Monitoring Adherence with Biofilms

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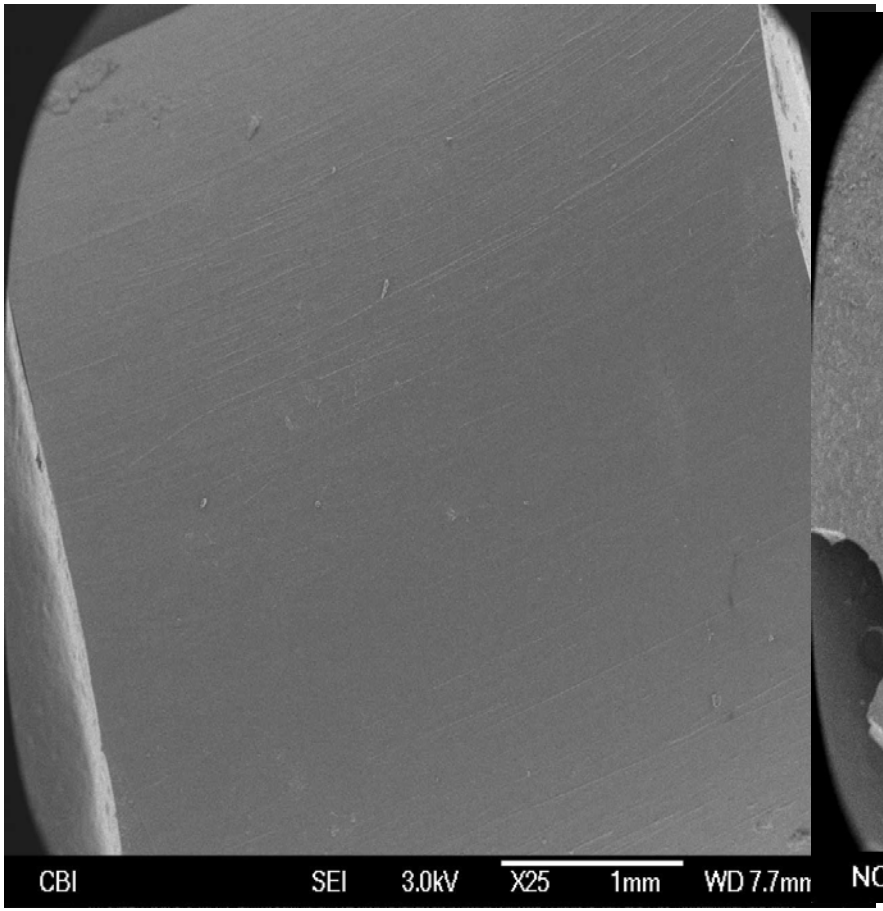
Why look at biofilms?

- A second method for measuring adherence that is independent of self-reporting
- The perfect method would be able to tell us how long the ring was worn
- There is some preliminary data suggesting the quantity of biofilm may correlate to time VR are worn

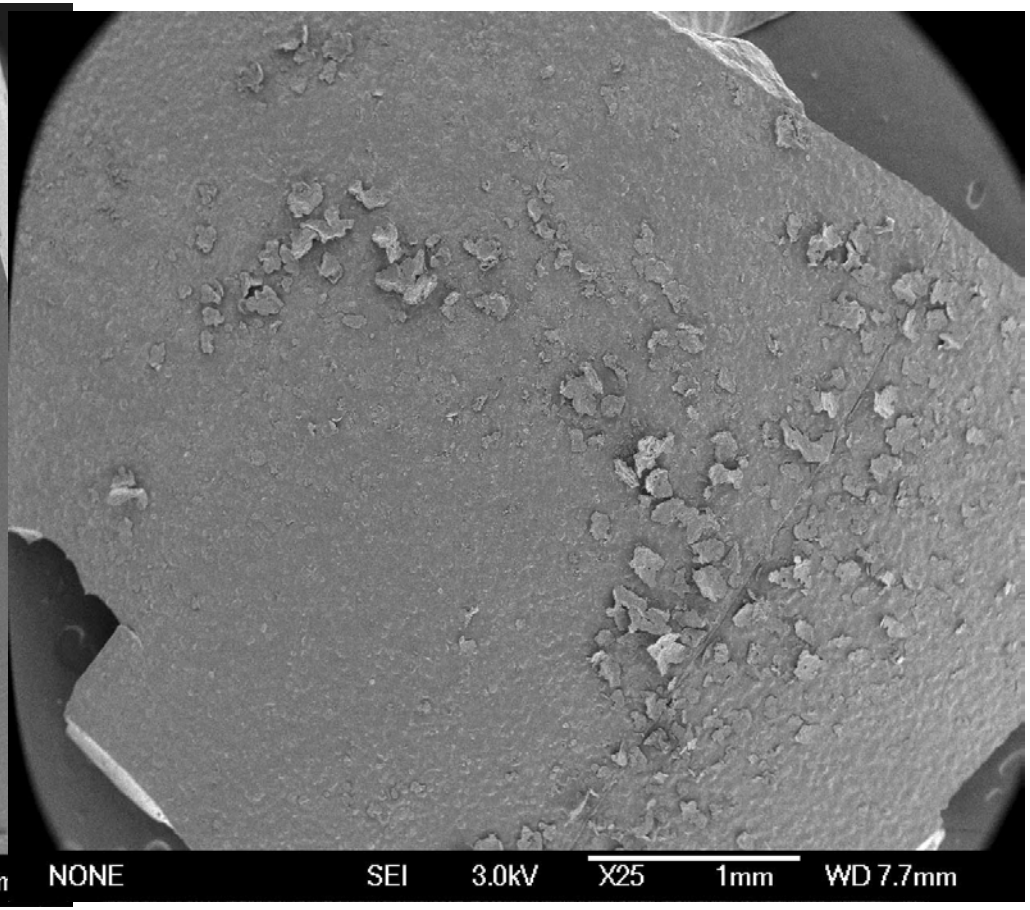
What are Biofilms

- Aggregates of bacteria within a matrix
- The bacterial secrete biopolymers to make the matrix, also called extracellular polymeric substrate (EPS)
  - Carbohydrates, proteins, extracellular DNA, lipids
- The matrix is responsible for adhesion to surfaces
- The biofilm become more complex over time

Detection of Biofilms with Scanning Electron Microscopy

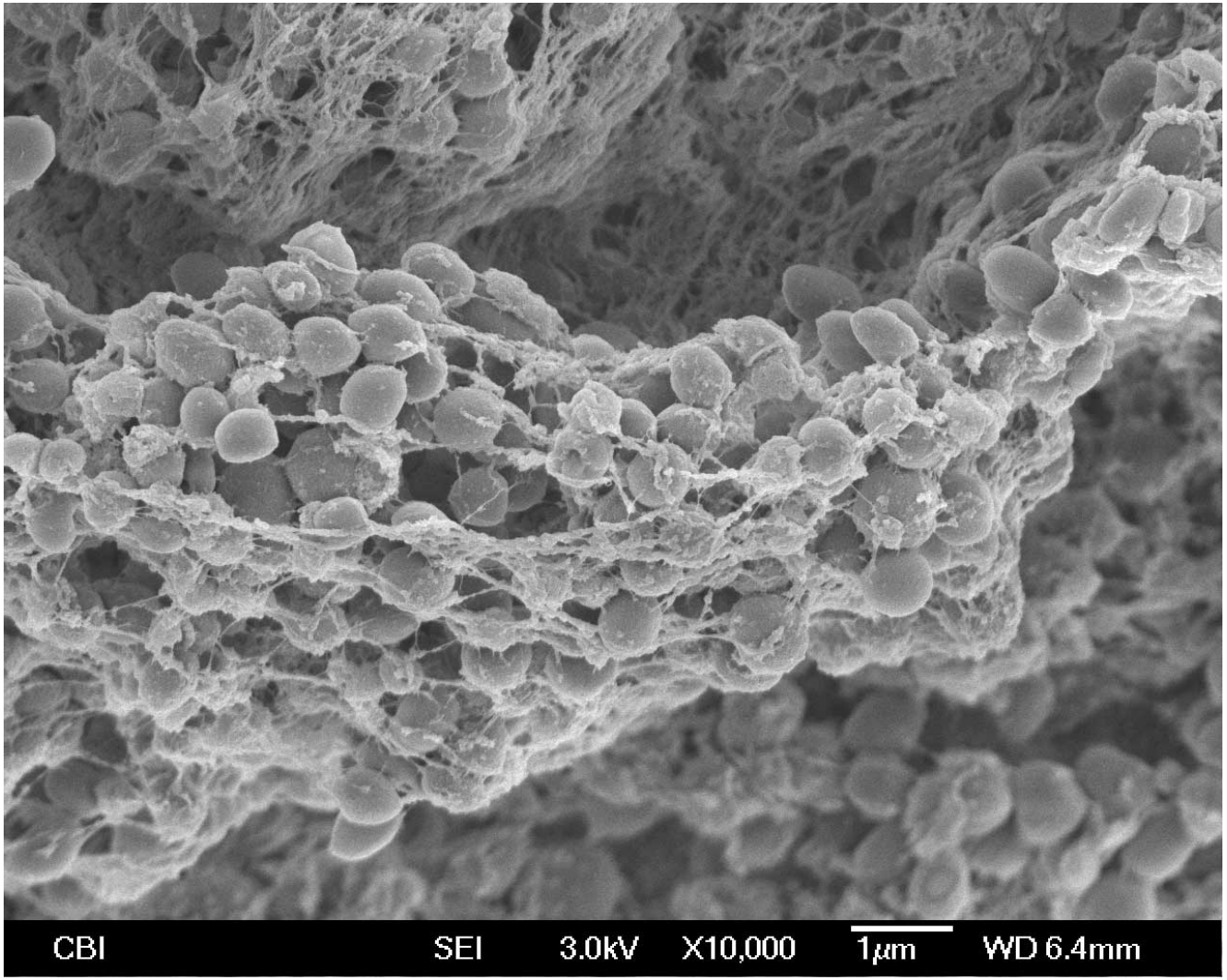


Unused ring

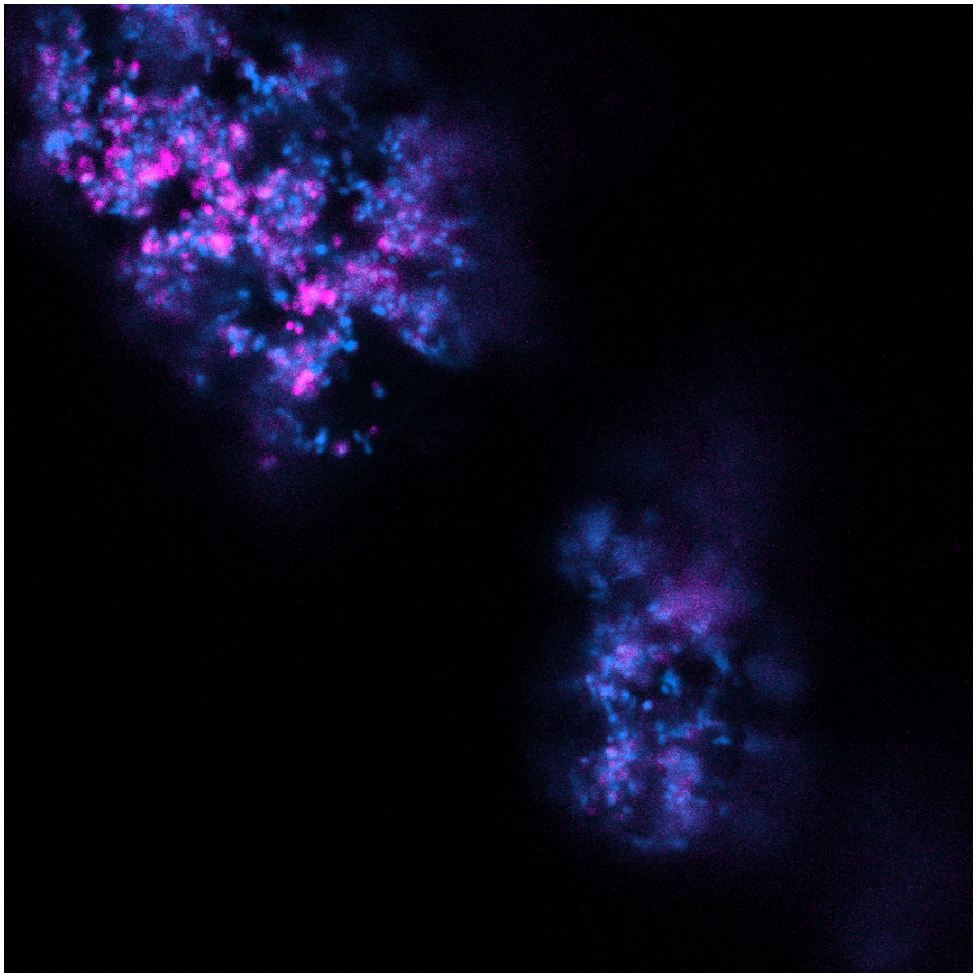


Ring worn for 28 days

Bacteria with biofilm



Detecting EPS with FISH



Gardnerella vaginal stained with DAPI and WGA

Blue: nucleus of cells
Pink: EPS

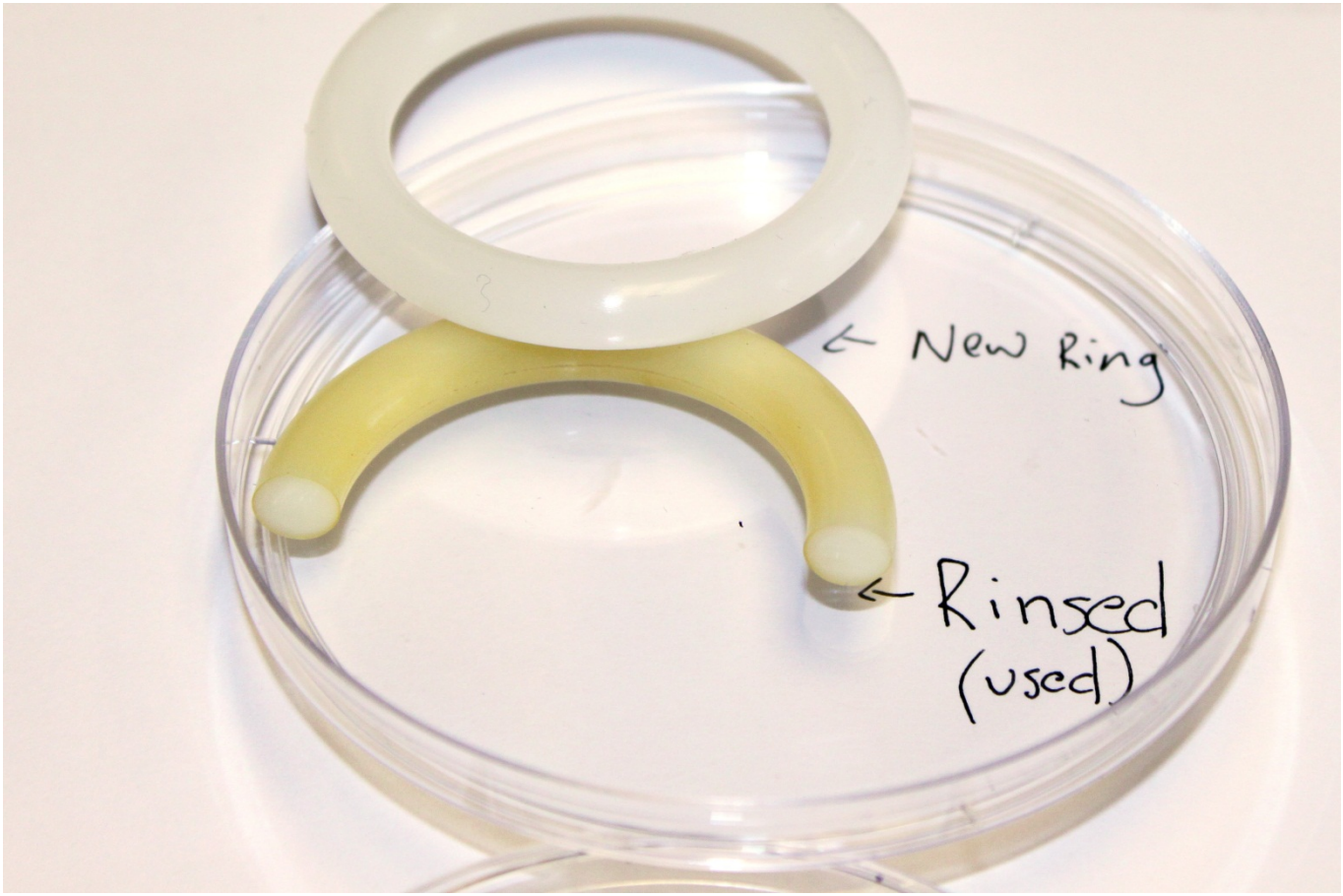
Quantifying Biofilm

- Study of Aciclovir delivery via vaginal ring for reduction of HSV transmission
- 6 women with recurrent genital HSV
  - 3 wore ring for 7 day, 3 women 14 days
- Comparison of the two groups showed the IVR worn for 14 days showed a more complex biofilm covering a larger portion of the ring than those worn for only 7 days. (Keller, MJ. J of Antimicrob. Chemotherapy 2012)

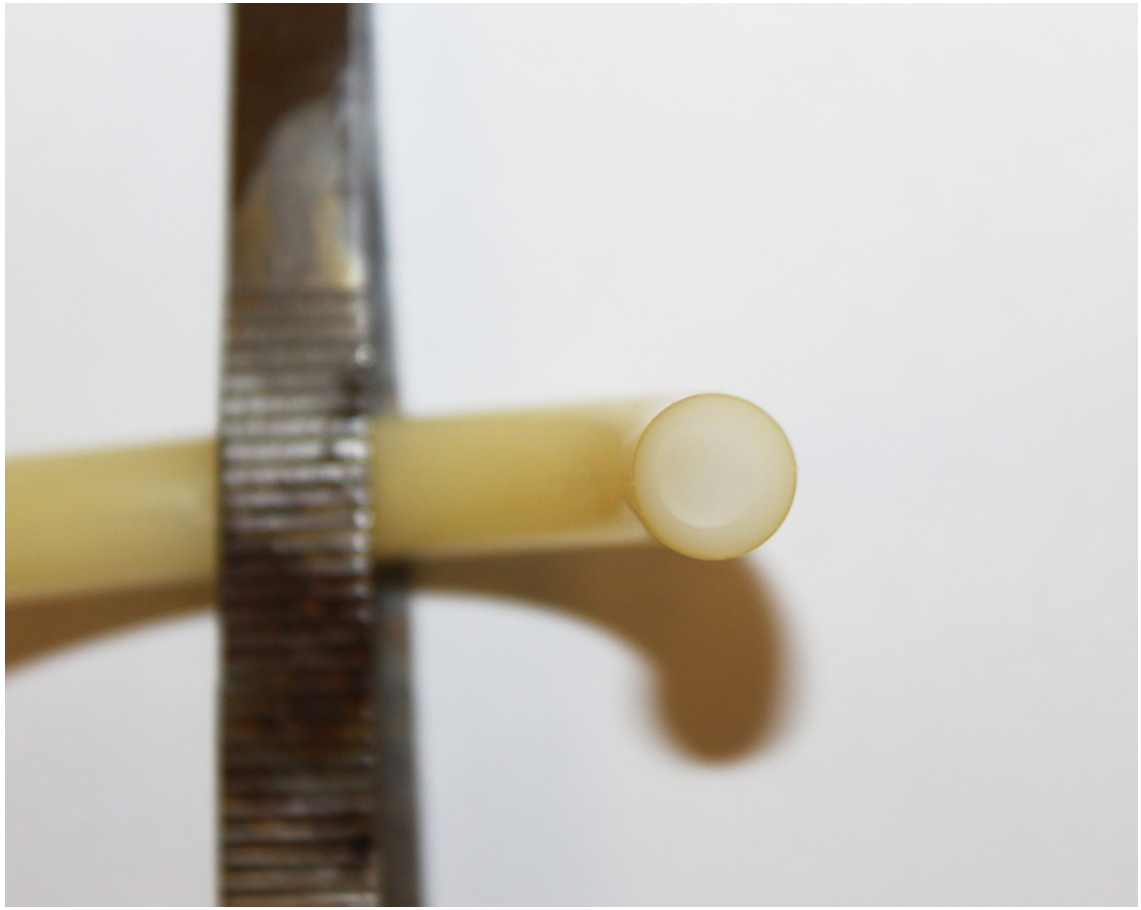
Processing Rings to Ensure Accurate Detection

- When removing the ring from the vagina limit the amount of twisting and handling
- Don’t wipe the ring
- Immediately place ring in container with 2.5% paraformaldehyde
- Must be fixed for 1 to 8 hours before the laboratory will prepare for storage and shipment
- If participant brings the ring into the clinic in a bag transfer it to the container with paraformaldehyde

Discoloration of ring

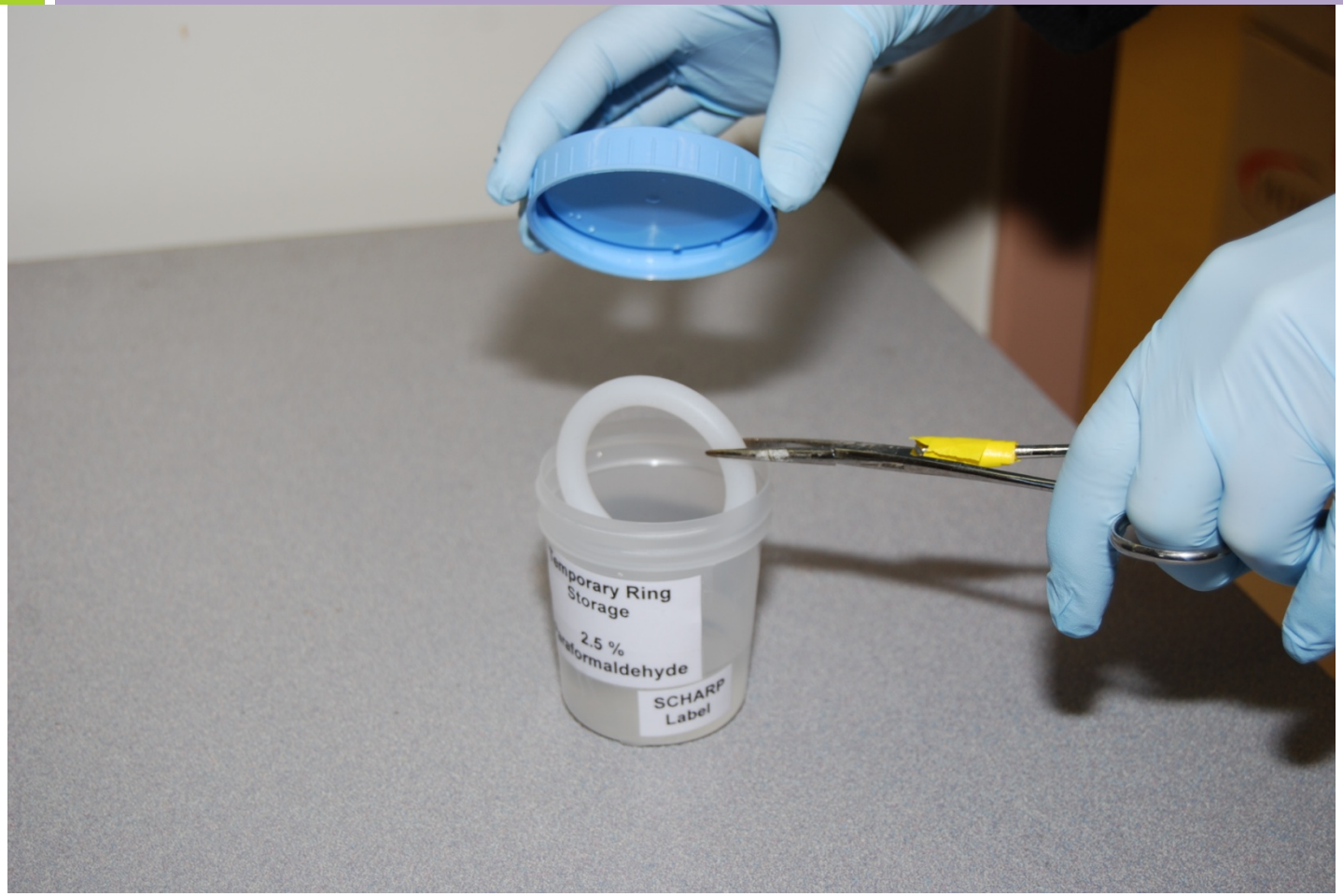


Ring is stained on the outside only



Any questions or comments?

Immediately place ring in container with 2.5% paraformaldehyde after removing from vagina



Cap tightly and transport the day of collection to the lab for processing and storage