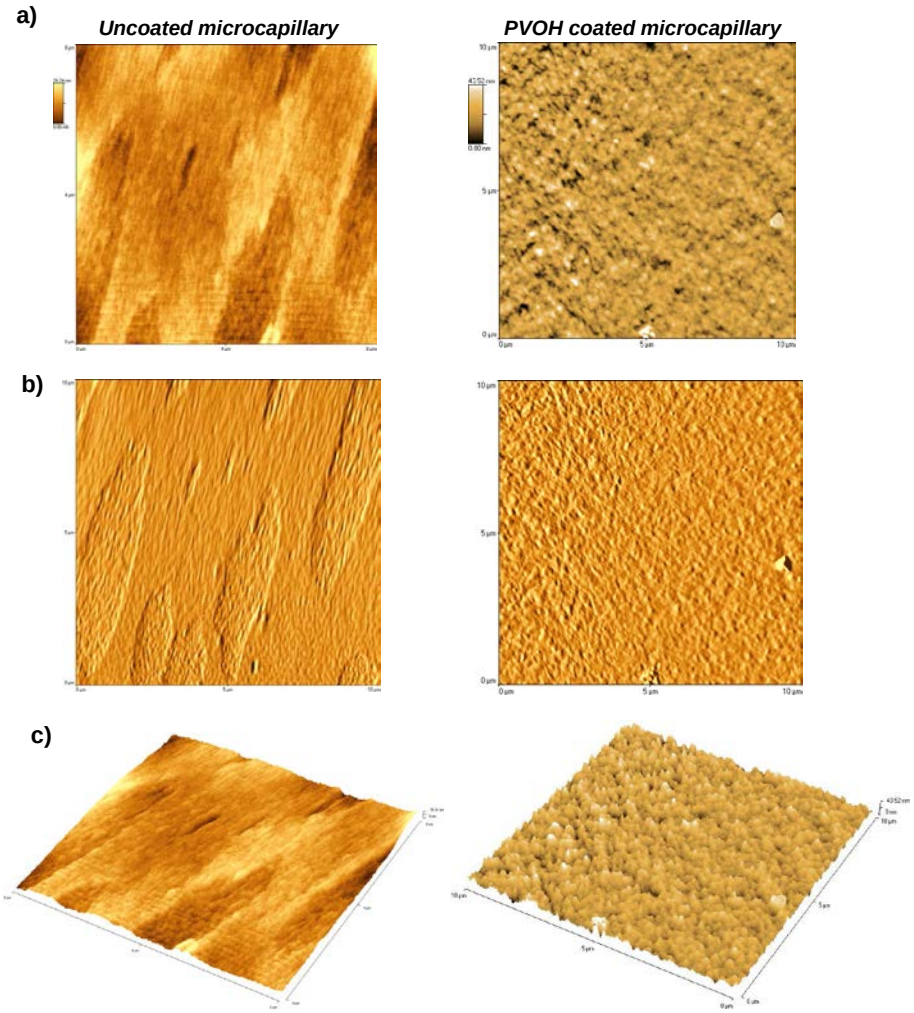


**Fig. S5** AFM images of 10 μm x 10 μm areas in the inner walls of uncoated and PVOH-coated microcapillaries in Tapping (i.e. contactless) Mode. a) 2D topography (true height). B) Error or Tip Deflection. C) 3D Topography.

**Clear optical detection of red blood cells agglutination in the microcapillaries**

The profile plots for positive and negative agglutination were remarkably distinct when agglutination happens within the capillaries (Fig. S6b), being very difficult so differentiate positive agglutination from sample drying when the test is performed on a flat surface like a glass slide or even Eldon card as shown in Fig. S6a. Note the very consistent capillary rise within the same strip, and the reduced capillary rise in the agglutinated sample (Fig. S6b), which explains the variability in capillary rise in the multiplexed blood agglutination strips in Fig. 4.

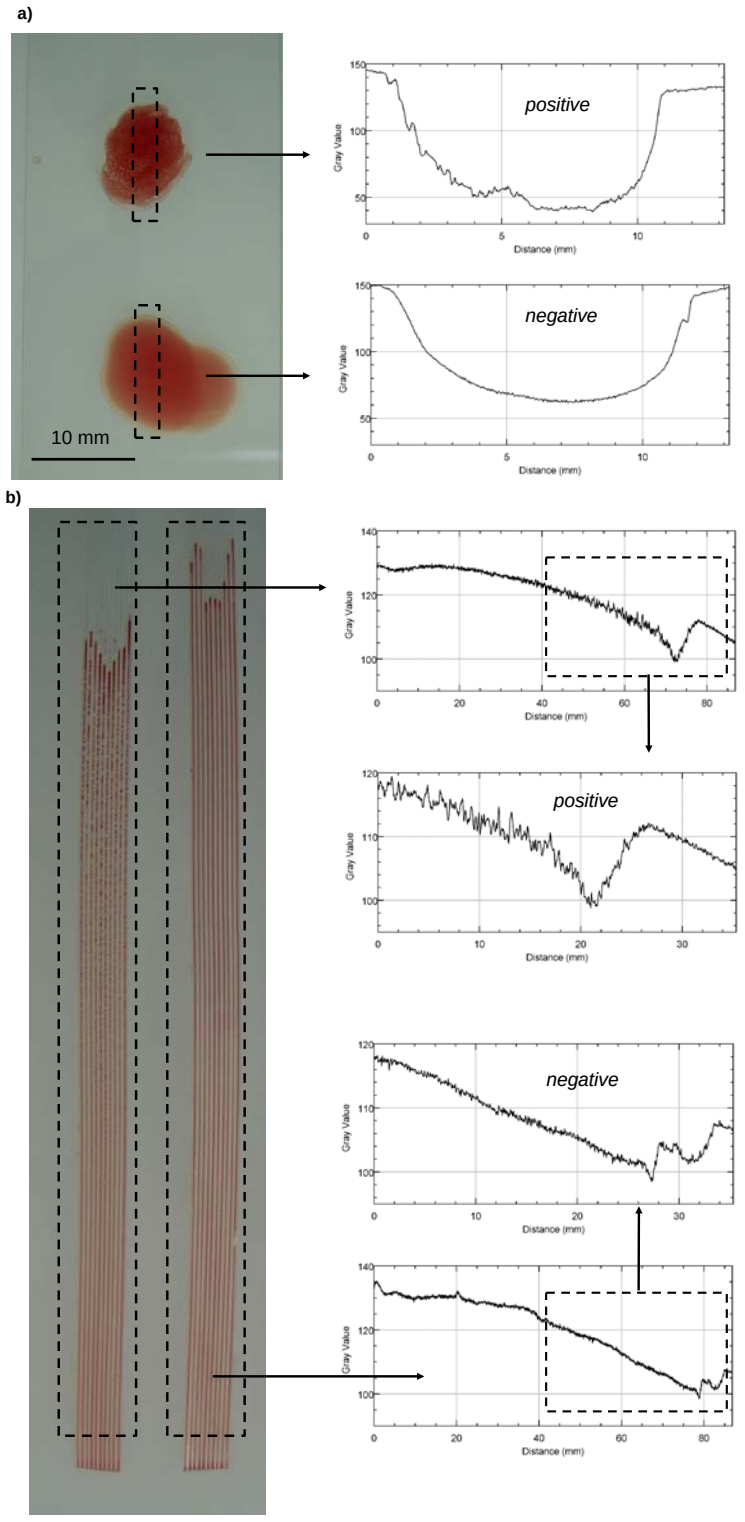


**Fig. S6** Greyscale profile plots obtained with agglutinated and non-agglutinated blood samples. a) Testing on a transparent glass slide, where anti-A antibody was mixed with blood sample using pipette tip. b) Testing in the PVOH-coated MCF; all 10 capillaries where pre-loaded with anti-A reagent which was released and mixed with the sample during rising of blood sample by capillary action.

**MIC assays in microtiter plates**

To compare the performance of antibiotic loaded MCF Lab-on-a-Stick test strips for MIC assays with current 'gold standard' microtiter plate MIC assays, the same samples of *E. coli* reference strain (ATCC ) and *E. coli* clinical isolate from a UTI patient were tested in parallel using the standardized protocol available in literature,[1] and also compared to the range of MIC values for the reference strain also published in Andrews[1] (Fig. S4 and Table S1). The MIC observed in the microplate correlated well with both the published reference values and with the values observed in the MCF test strips. However, it is important to note the even this standard reference protocol is inherently variable, and the published standard method indicates that even for reference strains significant assay-to-assay variation is expected:[1]

"*The MIC for the control strain should be within plus or minus one two-fold dilution of the expected MIC*"

In other words, there is an expectation of variation either one well higher or one well lower concentration than the published reference concentration. Indeed even with this 'gold standard' internationally agreed reference microplate method we do observe variation between replicate microplate tests, with the reference strain having 2-fold lower MIC in 2$^{nd}$ replicate than 1$^{st}$ replicate, and 4-fold lower MIC observed in 2$^{nd}$ replicate than first replicate (Fig. S7). Similarly, although resistant to 3 tested antibiotics, the UTI isolate shows 2-fold lower MIC in 2$^{nd}$ replicate than 1$^{st}$ replicate.

**Table S1.** Antibiotic concentrations used in microtitre plate MIC assays shown in Fig. S7, and comparison with threshold values and expected MIC values taken from Andrews[1]

| | | *Gentamicin* | *Tetracycline* | *Trimethoprim* | *Ciprofloxacin* |
|---|---|---|---|---|---|
| | *E. Coli* 25922 reference MIC (mg/L)[a] | 0.5 | 2 | 0.25 | 0.015 |
| | Resistant: | ≥ 2 | ≥ 2 | ≥ 4 | ≥ 2 |
| | Sensitive: | ≤ 1 | ≤ 1 | ≤ 0.05 | ≤ 1 |
| Concentration (mg/L) | Row 1 | 1 | 8 | 2 | 0.5 |
| | Row 2 | 0.5 | 4 | 1 | 0.25 |
| | Row 3 | 0.25 | 2 | 0.5 | 0.125 |
| | Row 4 | 0.125 | 1 | 0.25 | 0.063 |
| | Row 5 | 0.063 | 0.5 | 0.125 | 0.031 |
| | Row 6 | 0.031 | 0.25 | 0.063 | 0.016 |
| | Row 7 (no ABX) | 0 | 0 | 0 | 0 |
| | Row 8 (no cell control) | No cells | No cells | No cells | No cells |

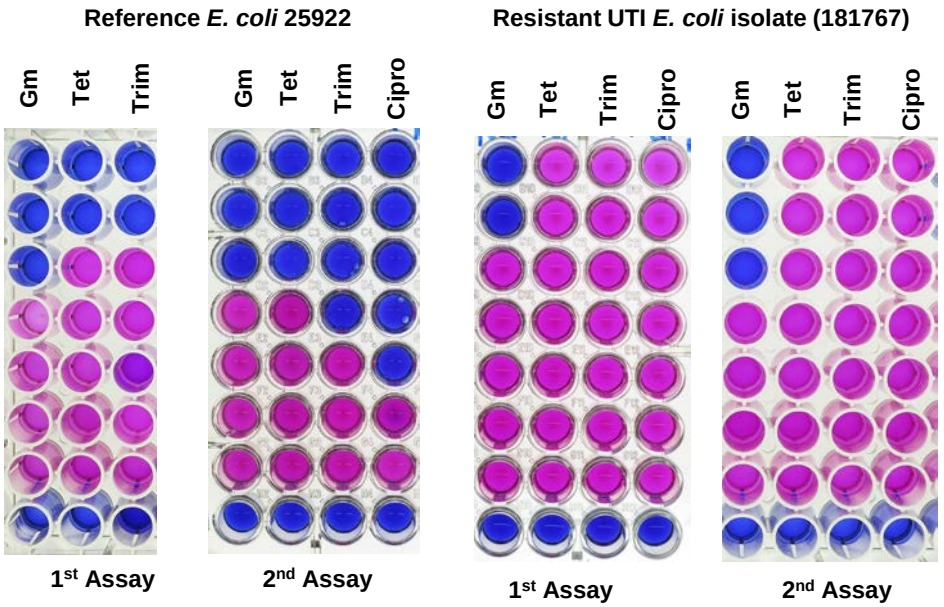


**Fig. S7** Example of conventional microtitre plate MIC assay results.

## Supplementary references

1 J. M. Andrews, *J. Antimicrob. Chemother.*, 2001, **48 Suppl 1**, 5–16.