Preparation of Metaphase Spreads for Karyotype: Giemsa stain

Collect 3-4 drops of blood in 5 mL of tissue culture medium (GIBCO PB-MAX Karyotyping Medium, Cat. # 12557-013) and incubate at 37^{o}C for 70 hours.
Add 0.05 mL of 37^{o}C Colcemid solution (GIBCO KaryoMAX Colcemid Solution, Cat. # 15212-012) to the cell culture, mix and return culture to incubator for an additional 2 hrs.

1) Resuspend culture and pour contents into a labeled 15 mL calibrated conical centrifuge tube.
2) Centrifuge for 8 min. @ 750-900 rpm.
3) Discard supernatant and resuspend cells in the remaining 0.25-0.5 mL of liquid using the vortex.
4) Add 6 mL of hypotonic (0.075M KCl) solution (GIBCO Potassium Chloride Solution, Cat. # 10575-090) – one pipetfull followed by mixing, then to 6 ml mark followed by mixing.
5) Incubate at 37^{o}C for 8 min.
6) Add 1 mL of cold fixative (Carnoy's: 3 parts methanol to 1 part glacial acetic acid) and gently mix by slow inversion of the tube.
7) Let sit at room temperature for 10 min.
8) Centrifuge for 8 min. @ 750-900 rpm.
9) Discard supernatant and resuspend cells in remaining 0.25-0.5 mL of liquid by flicking the tube.
10) Add 6 mL of cold fixative – one pipetfull followed by mixing, then to 6 ml mark followed by mixing.
11) Let sit at room temperature for 10 min.
12) Centrifuge for 8 min. @ 750-900 rpm.
13) Repeat steps 9-12.
14) Discard supernatant and resuspend in remaining 0.3 mL of liquid by flicking.
15) Remove a slide form cold 70% methanol and drain off excess methanol by touching unfrosted end to a paper towel.
16) From a height of 6 inches, drop half of the cell suspension onto the slide held at 45^{o} below horizontal.
17) Dry the back side of the slide with a paper towel, label it with your name, and dry by briefly running the slide over a Bunsen burner flame (quick strokes over the flame til dry).
18) Repeat steps 15-17 with the remaining cell suspension.
19) Place your slides in Giemsa stain (2 mL GIBCO KaryoMAX Giemsa Stain Stock Solution, Cat. # 10092-013, in 98 mL Gurr buffer (GIBCO Gurr Buffer Tablets, Cat # 10582-13)) for 6-8 min (maybe leave one slide in for 6 and one for 8 minutes)
20) Rinse slide gently under tap water and dry the back of the slide with a paper towel. (Slide may be viewed with 10X and 40X objectives at this point.)
21) When done, coverslip by dipping slide in citrisolv, blotting end on a paper towel, laying slide flat and placing one drop of mounting medium on the smear and dropping a coverslip on. Work out bubbles if necessary and let dry flat in hood for several days. Once mounting has had time to cure, you can view under oil immersion.