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NEW MOLECULAR ASSAY BASED ON NANOTECHNOLOGY FOR THE EARLY DETECTION OF HIV-1 P24

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Background

- 1,8 million new HIV-1 infections occur in the world each year; 150,000 in children.
- Early HIV detection (first six months) is key to control the HIV pandemic and to reduce the morbi-mortality associated with HIV infection.
- Primary infection comprises both acute and early infection, and acute infection includes 5 phases (eclipse and Fiebig stages I-V) until seroconversion.
- HIV-1 P24 capsid protein, the most abundant protein per virion, can be detected by 4th-5th generation screening immunoassays, detecting ≈ 10pg/mL of this viral protein (≈105 virions), allowing diagnosis 3-4 weeks after infection.
- New simple, rapid and sensitive diagnostic techniques are needed that allow the window period to be shortened, detecting P24 protein at the femtomolar level.

Methods

- A new plasmonic immunoassay based on nanotechnology (**Figure 1a**) was used to detect 23 plasma samples from patients in different HIV-1 early infection stages (4 eclipse and 4 of each Fiebig stage I-V; Panel 0800-0297-SeraCare) and in 6 paired plasma/DBS samples from subjects in chronic infection with viremia ranging from <1,6 (undetectable) to 4,15 log cop/mL). Sample lysis and antibody-antigen dissociation were done before testing. We also tested 25 culture supernatants with different HIV-1 variants (Eqapol Genetic Diversity Panel).
- Capture anti-P24-IBAB1 antibodies were used on the silicon surface and detection anti-P24-IBAB12 antibodies (Infinity-Biomarkers) conjugated to carboxyl-polymer coated 100nm-diameter gold nanoparticles (Nanopartz).
- The gold nanoparticles were optically identified, and their scattering was analysed to characterize, to classify and to count the nanoparticles present on the silicon Surface due to P24 detection with high specificity. The measurements by a duplicate of the plasmonic response were done with the AVAC scanner platform (Mecwins) **(Figure 1b)**

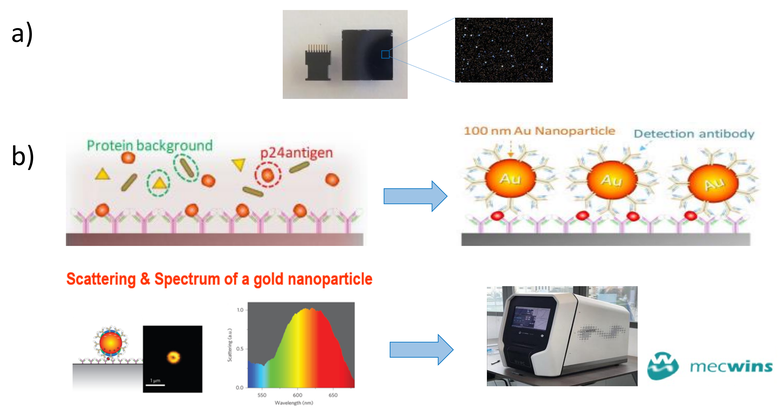

Fig 1. a) Biosensor chip developed by the bionanomechanics group of the CSIC. It is based on a silica plate with optoplasmonic detection technology. b) Capture antibodies attached to the Surface of the plate specifically recognize the P24 antigen. Subsequently, gold particles are added, which will stick to the retained P24 due to the detection of antibodies that cover its surface. Gold particles have optical resonances that make them scatter light very efficiently, letting the chip more sensitive than current commercial molecular detection techniques for HIV detection.

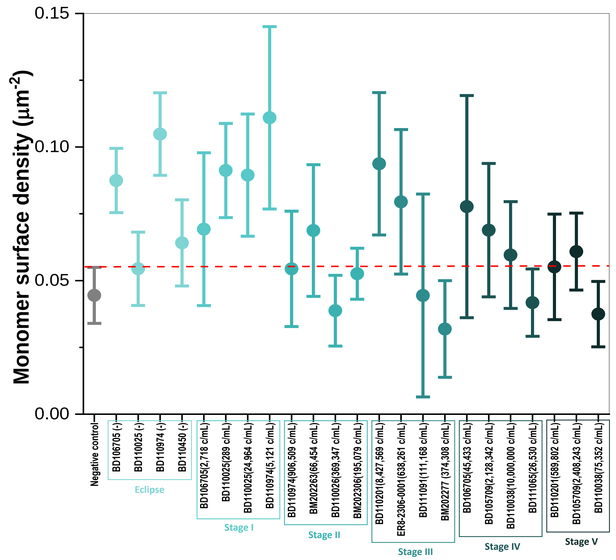

Fig 2. Detection capacity of the P24 HIV-1 protein of the biosensor chip after testing the SeraCare recent infection panel including 23 HIV-infected samples collected during the eclipse and the first five Fiebig stages from the primary infection.

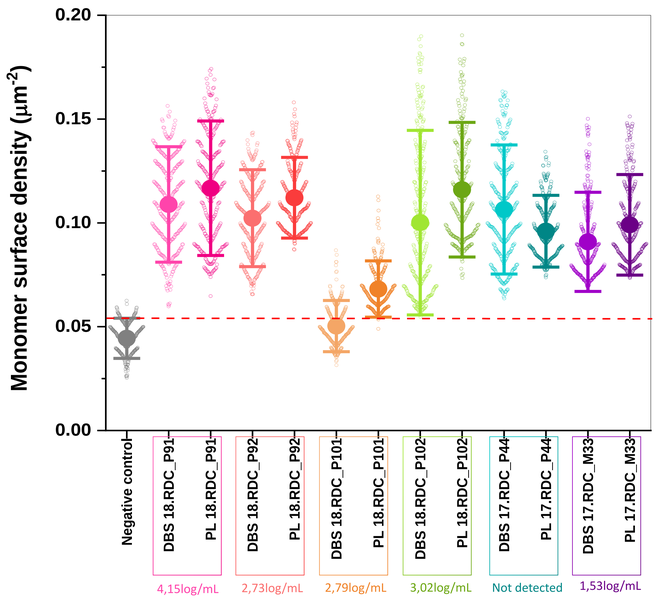

Fig 3. Detection capacity of the P24 HIV-1 protein of the biosensor in 6 paired plasma/DBS HIV-1 infected clinical samples collected during the chronic HIV infection phase, with variable viral load (log HIV-1 RNA copies/ml of plasma).

Results

- The new biosensor showed extreme sensitivity for HIV-1 P24 detection at early stages, undetectable by nucleic acid technologies (NAATs). It was able to detect P24 in 75% of samples in Eclipse stage and in all specimens in Fiebig stage 1, both within the acute infection (**Figure 2**). The rates of false-negative detection increased in stage II-V samples.
- The assay also detected P24 in all 6 plasmas and in 5 DBS collected during chronic infection (Fiebig stage VI), with no significant difference between samples types (**Figure 3**).
- The assay only detected 11 (44%) Eqapol samples, being the remaining not detected, undetermined or discarded by biosensor Surface contamination by analytes in supernatants. **(data not shown).**
- The LOD of the new P24 assay was 10 ag/mL (0,01fg/mL= 10^{-5}pg/mL=10^{-17}gr/mL), equivalent to one virion in 100µl of plasma
- This new sensitivity (1 virion/100µl) was 5 orders of magnitude higher than the first approved 5th immunoassay (7,02pg P24/mL, BioPlex-BioRad, 70.200 virions) and 2 orders of magnitude better than NAATs for HIV molecular diagnosis (20-50 HIV-1 RNA copies/mL or 10-25 virions/mL) (**Figure 4**).

Conclusions

- The new biosensor for HIV molecular diagnosis:
  - Detected P24 protein at atomolar levels, being able to diagnose HIV in plasma and DBS specimens from acute infection earlier than any commercial serological or molecular assay. This would allow the window period to be shortened, detecting viruses during the first week after primoinfection.
  - Presented a high rate of false negatives for samples in stages II-V, possibly due to the saturation of the chip with higher antibodies concentrations in later stages of the primary infection.
  - Let to diagnose HIV with the same efficiency in dried blood and plasma samples.
  - Was able to detect HIV-1 non-B subtypes and complex recombinants.
  - Could be an appropriate technology for the early diagnosis of the infection after its future development as a point of care assay.

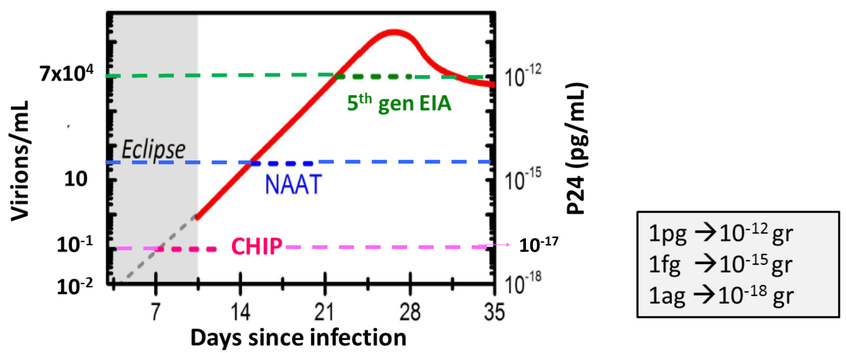

Fig 4. Sensitivity comparison of the biosensor chip under study and commercial assays to detect HIV. gr: gram; pg: pictogram; fg: femtogram; ag: attogram