Developing a novel gold nanoparticle-based colorimetric assay for the detection of cytomegalovirus (CMV) in pediatric urine

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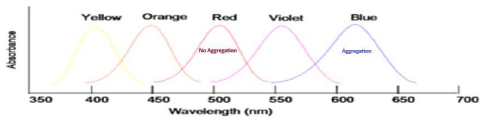
Abstract

- **Background**: One in five infants born with congenital CMV (cCMV) develop complications including hearing loss and developmental delays. Early detection of CMV during the treatable period is critical to prevent long-term complications. Due to the cost-prohibitive nature of testing modalities, most newborns are not tested for CMV if there are no signs of infection. There is a need for a cost-effective, rapid screening method to detect CMV in newborns.
- **Objective**: Developing of a simple gold nanoparticle (AuNP)-based colorimetric assay for the detection of CMV in pediatric urine.
- **Design/Methods**: A non-randomized, prospective experimental design with control group. IRB approval was obtained to collect 60 urine samples from pediatric patients at Nemours Children's Health in Wilmington, DE. All urine samples were confirmed by real-time quantitative PCR (qPCR). We tested 55 of the urine samples with our AuNP assay to assess accurate and sensitive CMV detection. Urine was mixed with AuNPs (CytoDiagnostics, Inc.) in the presence or absence of an anti-CMV antibody (Life Technologies). CMV-negative samples were distinguishable from positive samples via an immediately detectable color change from pink to blue. Positive samples appeared pink or purple immediately after mixing.
- **Results**: Of the 55 urine samples tested, 15 were CMV-positive by qPCR analysis. 11 of the qPCR-positive samples tested positive for CMV in the AuNP assay. There were 4 false negative results. There were 40 samples CMV-negative by qPCR. Of these, 26 samples tested negative for CMV with the AuNP assay. There were 14 false positive results. Compared with qPCR results, the assay has an estimated sensitivity of 73% and a specificity of 65%. The mean absorbance ratio for true positive samples and false positives was determined as greater than 1, whereas the mean absorbance ratio of true negatives and false negatives as less than 1. The break-point for sensitivity was determined at a mean Ct value of 32 i.e., 10,000 copies/mL.
- **Conclusions**: Preliminary results show the AuNP assay was not as sensitive as qPCR analysis. Detection at higher concentrations of CMV is robust. With further development, this urine-based detection assay may represent an alternative diagnostic method allowing point-of-care screening for CMV infection that is a simple, fast, and cost-effective compliment to current gold-standard methods.

Methods

- IRB approval was obtained for this study. Urine from 60 pediatric patients was collected and stored at -80C. Confirmatory quantitative real-time PCR (qPCR) testing was performed using the urine sample and the purified DNA extracted from 55 urine samples. Published primers and probe sequences targeting the CMV genes pp65 and AD1 were used.
- **Gold Nanoparticle assay**. Each urine specimen was aliquoted in triplicates. Each sample was mixed with a gold nanoparticle probe (CytoDiagnostics, Inc.) in the presence and absence of anti-CMV antibody (Life Technologies). The presence of CMV in urine was determined by measuring the absorbance at 530 nm and 630 nm wavelengths as a function of aggregation of CMV virions, anti-CMV antibody, and the gold nanoparticle probe. The colorimetric changes were evaluated over a 5-minute time point in the spectrophotometer (Spectramax iD3). The presence of CMV was determined by comparison of "control" (i.e., in the absence of antibody) to "test" (i.e., the presence of antibody) as shown in Figure 1.



Results

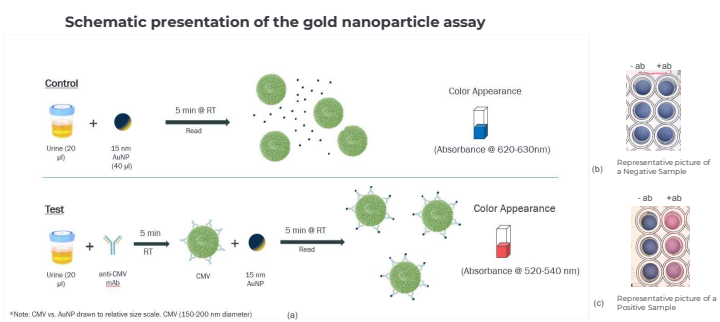


Figure 1. Gold nanoparticle (AuNP) assay for detection of CMV in urine. (a) Urine samples to be tested were treated with an anti-CMV antibody at room temperature (RT). After a brief incubation, gold nanoparticles were added to test (antibody-positive) and control (antibody-negative) samples. The absorbance of samples was measured at 530 nm and 630 nm wavelengths, and the absorbance ratio was calculated. (b) Representative picture of a negative urine sample. (c) Representative picture of a positive urine sample.

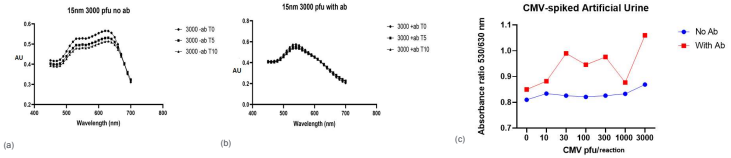


Figure 2. Gold nanoparticle (AuNP) assay limit of CMV detection in artificial urine. Artificial urine was prepared with aliquoted dry chemicals and mixed with DI water. (a) The spectrum (absorbance unit) of artificial urine spiked with CMV strain AD169 and no antibody. (b) The spectrum (absorbance unit) of artificial urine spiked with CMV strain AD169 and with antibody. (c) The plot of absorbance ratio (530/630 nm) and known (log) concentrations of CMV strain AD169, grown in-house, spiked into an aliquot of artificial urine and tested in the presence and absence of anti-CMV antibodies.

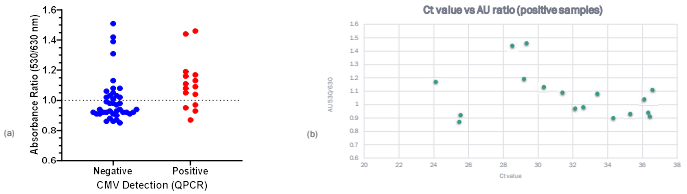


Figure 3. Gold nanoparticle (AuNP) assay analysis of patient samples. (a) Gold nanoparticle (AuNP) assay analysis of patient samples. Patient urine samples (n=55) were tested for the presence of CMV following the workflow in Figure 1. Absorbance values of each sample were measured at 530 nm and 630 nm wavelengths, and the absorbance ratio was calculated as 530/630 nm. (b) Plot of qPCR mean Ct value vs. absorbance ratio (530/630 nm) for positive samples.

Table 1. AuNP CMV colorimetric assay vs. CMV qPCR assay

	qPCR Present (+)	qPCR Absent (-)	Total
AuNP Colorimetry (+)	11	14	25
AuNP Colorimetry (-)	4	26	30
Total	15	40	55

Sensitivity = 73%
Specificity = 65%

Discussion

- Congenital CMV (cCMV) is the most common cause of non-genetic hearing loss in newborn babies. Due to a lack of effective vaccines and limited therapies, it is imperative to have an early diagnosis to allow early interventions. Failing the universal newborn screening initiates definitive testing for CMV, which helps 60% of pediatric patients only, whereas 40% develop hearing loss.
- There are no assays appropriate for large-scale newborn screening for CMV infections; the commercially available DNA PCR-based kits are expensive, time-consuming, and require skilled labor and time. Saliva-based testing is non-invasive but has the potential for false positives. Guthrie cards are sensitive but are an invasive procedure.
- We have previously evaluated 100 nm gold nanoparticles using them in a dynamic light scattering (DLS) assay. While sensitive and specific, measuring DLS requires a specialized instrument. Therefore, we evaluated 15 nm gold nanoparticles as the basis for a colorimetric assay that would not require a specialized instrument. This simple gold nanoparticle-based assay presents a better attribute as a routine, point-of-care method for screening cCMV. Urine is a better diagnostic medium as its collection method is non-invasive, and pediatric patients with CMV secrete a lot of virions in their urine.
- Our initial studies suggest that the colorimetric gold nanoparticles assay has the potential to be sensitive and specific, and we are in the process of optimizing the assay. To conclude, this diagnostic assay is simple, inexpensive, easy to use at point-of-care, and does not require a reference to external laboratories. Ultimately, we will develop the colorimetric assay into a lateral diffusion assay suitable for large-scale newborn CMV screening.

References

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