

Extraction, Concentration and Detection of *Salmonella* from Stainless Steel Surfaces

Byron Brehm-Stecher



Recap: Why Do We Need Rapid Methods?

- **Today's food processing and distribution networks are extremely efficient**
 - **Allow rapid dissemination of raw materials and finished products on a wide (global) scale**
- **“Classical” microbiology can't keep up with pace of harvest, production, distribution, consumption. Need new methods.**

Benefits of Rapid Testing

- Reduce likelihood that contaminated products will be released for sale and consumed
- Reduce costs associated with media, labor & storage of product pending results of “traditional” microbial testing
- Can yield increased time on shelves for products cleared for sale

Thinking Holistically About Going from Sample to Solution



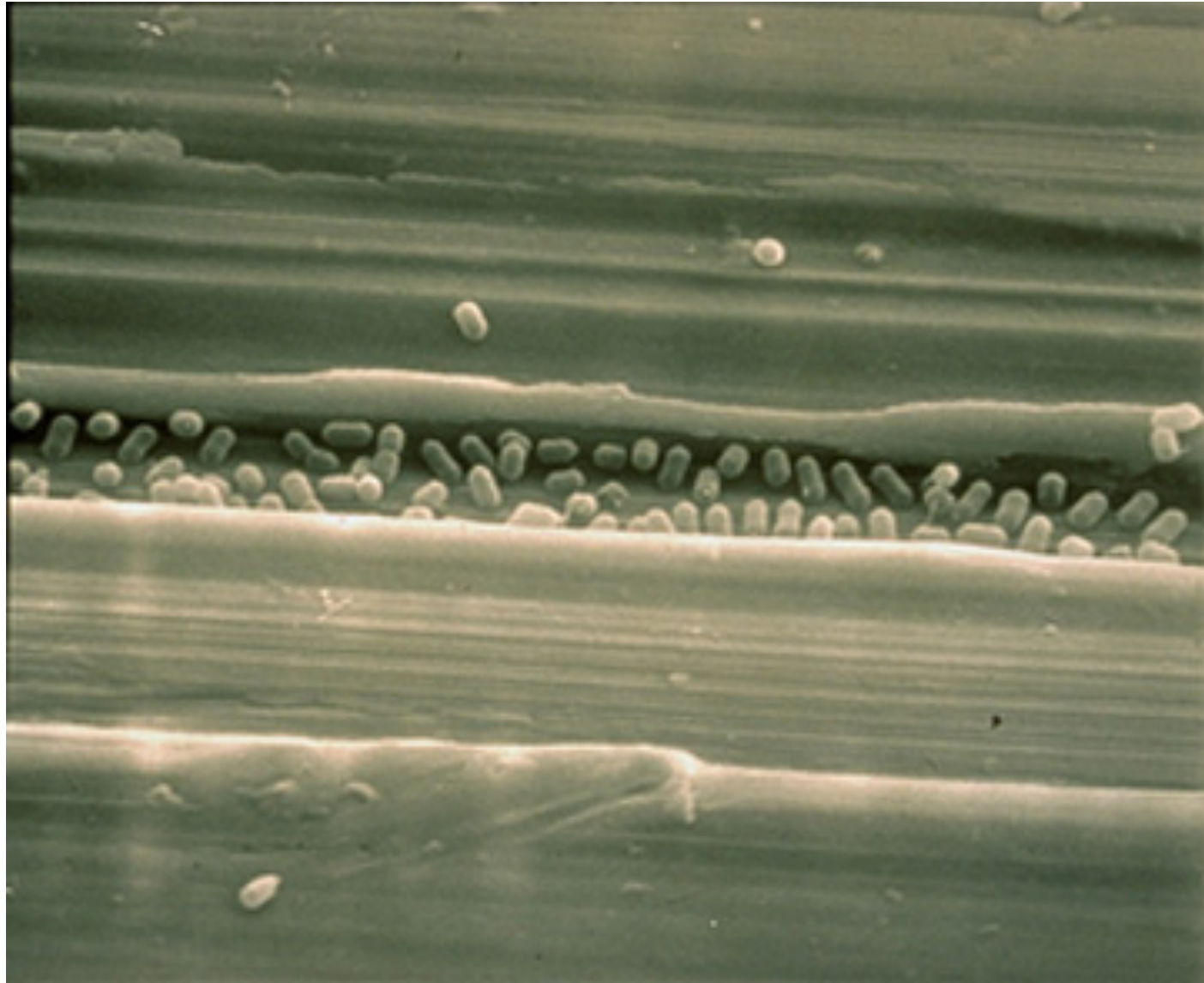
An *Ideal* Sample Prep Method Should

- *Separate* target from food or environmental matrix
- Increase the *concentration* of the target
- *Purify* target away from extraneous materials (or organisms)
- Achieve *volume reduction* in bulk samples
- Produce a *homogeneous* sample
- Extraction-Concentration-Purification methods serve as an *enabling bridge* to additional downstream steps – enrichment, detection, characterization, etc.

Extraction and Concentration of Bacteria from Food Preparation Surfaces

- Improved methods for surface testing may help *prevent pathogens getting into foods in the first place*
- Stainless steel is ubiquitous in food processing environments
- Pathogens such as *Listeria* and *Salmonella* can become trapped in the microscopically rough surfaces of macroscopically smooth steel
- These cells are difficult to dislodge/remove and may persist and contaminate foods

***Listeria monocytogenes* Embedded in Stainless Steel Surface**



Source: A. Wong, ASM Microbe Library

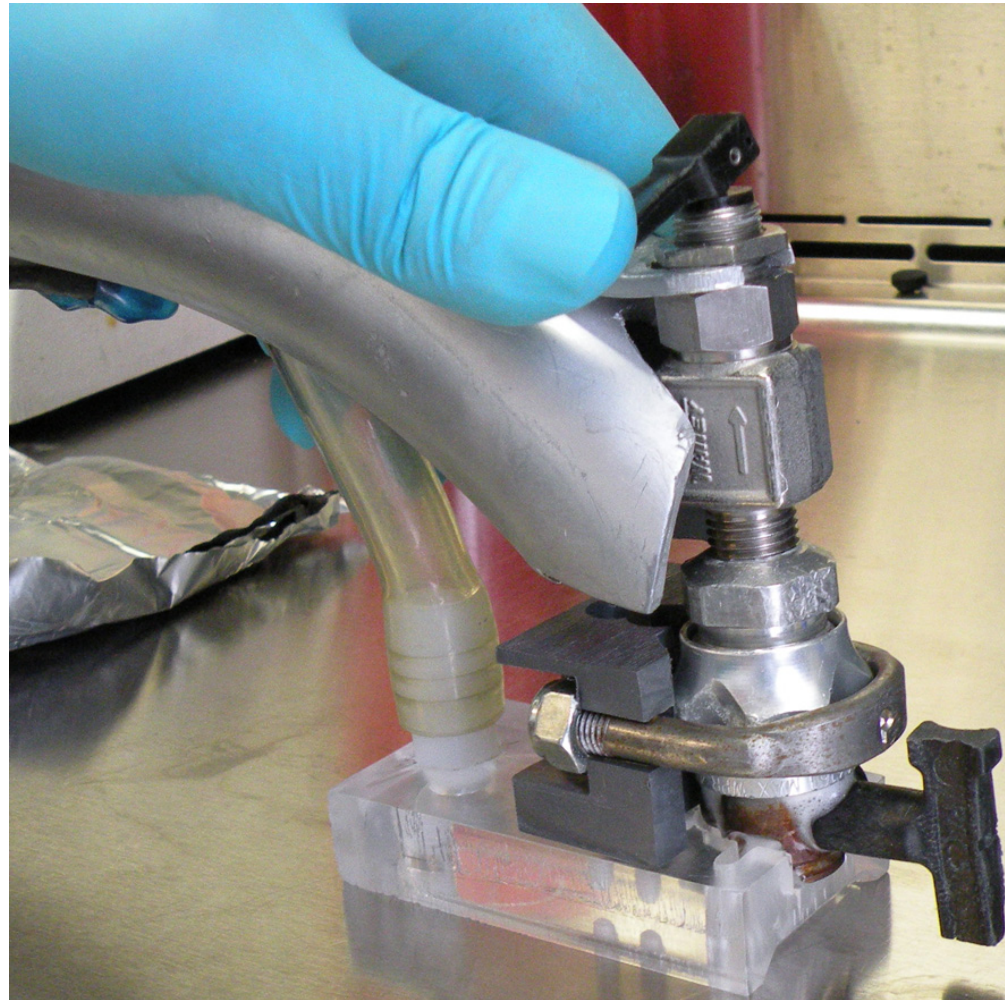
Combination of Vacuum-Based Foam Extraction and Hollow Fiber Concentration

- **Application of InnovaPrep instrumentation:**
 - **Stainless steel coupons contaminated with *Salmonella*, dried**
 - **Surfaces extracted with InnovaPrep surface extractor or cotton swab**
 - **DNA extracted from swab or concentrated extractate with PrepMan Ultra**
 - ***Salmonella* detected using quantitative RT-PCR (*invA* gene)**

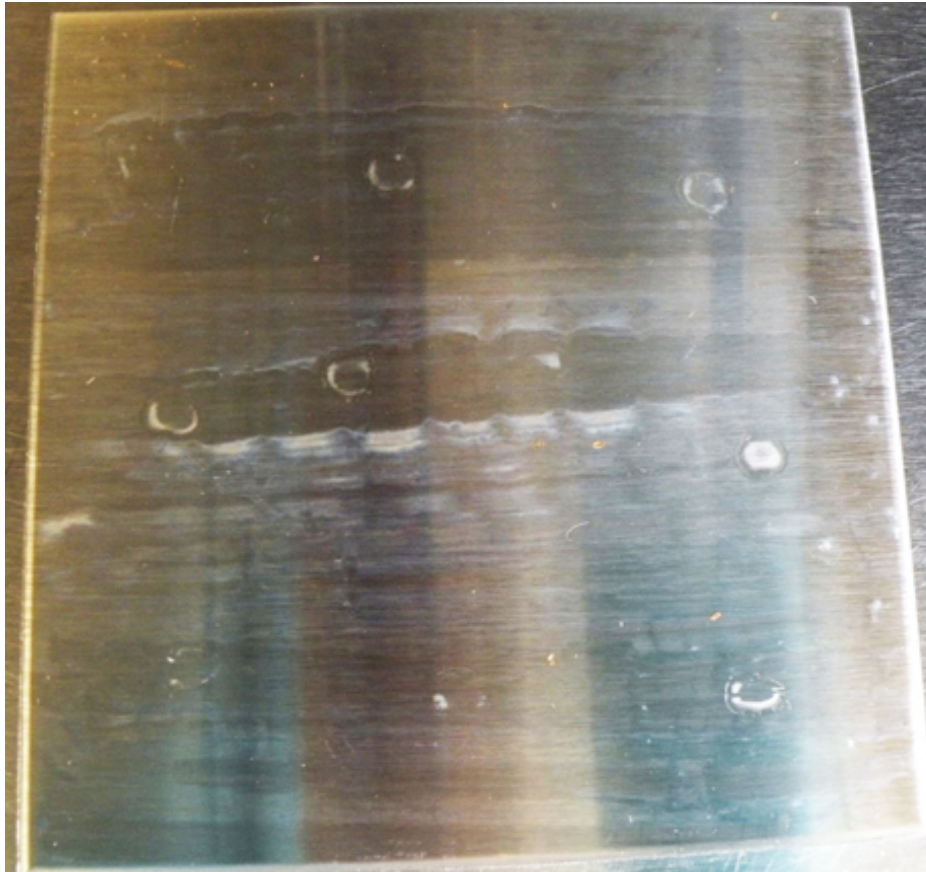
Contamination of Stainless Steel Coupons



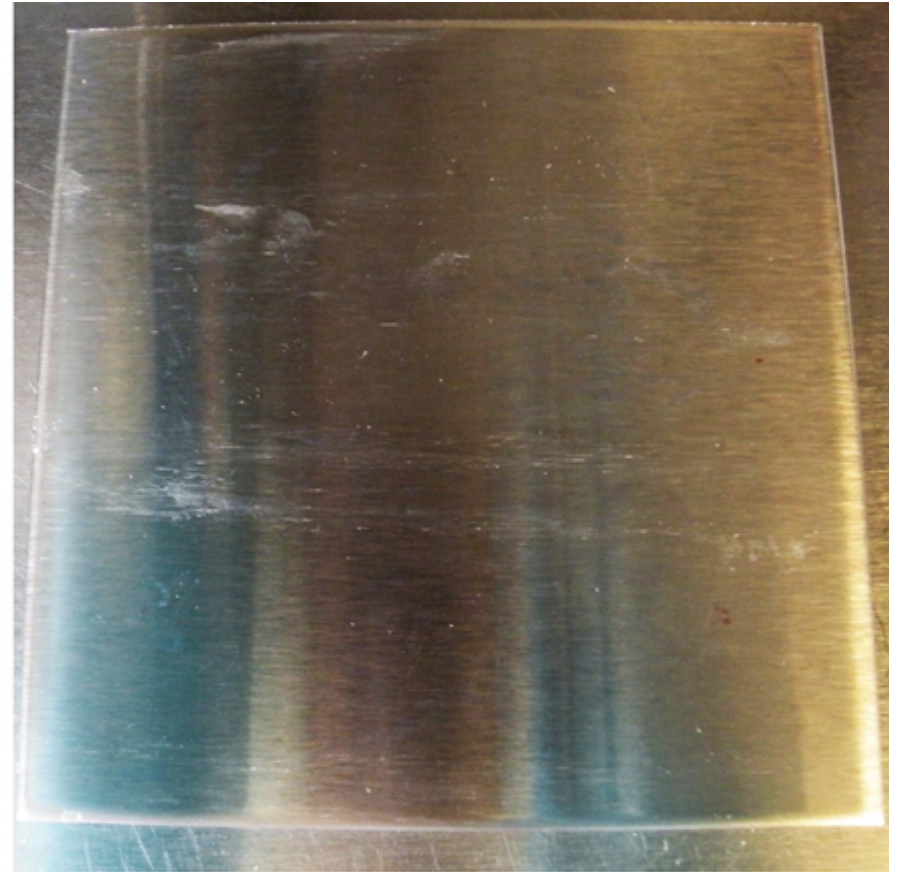
Foam-Based Vacuum Extraction (1 pass with grain, 1 pass perpendicular)



Examination of Coupons for Visible Residue After Sampling

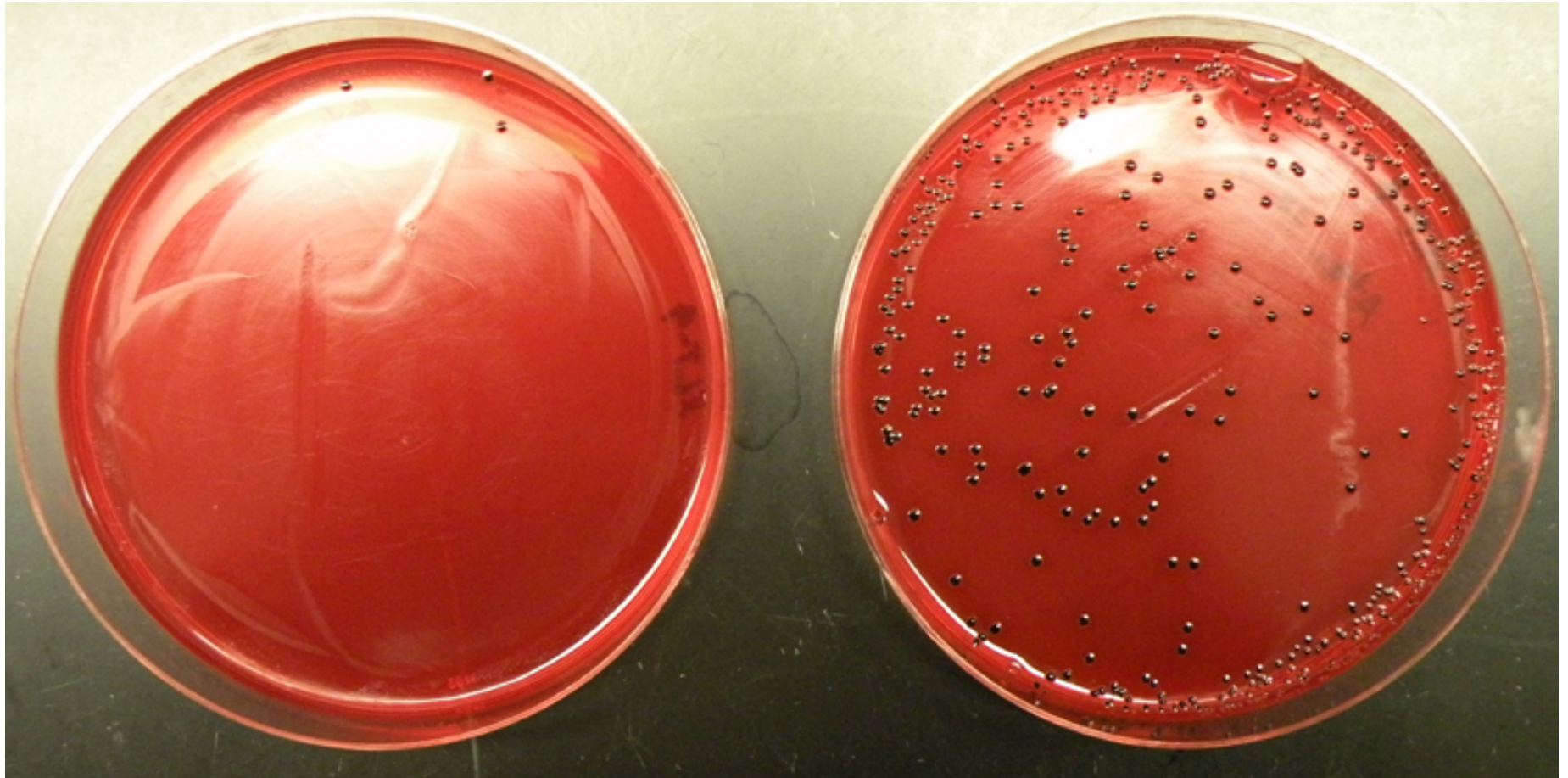


Swab



Vacuum

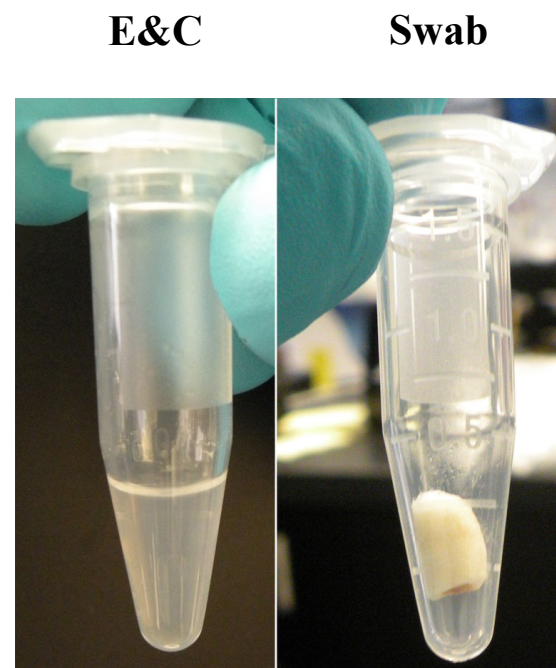
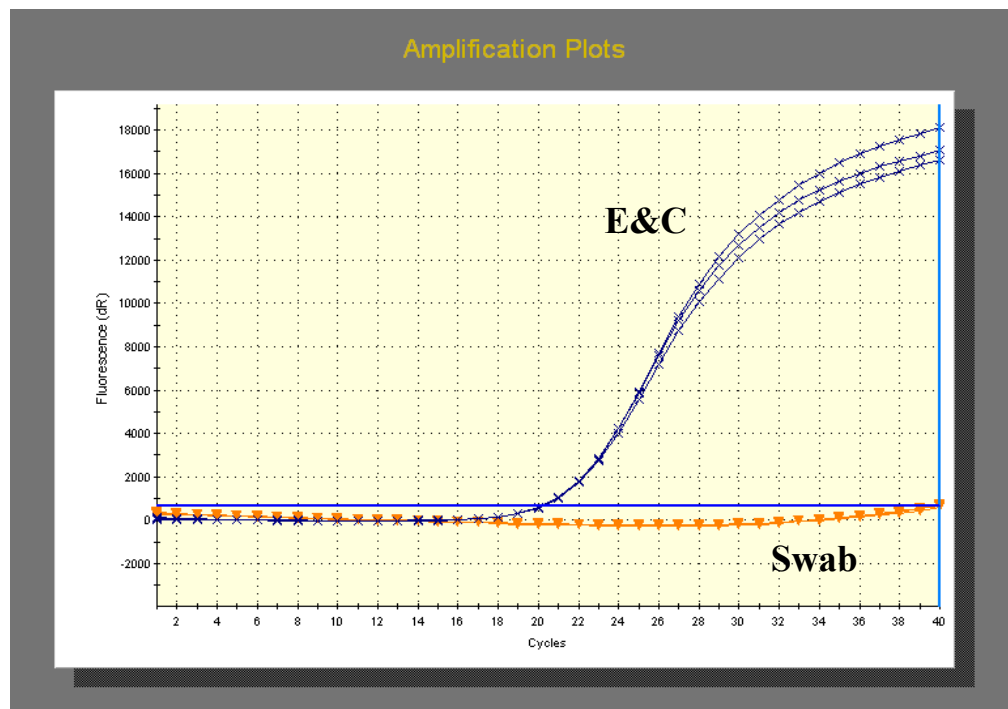
Sample Concentration with InnovaPrep HSC-40



Unconcentrated

Concentrated

Comparison of E&C vs. Swab via Quantitative RT-PCR



Swab sample

Ct value	# Copies	Dilution factor	Estimated # Salmonella
39.84	2.58	40	103.2
No Ct	No Ct	40	0
No Ct	No Ct	40	0

E&C sample

Ct value	# Copies	Dilution factor	Estimated # Salmonella
20.16	7.94E+05	20	1.59E+07
20.18	7.81E+05	20	1.56E+07
20.24	7.52E+05	20	1.50E+07

Results and Conclusions

- **More visible cell material seen on swabbed sample vs. foam-extracted sample**
- **HSC-40 concentration of foam extractate rapid and effective, verified by plating**
- ***Salmonella* readily detected in all three extracted and concentrated samples**
- **Only one swab replicate showed any *Salmonella* (~100 cells detected at cycle 39)**
- **Is swab simply inefficient, or are inhibitory substances present in swab?**
- **Study will be repeated and larger surfaces will be examined**

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