

A close-up photograph of a large quantity of bean sprouts, showing their characteristic white, elongated stems and yellowish-green cotyledons. The sprouts are piled together, creating a dense, textured appearance. The lighting is bright, highlighting the moisture on the surfaces of the sprouts.

Interventions to Improve Food Safety of Sprouted Seeds

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Sprouted Seeds

- Mung bean sprouts
- Alfalfa
- Soy bean

- Anti-oxidants
- Anti-carcinogens
- Anti-cholesterol



Foodborne illness Outbreaks Linked to Sprouts

	Number of outbreaks 1973-2001
USA	22
Canada	3
EU	3
Japan	3
UK	3

Pathogens Associated with Sprouts

Pathogen	Number of outbreaks (1973-2001)
<i>Salmonella</i>	22
<i>Escherichia coli</i> O157:H7	3
<i>Yersinia enterocolitica</i>	3
<i>Bacillus cereus</i>	3

Contaminated Seed

- Australia
- China
- Mongolia
- Burma
- United States

Sources of Seed contamination

- Contaminated irrigation water
- Grazing animals
- Manure
- Equipment

Mung Beans

- Seeds specifically produced for sprout production
- Australia: On-farm HACCP in mung bean production
- Traceability



Alfalfa

- Major alfalfa seed produced in the heart of cattle country
- Seeds not specifically produced for sprout production
- No motivation for on-farm HACCP



Sprout Production

- Pre-soak 3-16h
- Trays, drums or bins
- 25-30°C (>99% relative humidity) for 4-5 days

- Irrigation

Alfalfa: 15 second spray every 2h (4 liters per min)

Mung beans: Shower every 3-4h (40 liters per min)



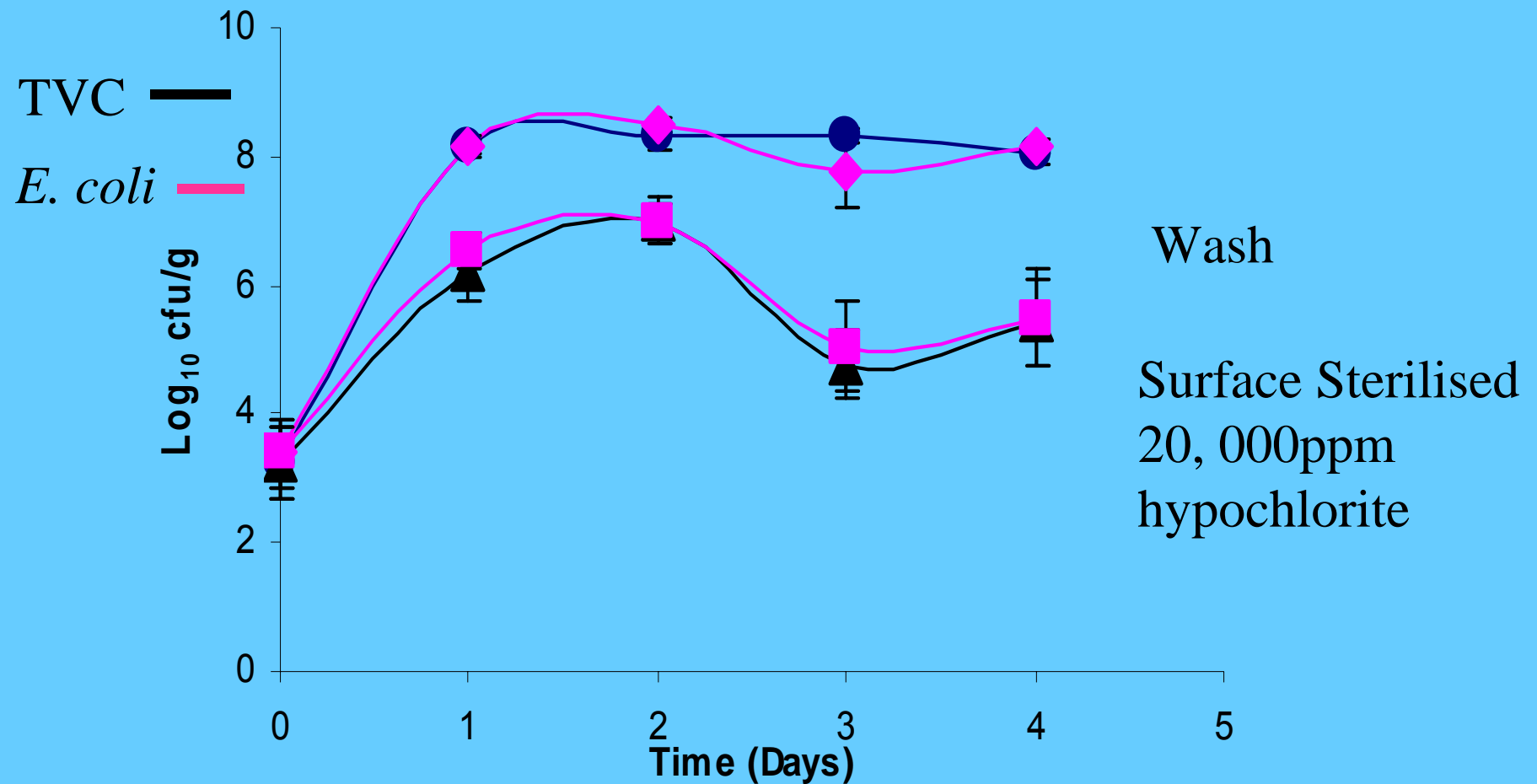
Seed Production, Storage and Treatment





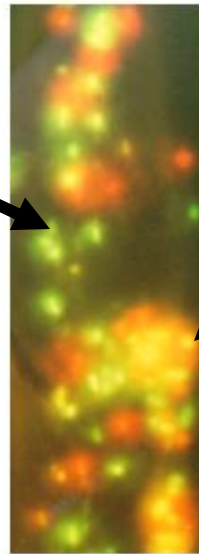
- 25-70kg mung bean batches
- 24-30°C for 4-5 days
- Irrigated every 3 h

Growth of *E. coli* during mung bean sprouting



Apoplastic Fluid from Surface Sterilised Bean Sprouts

Live Bacteria



Dead Bacteria

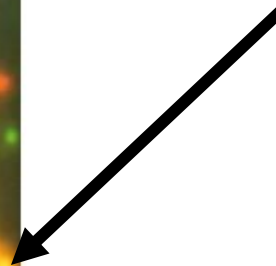
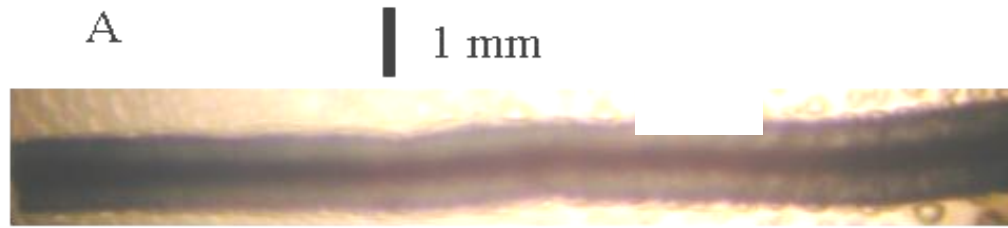
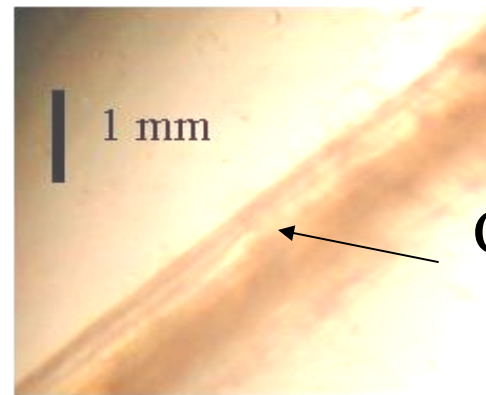
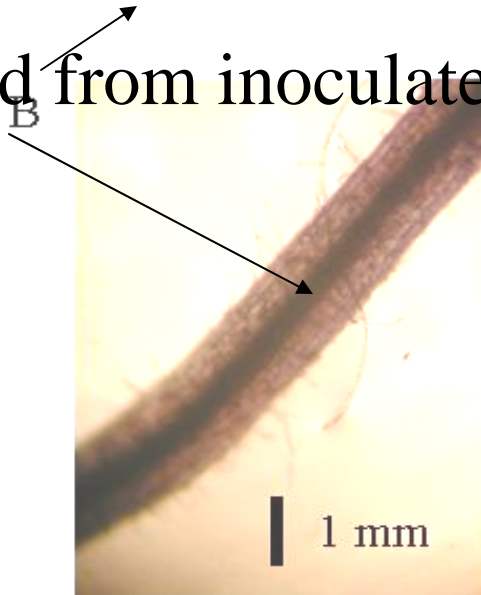


Fig 5



Sprouts derived from inoculated beans



Control

GUS Assay

Guidelines

Microbial Safety Evaluations and Recommendations on Sprouted Seeds (NACMCF) - Adopted May 28, 1999

<http://vm.cfsan.fda.gov/~mow/sprouts2.html>

- Health Canada Policy on Managing Health Risk Associated with the Consumption of Sprouted Seeds and Beans

• http://www.hc-sc.gc.ca/fn-an/legislation/pol/sprouts_pol_pousses_e.html

- **Educational video for sprout producers (FDA).**

<http://www.cfsan.fda.gov/~dms/sprouvid.html>

Recommendations

- Sanitation and GMP
- Seed screening
- Seed decontamination
- Spent irrigation water testing

Sprout Outbreaks 1996-2004

Year	Alfalfa	Clover	Mung Bean	Cases
1996	1	1		650
1997	3	1		277
1998	3	1		48
1999	5	2		389
2000	-----	-----	1	75
2001	1		2	88
2002	1		1	21
2003	5			52
2004	2			33
2005			1	632
2006	1			Recall

2004 - Sanitation

- **50% of firms were described as having deficiencies including:**
 - **unsanitary food contact surfaces (38%)**
 - **evidence of pests (33%)**
 - **lack of personnel cleanliness (25%)**
 - **water quality problems (8%)**

- **Seed Screening**

1. Seed sampling

- 25 g subsamples from each bag . At least 3 kg per seed lot

2. Seed inspection

3. Sprout seeds (3kg batch)

4. Spent irrigation water sampling after 48h

5. Enrichment of sampled water

6. Pathogen testing

- Prevented at least one potential outbreak of *E.coli* O157:H7 and prevented shipment of contaminated seeds.
- No records maintained on test results
- Irrigation water sample: \$50 – 100 per test
- \$100, 000 per year

Seed Decontamination

- 5 log reduction required
- 20, 000 ppm Calcium hypochlorite

Problems

- Not totally effective
- Worker safety
- Incompatible with organic production
- No other sanitizer listed

2004 - Seed Treatment

- **70% of sprouts were treated with $\text{Ca}(\text{OCl})_2$**
- **Sodium hypochlorite (25%), ozone, and peroxyacetic acid were also used.**
- **only 2 used the recommended 20, 000ppm**

Seed Decontamination

- Eliminate pathogens
- Maintain seed viability
- Low cost and practical

Seed Decontamination

- Alfalfa seeds more difficult to decontaminate than mung beans
- 5 log reduction vs complete elimination
- Majority of methods reduce but do not eliminate pathogens

Successful Seed Decontamination Methods

Gaseous Acetic Acid – Mung Bean Seed

Gaseous acetic acid (2500ppm, 12 h, 45°C)

- Inactivates *Salmonella* and *E. coli* O157 on mung beans.
- Reduces but does not eliminate *L. monocytogenes*
- % germination reduced from 96 to 88%

Reduces viability of alfalfa seed

Delaquis et al. 1999. J. Food Prot. 62: 953-957.

Dry Heat – Mung Bean Seed

Dry heat (55°C 4-7 days)

- Eliminates *Salmonella* and *E. coli* O157
- No effect on mung bean germination
- Alfalfa viability reduced

Hu, *et al.* 2004. J. Food Prot. 67: 1257-1260.

Hot Water – Mung Bean Seed

- Hot water (5g seed/250 ml)
 - 55°C/20 min → 5 log reduction of *Salmonella*
 - 60°C/10 min → 5 log reduction of *Salmonella*
 - 70°C/5 min → 5 log reduction of *Salmonella*
 - 80°C/2 min) → 6 log reduction of *Salmonella*

 - No effect on seed germination

Daisy Company Japan

- Hot water pasteurization
- Unreliable
- Reduced seed viability



Combinations – Mung Bean Seed

Dry heat (50°C, 1 h) followed by gamma irradiation (2.0 kGy)

→ 4.6 log reduction of *E. coli* O157:H7 (no survivors), no effect on germination, reduced sprout growth rate

Bari *et al.* 2003. J. Food Prot. 66: 767-774.

Ozone Tsunami-100

- 30, 000ppm Ozone
RH 65% 24h
- 3% Tsunami-100
20 mins
- No survivors

Table 2. Peracetic acid/hydrogen peroxide sanitizing treatment of *Salmonella* inoculated alfalfa seeds for 20 minutes.

<u>% Sanitizer</u>	<u>log CFU/g Recovered</u>	
	<u>Non-Treated</u>	<u>Ozone-Treated</u>
0	6.05	3.64
1	4.30	2.0
2	4.19	1.5
3	3.40	NG*

hypochlorite	5.21	

* NG after plating and MPN recovery.

Fatty Acid Based Sanitizer: Alfalfa

Composition	ppm
Peroxyacetic acid	3, 750
Caprylic and capric acid	15, 000
Lactic acid	15, 000
Glycerol monolaurate	7, 500

5 min treatment: No survivors *L. monocytogenes*,
Salmonella, *E. coli* O157

Seeds tested for pathogens

Pierre PM and Ryser ET (2006). J Food Prot 69 (3): 582-590

Germin-8-or

- Germin-8-or is Phyto-compatible and can be introduced into steep water used for germinating seeds.
- Hora, R., Kumar, M., Kostrzynska, M., Dixon, M. A. and Warriner, K.(2007). Lett Appl Microbiol 44 (2): 188-193.
- M. Kumar, R. Hora, M. Kostrzynska and K. Warriner. (2007). J. Appl. Microbiol. 44: 188-193.
- Kumar, M. Hora, R., Kostrzynska, M., Waites W. M. and Warriner K. (2006). J. Food Prot. 69 (7): 1571-1578

Methods

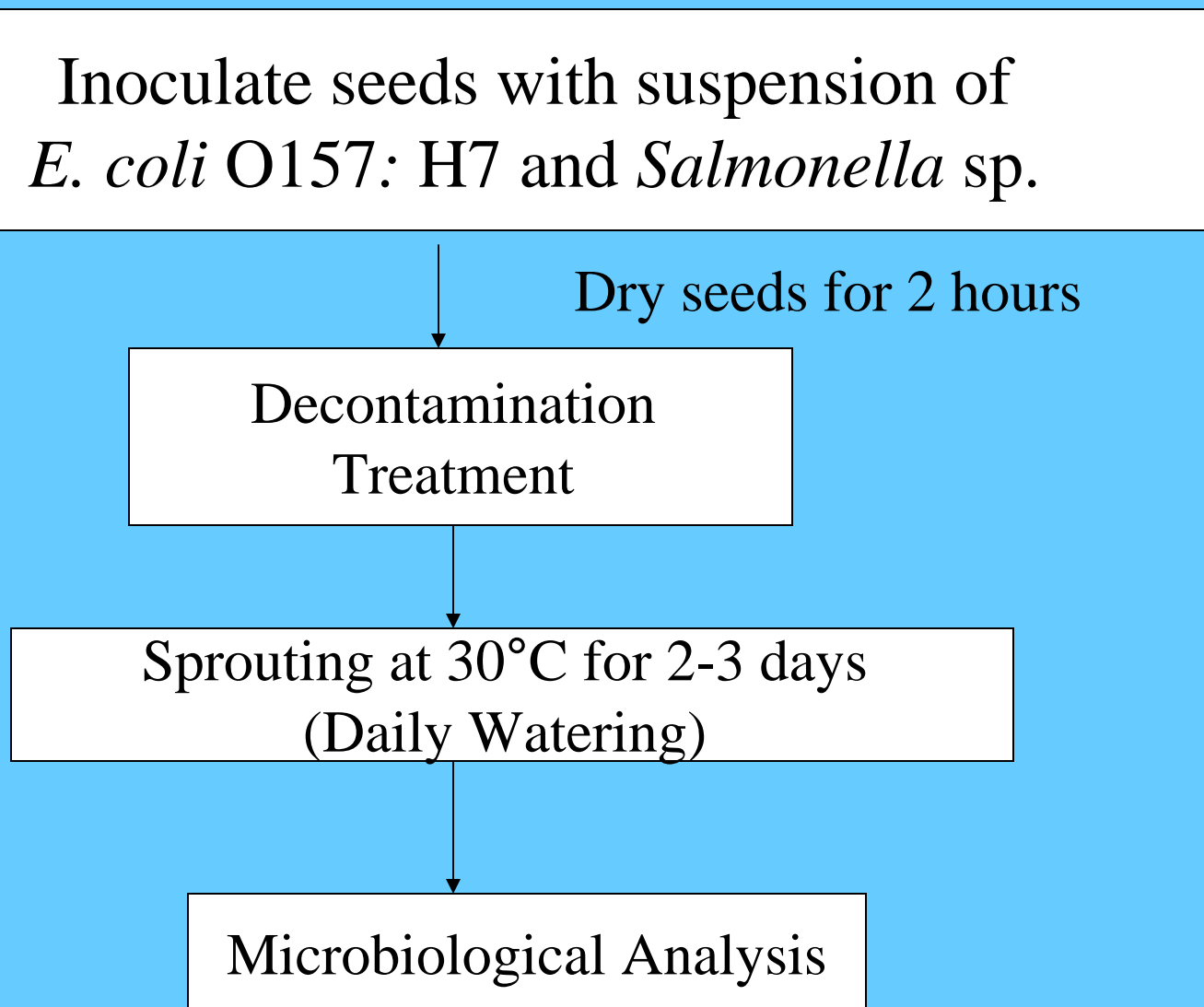
Inoculate seeds with suspension of
E. coli O157: H7 and *Salmonella* sp.

Dry seeds for 2 hours

Decontamination
Treatment

Sprouting at 30°C for 2-3 days
(Daily Watering)

Microbiological Analysis



Effect of Germin-8-or concentration on seed decontamination efficacy

	Counts on bean sprouts (log cfu/g)		
Germin-8-or Concentration	TAC	<i>E. coli</i> O157:H7	<i>Salmonella</i>
0	9.72	9.12	9.10
50ppm	9.80	9.09	8.87
100ppm	8.64	ND	5.30
150ppm	8.28	ND	4.90
200ppm	8.82	ND	ND

Calcium Hypochlorite (20, 000ppm) Vs Germin-8-or (200ppm)

Treatment of mung beans	<i>E. coli</i> O157:H7		<i>Salmonella</i>	
	Count Log cfu/g	Enrichment	Count Log cfu/g	Enrichment
Calcium hypochlorite (20, 000ppm, 20mins)	8.59	NT	7.96	NT
Germin-8-or 200ppm	ND	ND	ND	ND

Initial loading: 3-4 log cfu/g

ND <1 cfu/25g

Naturally contaminated seeds

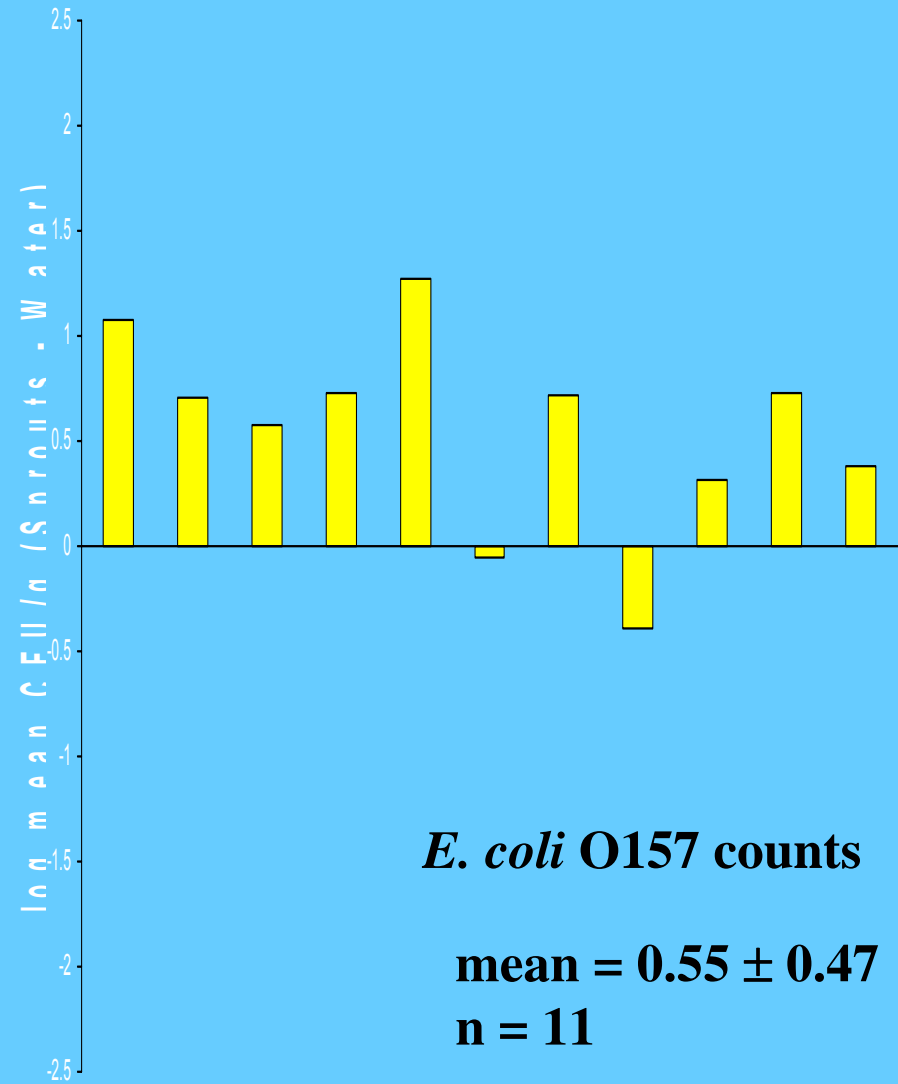
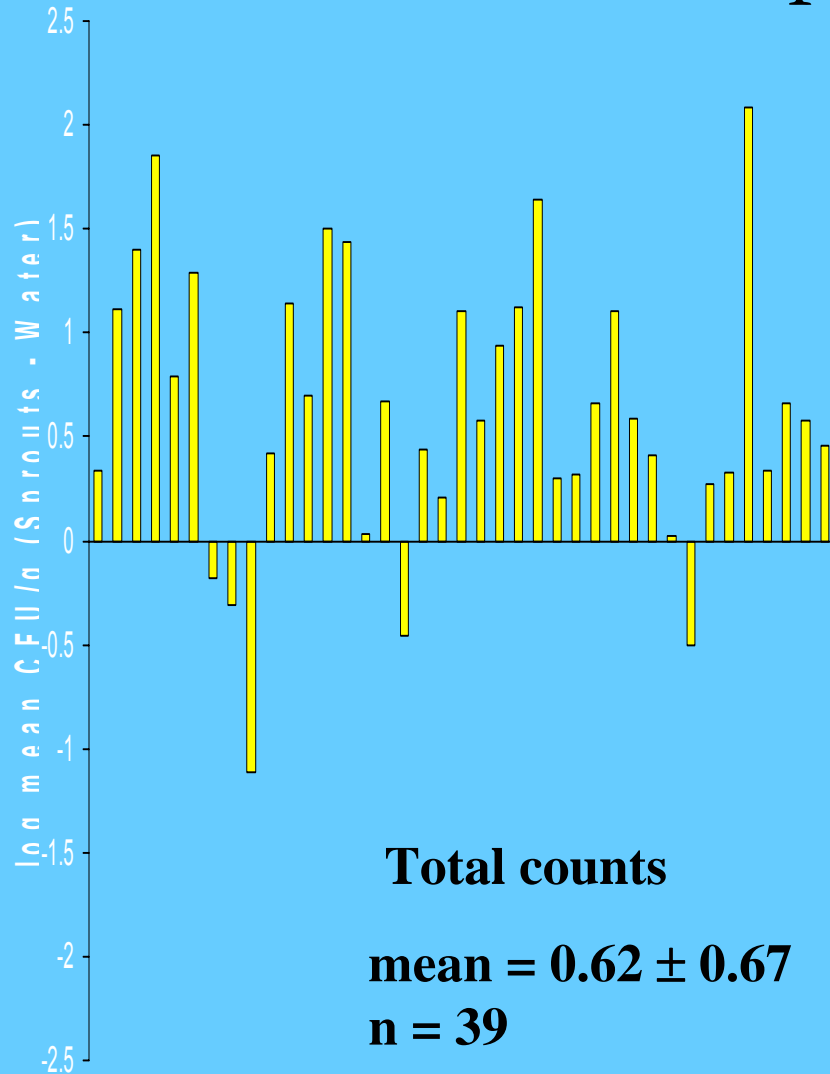
- Inoculated flowers with either *Salmonella* or *E. coli* O157:H7
- 10/10 seed batches contaminated with *Salmonella*
- *E. coli* O157:H7 present on 3/10 seed batches tested
- Effectively decontaminated with Germin-8-or.



Spent Irrigation Water Testing

- Guidelines based on studies with alfalfa seed.
- Growth of pathogens during sprouting: 48h
- Screen water as opposed to sprouts directly

Difference in microbial counts between sprouts and water

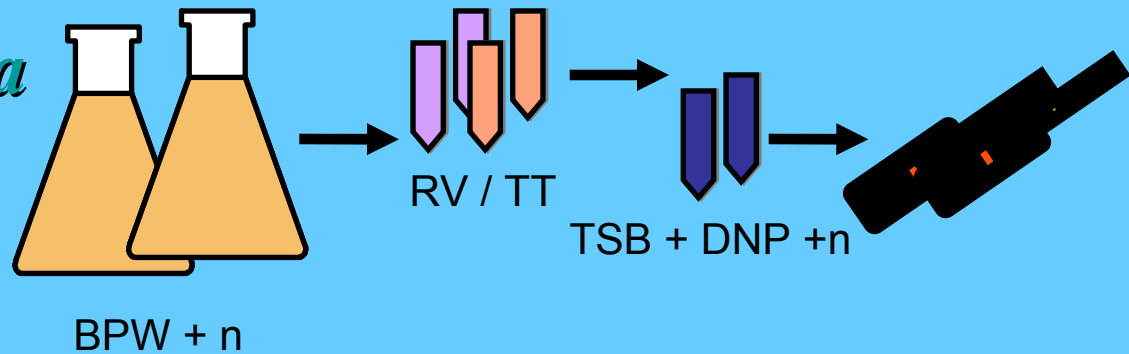


Testing of Water from Each Production Batch

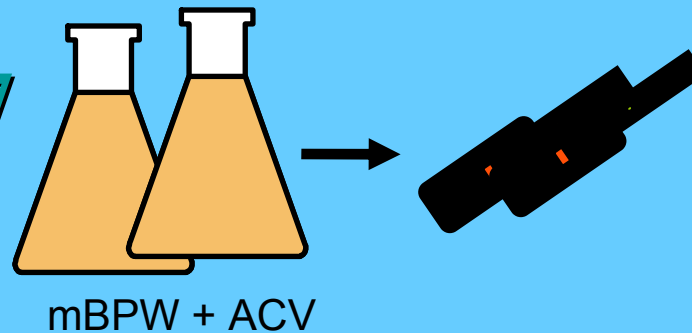
Salmonella



and



E. coli O157:H7



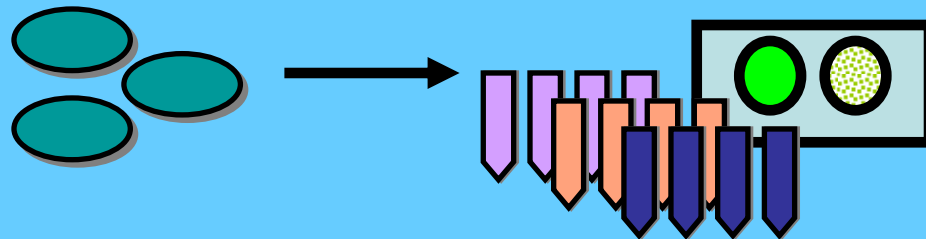
....Testing of Water from Each Production Batch

If positive results, then:

- discard sprouts and seed; disinfect contact surfaces

or

- run confirmatory tests from enrichment



2004 - Sampling and Microbial Testing of Spent Irrigation Water

- **71% of firms collected spent irrigation water for microbial testing**
- **Almost all firms tested for *Salmonella* spp. and *E. coli* O157:H7 but the testing method varied greatly.**
 - **“Pooling of composite samples**

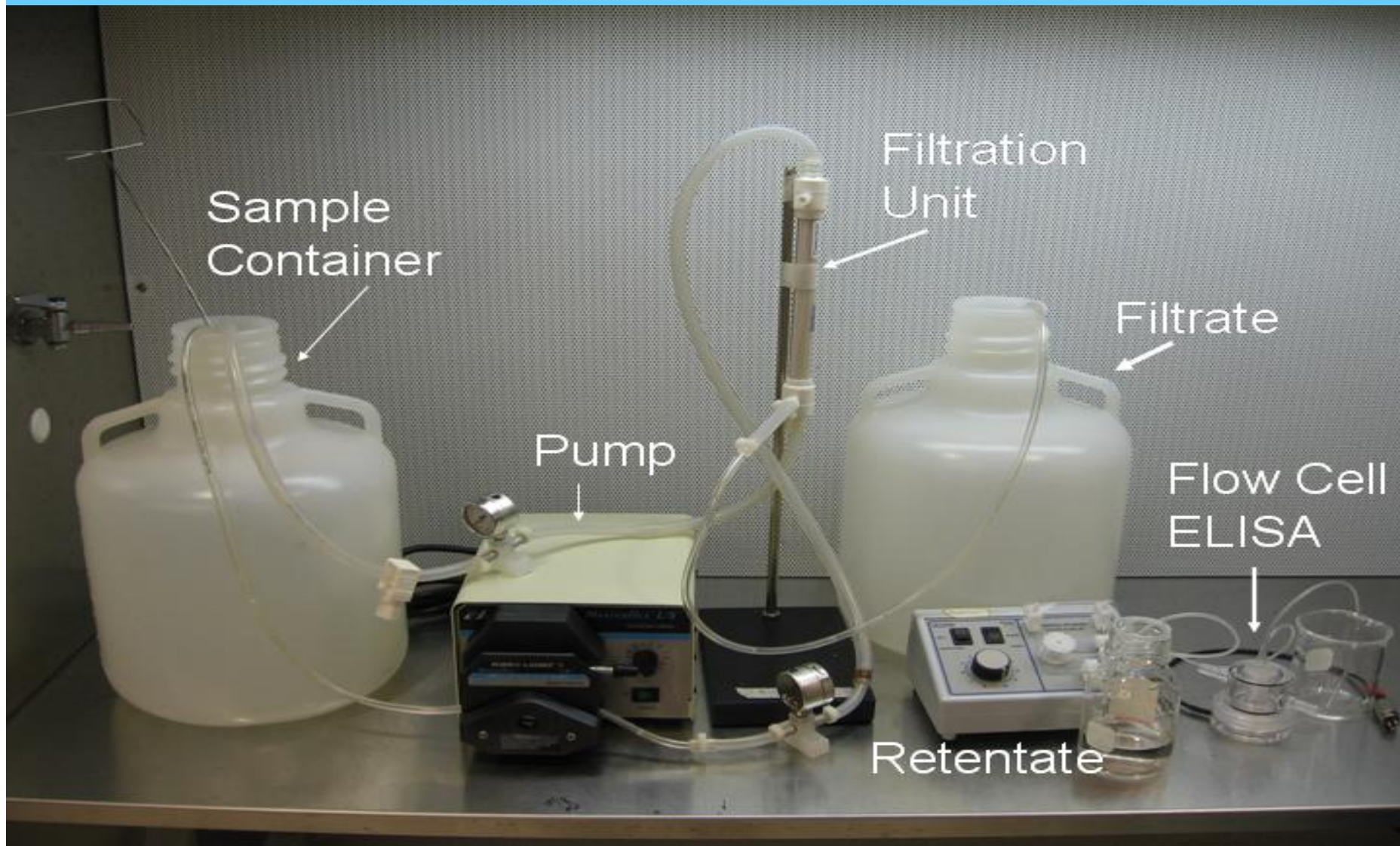
2004

Testing Spent Irrigation Water

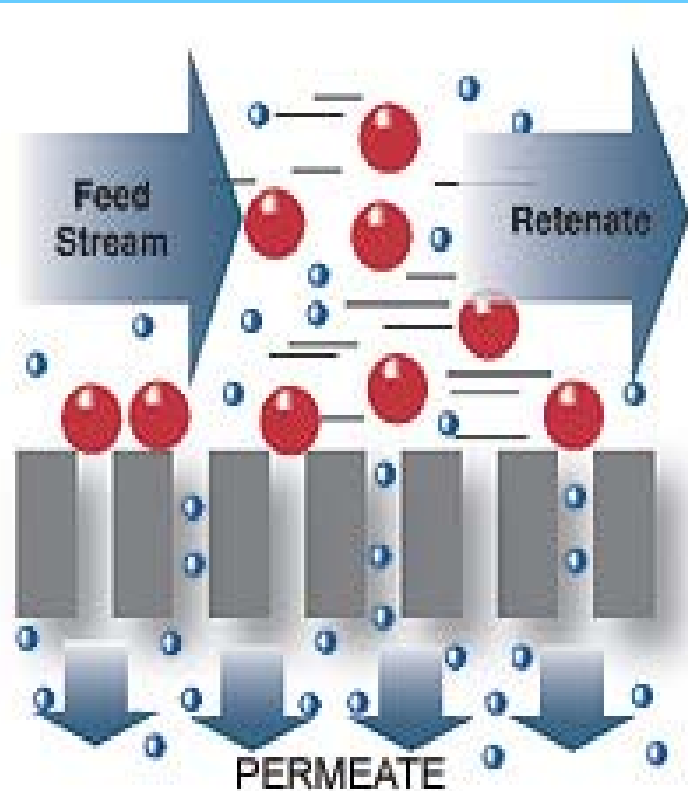
Record Keeping

- 88% that conduct testing maintain records of test results from 48 hour spent irrigation water samples.
- 6% maintains a record of 48 hour spent irrigation water samples collected but not the results.
- A majority of firms (92%) do not have a corrective action or product recall plan.

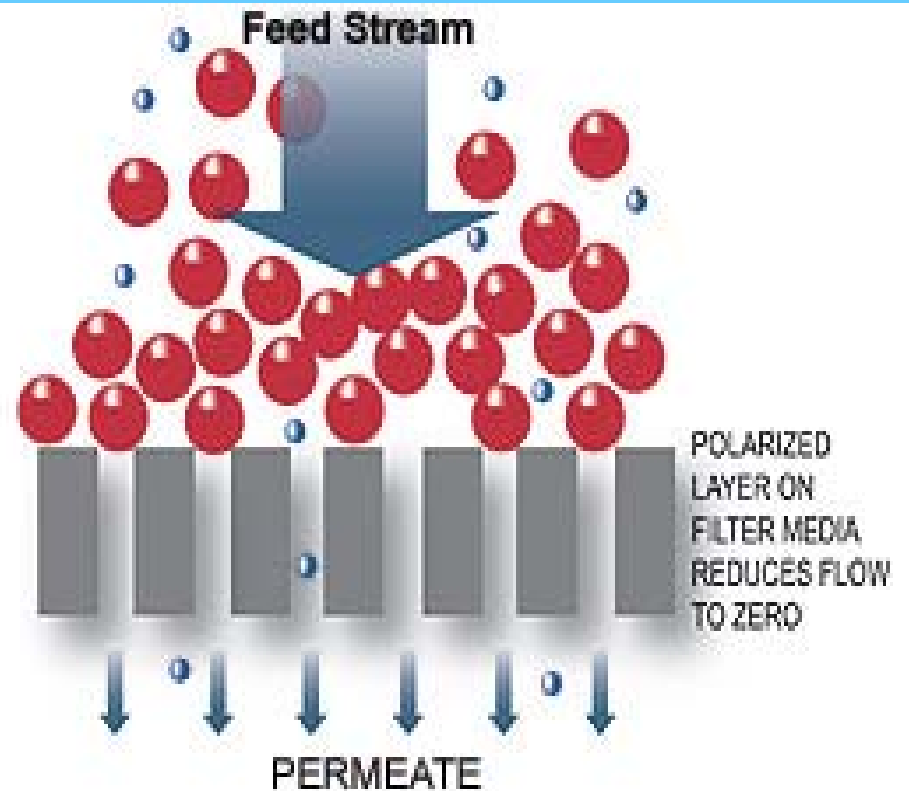
Advances in Spent Irrigation Water Testing



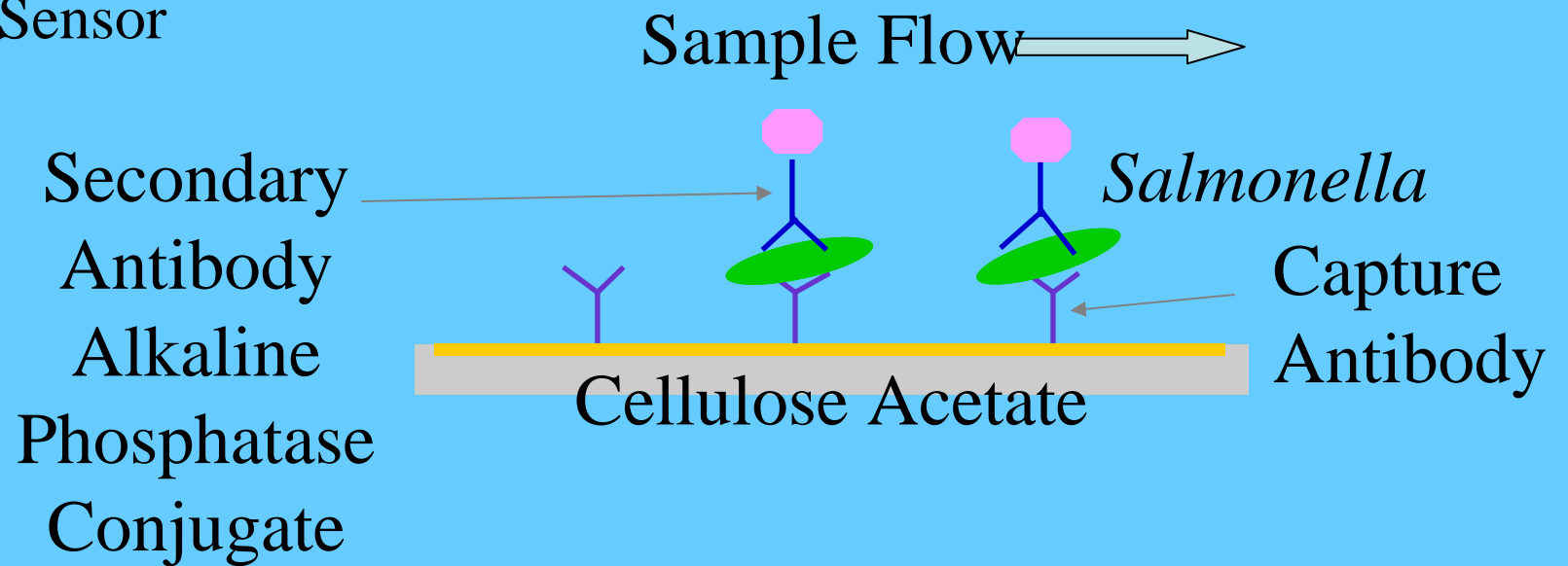
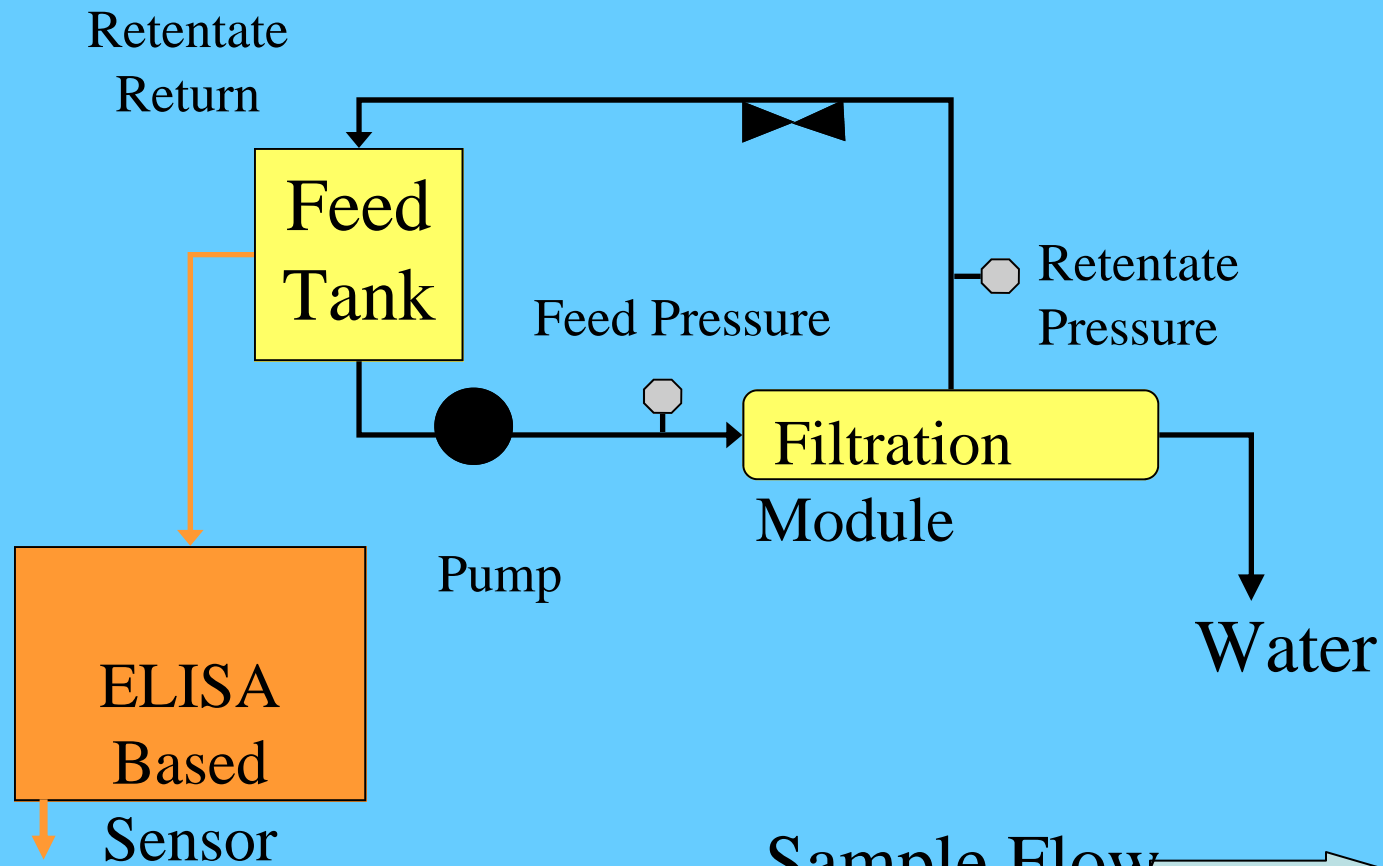
TFF Cell Concentration

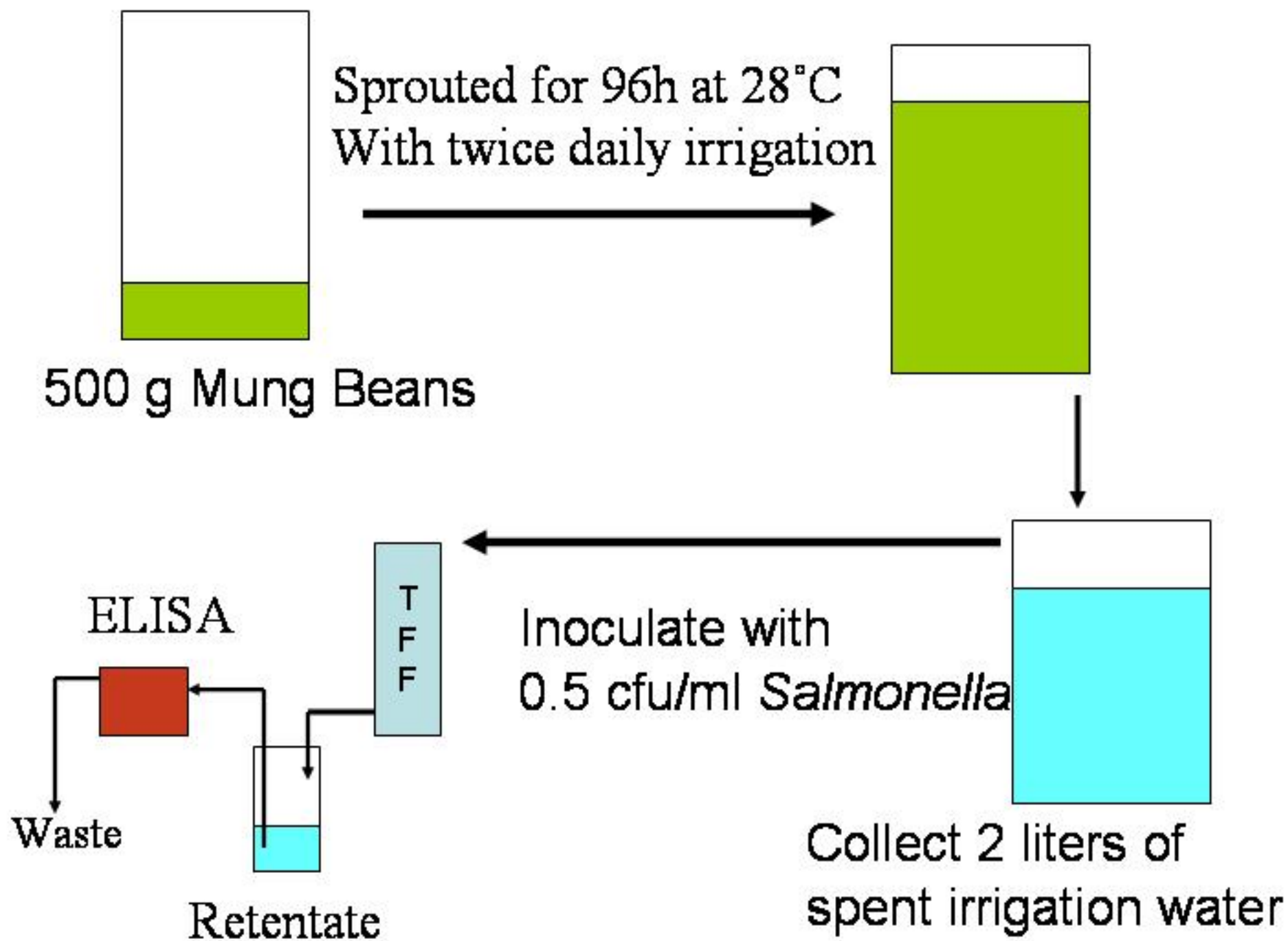


Tangential Cross-Flow Filtration
(high permeate rate)



Dead-End Filtration
(low permeate rate)





System Performance

10min Room Temperature

Antibody modified membrane



Sample In



- Detection of Salmonella at 0.3 log cfu/ml in 10 liter sample volumes
- Time for analysis: 3.5 h
- Re-usable and stable to CIP

Research Needs

- Standardized methods for validating and verifying seed decontamination methods.
- Introduction of alternative seed decontamination methods
- Rapid and reliable screening methods
- Seeds produced specifically for sprout production

- Detailed food safety guidelines
- Consider different seed types
- Organic sector
- Regulation?
- Focus on health benefits of sprouted seeds

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