

# Sampling for Microbial Analysis



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## Microbial Testing

- One of the most apparent but poorly understood parts of food microbiology and quality assurance

## Why test?

- Regulatory
  - Recommendation
  - Requirement
  - Testing Program
- Buyer Requirement
- Product history
- In-house Specifications for Ingredients
- HACCP Validation/Verification
- **Everybody is Doing It**
- **It Seemed Like a Good Idea**

## Why not test?

- No scientific validity
- Too costly
- Test not available
- **Don't want to know**



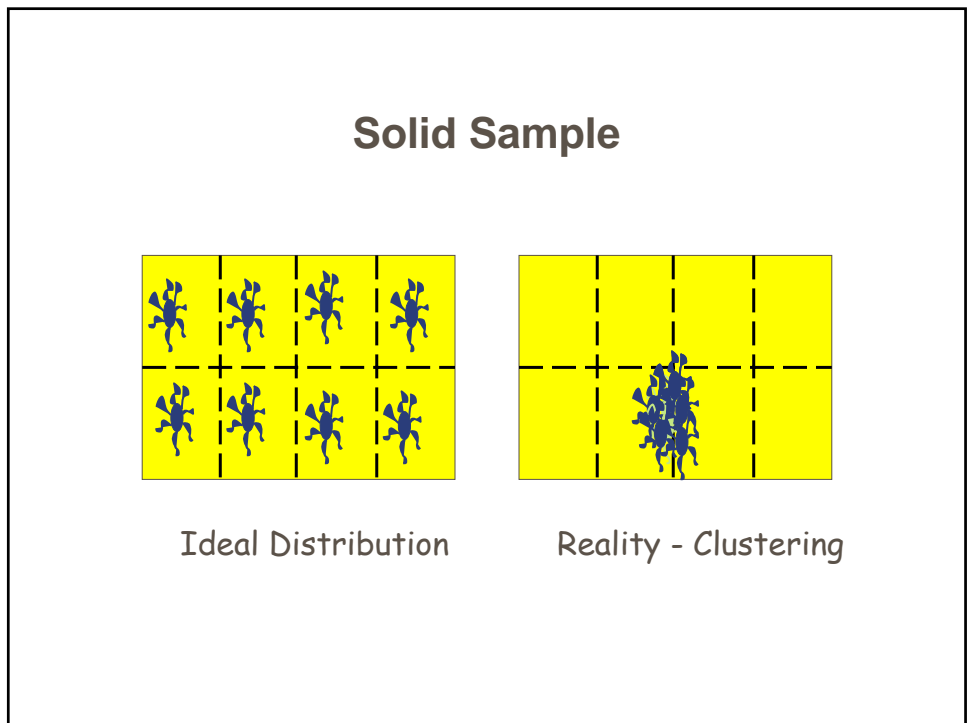
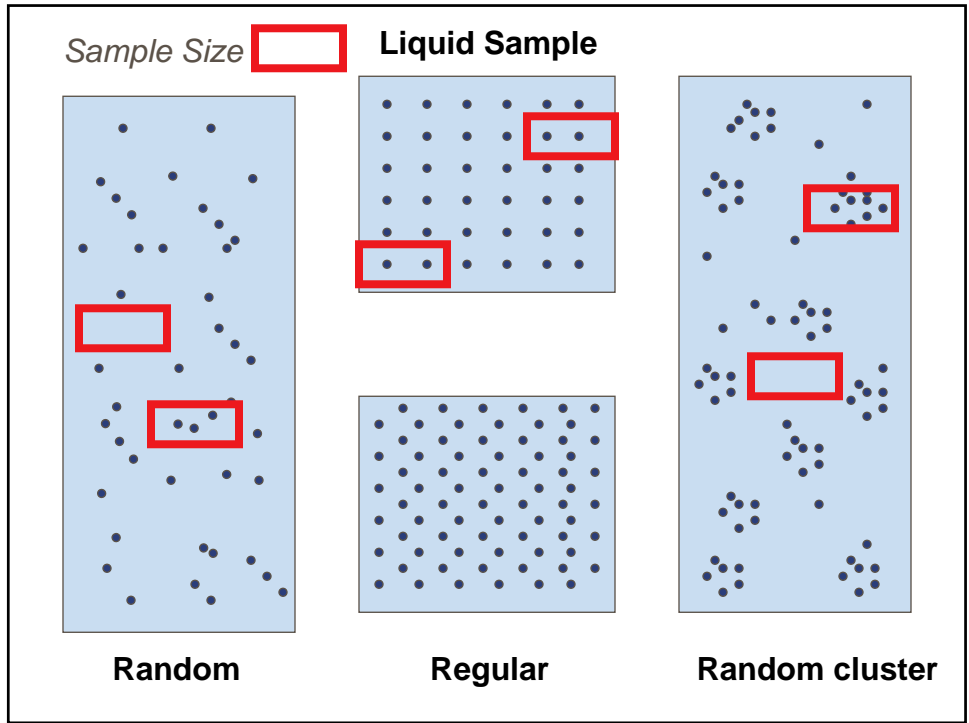
## Microbial Testing

- Effective use requires a clear understanding of the goals, assumptions, and characteristics of the testing program



## Collecting Samples

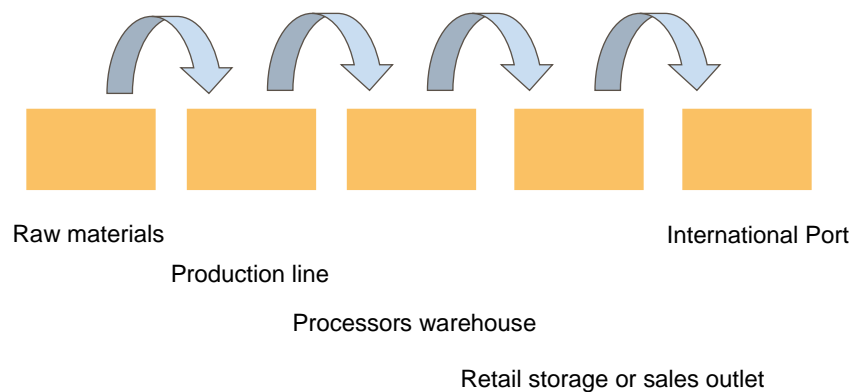
- Lot must be defined, samples selected randomly
- Microbial sampling is destructive
- Results may be delayed for several days
  - Perishable product may be impacted



## Sample Size

- Based on standard methods for test organism
- For non-pathogens, typically 10 g
  - Could also be surface area 100 cm<sup>2</sup>
- For pathogens, typically 25 g, may be higher
  - These samples can be pooled.  
60 samples of 25 g can be 4 samples of 375 g.

## Points of Sampling



## How? - Sampling Plan

- Written Sampling Plan
  - Objectives or goals
  - Who will sample?
  - How to sample? (Aseptic Technique)
  - What to sample?
  - How much to sample?
    - How many? How large? How often?
  - How to store/transport?

## What organism?

- Utility organisms
  - Aerobic plate count
  - Spoilage microorganisms
- Indicator organism
  - Coliform
  - Thermotolerant coliform
  - *E. coli*
- Specific pathogen



## Problems with Testing for Pathogens

- Contamination non-uniform
- Levels are typically low
- Negative results give no real assurance

## How? Qualitative



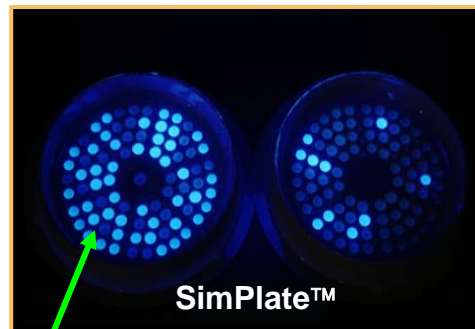
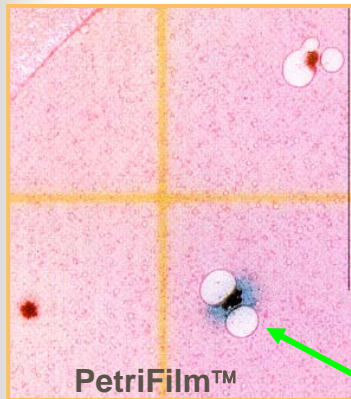
Coliform/*E. coli* Presence/Absence



Colilert

## How? Qualitative

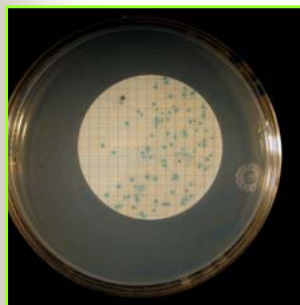
Coliform/TTC/*E. coli* - Enumeration



Nonpathogenic *E. coli*

## Coliform/*E. coli* Enumeration

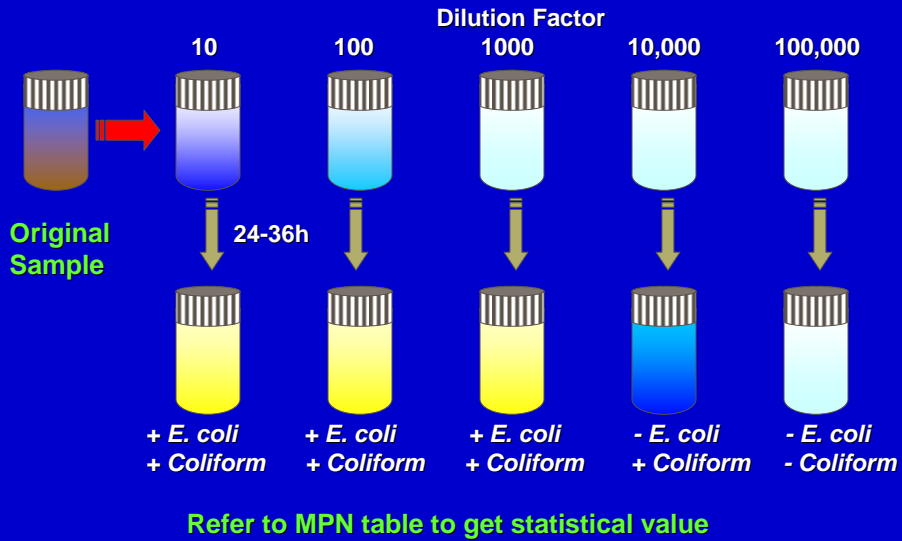
Membrane Filtration



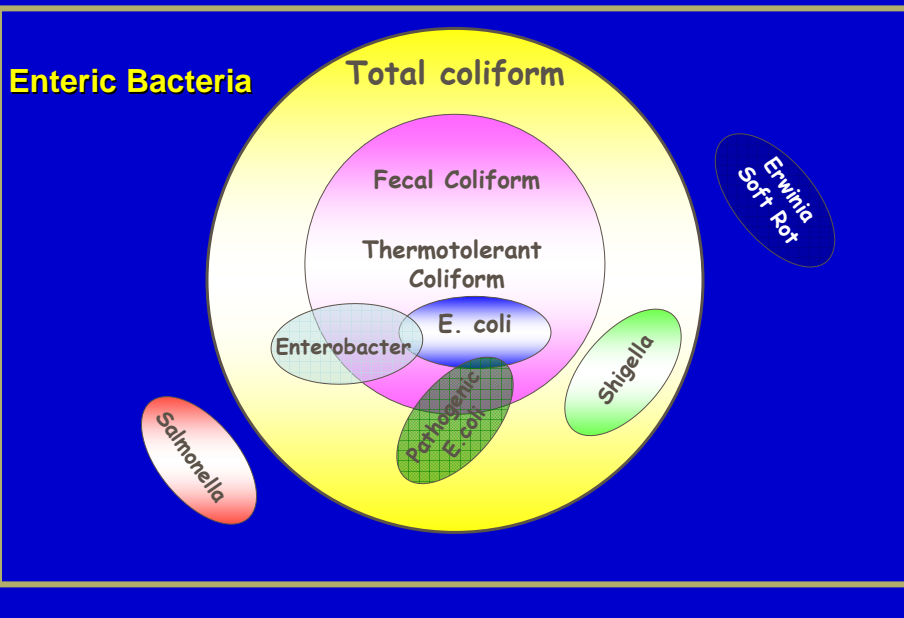
Quantitray



## How Is MPN Testing Done?



## What Do the Tests Tell us About Food Safety?



**Approximate Distribution of Phyllosphere and Rhizosphere Thermotolerant Coliforms that Test Positive in Fecal Coliform Assays**

Possible fecal origin

*Citrobacter freundii*  
*Citrobacter* spp.  
*Klebsiella pneumoniae*  
*Klebsiella* spp.  
*Enterobacter cloacae*  
*Enterobacter aerogenes*

Freq of isolation from plants

70-100%  
 70-100%  
 50-80%  
 50-100%  
 50-100%  
 50-70%

Unlikely fecal origin

*Pantoea agglomerans*  
*Pantoea* spp.  
*Enterobacter sakazakii*  
*Serratia marcescens*  
*Serratia liquefaciens*  
*Serratia odorifera*  
*Klebsiella trevisanii*

highly common  
 highly common  
 highly common  
 very common  
 very common  
 common  
 common

**Absence of Evidence  
 is not  
 Evidence of Absence**

Probability Of Microbial Detection

Percent Contaminated	Number of Samples Analyzed					
	5	10	15	20	30	60
10%	41	65	79	88	96	>99
5%	33	40	54	64	79	95
2%	10	18	26	33	45	70
1%	5	10	14	18	26	45
0.1%	1	1	2	2	3	6

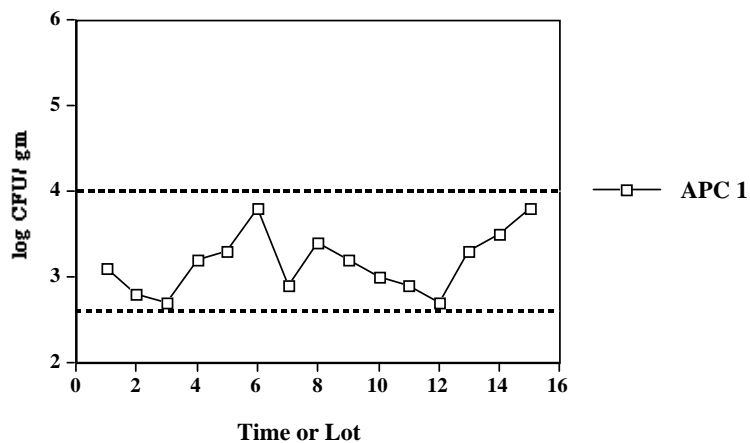
## Product Testing

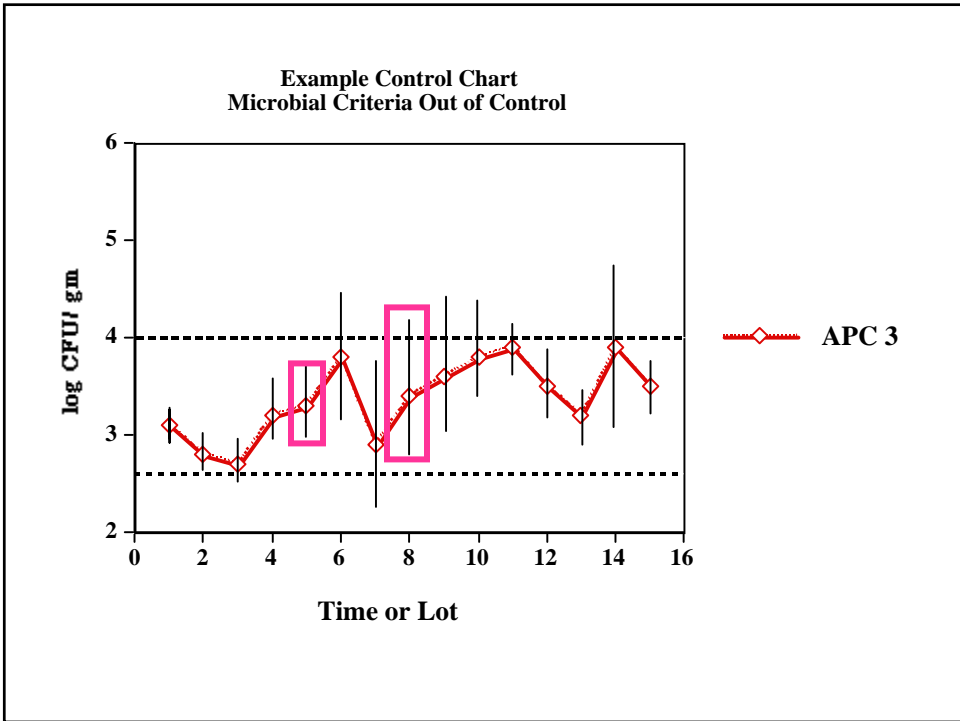
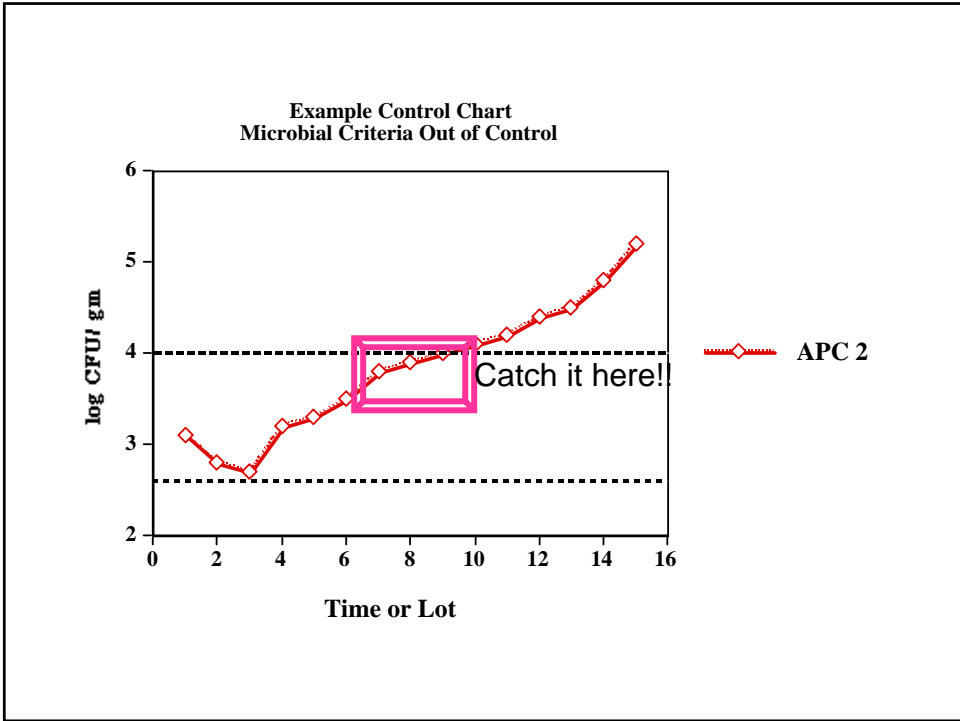
### ■ Results

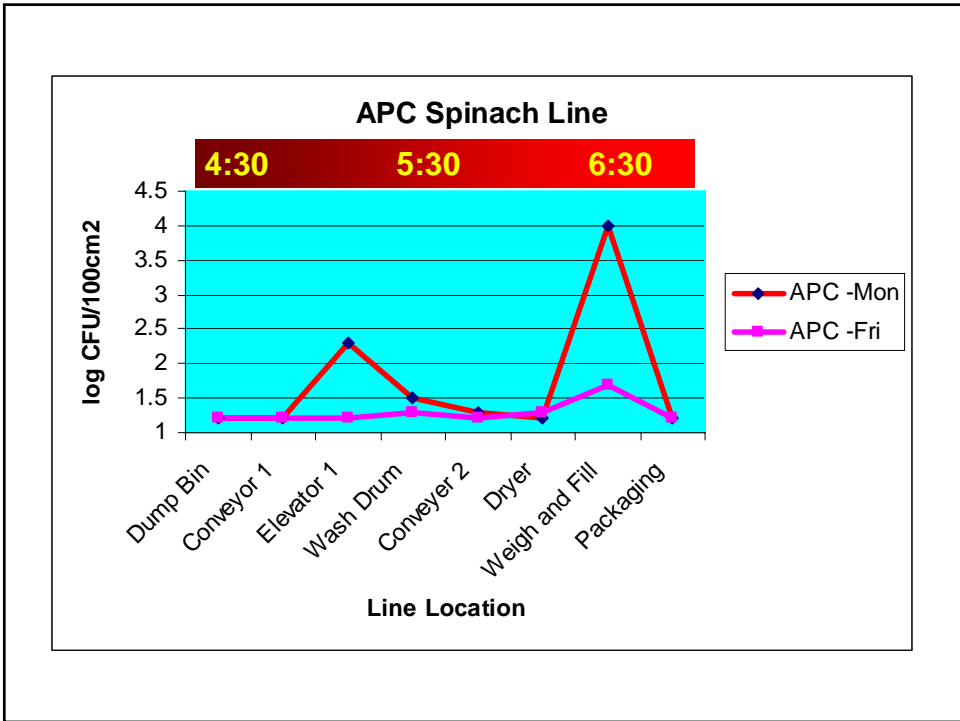
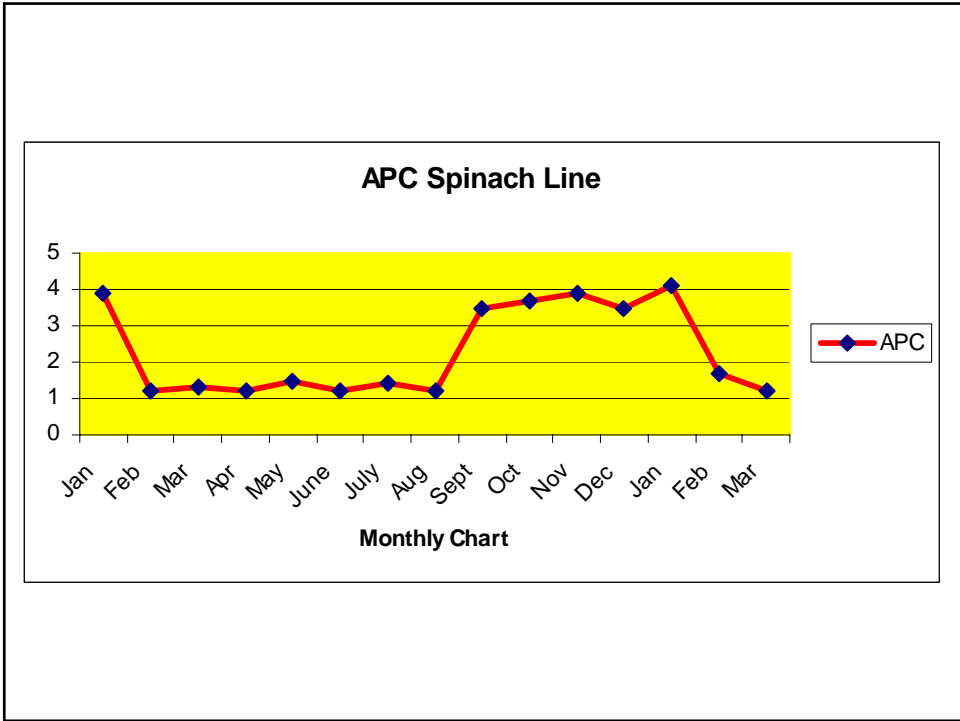
- Review the objectives
- Use the data.
- Understand the limitations.
- Define Critical Limits **in Writing**
  - What is unacceptable?
- Define Corrective Action **in Writing**
  - Action for unacceptable result?
- **Written** data disposition policy.

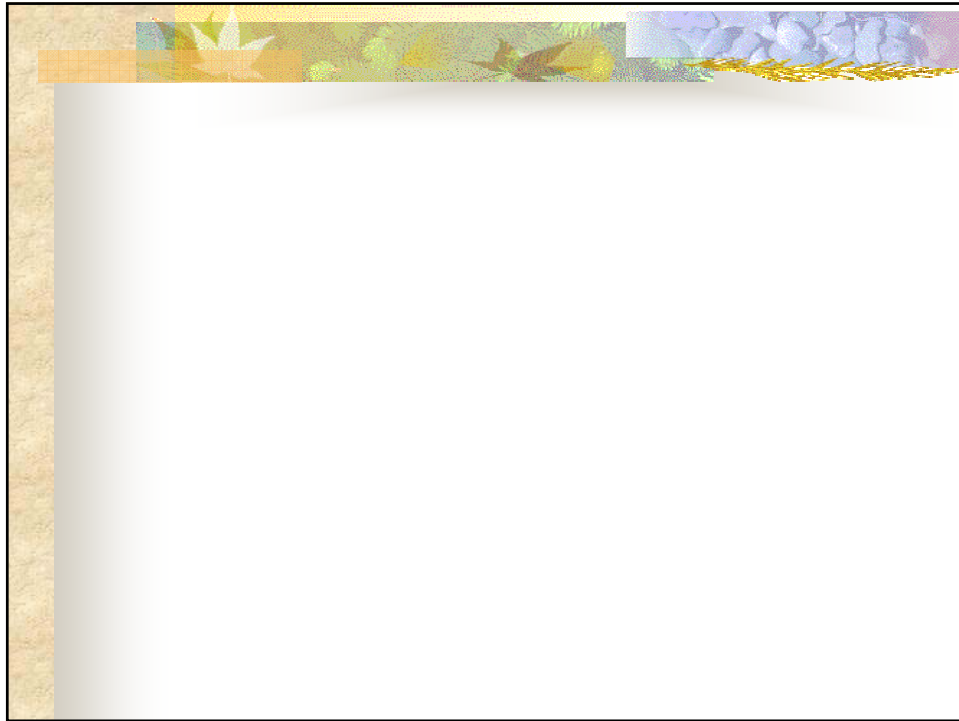
## General Microbe Testing: Counts, Cleanliness, Pathogen Detection

Example Control Chart  
Microbial Criteria Under Control

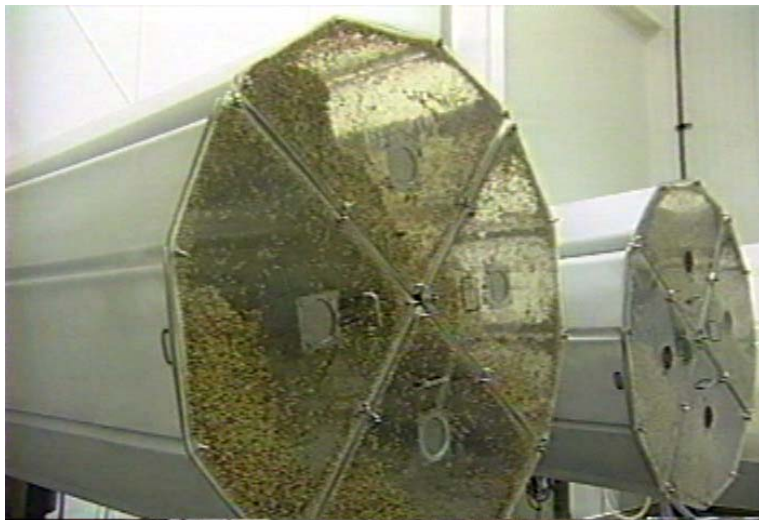




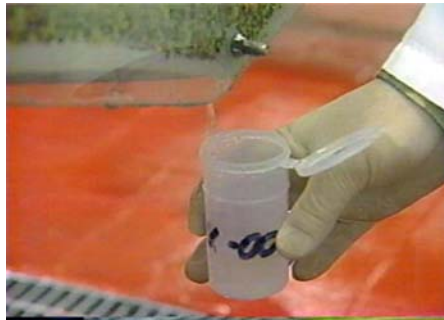




## Sprout Production - Exception for pathogen testing



## FDA Recommended Screening



- Every batch
  - Lot started at same time with single lot of seeds
  - 48 h after soak and at least 48 h before harvest
  - *E. coli* O157:H7 and *Salmonella*

## Environmental Testing for Pathogens

- Tiered Approach for LM in RTE foods
  - First Tier
    - Sampling non-contact locations in high potential areas after cleaning and sanitizing
  - Second Tier
    - Sampling non-contact locations in high potential areas during operation
  - Third Tier
    - Sampling non-contact locations in moderate potential areas after cleaning and sanitizing
  - Fourth Tier
    - Sampling non-contact locations in moderate potential areas during operation
  - Fifth Tier
    - Sampling contact surfaces after cleaning and sanitizing