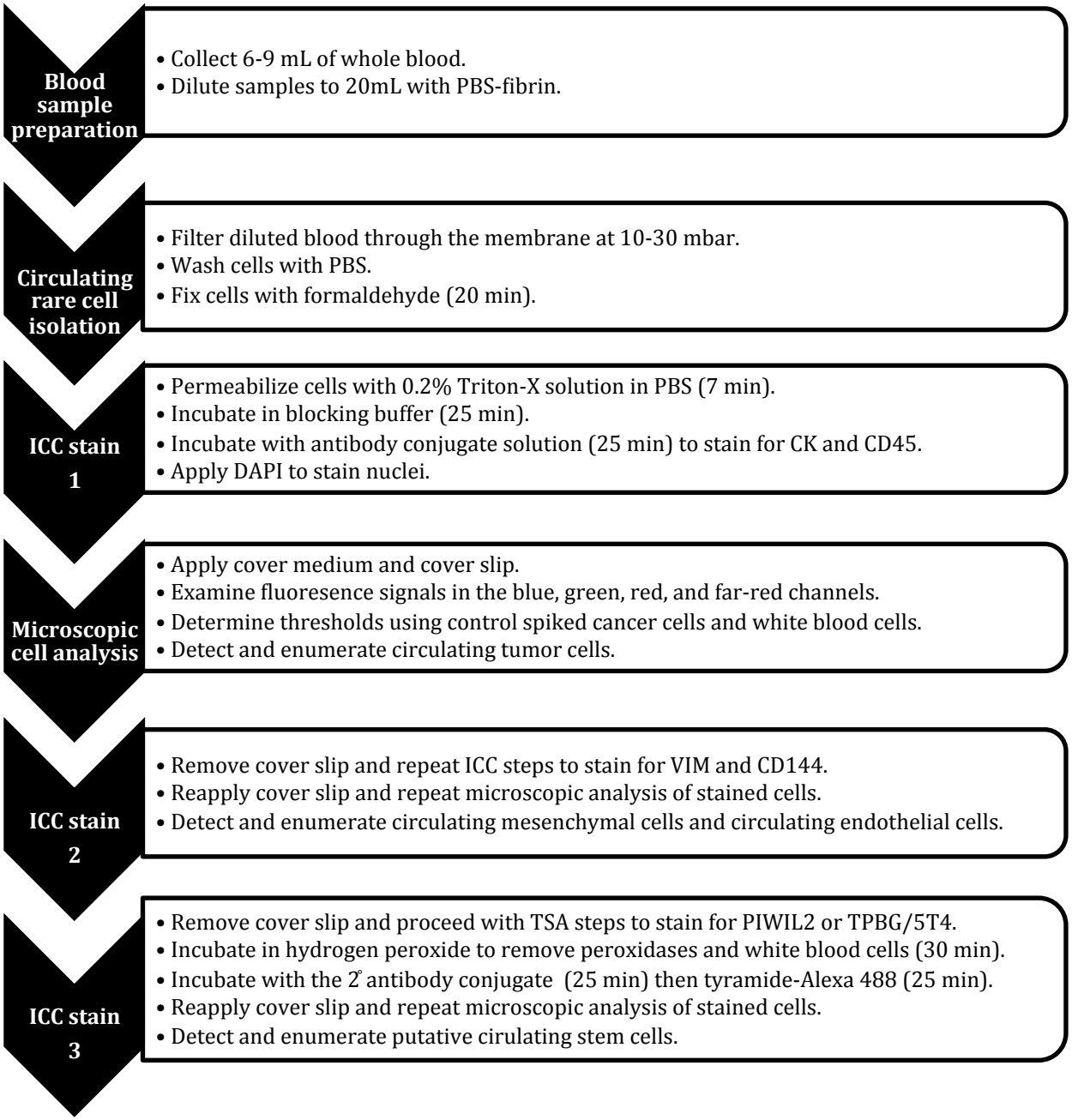
Blood sample preparation
• Collect 6-9 mL of whole blood.
• Dilute samples to 20mL with PBS-fibrin.
Circulating rare cell isolation
• Filter diluted blood through the membrane at 10-30 mbar.
• Wash cells with PBS.
• Fix cells with formaldehyde (20 min).
ICC stain 1
• Permeabilize cells with 0.2% Triton-X solution in PBS (7 min).
• Incubate in blocking buffer (25 min).
• Incubate with antibody conjugate solution (25 min) to stain for CK and CD45.
• Apply DAPI to stain nuclei.
Microscopic cell analysis
• Apply cover medium and cover slip.
• Examine fluoresence signals in the blue, green, red, and far-red channels.
• Determine thresholds using control spiked cancer cells and white blood cells.
• Detect and enumerate circulating tumor cells.
ICC stain 2
• Remove cover slip and repeat ICC steps to stain for VIM and CD144.
• Reapply cover slip and repeat microscopic analysis of stained cells.
• Detect and enumerate circulating mesenchymal cells and circulating endothelial cells.
ICC stain 3
• Remove cover slip and proceed with TSA steps to stain for PIWIL2 or TPBG/5T4.
• Incubate in hydrogen peroxide to remove peroxidases and white blood cells (30 min).
• Incubate with the 2° antibody conjugate (25 min) then tyramide-Alexa 488 (25 min).
• Reapply cover slip and repeat microscopic analysis of stained cells.
• Detect and enumerate putative cirulating stem cells.