Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting Information

Gold on Paper – Paper platform for Au-nanoprobe TB detection

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1. Paper platform production and characterisation

In this work we used Whatman No. 1 chromatography paper (Whatman Internacional Ltd., Florham Park, NJ) without any previous treatment. Before producing the paper micro-plates the paper and the wax were characterised in terms of thermal, structural and morphological properties.

To evaluate the thermal decomposition of the paper, thermogravimetry/DSC measurements of the paper were recorded and the residual mass (wt%) plotted as function of temperature – Figure S1a. Two main weight loss regions were detected, one around 90-100 °C and another in the 300-370 °C region. The first decomposition region is associated with moisture and desorption of low molecular weight compounds remaining from the isolation manufacture process. The main decomposition process occurs in the 300-370 °C range, where cellulose decomposition takes place.

Thermal analysis of wax reveals an endothermic reaction at 81.1 °C (maximum) to which corresponds the melting point of the wax and that the wax is stable up to 300 °C – Figure S1b

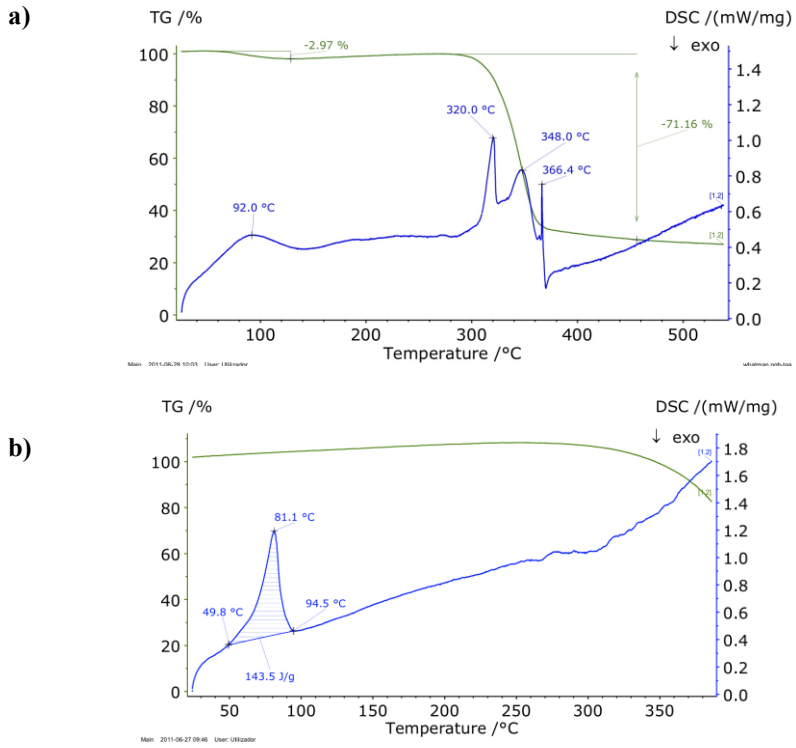


Figure S1 - Thermal gravimetry and differential scanning calorimetry for a) Whatman No. 1 chromatography paper; and b) Solid wax (Xerox ColorQube).

To analyse the structural properties of the paper platform, X-ray diffractometry was carried out. Data in Figure S2a shows that Whatman paper is constituted solely by cellulose as the diffraction spectrum obtained is characteristic of native cellulosic materials (cellulose type I) with its three characteristic peaks: the peak between 14.8º and 16.5º corresponds to the crystalline plane (101), which are possible to identify because this substrate has a high content of cellulose; and the peaks with angles 22.6º and 34.4º corresponding to the crystallographic planes (002) and (004), respectively. No other peaks where identified in this spectrum.

Figure S2b shows the XRD analysis for the Xerox solid wax and paraffin wax. From the analysis of the spectra it was possible to verify that paraffin wax is the major component of solid ink, since all crystalline characteristic peaks (phases) are also found in solid ink spectrum.

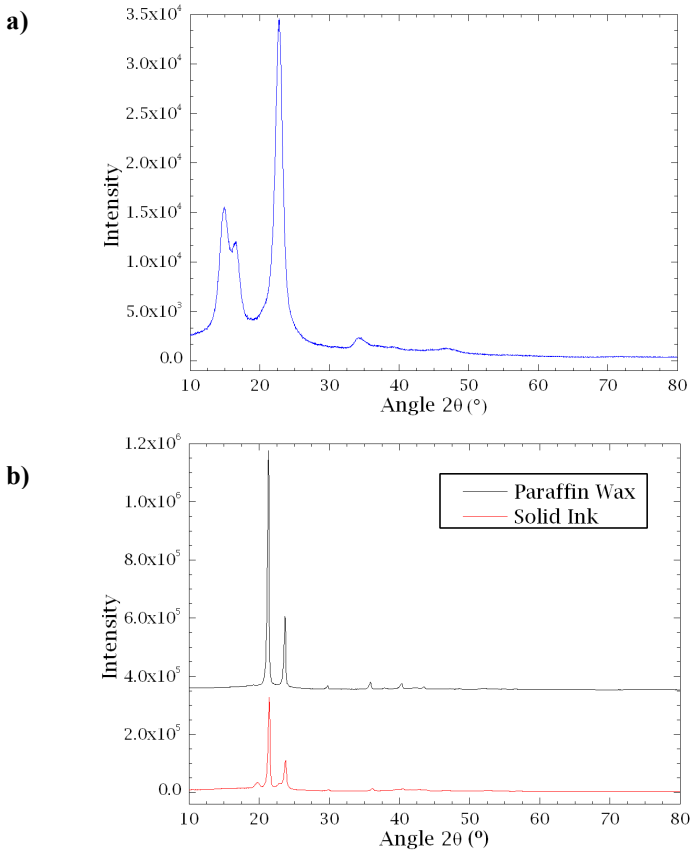
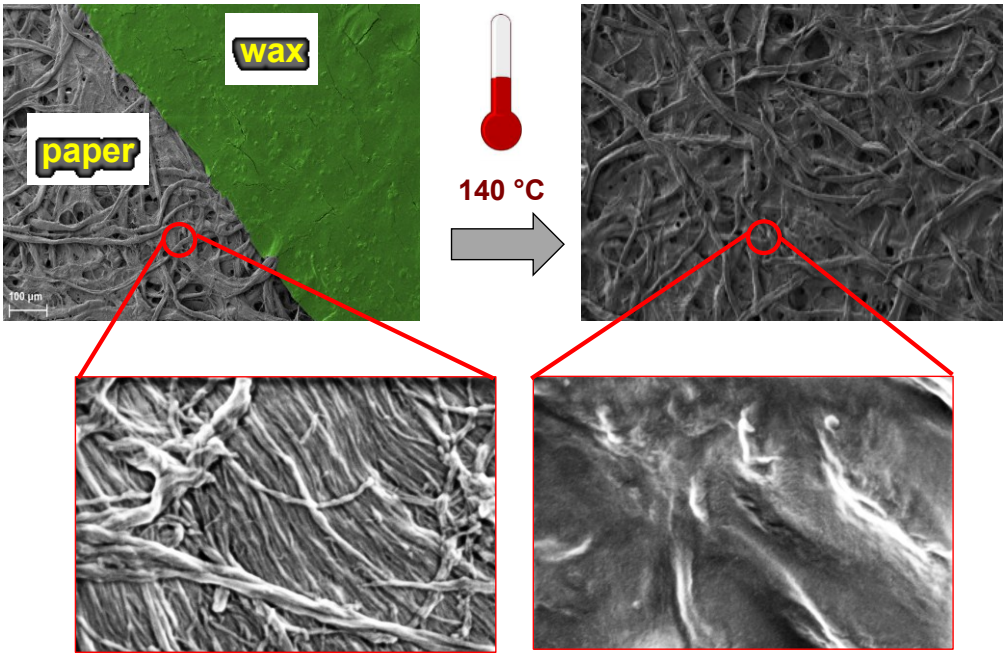


Figure S2 – XRD analysis for a) Whatman No. 1 chromatography paper and b) Solid wax (Xerox).

Scanning Electron Microscopy (SEM) allows the observation of the morphological surface of materials. Since paper is formed in a filtration process, the fibres are approximately layered in the horizontal plane and a typical sheet of paper is ten fibres thick (vertical plane). Cellulose is the building block of paper, and they consist of fibres with approximately 1.5 mm long, 20 μm wide and with a wall thickness of approximately 2 μm. Half of these fibres will be exposed at some point in the paper surface while the others are completely inside the fibre structure. SEM images of Whatman no. 1 paper are shown in fig. S3 - without wax (a) and with diffused wax (b).



a) **b)**

Figure S3 – SEM image of the surface of Whatman no. 1 paper a) with solid ink (green layer); and b) without solid ink diffused. Bottom images are the same as above but with a higher magnification.

2. Wax-printing technology

To produce the paper platforms, commercially available wax-printing technology was used. This approach uses a wax printer and the ink is supplied as solid wax, which is melted before being ejected from the print head. Upon reaching the paper, the wax solidifies instantly. The printed paper is heated up on a hot plate, allowing the wax to move vertically through the porous paper, creating hydrophobic barriers that define hydrophilic channels and reaction zones (fig. S4).

Figure S4 – Equipment used for the fabrication of paper micro-plates: Xerox ColorQube solid ink Printer (left) and a thermal plate (right)

Optimisation of the printing method and the diffusion process for making the hydrophobic barriers was carried out by testing different temperature for wax diffusion. The dependence of the effectiveness (yield) of the line with as a function of the wax diffusion temperature is shown in Figure S5. From the data it is possible to conclude that a yield of 100% is obtained for a temperature of 140 °C and line width of 200 µm.

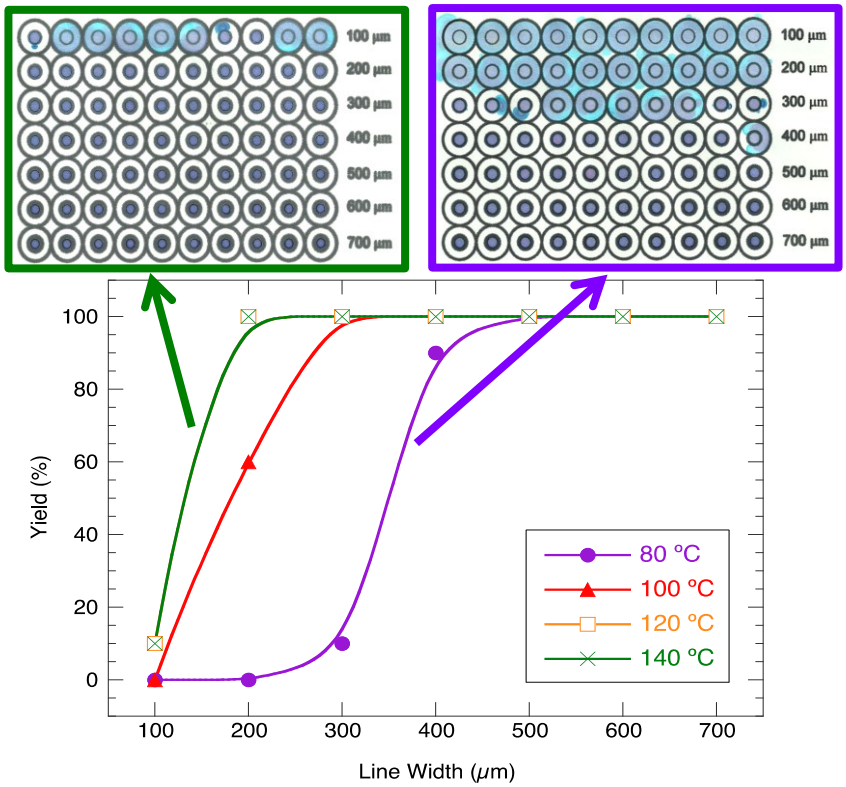


Figure S5 – Dependence of the yield as a function of line width for different diffusion temperatures.

Fig. S6 shows a typical optical micrograph of the cross section of Whatman no. 1 paper, were it is possible to distinguish the hydrophobic regions from the hydrophilic path (coloured with red dye).

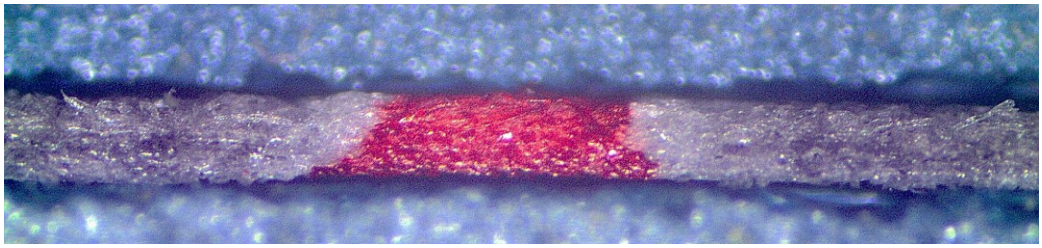


Figure S6 – Optical micrograph of the cross section of Whatman no. 1 paper.

3. Au-nanoprobe sequence specificity (Supplementary table 1)

Sample	rpoB region sequence	Sequence identity to MTBC probe
MTBC sample *M. tuberculosis* H37RV GenBank Ac. No. L27989	AGCGGATGACCACCCA**GGACGTGGAGGCGATC**ACACCGCAGACGTT	16/16
non-MTBC sample *M. kansasii* GenBank Ac. No. AY544934	AACGGATGACCACTCA**GGACGTcGAGGCGATC**ACGCCGCAGACACT	15/16
Non-related sample *Plasmodium berghei* GenBank Ac. No. M14599.1	-	0/16

MTBC: *Mycobacterium tuberculosis complex species. Small caps "c" points to the sequence mismatch to that of the Au-nanoprobe*

4. Au-nanoprobe assay UV-visible absorption spectra

Comparison of a typical absorption spectra for Au-nanoprobe assay in a standard plastic micro-plate shows comparable aggregation profiles following salt induced aggregation to that of the proposed paper micro-plate methodology.

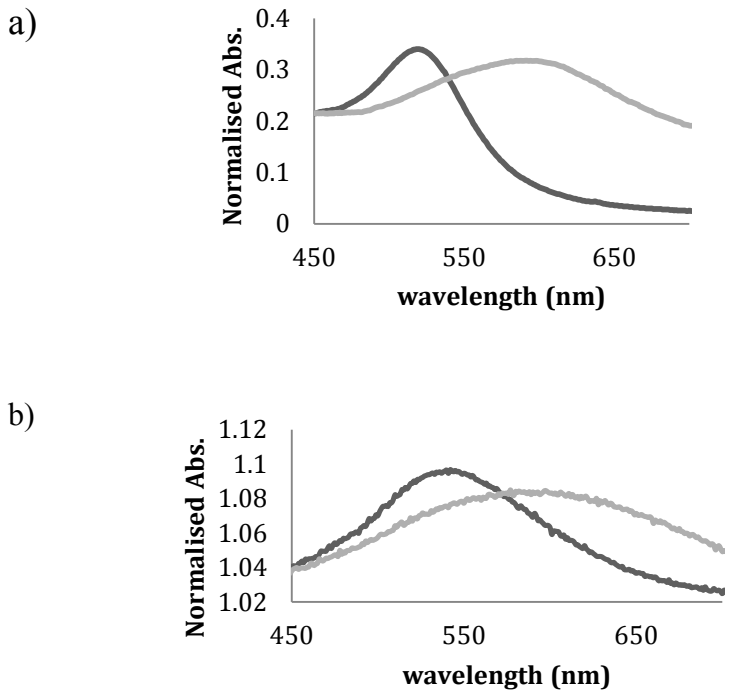


Figure S7- UV-visible spectra of Au-nanoprobe assay attained via: a) standard 384 well micro-plate versus b) paper micro-plate – *Gold on Paper*. Black line - Positive sample; grey line - Negative sample.

5. Limit of detection

Comparison of the Au-nanoprobe detection analysis via the *Gold on paper* platform and the standard microplate reader. The response of the detection strategy as function of DNA sample concentration was evaluated (Fig. S8) and differences between positive and negative sample are statistically significant (ST2 and 3)

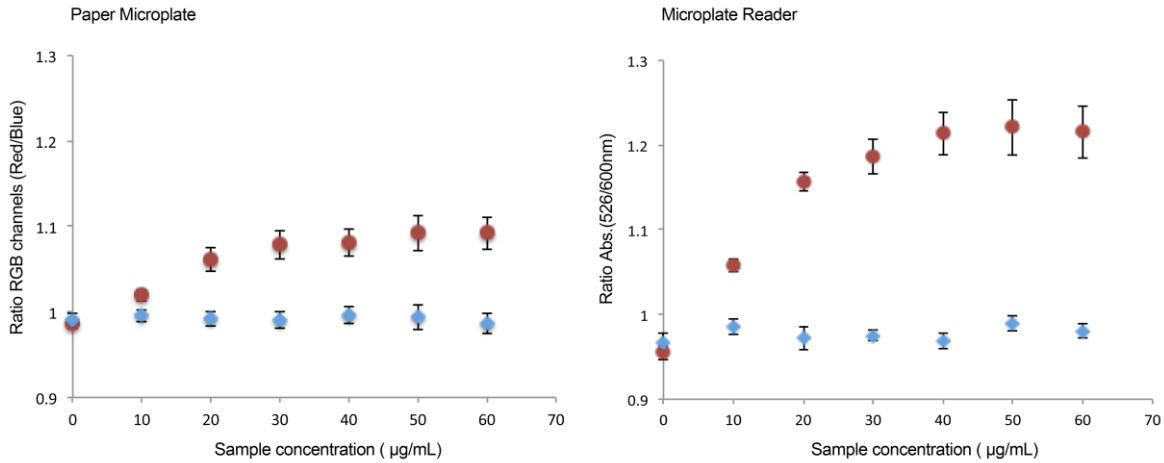


Figure S8 – Signal variation as function of DNA sample concentration. Comparison between A) *Gold on paper* platform and B) microplate reader. Each assay is the average of three independent measurements and the error bars represent standard deviation. Red – positive (MTBC) sample and Blue – negative (non-MTBC) sample.

Supplementary table 2 - Tukey's Multiple Comparison Test for *Gold on paper* platform

	Mean Diff.	q	Significance P < 0.001	Summary	99.9% CI of diff
MTBC sample 10ng/uL vs MTBC sample 20ng/uL	-0.01735	2.669	No	ns	-0.06732 to 0.03262
MTBC sample 10ng/uL vs non-MTBC sample 10ng/uL	0.07018	11.54	Yes	***	0.02343 to 0.1169
MTBC sample 10ng/uL vs non-MTBC sample 20ng/uL	0.069	11.87	Yes	***	0.02430 to 0.1137
MTBC sample 20ng/uL vs non-MTBC sample 10ng/uL	0.08753	14.39	Yes	***	0.04078 to 0.1343
MTBC sample 20ng/uL vs non-MTBC sample 20ng/uL	0.08635	14.85	Yes	***	0.04165 to 0.1310
non-MTBC sample 10ng/uL vs non-MTBC sample 20ng/uL	-0.001181	0.2211	No	ns	-0.04224 to 0.03987

Supplementary table 3 - Tukey's Multiple Comparison Test for standard microplate reader

	Mean Diff.	q	Significance P < 0.001	Summary	99.9% CI of diff
MTBC sample 10ng/uL vs MTBC sample 20ng/uL	-0.09902	15.9	Yes	***	-0.1549 to -0.04312
MTBC sample 10ng/uL vs non-MTBC sample 10ng/uL	0.07231	11.61	Yes	***	0.01641 to 0.1282
MTBC sample 10ng/uL vs non-MTBC sample 20ng/uL	0.08604	13.82	Yes	***	0.03013 to 0.1419
MTBC sample 20ng/uL vs non-MTBC sample 10ng/uL	0.1713	27.52	Yes	***	0.1154 to 0.2272
MTBC sample 20ng/uL vs non-MTBC sample 20ng/uL	0.1851	29.72	Yes	***	0.1292 to 0.2410
non-MTBC sample 10ng/uL vs non-MTBC sample 20ng/uL	0.01372	2.204	No	ns	-0.04218 to 0.06962

6. Gold on paper assay

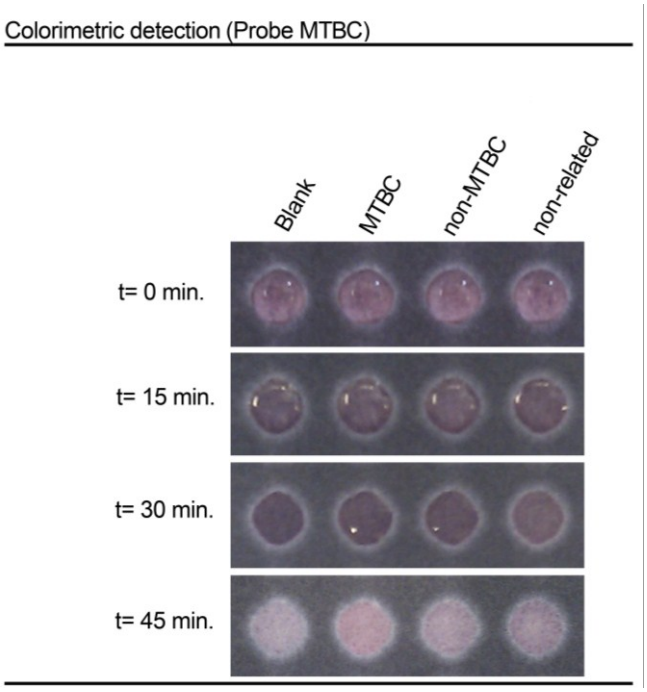


Figure S9 – Colour variation of spotted assay on *Gold on paper* platform as function of time. Drying the spotted reaction mixture onto the paper platform allows colour revelation. Optimal colour discrimination is attained after 45 minutes (complete evaporation of solvent).