**Introduction**

- Current invasive methods of prenatal diagnosis are chorionic villus sampling (CVS) and amniocentesis (AC) at 10-13th and 15-20th week gestation, respectively.  
- Non-invasive detection of circulating fetal cells in maternal peripheral blood is advantageous for early prenatal diagnosis.  
- Circulating trophoblasts or extravillous trophoblasts (EVT) is the main fetal cells which shed from the placenta into maternal circulation during early pregnancy.  
- Human leukocyte antigen-G (HLA-G) is a trophoblast specific biomarker which allows separation of trophoblast from other maternal cells.  
- In this Application Note, we applied CytoQuest™ CR positive selection microfluidic system to capture and detect EVT in maternal peripheral blood.

**Materials & Methods**

- Peripheral blood of pregnant donor was collected in Heparin Tube (02-689-6, BD)  
- Nine mL blood was prepared for collecting the peripheral blood mononuclear cell (PBMC) by density gradient centrifugation using Leucosep® (163290P, Greiner Bio-One) and Histopaque®-1077 (10771, Sigma-Aldrich).  
- The PBMC fraction was harvested and resuspended in Wash Medium.  
- Resuspended PBMC was loaded into the CytoQuest™ CR System and CFC was captured by HLA-G (KA4515, Abnova) immobilized CytoChipNano (U0095, Abnova).  
- Immunofluorescence staining for detecting CFC was performed using TBA, CD45 (KA4515, Abnova), DAPI as the instruction protocol.  
- Imaging was performed using Nikon Eclipse Ti-E fluorescent inverted microscope.

**Results**

- CFC Counts: In 9mL blood of pregnant donor, 4 cells are counts as CFC (TBA+, CD45-, DAPI+)

![Figure 1](image-url)  

**Discussions**

- CytoQuest™ CR successfully captures EVT from maternal circulation via biotinylated HLA-G monoclonal antibody on a streptavidin coated CytoChipNano substrate.  
- Abnova has developed a proprietary trophoblast antigen (TBA) monoclonal antibody to detect and validate EVT after HLA-G monoclonal antibody capturing.  
- This is the first known demonstration of EVT capture and detection using an antibody-based, positive selection microfluidic system.  
- Isolation of EVT allows further downstream single cell analyses such as short tandem repeat (STR) genotyping and whole genome amplification (WGA) for array comparative genomic hybridization (aCGH) and next generation sequencing (NGS).  
- Circulating fetal EVT could be utilized to perform noninvasive prenatal diagnosis in early pregnancy which obviates the risks of invasive procedures such as CVS and AC.
References


