24 January 2025

A NEW METHOD FOR CONCURRENT ANTIBODY & RNA VIRAL-LOAD DIAGNOSTICS

TD, TC, DMG, BOF, NO, DMC, CK, JOD, MMC, JZ, XC, KO, JW, YZ, PFF, LW, MA
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ABSTRACT

We introduce a novel method of molecular testing which replaces **Optical Detection** with **Electronic Detection.** Our assay utilizes synthetic peptide nucleic acid (PNA) probes for NA capture direct from raw samples, antigenic peptide sequences for antibody capture, superparamagnetic beads, electromagnetics, microfluidics and our proprietary CMOS silicon chip detector. This enables simultaneous genetic and serology testing.

Results are presented for detection of HIV-1 & HIV-2 antibodies from HIV-positive patient plasma; and detection of SARS-CoV-2 RNA virus from human saliva. Our technology, when encapsulated in inexpensive single use cartridges, will enable complex molecular & serology testing to be undertaken cost-effectively[1] at the point of need.

PROPRIETARY MATERIALS

Digital CMOS Sensor Chip[2]:

Single superparamagnetic beads are specifically tethered by the target to our sensor & quantitatively detected by employing fringe-field sensing:

Fig.1: The CMOS sensor 'fringe-field' capacitive sensing principle, and electronic signal amplification:

PNA Probes[3] (for NA capture):

Altratech-designed PNA probes for specific target capture, directly from samples. We have co-developed chiral PNAs synthesized in FMOC chemistry with the US National Institute of Health[3]. PNA probes are known for their excellent specificity[4], e.g. Single-Base-Mismatch specificity:

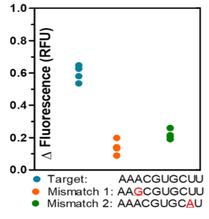


Fig.2: SBM specificity of SARS-CoV-2 capture by PNA probes from human saliva (BioIVT). (Evaluated by plate-reader for benchmarking.)
Specific capture (> 95%) of SARS-CoV-2 from human saliva (BioIVT) was also demonstrated, with no false positives (40/40) with background of Flu-A/B, RSV, 229E, OC43, NL63, HKU1 cross-reactives.

Antigenic Peptides (for Antibody capture):

We synthesize these with the same FMOC chemistry

Superparamagnetic Beads:

PNA-coated superparamagnetic beads with captured Target are magnetically removed from the sample.

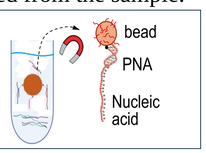


Fig.3: (a) Human saliva sample in vial.
(b) Capture & extraction of SARS-CoV-2 RNA

PROTOTYPE & HARDWARE

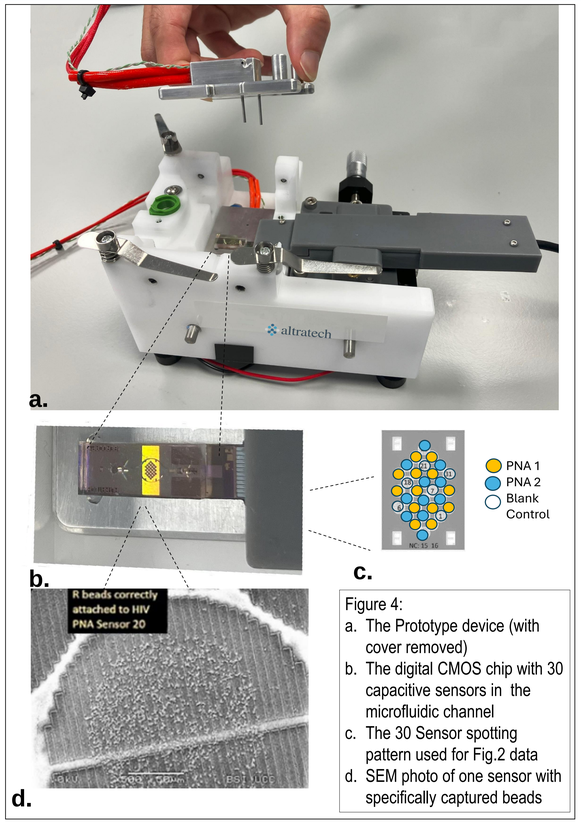


Figure 4:
a. The Prototype device (with cover removed)
b. The digital CMOS chip with 30 capacitive sensors in the microfluidic channel
c. The 30 Sensor spotting pattern used for Fig.2 data
d. SEM photo of one sensor with specifically captured beads

2025 FULLY INTEGRATED DEVICE

- Full integration of sample-input with detection[4] underway in 2025, in conjunction with Cambridge Design Partners (UK)
- Clinical validation with 140 HIV patient samples planned to begin in Q4'2025 at St. Cecilio Hospital, Granada, Spain.

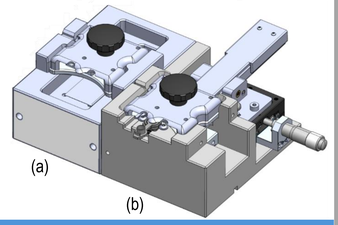
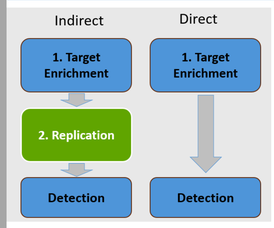


Fig.5: (a) Sample loading & processing (integration of Fig 2)
(b) Detection & Quantification unit of Fig 3 above.

2026 HIGH VOLUME PRODUCT

Fig. 6: Single-use disposable per test (low-cost injection-moulded) Reusable Base Reader Unit with encryption & wireless upload.

ASSAY METHOD

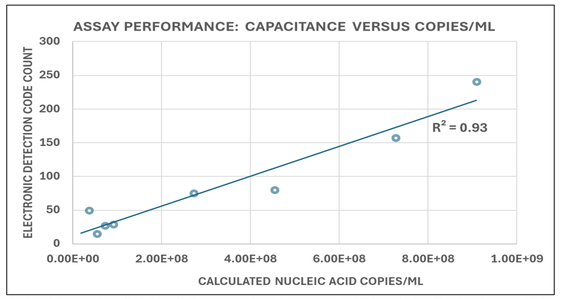


Indirect: incorporates a rapid enzymatic step, which tags and replicates the target. This provides for improved sensitivity, which is critical for applications assessing low-titre samples. Quantitative ability is limited.

Direct: Allows direct detection of target molecule and quantification (current R^2=0.93) using 'detection by proxy' where the bead being detected is a 'proxy' for the RNA or analyte attached to it.

NUCLEIC ACID DATA

Fig.7: **Direct** method: Linear quantification of synthetic SARS-CoV-2 in human-saliva:



Limit-of-Detection (LOD) of **direct** method is currently1E6 cp/mL. Reduction to 1E3 (1000 cp/mL) is planned with full assay integration & miniaturisation, see Fig.8:

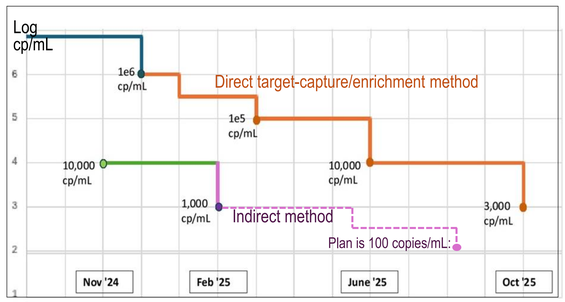


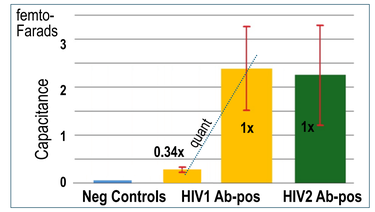
Fig.8: Analytical Sensitivity: LOD progress & roadmap of direct & indirect assay methods:

ANTIBODY DATA

The key principle of our technology, **Detection by Proxy[4],** enables this hardware and **direct** assay to also **detect and quantify protein targets:**

Fig 9:
Capacitance-vs-HIV1 & HIV2 immuno-assay **Antibodies** (n=2)

→HIV 1 & 2 antibodies detected in HIV-positive-patient plasma (NIBSC pos-control).



In this data we demonstrate the replacement of the enzyme in a commercially-available HIV antibody test with our superparamagnetic PNA coated reporter beads. This allows specific detection by complementary PNA's on the sensor chip.

DISCUSSION

Analytical specificity:

PNA's neutral charge enables them to bind targets in raw samples, with excellent specificity (Fig.2). This eliminates centrifuging and sample-prep steps from this assay.

Qualitative & Quantitative performance:

The synthetic PNA-probe and CMOS-sensor foundations of the assay are the same for both methods. The direct method gives quantification with excellent linearity, and has potential applications in genotyping and drug development. The indirect method (with extra replication step) is currently qualitative, has lower LOD, and is facilitating rapid hardware improvement.

As upstream advances in Target Enrichment are implemented, the direct method with no replication step will supplant the indirect approach when the sensitivity levels are comparable.

CONCLUSION

We have introduced a first-of-kind multi-analyte combo DNA/RNA/Antibody/Antigen diagnostic. Our objective is to deliver in 2025 a portable prototype with digital molecular diagnostic capability at an LOD of ≤1000 cp/mL[5]; in 2026 a smaller battery-operated portable version to meet the WHO 'REASSURED' criteria[6], and in 2027 the entire multi-analyte capability in portable kit format.

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4. Altratech patents *US11274291/10738348/11796498, US11459601* and EU/CN/JP equivalent granted patents. ***(Altratech Ltd owns an extensive patent portfolio covering all aspects of this product & technology).***
5. HIV Diagnostic rec: *WHO, ISBN 978-92-4-151621-1.*
6. https://www.nature.com/articles/s41564-018-0295-3

ACKNOWLEDGEMENTS

- Tech-transfer of PNA synthesis from NIH to Altratech & SARS-CoV-2 capture from saliva was funded by **BARDA contract 75A50122C00067.**
- End-to-end assay proof-of-principle was funded by **EU-H2020 'Easiviral' grant 768090** & Enterprise Ireland.
- The 2025 'Triple-H' HIV-HCV-HBV productization is being funded by **EIC-Accelerator 'UbiHealth'** grant (101188322) & EU public-private matched equity investment.