Use of Nanotrap® particles for capture and enrichment of febrile-illness-causing pathogens including Zika, Chikungunya, Dengue and Influenza viruses

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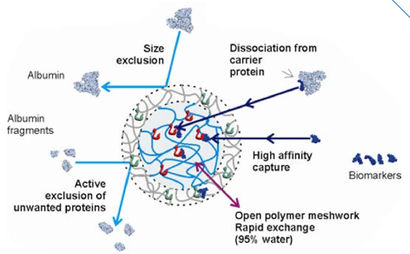
Nanotrap® Particle Technology

Nanotrap® particles are engineered hydrogel particles developed for target analyte separation and discovery applications.

Nanotrap® particles consist of cross-linked N-isopropylacrylamide (NIPAm) copolymers that are functionalized with chemical affinity baits to enable broad-spectrum collection and retention of target proteins, peptides, nucleic acids, and pathogens.

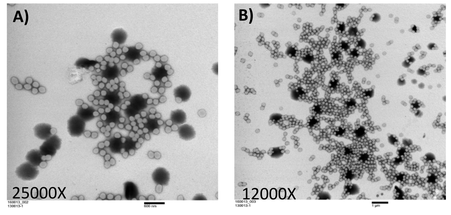


Viral Capture & Enrichment

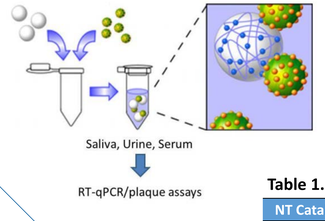
- Increasing geographic distribution of mosquito vectors and subsequent transmission by international travel has created a need for a technology that is capable of measuring low-concentration viral pathogens in biological samples.
- Nanotrap® particles can capture and enrich human retroviruses such as Human Immunodeficiency Virus (HIV), Rift Valley Fever Virus (RVFV) and Venezuelan Equine Encephalitis Virus (VEEV).

Nanotrap® particles concentrate viral pathogens in biological fluids for improved detection by downstream diagnostic assays such as enzyme-linked immunosorbent assay (ELISA) and quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Electron Microscopy images of VEEV TC83 and Nanotrap® particle interaction



Nanotrap® particle enabled viral capture workflow



Nanotrap® particles are added to biological fluids (e.g. urine, saliva) containing viruses. Unbound material is removed after a capture incubation period. Viral binding is evaluated via RT-qPCR

Table 1. Nanotrap® Particles for Viral Capture

NT Catalog No.	Product Description
CN1030	Reactive Red 120 Core Hydrogel Particles
CN2030	Acrylic Acid Core-Shell Hydrogel Particles
CN2010	Blue Core-Shell Hydrogel Particles
CN4000	Custom Mixture – CN1030, 2030, 2010

Experimental Overview

We assessed the utility of Nanotrap® particles for capture and enrichment of febrile-illness causing pathogens, including Zika (ZIKV), chikungunya (CHIKV), dengue (DENV) and influenza (FLU) viruses. Through use of qRT-PCR methods, we have demonstrated that Nanotrap® particle type CN1030 provides at least 8-fold enrichment of ZIKV, CHIKV and DENV from urine biospecimens. We demonstrated detection of ZIKV, CHIKV and DENV at viral concentrations as low as 0.1 PFU/mL.

Nanotrap® Particles Enrich CHKV, DENV and ZKV from Urine

Nanotrap® particles enrich febrile illness pathogens

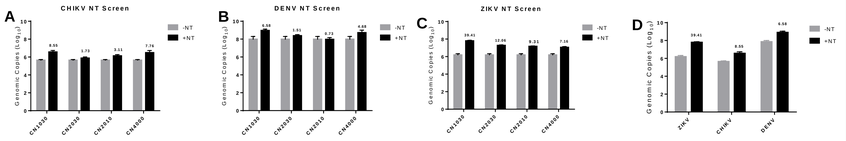


Figure 1. Febrile illness pathogen screen with Nanotrap® particles CN1030, CN2030, CN2010 and CN4000 in human urine. In urine, CHIKV, DENV and ZIKV were captured and enriched by all four Nanotrap® particle types with optimal enrichment provided by CN1030 (A, B, C). Capture and enrichment with use of CN1030 (n=3) was observed with all three viral pathogens (D). Nanotrap® positive (NT+) and Nanotrap® negative (NT-) are compared for calculation of fold-change enrichment.

Nanotrap® particles improve limit of detection for ZIKV, CHIKV and DENV

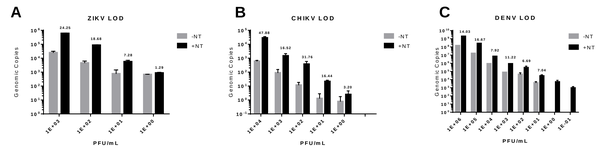


Figure 2. The limit of detection (LOD) of Nanotrap® CN1030 was determined for ZIKV, CHIKV and DENV in human urine. The LOD was determined through use of decreasing concentrations of viral PFU/mL in human urine. It was observed that CN1030 could enrich ZIKV and CHIKV at viral concentrations as low as 1 PFU/mL (A, B). CN1030 was capable of enriching DENV in urine as low as 10 PFU/mL (C).

Nanotrap® particles enable simultaneous enrichment of viruses in co-infection models

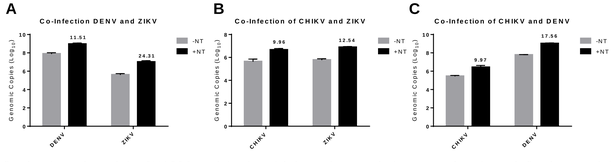


Figure 3. In co-infection based models, CN1030 enriched multiple pathogens in human urine. In co-infection models of various combinations of DENV, CHIKV and ZIKV, CN1030 enriched viral pathogens and displayed no viral preference for enrichment. With dual pathogens present there was greater than 9-fold enrichment observed (A, B, C).

Magnetic Nanotrap® Particles

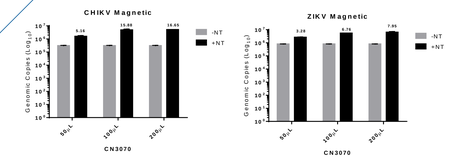


Figure 4. Nanotrap® magnetic particles (CN3070) are capable of capturing and enriching ZIKV and CHIKV in human urine. Increasing volumes of Nanotrap® particles were used for pathogen capture. 100µL and 200µL volumes of Nanotrap® particles yielded the greatest enrichment for both CHIKV and ZIKV. A 16-fold enrichment of CHIKV in urine using CN3070 particle processing was observed (A). CN3070 yielded a 7-fold enrichment of ZIKV when compared to Nanotrap® negative samples (NT-)

Magnetic Nanotrap® particles are suitable for automated high-throughput workflows. These particle types provide similar virus enrichment performance to non-magnetic Nanotrap® particles.

Nanotrap® Particles Enrich H1N1 from Saliva

Pre-concentration using CN1030 particles resulted in a 32-fold increase in detection of genomic copies of Influenza A virus in human saliva.

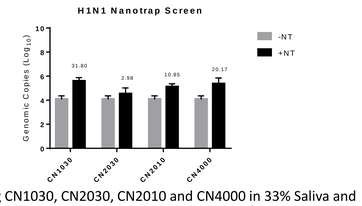


Figure 5 H1N1 Influenza Nanotrap® particle screening using CN1030, CN2030, CN2010 and CN4000 in 33% Saliva and 66% Puritan® UniTranz-RT® Transport System. H1N1 in 33% saliva was enriched by all four Nanotrap® particle types with the highest degree of enrichment occurring with CN1030 particle processing. Nanotrap® positive (NT+) and Nanotrap® negative (NT-) are compared for calculation of fold-change enrichment.

Conclusions & Future Directions

- Nanotrap® particle pre-concentration can help overcome current limitations of existing assays associated with low viral concentrations.
- Nanotrap® particles can enable a universal sample enrichment approach to support both lab-based and automated high-volume testing approaches with non-invasively collected biospecimens.

Acknowledgements

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- Army Disclaimer: Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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