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Purification and Conservation of Cytomegalovirus (CMV) Antibody in Oral Fluid Using NanoTrap® Particle Technology

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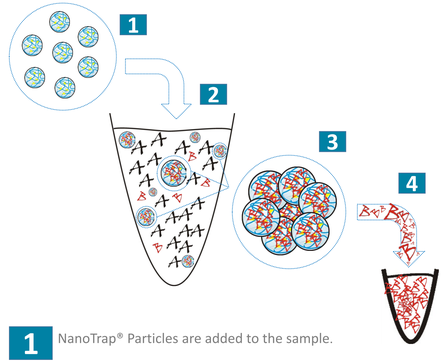
I. Background

Routine serological testing for antibodies to detect current or past exposure to infectious agents requires the collection of a blood sample by venipuncture, limiting sample collection to conventional healthcare settings. Using non-invasive samples for such testing is highly desirable, since it enables expansion of test access to non-conventional settings, such as health fairs and donor drives. Oral fluid, specifically mucosal transudate, contains measurable concentrations of immunoglobulins and has been used successfully as an alternative to serum for a limited number of applications. The content of oral fluid collections is highly variable, however, and the presence of interferents (e.g. mucous, saliva, microbes) can impact both the specificity and sensitivity of immunoassays optimized for serum or plasma samples. This has typically necessitated the development of assays that are specifically designed for using oral fluid as the test matrix. The availability of a pre-analytical technology that eliminates background interferents whilst simultaneously enriching the sample for the target analyte could potentially allow oral fluid to be widely utilized as a sample type for enzyme immunoassays (EIA) developed originally for blood-based testing. In this study, we demonstrate the ability of such a technology, namely the NanoTrap® system (Ceres Nanosciences; Manassas, VA), to concentrate and purify Cytomegalovirus (CMV) IgG from oral fluid collections enabling successful identification of seropositive individuals without the need for blood collection.

Figure 1: NanoTrap® Particle Workflow



1 NanoTrap® Particles are added to the sample.

2 NanoTrap® Particles rapidly collect the valuable biomarker while repelling interfering substances.

3 NanoTrap® Particles and the biomarker are extracted from the sample.

4 Biomarker is eluted from the NanoTrap® Particles.

Images courtesy of Ceres Nanosciences

II. Methods

Blood and oral fluid was collected from each of 102 volunteers. The blood was spun down and the serum tested for CMV IgG using the Bio-Rad CMV IgG kit according to package insert. Approximately 1 milliliter of oral fluid was collected using the Oasis Diagnostic Super-Sal™ saliva collection device (Catalog # SSAL-601). Oral fluid samples were incubated with NanoTrap® particles specific for CMV IgG, concentrated, and CMV IgG was purified and eluted from the particles. The eluate was tested using the Bio-Rad CMV IgG kit with minor modifications to Incubation times and temperatures. Next, the same group of volunteers self-collected oral fluid with Copan FLOQSwabs™ (Catalog # 552C). Briefly, the swab was dried and then eluted in 500uL of 0.25XPBS. NanoTrap® particles specific to CMV IgG were added to the eluate and then concentrated and CMV IgG was eluted from the particles. The eluate was tested using the Bio-Rad CMV IgG kit with minor modifications to incubation times and temperatures.

III. Results

A total of 102 patients volunteered to donate blood and oral fluid for initial CMV testing, 93 of who also collected FLOQSwabs™ for a second round of testing. A total of 39 volunteers were seronegative while 63 volunteers were seropositive. Only 46 patient samples collected with the Super-Sal™ device were positive for CMV IgG, resulting in a sensitivity of 73%, and a specificity of 100%. When FLOQSwabs™ were used to collect oral fluid samples a sensitivity of 84% was achieved while maintaining 100% specificity. Note, unpurified oral fluid was also tested, however as expected the sensitivity and specificity were not ideal due to high background (data not shown).

IV. Conclusions

Use of the NanoTrap® technology enables oral fluid to be analyzed for CMV IgG using a commercially available assay designed for testing serum samples. In this pilot study the use of the COPAN FLOQSwab™ appeared to afford a further increase in sensitivity of detection of CMV IgG, as compared with a standard saliva collection device. The combination of an inexpensive, readily transportable, non-invasive collection method with the NanoTrap® pre-analytical processing technology could enable the CMV serostatus of potential hematopoietic stem cell donors to be determined at the time of initial enrollment.

Super-SAL	Serum	
	POS	NEG
POS	46	0
NEG	17	39

Table 1.

Comparison of CMV EIA results for Super-SAL™ collected oral fluid concentrated and purified using NanoTrap® particles against serum tested via standard procedures.
Specificity: 100% (39/39) Sensitivity: 73% (46/63)

FloQ Swab	Serum	
	POS	NEG
POS	47	0
NEG	9*	36

Table 2.

Comparison of CMV EIA results for FLOQSwabs™ collected oral fluid concentrated and purified using NanoTrap® particles against serum tested via standard procedures.
Specificity: 100% (36/36) Sensitivity: 84% (47/56)
*8 of 9 congruent with saliva results

Figure 2: Comparison of Serum and Oral Fluid Results

Absorbance values of serum samples (without NanoTrap® particles—standard process) versus oral fluid (with NanoTrap® particles). Cutoff equals Mean absorbance of seronegative samples +5SD.