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

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

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ABSTRACT

Altratech introduce a novel method of molecular detection. This method utilizes synthetic peptide nucleic acid (PNA) probes for NA capture, antigenic peptides for antibody capture, super-paramagnetic beads, electromagnetics, microfluidics and CMOS silicon chip technology. Optical detection is replaced with electronic detection. This novel assay enables simultaneous genetic and serology testing.

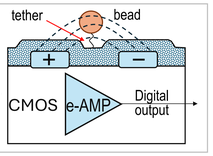
Results are presented for detection of HIV-1 & HIV-2 antibodies from HIV-positive patient plasma; and detection of SARS-CoV-2 RNA virus from human saliva. Altratech's technology, when encapsulated in inexpensive single use cartridges, will enable complex molecular testing to be undertaken at the point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

PROPRIETARY MATERIALS

Digital CMOS Sensor Chip[3]:

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

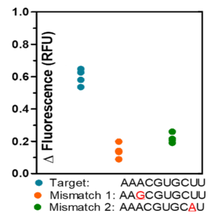
Fig 1: The CMOS sensor 'fringe-field' capacitive sensing principle, and electronic signal amplification.



PNA Probes (for NA capture):

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



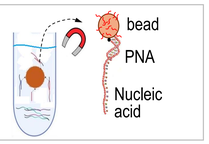
Antigenic Peptides (for antibody capture):

Synthesized with the same FMOC chemistry as PNA.

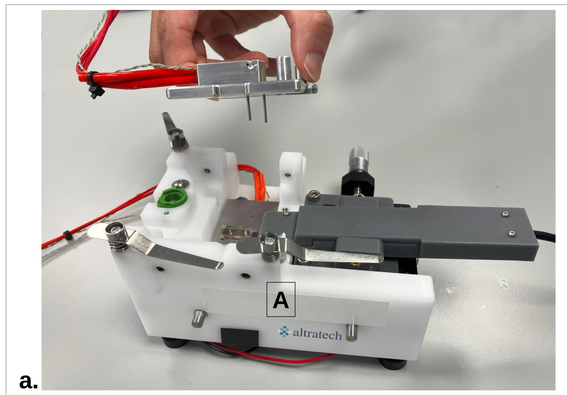
Superparamagnetic Beads:

PNA-coated superparamagnetic beads with captured Target are magnetically removed from the sample[5].

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



PROTOTYPE & HARDWARE



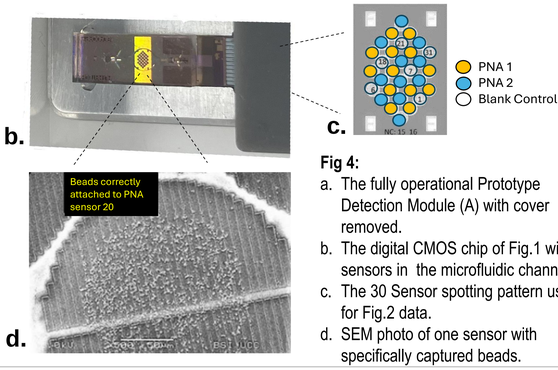
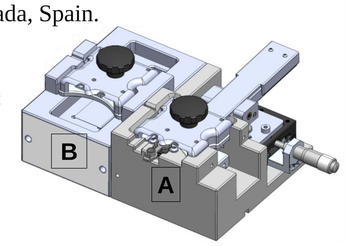


Fig 4:
a. The fully operational Prototype Detection Module (A) with cover removed.
b. The digital CMOS chip of Fig.1 with 30 sensors in the microfluidic channel.
c. The 30 Sensor spotting pattern used for Fig.2 data.
d. SEM photo of one sensor with specifically captured beads.

2025 FULLY INTEGRATED DEVICE

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

Fig.5: (a) Detection & Quantification unit of Fig 4 above.
(b) Sample loading & processing (integration of Fig 3)



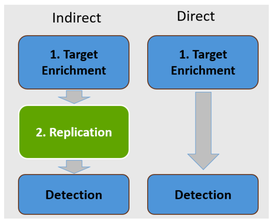
2026 HIGH VOLUME PRODUCT

The final product will be a small form-factor, low-cost cartridge and wireless reader unit (Fig 6). This miniaturisation is made possible through the solid-state nature of the device and by replacing Optical Detection with our CMOS enabled Electronic Detection:

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

ASSAY METHOD[5]

There are two assay variants: **(1) Indirect** and **(2) Direct**.



Indirect Detection: Incorporates a single enzymatic step, which tags and replicates the target. This provides for improved sensitivity, which is critical for applications assessing low-titre samples.

Direct: Without the requirement for an enzymatic step, this variant allows direct detection of target molecule and quantification (currently R^2 = 0.93), using 'detection by proxy; where the bead being detected is a 'proxy' for the RNA or analyte attached to it.

The indirect approach facilitates rapid progress towards improved assay sensitivity, through electromagnetic mixing and hardware improvements. As upstream advances in Target Enrichment are implemented, the direct method (no replication step) will supplant the indirect approach when the sensitivity levels are comparable.

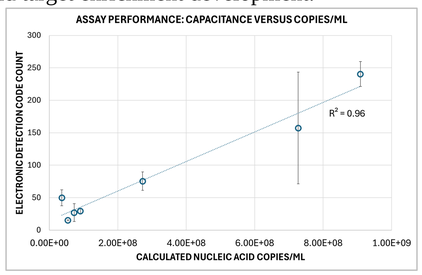
INDIRECT: NUCLEIC ACID DATA

- **Current LOD** 1E4 copies/ml using SARS-COV-2.
- **Targeting** 1E3 copies/ml by end of Q1 2025. This is the WHO clinically relevant 1000cp/mL level for portable non-laboratory RNA viral-load testing[1].
- **Capable** of achieving 1E2 copies/ml sensitivity with further engineering refinement.

DIRECT: NUCLEIC ACID DATA

- **Current LOD** 1E6 copies/mL using synthetic SARS-COV-2.
- **Targeting** 1E4 cp/mL by end of Q3 2025.
- **Capable** of achieving 100 cp/mL sensitivity with further engineering and target enrichment development.

Fig 7: Linearity of direct 'detection by proxy' demonstrates the quantitative ability of this approach.

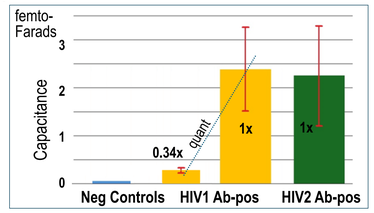


DIRECT: ANTIBODY DATA

The key principle of our technology, **Detection by Proxy[5],** enables this hardware and **direct** assay to also **detect and quantify protein targets:**

Fig 8:
Capacitance-vs-HIV1 & HIV2 immuno-assay **Antibodies** (n=2).

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



Here 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 allows for sequence-specific extraction and eliminates centrifuging and complicated sample-prep steps from this assay. Many different infectious diseases can be detected simply by changing the PNA probe sequences.

Analytical sensitivity & LOD:

The *indirect* method in Q1'2025 is facilitating rapid progress to improved sensitivity. The *direct* method will enable further improvements and cost-reduction, to target compliance with the challenging demands of the WHO REASSURED criteria[6], most especially in providing a commercially viable diagnostic tool for the developing world.

CONCLUSION

- We have introduced a first-of-kind multi-analyte combo DNA/RNA/Antibody/Antigen diagnostic.
- This technology can be used for both Serology and Nucleic Acid detection and quantification. Furthermore, it offers capability to multiplex multiple targets in the same assay.
- Its stand-alone portability makes it ideal for point of care triage and point of need testing.
- Applications for this technology are in molecular diagnostics, companion diagnostics and vaccine development.

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4. Cyclopentane Peptide Nucleic Acid (PNA*)", D. Appella, B.O'Farrell, K.Oshaben et al, Biopolymers, (doi.org/10.1002/bip.23481, Biopolymers 2021)*
5. Altratech patents *US11274291/10738348/11796498, US11459601* and EU/CN/JP equivalent granted patents. ***(Altratech Ltd owns an extensive patent portfolio protecting all aspects of this product & technology).***
6. https://www.nature.com/articles/s41564-018-0295-3

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