Electronic Supplementary Material (ESI) for Analytical Methods.


## Supplementary Data

*Electronic supplementary information*

**Movie S1** Video showing trapping of hydrogels in microfluidic chip.

**Fig. S1.** (A) Photograph of the toaster-sized reader used for flow automation and fluorescence detection (B) Fluidic mechanism showing how liquids are dispensed into the channel and flown through suction pumping. (C) Device for hydrogel fabrication off chip.

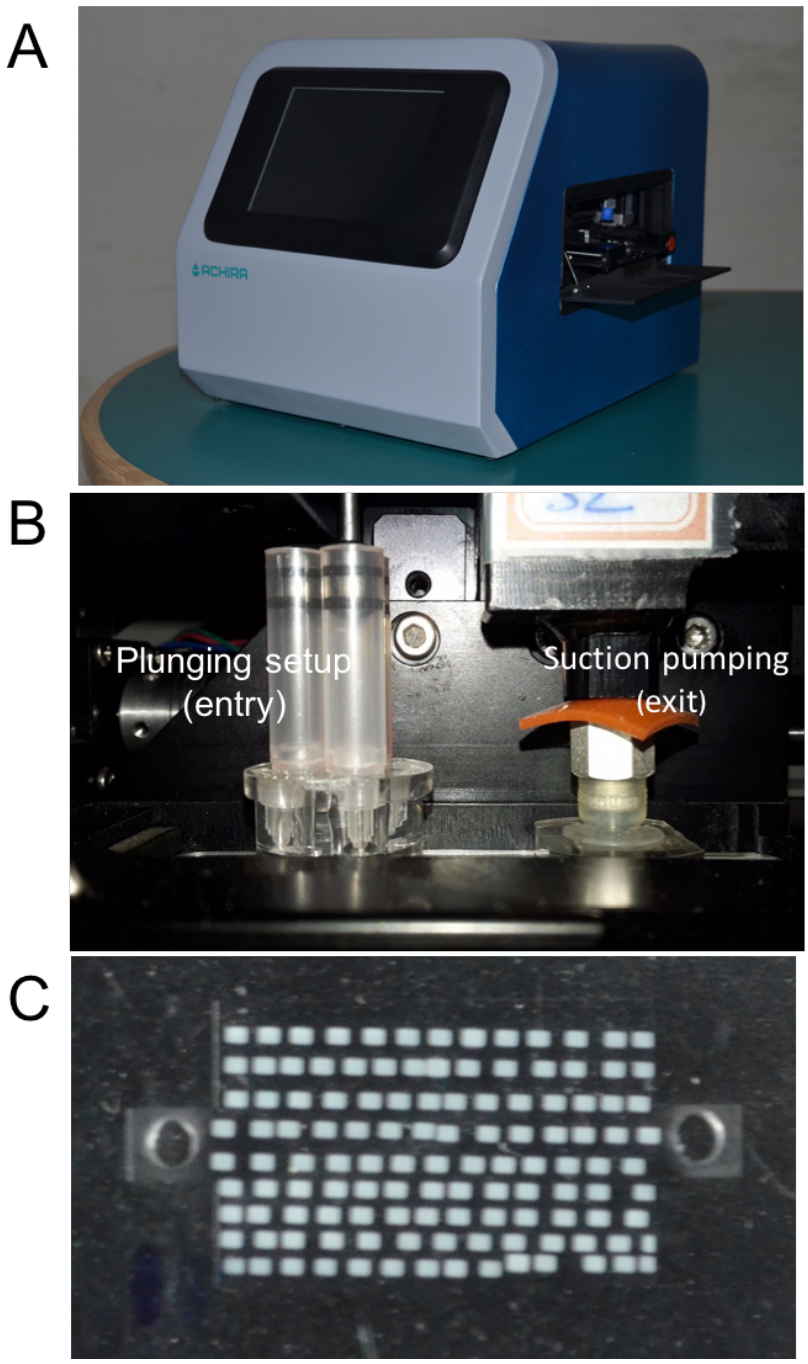
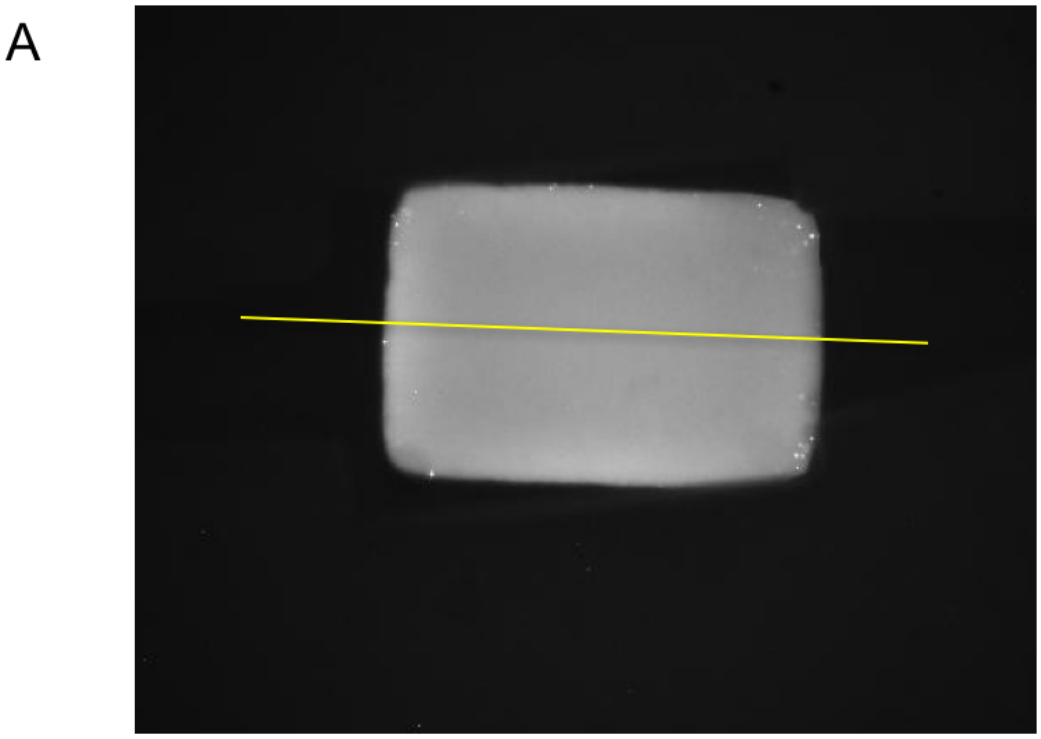


**Fig. S2.** (A) Image of a hydrogel sensor captured at the end of the assay. (B) A uniform signal is seen across the length of the gel as measured by the fluorescent intensity from end-to-end.



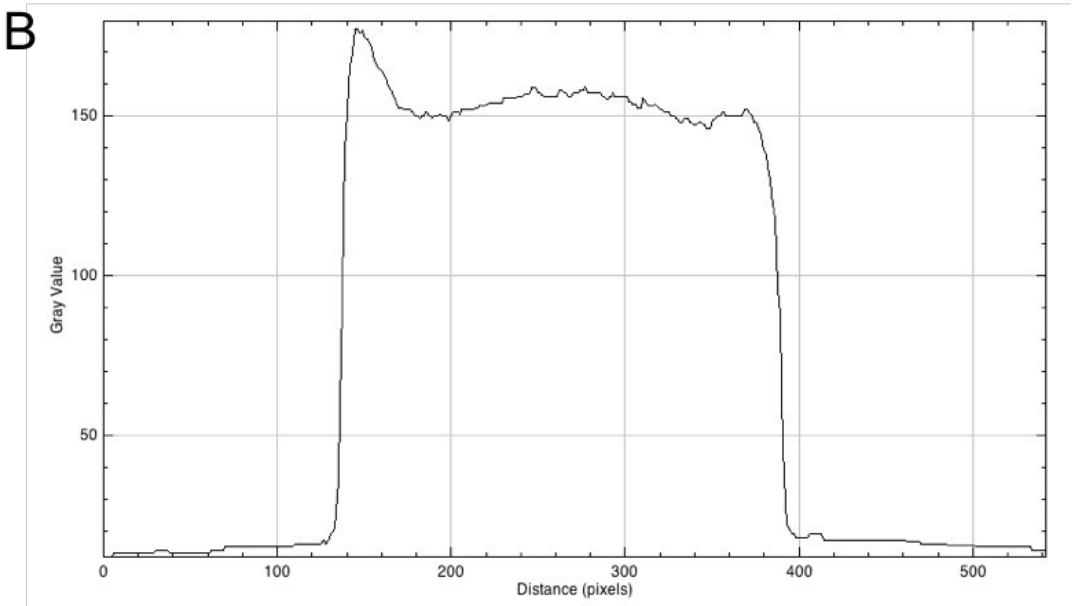



**Fig. S3.** Bland altman analysis of (A) β-HCG (B) FSH (C) LH (D) PRL hormone tests.

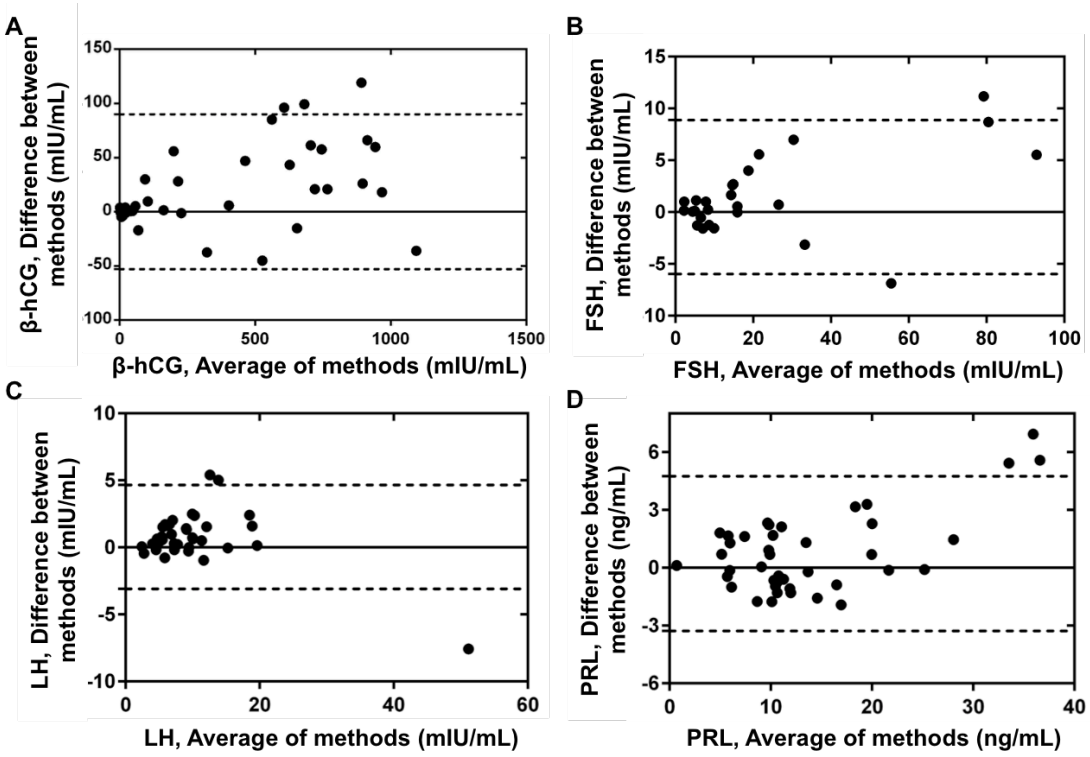


**Table S1**. Size recovery of hydrogels post drying and hydration.

| | **Hydrogel size (μm)** | | |
|---|---|---|---|
| **β-HCG** | Before drying | After drying and hydration | Recovery (%) |
| Length | 525 | 505 | 96.2 |
| Width | 356 | 354 | 99.4 |
| Height | 140 | 129 | 92.1 |
| **FSH** | | | |
| Length | 510 | 497 | 97.5 |
| Width | 343 | 336 | 98.0 |
| Height | 135 | 125 | 92.6 |
| **LH** | | | |
| Length | 487 | 490 | 100.6 |
| Width | 345 | 337 | 97.7 |
| Height | 139 | 136 | 97.8 |
| **PRL** | | | |
| Length | 510 | 476 | 93.3 |
| Width | 363 | 339 | 93.4 |
| Height | 143 | 139 | 97.2 |
| **Negative** | | | |
| Length | 510 | 470 | 92.2 |
| Width | 355 | 334 | 94.1 |
| Height | 135 | 131 | 97.0 |

Note: Hydrogel size recovery was tested after 1 week of storage of dried hydrogels at 4$^{o}$C in vacuum. N=3, Standard deviation was ≤ 10%.

**Table S2.** Intra-batch precision: assay precision tested within same batch of hydrogels for three different hormone concentrations in triplicate.

| FSH (mIU/mL) | X | σ | C.V. |
|---|---|---|---|
| 5 | 4.9 | 0.2 | 4.6 |
| 10 | 8.3 | 0.8 | 1.6 |
| 50 | 43.4 | 9.7 | 3.6 |
| LH (mIU/mL) | | | |
| 5 | 5.35 | 0.4 | 8.1 |
| 25 | 22.4 | 1.2 | 5.5 |
| 100 | 99.5 | 9.5 | 9.6 |
| PRL (ng/mL) | | | |
| 10 | 9.77 | 0.4 | 3.7 |
| 25 | 22.9 | 0.2 | 0.9 |
| 100 | 104.73 | 10 | 9.5 |
| β-hCG (mIU/mL) | | | |
| 25 | 30.45 | 0.63 | 2.08 |
| 500 | 495.33 | 39.2 | 7.91 |
| 1000 | 998.71 | 11.26 | 1.12 |

**Table S3.** Inter-batch precision: Assay precision tested within different hydrogel batches for one hormone concentration in triplicate.

| Hormone | Concentration | N | X | σ | C.V. |
|---|---|---|---|---|---|
| FSH | 50 | 11 | 99.9 | 7.77 | 7.78 |
| LH | 100 | 7 | 101 | 9.96 | 9.86 |
| PRL | 100 | 5 | 98 | 8.86 | 9.04 |
| β-HCG | 100 | 7 | 97.71 | 7.38 | 7.56 |

**Table S4.** Cross-reactivity (%) of capture antibody was tested using highest concentration of hormones used in the study.

| | | Hormone used | | | | |
|---|---|---|---|---|---|---|
| Capture antibody | | FSH | LH | TSH | β-HCG | PRL |
| | FSH | 100 | 0.7 | 1.25 | 0.62 | ND |
| | LH | 0 | 100 | 1.25 | 0.28 | ND |
| | PRL | 0 | 0 | 0 | 0 | 100 |
| | β-hCG | 0 | 0 | 0 | 100 | ND |

N=3, Standard deviation was ≤ 10%.

**Table S5.** Effect of hemoglobin interference on assay.

| Hormone | X | σ | C.V. |
|---|---|---|---|
| β-hCG | 452 | 5.65 | 1.25 |
| FSH | 45.42 | 1.34 | 2.94 |
| LH | 83.3 | 0.91 | 1.09 |
| PRL | 161.73 | 8.91 | 5.51 |

Calibrator concentration used for interference study was β-hCG- 500 mIU/mL, FSH- 50 mIU/mL, LH- 100 mIU/mL and PRL- 150 ng/mL.

**Table S6.** Stability of β-HCG, FSH, LH, and PRL capture antibody in hydrogels stored and dried in the presence of stabilizers.

| | Stabilizer and hydrogels containing capture antibody | | | |
|---|---|---|---|---|
| Day | Liquid plate sealer | 0.45 M Trehalose + 1% PEG 3.4 K | | |
| | HCG | FSH | LH | PRL |
| 0 | 100 | 100 | 100 | 100 |
| 7 | 105 | 97 | 98 | 108 |
| 10 | ND | 100 | 101 | 80 |
| 15 | 97 | 92 | 85 | 83 |
| 21 | ND | 88 | 94 | 76 |
| 30 | 99 | ND | ND | ND |
| 45 | 95 | ND | ND | ND |

N=3, Standard deviation was ≤ 10%. ND: not determined.

**Table S7.** Stability of FSH, LH and PRL detection antibody on conjugate pad stored in vacuum desiccator at 4$^{o}$C.

| | FSH | | | LH | | | PRL | | |
|---|---|---|---|---|---|---|---|---|---|
| Days | X | σ | C.V. | X | σ | C.V. | X | σ | C.V. |
| 80 | 95.5 | 4.9 | 10.7 | 94.6 | 6.2 | 6.4 | 86.7 | 11.7 | 13.5 |
| 120 | 99.3 | 0.8 | 1.8 | 105.1 | 3.3 | 3.9 | 103.6 | 13.1 | 12.7 |
| 180 | 75.9 | 1.8 | 4.6 | 83.5 | 4.2 | 4.5 | 113.1 | 2.6 | 2.3 |

**Table S8.** Cost of different cartridge elements for a batch of 20,000 cartridges

| Item | Cost (USD) |
|---|---|
| Plastic disposables (including cartridge and other consumables) | 0.53 |
| Antibodies and other reagents (for 3 tests FSH, LH, Prolactin) | 0.59 |
| Packaging | 0.09 |
| Total | 1.21 |