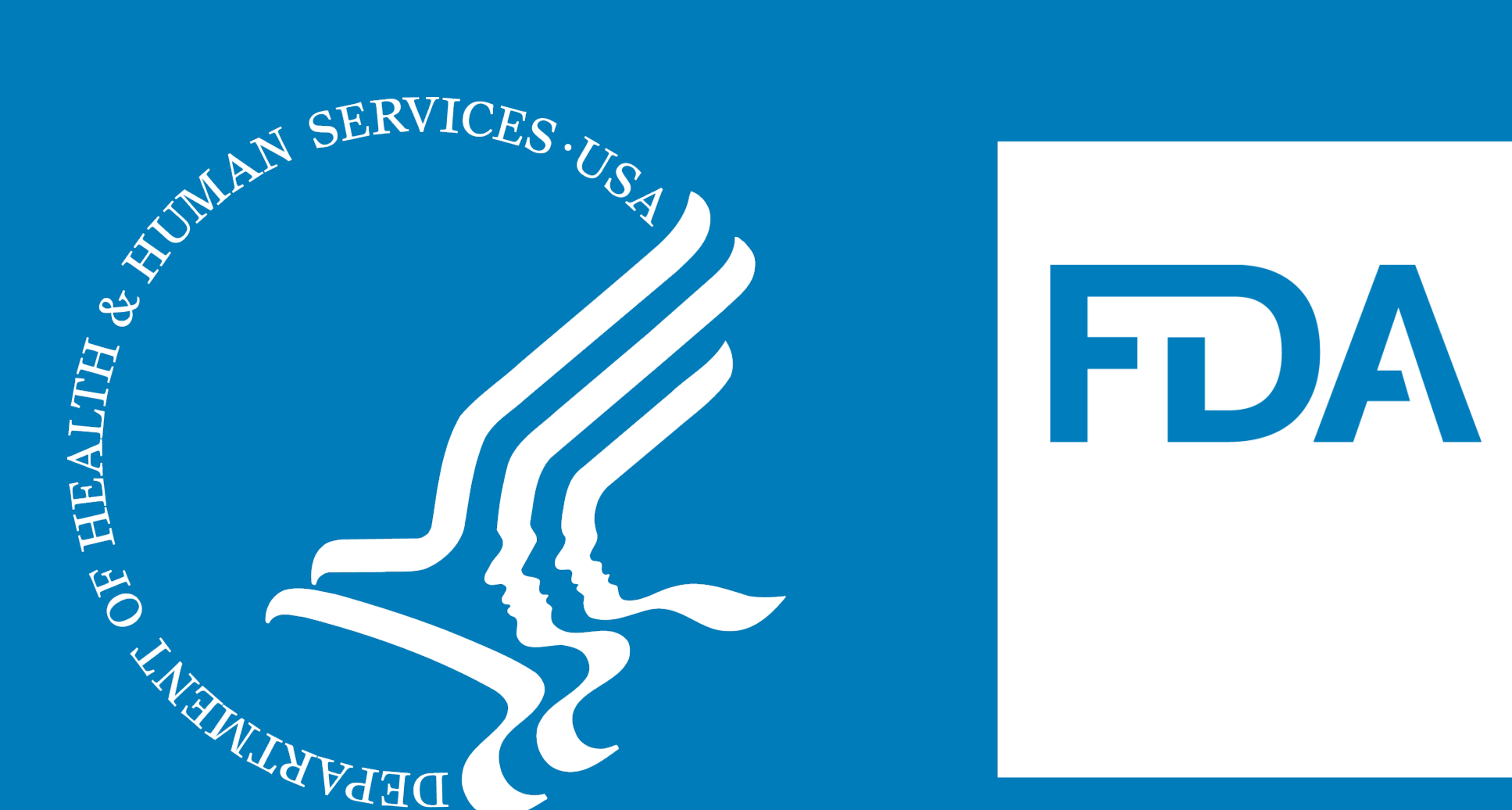


Commercial poultry litter particulates as a vehicle for *Salmonella enterica* contamination in cucumber fruit



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Introduction

Fresh fruits and vegetables are important foods for a healthy and balanced diet. Cucumbers have recently emerged as recurrent vehicles of widespread foodborne illness outbreaks caused by bacterial pathogens, including *Salmonella*. *Salmonella enterica* cause an estimated 1.2 million illnesses annually (Scallan et al., 2011) and is the primary bacterial etiological agent responsible for produce-related outbreaks in the U.S. (Hanning et al., 2009). Pre-harvest contamination of cucumber with *Salmonella* has been previously examined (Burris et al., 2020). While the means by which produce contamination occurs in the field is mostly unknown, research points to blossoms as a direct route by which fruit can become contaminated with pathogens (Zheng et al., 2013; Gautam, 2011, 2012; Erickson et al., 2018; Burris et al., 2020, 2021).

Poultry house dust is composed of feathers, skin debris, feed, litter, and feces; all of which can carry microorganisms (Madelin and Wathes, 1989). Poultry dust is considered an aerosol as it has fine particulate characteristics and is capable of dispersing easily in the environment (Al Homidan et al., 2003). Poultry-associated microorganisms from animal operations have been shown to transfer short distances into adjacent agricultural production environments (Theofel et al., 2020; Glaize et al., 2021). Therefore, the purpose of this work was to investigate the ability of *Salmonella* to colonize and internalize cucumber fruit when applied to blossoms via contaminated poultry litter.

Materials and Methods

Cucumber BSL-3P Layout

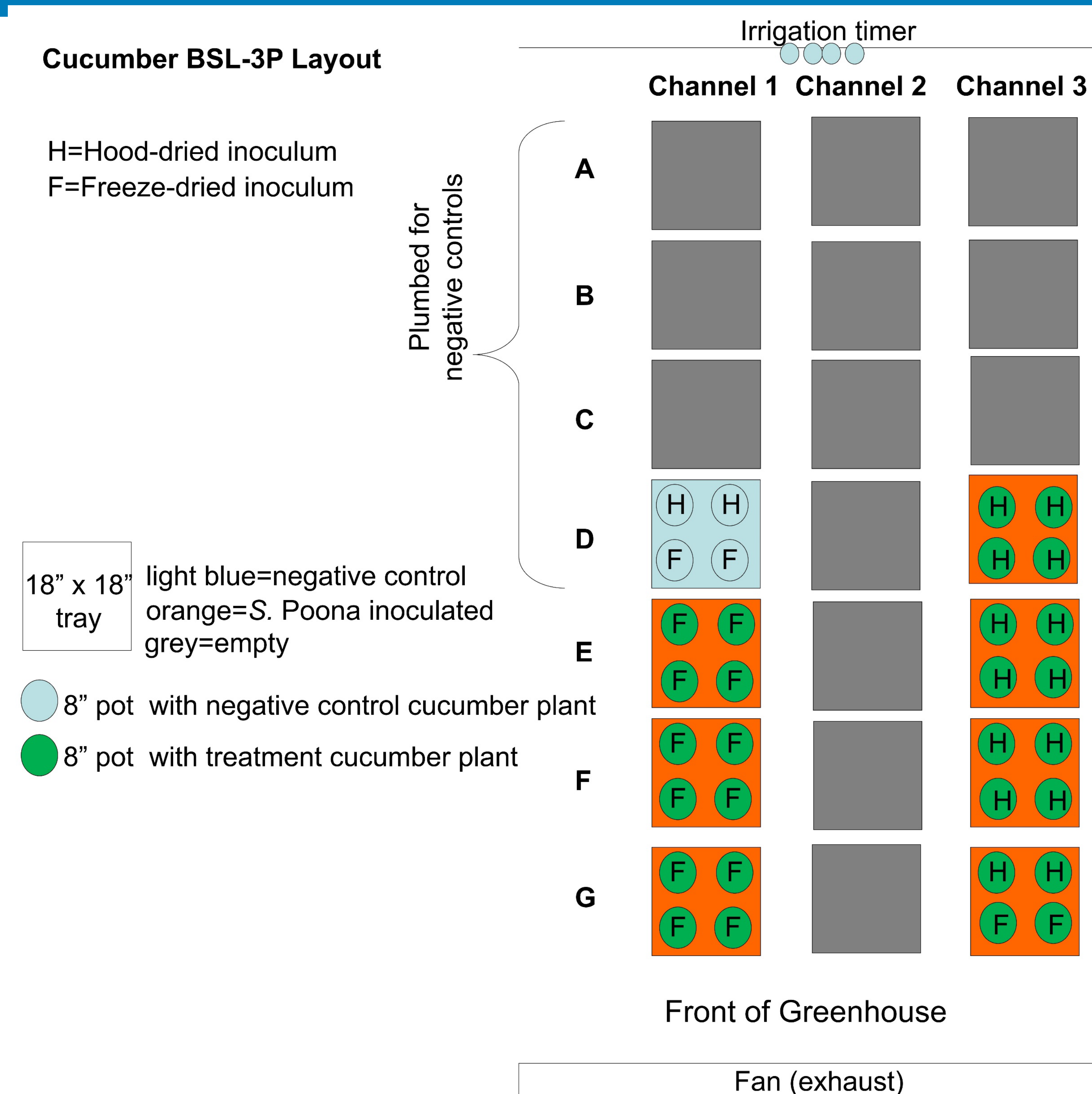


Figure 1. Schematic showing layout of NCSU BSL-3 Phytotron greenhouse during this study. *Salmonella* Poona contamination (orange) was introduced via blossoms of a single cultivar of cucumber plants (Marketmore76) at ca. 5.9 log CFU/blossom (F, freeze-dried method) or 3.2 log CFU/blossom (H, hood-dried method) via contaminated poultry litter. In total, 32 cucumber plants at the blossom stage were divided into three treatment groups: a negative control group [dosed with untreated poultry litter (PBS or skim milk, blue); n=4] and two treatment groups [orange, inoculated with freeze-dried *S. Poona* litter (n=14) or hood-dried *S. Poona* litter (n=14)].

Harvest individual fruits at maturity (4-55 dpi)

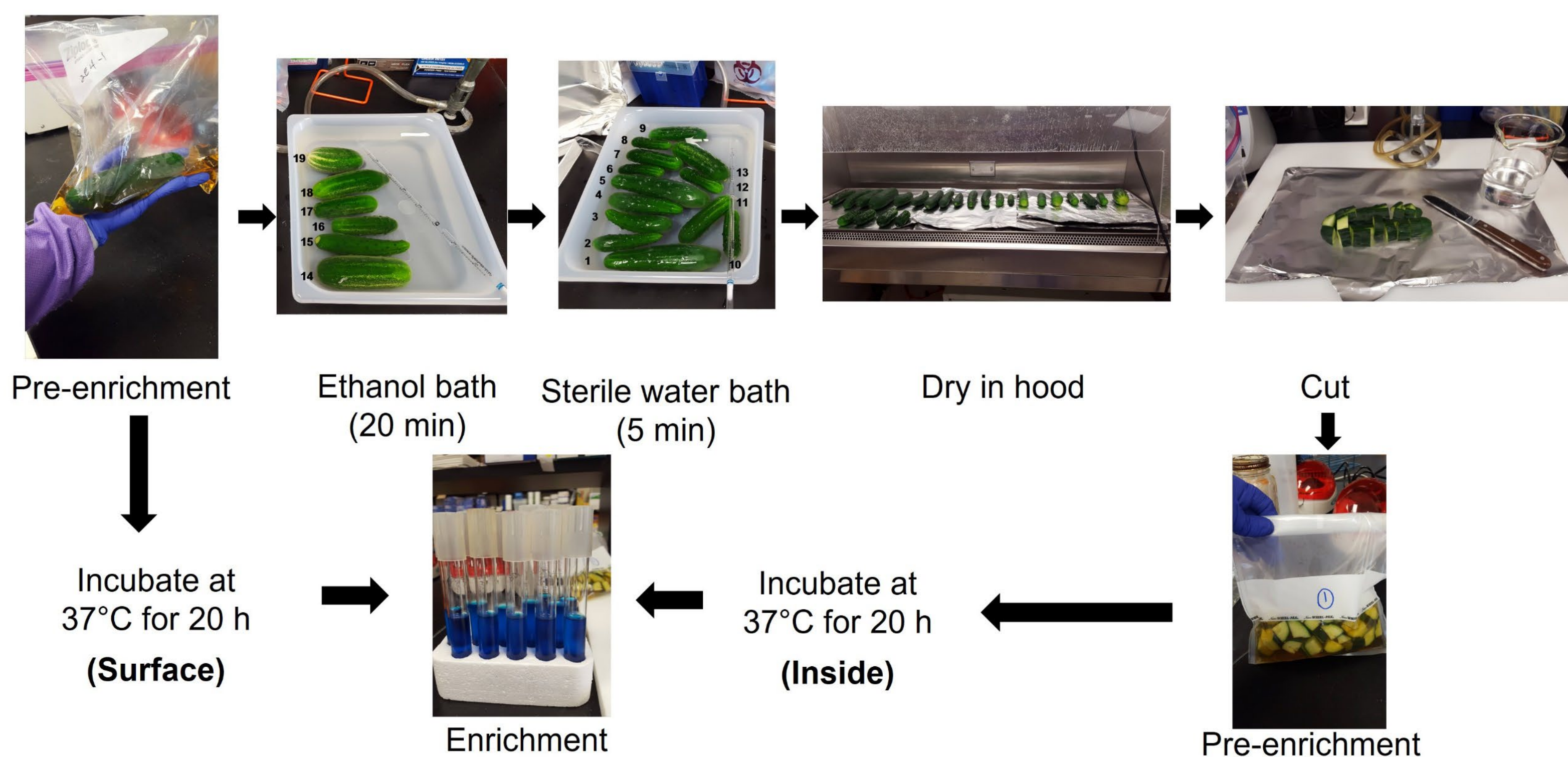
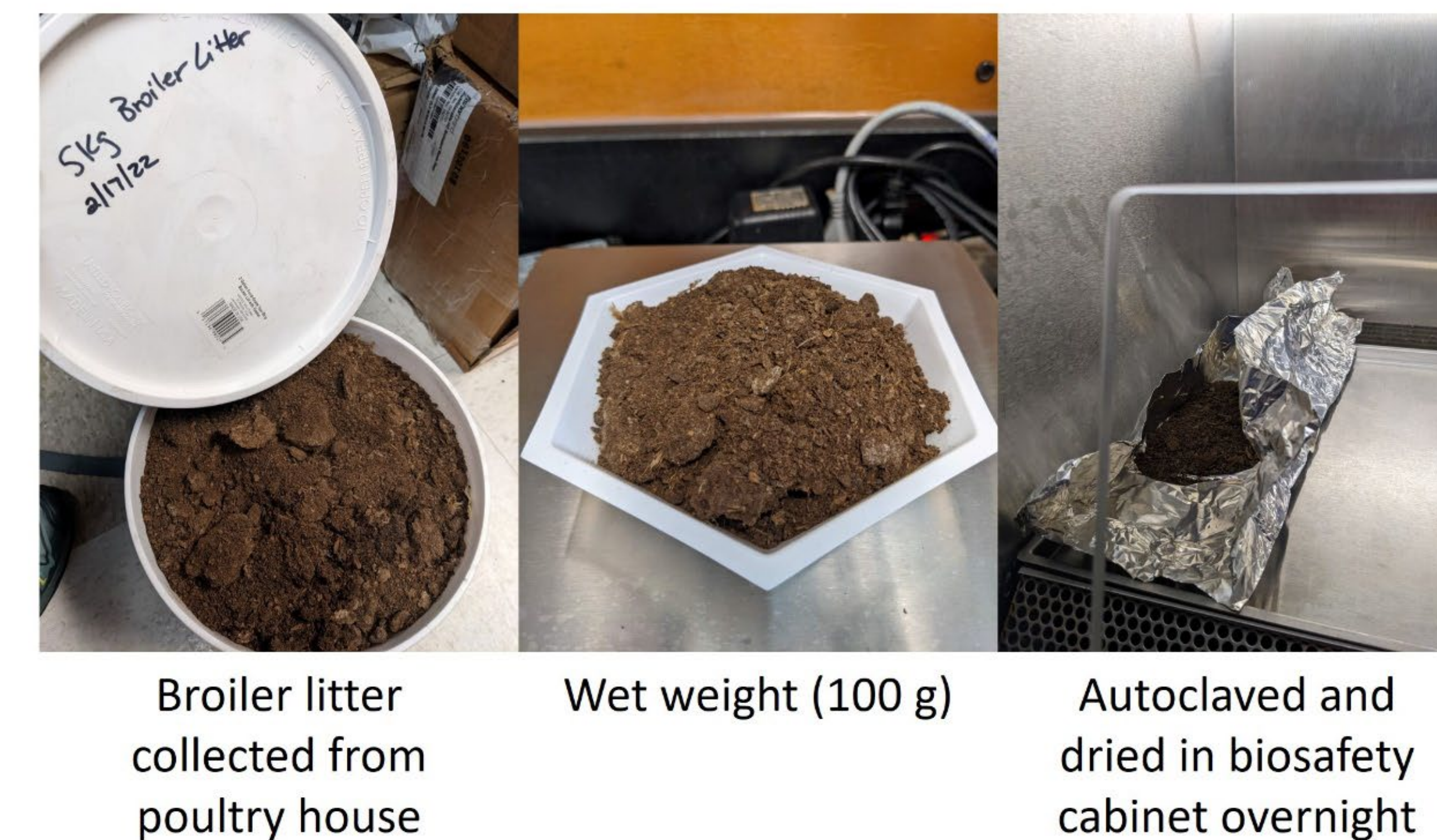


Figure 2 Schematic of mature fruit processing and *Salmonella* detection. Steps include sterilization, drying, cutting, pre-enrichment (surface and internal), enrichment, selection, isolation and molecular detection.

A. Preparation of broiler litter



B. Hood-dried method



C. Freeze-dried method

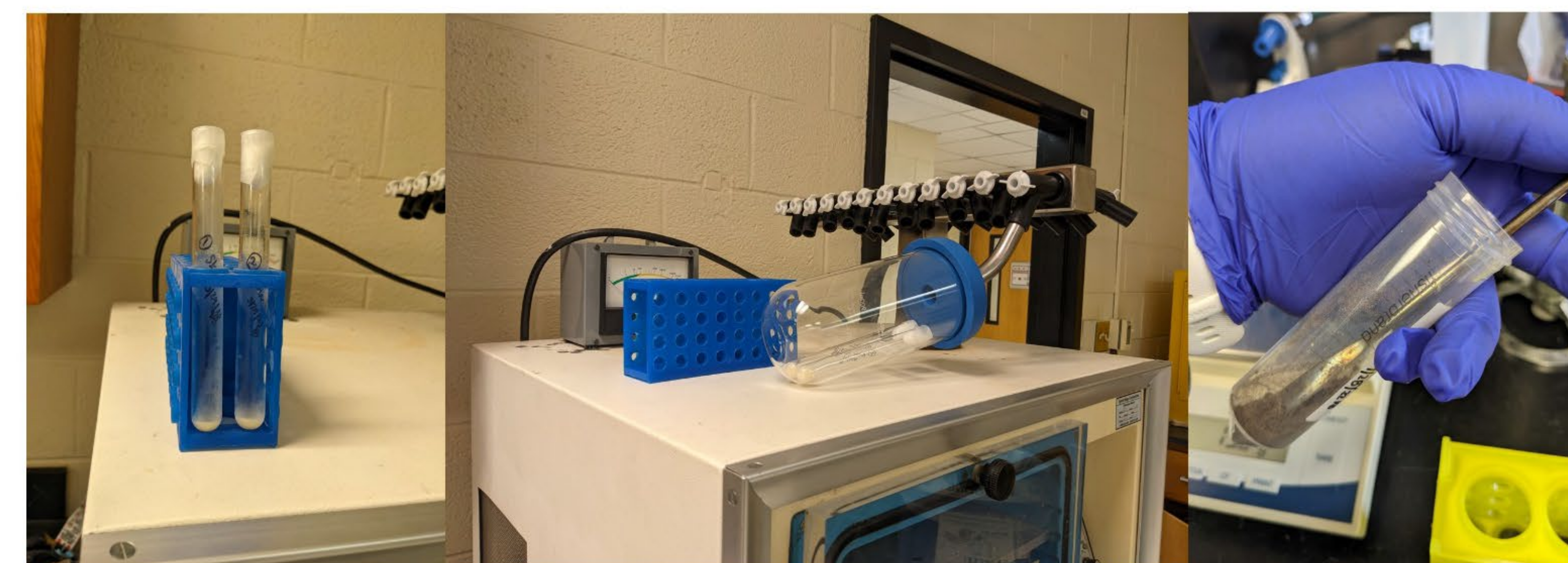


Figure 3. Schematic of commercial broiler litter inoculum preparation. Autoclaved dry litter (A) was mixed with liquid inoculum and dried overnight in biosafety cabinet (termed hood-dried method, B) or overnight inoculum was processed and washed via centrifugation, resuspended in skim milk, frozen at 20 °C and freeze-dried for 5.25 hours (termed freeze-dried method, C) and applied to female cucumber blossoms using forceps (ca. 10 mg, D).

D. Blossom inoculation



Results

Table 1. *Salmonella enterica* contamination of mature cucumber fruit when introduced through blossoms via contaminated poultry litter.

Method (ca. log ₁₀ CFU/blossom)	# colonized/total # of fruit (%)	# surface only/total # of fruit (%)	# surface and inside/total # of fruit (%)
Air-dried (3.6)	10/37 (27.0)	7/37 (18.9)	3/37 (8.1)
Freeze-dried (6.2)	15/24 (62.5)	13/24 (54.2)	2/24 (8.3)

Prevalence of internal contamination was equivalent when comparing inoculum methods ($X^2=0.001$, $P=0.9750$). Surface contamination was significantly lower in air-dried versus freeze-dried method ($X^2=7.573$, $P=0.0059$).

Conclusion

These results identified contaminated poultry litter as a means for *Salmonella* to colonize and internalize mature fruit when introduced to blossoms during pre-harvest.

Acknowledgements

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