Assessing the Enhanced Sampling Power of a Penetrative Sampling Device, MicroTally® MicroBroom™ Sampler, Over Conventional Tissue Sampling for Preharvest Microbial Assessment of Romaine Lettuce

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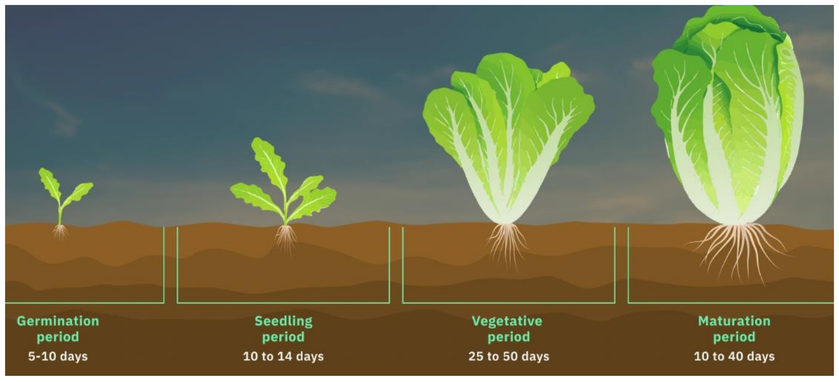
MicroTally® by FREMONTA

Why Penetrative Sampling of Romaine?

- Confirmed outbreaks were missed
- The head opens to the soil
- Contamination hides inside
- Middle third more likely to test positive



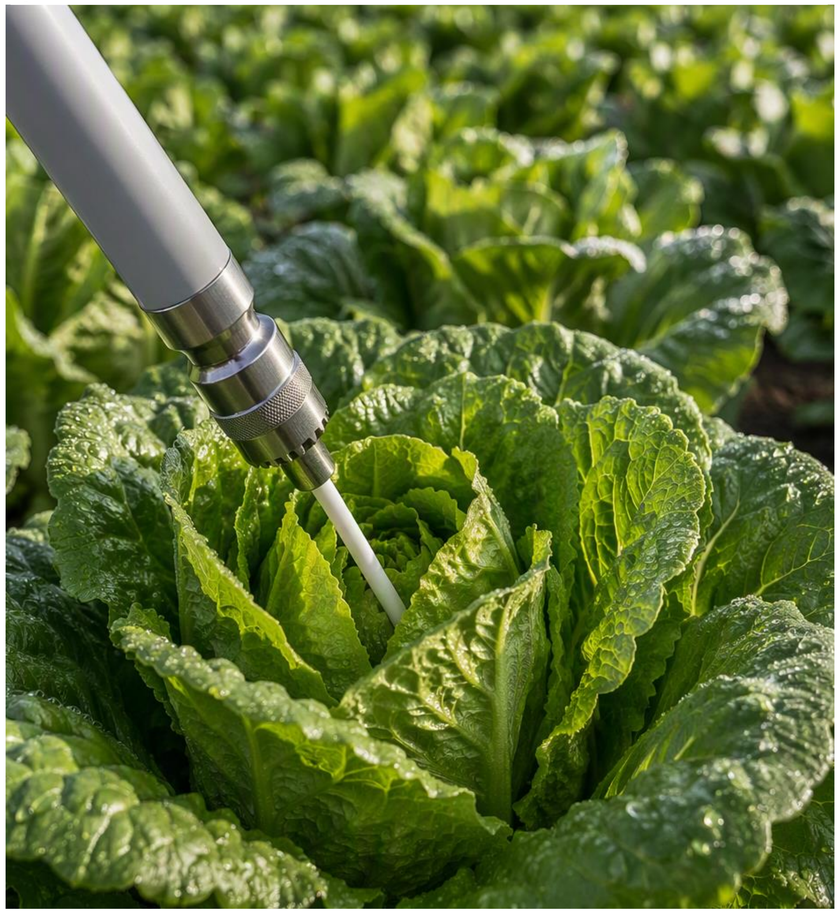




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Cloth-based Penetrative Sampling

Using MicroTally® MicroBroom™ sampler, a shaft mounted cloth sampler, penetrative aggregated samples of 60 heads of Romaine lettuce were generated and tested for markers: *ybb*W, *fli*C, *tet*A, *tet*B, *pho*E, *ygf*E1, *rps*U-MT1.



Marker Observations

Marker	Description	% Cloth	% Tissue	P-value Fisher	P-value Psi
Entero-set 1	*Enterobacteriaceae*	100	92.40	0.01	Error
***ybb*W**	Generic *E. coli*	31.50	23.90	0.32	0.11
***fli*C**	H7 flagella antigen	5.40	3.30	0.72	0.33
***tet*A**	Tetracycline resistance (Broad indicators of *E. coli* and *Salmonella*)	8.70	7.60	1	0
***tet*B**	Tetracycline resistance (Broad indicators of *E. coli* and *Salmonella*)	30.40	31.70	0.81	0.25
***pho*E**	*Klebsiella pneumoniae*	54.30	31.50	0.01	0.91
***ygf*E1**	*Salmonella* & *Citrobacter*	94.60	90.20	0.41	0.55
***rps*U-MT1**	*Listeria* & *Brochothrix*	0.00	0.00	1	Error

Summary of MicroTally® MicroBroom™ Samplers Performance

- Overall Cloth outperformed tissue (P~0.004, N=736).
- Cloth outperformed tissue in detecting Entero-set 1, phoE (P~0.01).
- Cloth trended better or matched tissue in detecting the remaining six markers.
- Cloth achieved 100% detection of *Enterobacteriaceae*, but tissue did not.

Is Aggregated Penetrated Sampling Validated?

- **No**, it has been shown to be as good or better at detecting the selected markers.
- The ideal comparison: equal or better rare-pathogen prevalence vs. tissue grabs
- FREMONTA developed a panel of genetic markers
  – Target attributes common to pathogens and related organisms
- Best comparison prevalences in enriched samples: ~20–80%
- TF collected 92 matched tissue grabs (375g) and cloth-based samples in Romaine preharvest testing
- Samples enriched, DNA extracted, and marker presence assessed by PCR
- Validates the sampling approaches

Abstract

Introduction: Romaine lettuce has been identified as causative in many fresh-cut produce related outbreaks. Pre-harvest testing data indicates that conventional tissue sampling may underreport pathogen presence. It is speculated that solarization is an important mitigation which does not impact the interior of the head. Penetrative sampling can potentially overcome this limitation.

Purpose: To compare a penetrative cloth-based aggregative sampling to conventional composite tissue grabs for microbial assessment of Romaine lettuce given the expected absence of pathogens.

Methods: During the regular pre-harvest assessment, 88 penetrative aggregative samples where 60 heads were spiked and sampled with the cloth sampler in parallel to 88 375g (a correction) tissue composites where 150 (a correction) 2.5g specimens were collected. Samples were enriched and screened for pathogens (stx and invA). Purified DNA was assayed for 6 rare markers (tetA, tetB, ybbW, phoE, EB-set1, and fliC) similar to those used for validating cloth sampling of protein foods. The markers are loosely associated with pathogens making suitable surrogates for the extremely rare pathogens to examine sampling efficacy. Prevalences, tissue versus cloth, for each marker were compared with Fisher's exact test for difference and PSI correlation coefficients were examined pairwise to assess correlation.

Results: No pathogens were detected as expected in this number of samples. Prevalence for the markers reached up to 100%. Cloth sampling did as good or better for all markers with no significant differences (P's > 0.2). The results for fliC and tetA showed significant positive correlations (P's < 0.05).

Significance: These results suggest that sampling the interior of Romaine lettuce would better detect pathogens than conventional tissue grabs. The MicroTally® MicroBroom™ sampler is on approach to sampling the interior of Romaine lettuce.