Determination of Synovial Fluid Leukocyte Count and Differential
Standard operating procedures (SOP), 1 June 2015, by Andrej Trampuz, Charité

Cell count of the synovial fluid is a basic procedure in the assessment of the degree of inflammation present in a joint. Although manual count of the synovial fluid is generally used, automated counters have higher precision, are easier and faster performed. Here, the standard operating procedure (SOP) of joint aspiration and synovial fluid leukocyte analysis is described.

Joint aspiration (arthrocenthesis)
- After skin preparation and local anesthesia with 1% lidocaine, synovial fluid is aseptically collected using a sterile 18-gauge spinal needle and a 10-ml syringe.
- If no synovial fluid was initially obtained, the needle is repositioned without withdrawing it through the skin; no fluid is injected into the joint.
- Volume of synovial fluid and presence or absence of purulence is noted. The aspirate is transferred into 2 vials (one, containing EDTA for cell count, and one, without additives for culture) and transported to the laboratory within 6 hours.
- The EDTA vial is gently turned by hand-mixing 5 times.
- Synovial fluid specimen is described by the laboratory technician as serous (straw-colored and transparent), cloudy (opaque) or bloody (orange or red).

Synovial Fluid Analysis (determination of leukocyte count and differential)
- Leukocyte count and differential can be determined by microscopic (visual) or automated examination.
- Clotted specimens were treated with 10 µl hyaluronidase (Sigma) for 10 minutes at room temperature.
- Dilution of synovial fluid: 10 µl of synovial fluid is mixed with 200 µl normal saline (3% saline for bloody specimens) containing 0.001% benzentonium chloride (Hyamin 2389), an erythrocyte lysing agent.
  - Microscopic examination: Diluted synovial fluid specimens are plated on a hemacytometric chamber (Neubauer) and examined microscopically. Specimens with leukocyte counts >10 x 10⁹/L were diluted 1:100 in normal saline and re-examined.
  - Automated counters: According to manufacturer's recommendation.

Literature: