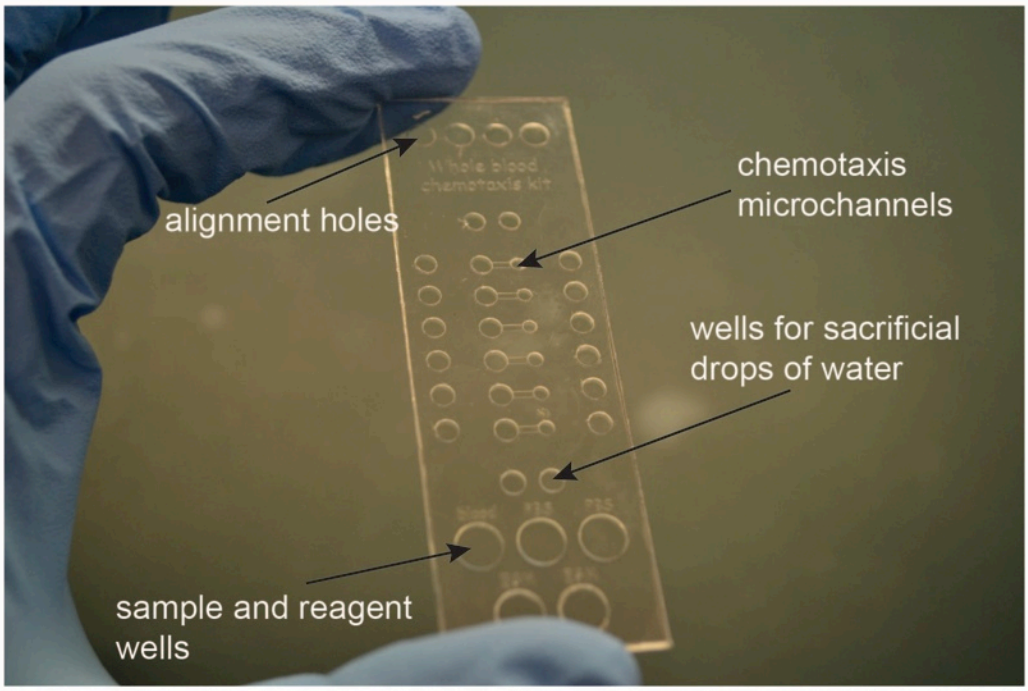
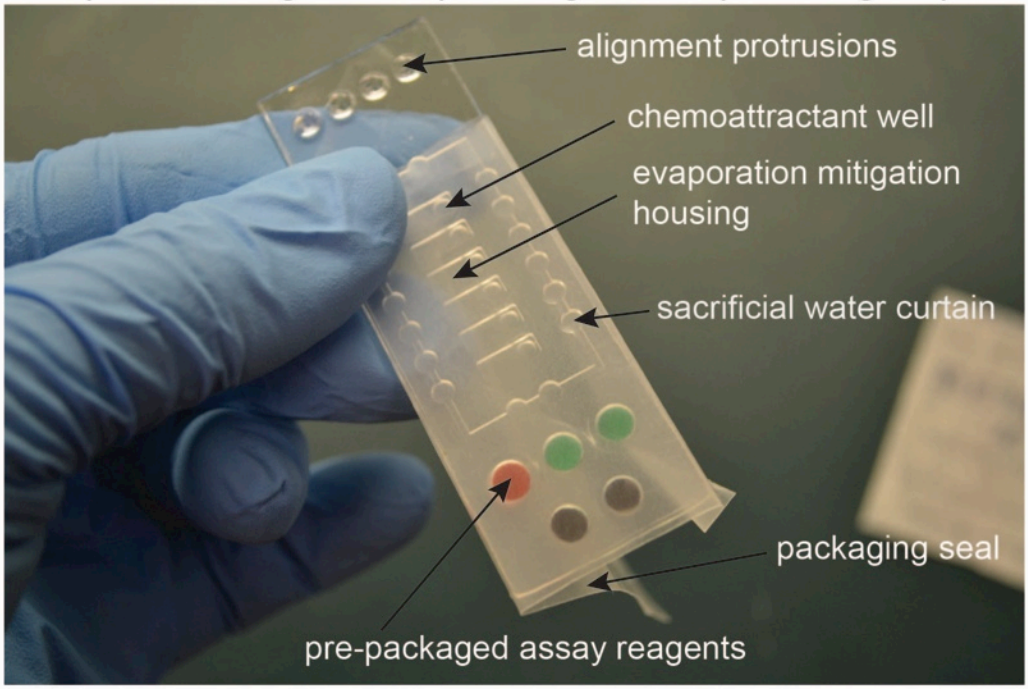
Diagnostic chip base microchannels for neutrophil sorting & chemotaxis

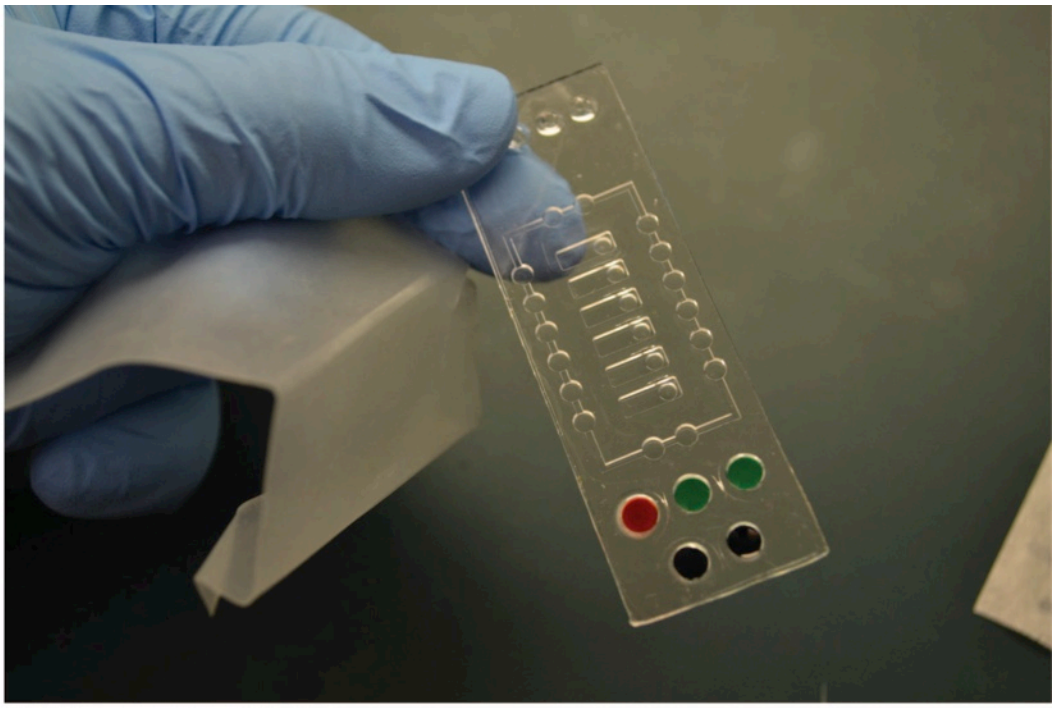


A) Image shows the features of the "base" component of the asthma diagnostic chip. Prior to performing the neutrophil sorting and chemotaxis, the microchannels should be coated with recombinant human P-selectin (see Materials and Methods). The channels should be rinsed with two replacements of PBS prior to flowing in dilute blood and initiating the assay.

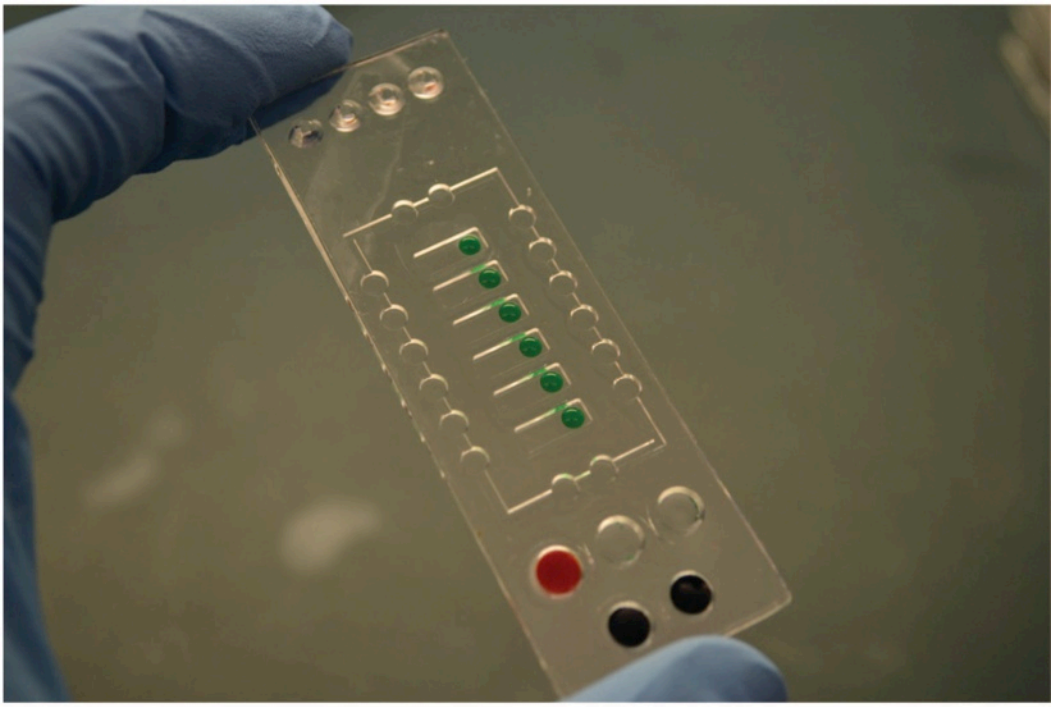
Lid component of diagnostic chip for reagents and preventing evaporation



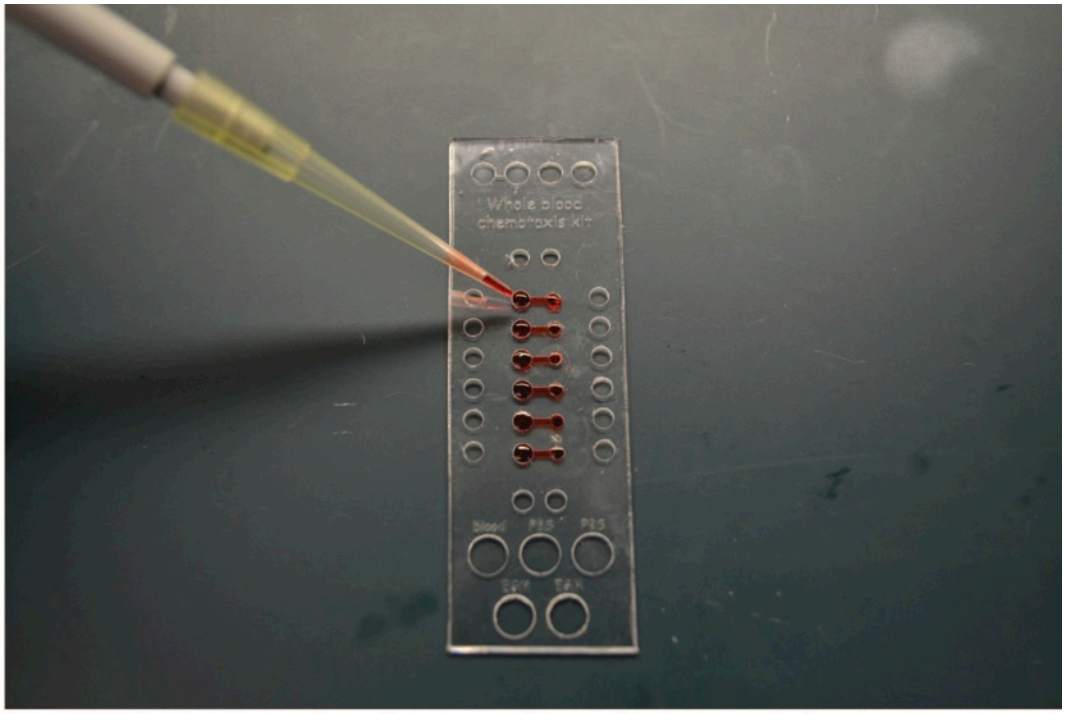
B) Image shows the features of the "lid" component of the asthma diagnostic chip, with the dyed liquid representing different reagents in the assay. The diagnostic assay can be pre-packaged by filling the lid with the required reagents to run the diagnotstic test. Reagents can be frozen down and sealed, allowing the user to simply remove the seal and thaw the reagents prior to initiating the assay.



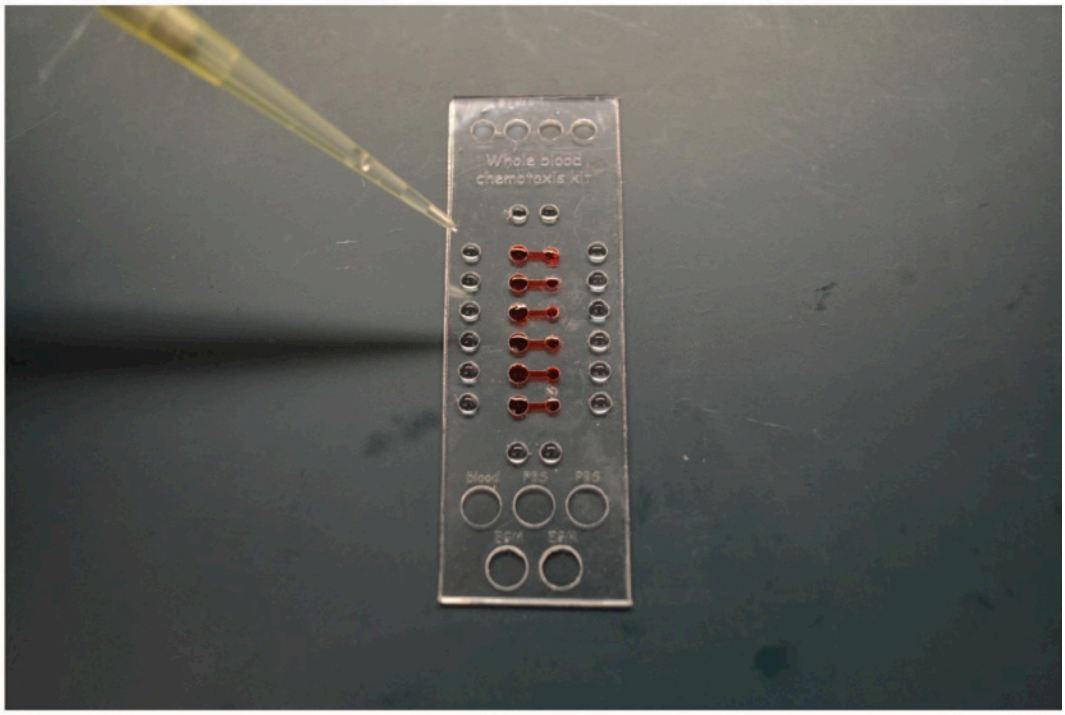
C) Remove the seal and allow the reagents to thaw at room temperature (1-2 minutes). The seal should be removed before the media has thawed to prevent reagents from adhering to the seal following the solid-to-liquid phase change.



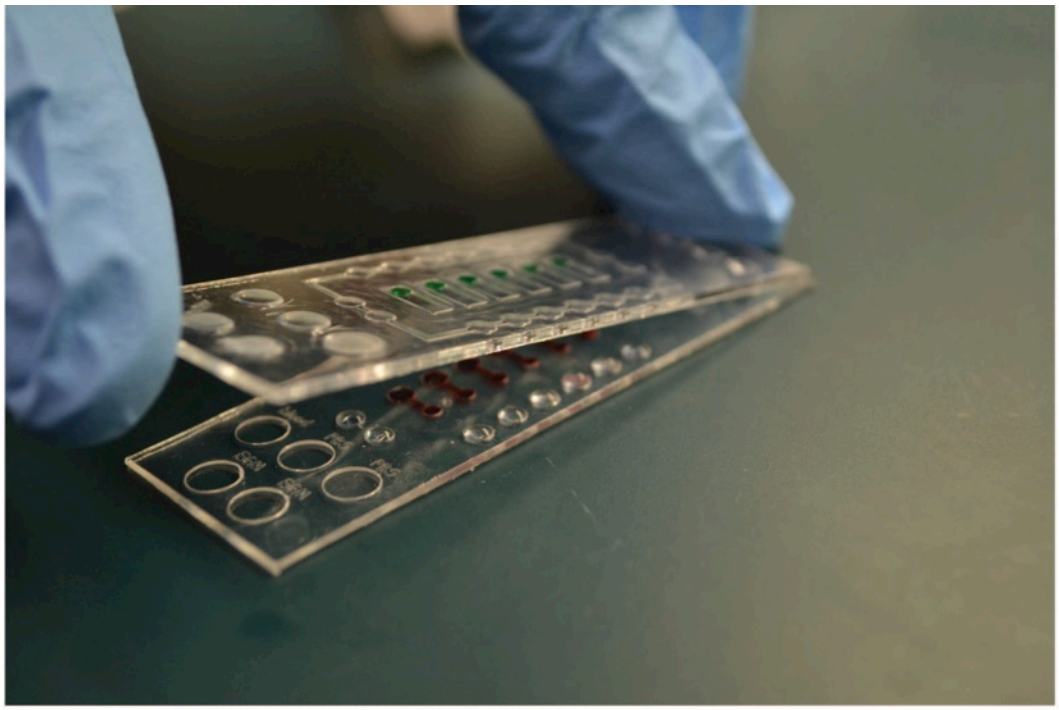
D) Pipet 3 μL drops of the hydrogel-chemoattractant (H-CA) mixture into the "chemoattractant well." If 3 μL drops do not realiably make contact to the output port drops of media in the base microchannels, larger volumes of H-CA should be used. The H-CA mixture is represented by the green liquid.



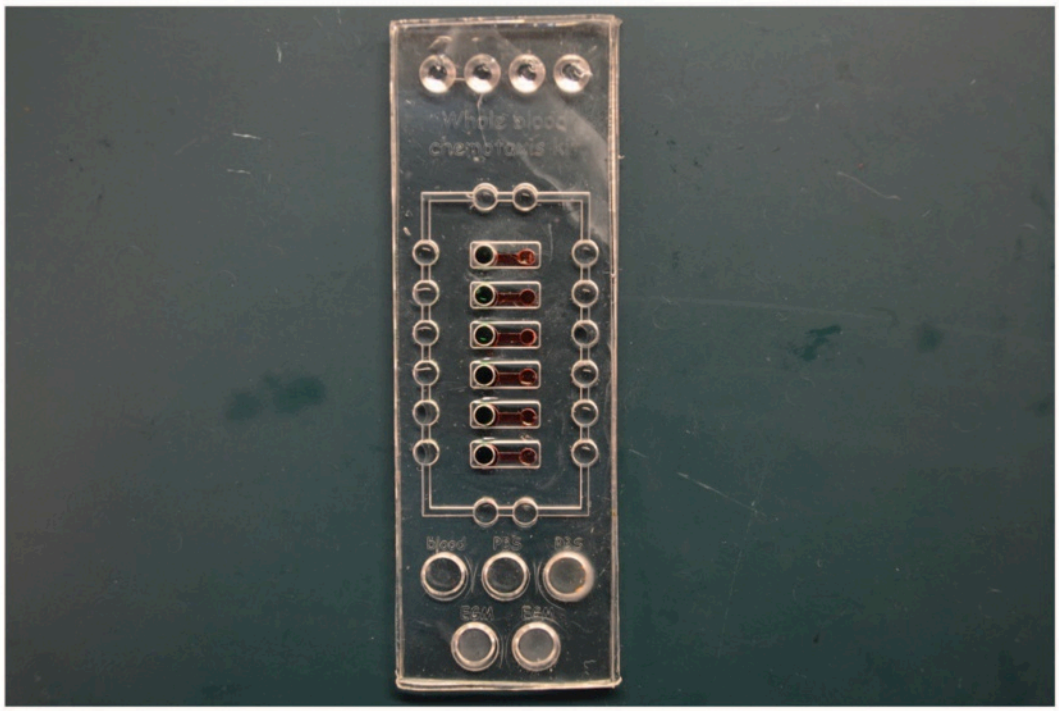
E) After performing a lancet puncture on the finger of the patient, dilute the whole blood into PBS in the vacant “blood” well (see Methods and Materials). Pump 1 μL of dilute blood into the microchannel for neutrophil capture. Blood can be passed through the microchannels multiple times to further enrich the surface with neutrophils. Perform several wash steps to remove erythrocytes from micorchannels.



F) Pipet 6 μL drops PBS into the wells for sacrificial liquid. These drops help to mitigate evaporation during the timelapse imaging portion of the assay.



G) After heating up the lid and base of the diagnostic assay to 37 degrees celcius in an humidified chamber (~3 minutes), use the alignment protrusions and wells to place the lid onto the base. The H-CA (shown in green) should make contact with the media in the base microchannels, allowing for diffusion of the chemoattractant into the microchannels to form a chemical gradient.



end

H) Image shows lid-base combination of the diagnostic assay. The liquid droplets of PBS in the sacraficial liquid wells will wick into the hydrophilic channels in the lid to form a “liquid curtain” to mitigate evaporation. Once the lid has been placed onto the base, the chip can be placed on a stage for timelapse imaging.