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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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NIH NIDDK CRADA

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KERNEL CAPITAL

INFINITY CAPITAL

ABSTRACT

We introduce a completely new method of molecular testing. We replace the need for biological amplification with **electronic signal amplification.** The method does not require enzymes, reverse transcriptase steps or optical detection. It utilizes synthetic peptide-nucleic-acid (PNA) probes, paramagnetic beads, electromagnetics, microfluidics and digital semiconductor CMOS silicon chip technology to detect and quantify genetic material, proteins, and other complex molecular targets. This is achieved without the use of direct genetic amplification. The method is suitable for DNA/RNA/Antibody & Antigen combination tests.

Results are presented for detection of HIV-1 & HIV-2 antibodies from HIV-positive patient plasma; and atto-mole specific capture of SARS-CoV-2 from human saliva. Limit-of-detection is being steadily reduced by automation and integration. Our technology facilitates rapid inexpensive development of tests for new targets with a roadmap to WHO level of 1000cp/mL for our HIV-VL tests[1] in non-clinical settings, with end user cost-per-test of less than $5[2]. The ability to undertake serology and viral-load testing of infectious diseases, with single-use inexpensive cartridges in non-clinical settings, facilitates immediate patient treatment.

MATERIALS

PNA Probes:

Altratech designed PNA probes for specific target capture, direct from samples. We have co-developed chiral PNAs synthesized in FMOC chemistry with the US National Institute of Health (NIH)[3].

Paramagnetic Beads:

PNA-coated paramagnetic beads with captured RNA are magnetically removed from the sample.

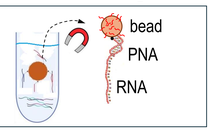


Fig.1: (a) Human saliva sample in vial. (b) Capture & extraction of SARS-CoV-2 RNA

Digital CMOS Sensor Chip[4]:

Single Paramagnetic Beads are specifically tethered by target RNA to our sensor, and quantitatively detected by fringe-field sensing.

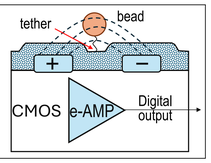


Fig.2: The CMOS sensor 'fringe-field' capacitive sensing principle, and electronic signal amplification:

ASSAY METHOD & PROTOTYPE

Target capture is integrated in the sample vial (Fig.1). The bead assay and CMOS sensor chip are integrated in a lunch-box size prototype (device 2, Fig.3a), which includes heaters and electromagnetics for assay control and automation.

Sample-to-result assay operation:

- Beads with PNA probes **capture the RNA virus directly in the sample** after a lysis step (Fig.1a).
- The PNA-RNA-bead complex is magnetically removed from the sample (Fig.1b), into the device microfluidic channel. The beads progress under electromagnetic control through washing and further specificity tethering steps[5], where the beads progress to the CMOS sensor surface (Fig.2), and are detected and quantified by the CMOS sensor, giving a digital readout of "capacitance-vs-RNA", i.e. **benchtop RNA viral-load quantification.** This is *'detection-by-proxy'*, where the bead being detected is a 'proxy' for RNA or analyte attached to it.
- The CMOS electronic signal amplification of 10^{10} to 10^{12} is roughly equivalent to 30 to 40 PCR cycles

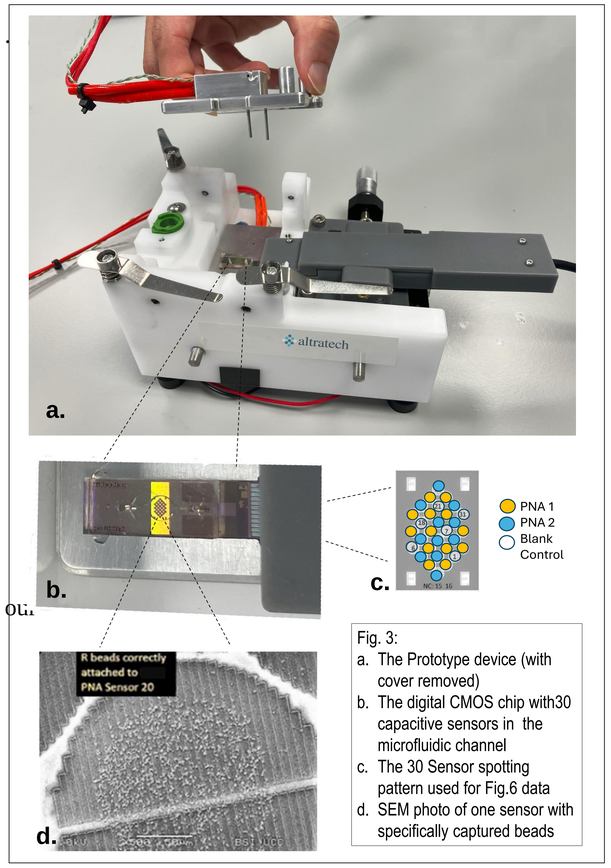
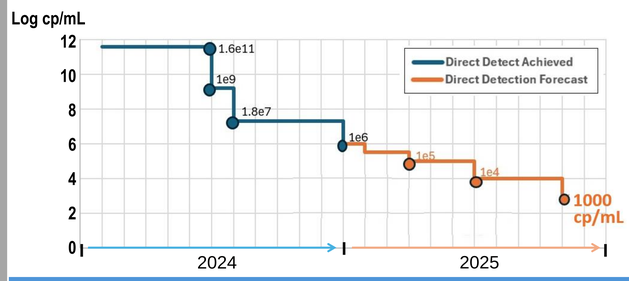


Fig. 3:
a. The Prototype device (with cover removed)
b. The digital CMOS chip with30 capacitive sensors in the microfluidic channel
c. The 30 Sensor spotting pattern used for Fig.6 data
d. SEM photo of one sensor with specifically captured beads

VIRUS DETECTION DATA

Fig.4: Analytical Sensitivity: **Limit-of-Detection** (LOD) progress & roadmap.

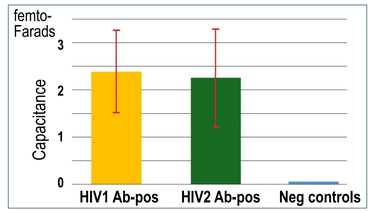


ANTIBODY & ANTIGEN DATA

The key principle of the technology, **Detection by Proxy,** enables the same assay to also **detect & quantify protein targets:**

Fig 5:
Capacitance-vs-HIV1 & HIV2 immuno-assay **Antibodies** (n=2)

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



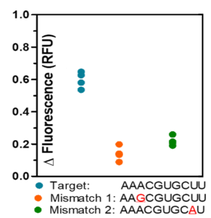
DISCUSSION

Analytical specificity:

PNA probes can target any point on an RNA target, enabling rapid design for any new virus. PNAs are also known for their excellent diagnostic properties[3], including ability to bind their target in crude samples, eliminating centrifuging and sample-prep steps. This is demonstrated with single-base-mismatch specificity in Fig.6:

Fig.6: Single-base-mismatch 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 (data not shown here).



Analytical sensitivity:

The digital readout is highly linear (R^2=0.987), which with the signal amplification, gives this assay the potential resolution capability of digital PCR.

In August 2024, working with the Cambridge Design Partnership (UK) we designed our product prototype, Fig.3 . This delivers a semi automated process and is significantly improving detection levels (Fig.4), through electromagnetic mixing and chemistry, hardware, and software improvements. The next iteration (Fig.7) will further integrate and miniaturize the assay. Our objective is to deliver in 2025 a portable digital molecular diagnostic capability with an LOD of 1000 cp/mL and a target cost per viral test of < $5.

2025 TRIPLE-H PRODUCT PLAN

In November 2024, following a competitive process Altratech was approved for up to $10.5M investment from the European Innovation Council to productise this innovation. Our exemplar product will be a novel rapid Triple-H combination serology and viral diagnostic, in a portable test-kit format.

This will address the vital triage need of rapid detection and viral DNA/RNA quantification of past and present HIV-HCV-HBV infections in ill patients presenting in community and outreach clinics, facilitating immediate diagnosis, intervention and patient treatment.

Encryption-at-source in the CMOS detector will enable strong GDPR and HIPAA compliance. Digital wireless connectivity and immediate data upload to databases will facilitate inclusion of local and remote clinics in real-time public health monitoring programs.

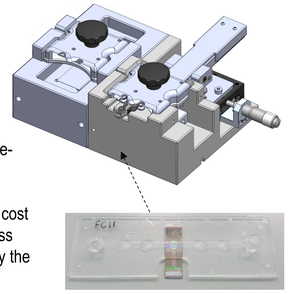


Fig.7(a):
The 2025 fully-integrated device design (sample-loading & analyser).

Fig.7(b): photo of the single-use-disposable slide, with beads, reagents, and sensor chip.
Its high-volume manufacturing cost target is less than $3, to address the unmet need as called for by the MSF 'Time for $5' coalition[2].

REFERENCES

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3. Cyclopentane Peptide Nucleic Acid (PNA)", *D. Appella, B.O'Farrell, K.Oshaben et al, Biopolymers, (doi.org/10.1002/bip.23481, Biopolymers 2021)*
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5. 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).

ACKNOWLEDGEMENTS

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