Protocol for isolation of porcine neutrophils (from Zahra Bond USDA)	PI	Dr. Chris Tuggle
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Up to 120ml of blood was drawn into BD Vacutainer acid citrate dextrose (ACD) solution A tubes (Catalog N. 364606, BD). Neutrophils were isolated by dextran sedimentation using 6% Dextran / 0.9% NaCl solution at room temperature for 45-60 minutes, or until separation is complete. Supernatant was transferred to a conical tube and centrifuged for 12 minutes at 300 RCF, 4ºC with low brake. Supernatant was discarded and the pellet was lysed with ACK Lysing buffer (Catalog No. A10492-01, ThermoFisher) per manufacturer instructions to lyse contaminating red blood cells. The sample was centrifuged at 300 RCF for 5 minutes, and the supernatant containing lysed red blood cells was discarded. The pellet was resuspended in phosphate buffered saline (PBS) and the cell suspension was layered over Ficoll-Histopaque-1077 (Catalog No.1077, Sigma) and centrifuged for 30 minutes at 450 RCF, room temperature, low brake. Post centrifugation, the peripheral blood mononuclear cell (PBMC) layer was removed and discarded, and the pellet (containing granulocytes) was further processed. The granulocyte pellet (primarily neutrophils) was rinsed with 4 ml of PBS, centrifuged at 450 RCF for 5 minutes and resuspended in 2 ml HBBS. Cell count and viability data were obtained using the MUSE cell analyzer system (Millipore).