1. Turn on the Z1 by flipping the switch on the top left of the instrument to the "ON" position
2. Open the door to the instrument and carefully lower the black platform to the bottom position
   - *The platform has a safety latch that must be pressed to allow the platform to move*
3. Remove the Z1 cup containing the blue Coulter Clenz solution
   - *Save this cup and the Coulter Clenz solution for when you are finished*
   - *Periodically, this solution should be replaced with fresh Coulter Clenz solution from the jug next to the sink*
4. Fill the "FLUSH" Z1 cup with 10 ml Isoton II Diluent
   - *The pump on the Z-Pak fill dispenser automatically dispenses 10 ml*
   - *Be sure to remove the screw cap on the spigot before trying to dispense*
5. Put the "FLUSH" Z1 cup on the black platform of the instrument and raise the platform to the top position
   - *The platform will stop before the glass probe hits the bottom of the Z1 cup, so do not worry about smashing the probe*
6. On the instrument keypad, press "Function" and scroll down until "Prime Aperture" is displayed
7. Press "Start" twice to begin priming the aperture
   - *Aperture priming replaces the fill solution in the probe with fresh Isoton II Diluent*
   - *The priming procedure takes ~5 min*
8. While the instrument is priming, prepare another Z1 cup with 10 ml Isoton II Diluent and 50 μl of the cell suspension to be counted
   - *This 200-fold dilution is coded into the instrument and is factored into the cell concentrations shown*
   - *If another dilution is used, then the concentrations returned will not be accurate*
9. After the priming has completed, lower the black plaform of the instrument to the bottom position and discard the remaining Isoton II Diluent from the "FLUSH" Z1 cup
   - *If measuring more than one cell suspension, refill the "FLUSH" Z1 cup with fresh Isoton II Diluent (the priming procedure leaves some residual Coulter Clenz in the "FLUSH" Z1 cup)*
10. Put the Z1 cup containing the diluted cell suspension on the black platform of the instrument and raise the platform to the top position
    - *To ensure that the cells have not settled, invert the cup once or twice before removing the cap and putting the cup on the platform*
11. On the instrument keypad, press "Output" and then "Start" to begin counting
    - *Be sure to inspect the particle counts when the instrument is counting*
    - *The most-accurate readings occur when the number of counted particles is between 500–5000, which corresponds to cell concentrations between $2 \times 10^5 – 2 \times 10^6$ per ml (each count draws 0.5 ml of the Isoton solution)*
    - *If your cell concentration is much lower or higher, the cell suspension should be diluted or concentrated and then recounted*
    - *The counting procedure should take ~15 sec*
12. The concentration shown on the instrument is the concentration of the original cell suspension
13. If measuring additional cell samples, lower the black platform of the instrument about halfway to expose the probe, rinse the probe with a squirt bottle, and remove the Z1 cup containing the counted cell suspension
14. Put the "FLUSH" Z1 cup (containing fresh Isoton II Diluent) on the black platform and raise the platform to the top position
15. On the instrument keypad, press "Function" and scroll down until "Flush Aperture" is displayed
16. Press "Start" once to begin flushing the aperture
    - *Aperture flushing draws a volume of clean Isoton II Diluent through the aperture of the probe*
    - *The flushing procedure should take ~15 sec*
17. Repeat steps 10–16 for each additional cell suspension to be counted
    - *Once the "FLUSH" Z1 cup has been replaced with fresh Isoton II Diluent after the priming step, this solution can be reused for all subsequent aperture flushes without replacement of the solution*

18. When finished for the day, Prime the Aperature (complete cycle) using a non-lytic cleaner (e.g. Beckman Coulter CLENZ) and leave the cleaning agent around the aperature overnight. Ensure that the Aperature and the electrode are submerged in the cleaning agent before setting the Power Switch to O (off).
19. Used Z1 cups should be rinsed with DI water, sprayed with 70% EtOH, and left to dry for the next user
20. The Counter should be disinfected periodically following Appendix 7 in the User's Manual.

Troubleshooting the Z1 instrument

- Counting takes longer than ~15 sec
  - Diagnosis: The aperture is blocked (you can see the blockage in the window on the upper right-hand side of the instrument)
  - Solution: First, try flushing the aperture (Steps 14–16); if flushing does not work, then use the red scrub brush on the stop of the instrument to clean the pink aperture on the probe. System may need to be drained, disinfected, and refilled (see Appendix 7 of the Z1 manual).
- Error message on instrument reads "Check diluent vessel"
  - Diagnosis: Either the Isoton II Diluent bottle is empty or the waste bottle on the back of the Z-Pak is full
  - Solution: Check the two bottles, emptying the waste bottle in the sink and refilling the Isoton II Diluent bottle with the 20 L jug or the refill containers underneath the counter (replacement jugs under counter)
- Error message on the instrument reads "High masking"
  - Diagnosis: Build up of contaminants within the Z1 fluidics system requiring routine maintenance
  - Solution: Alert Cheryl about the error and she will follow Appendix 7 of the Z1 manual to drain, disinfect, and refill the system.