



International Specialty Supply  
Supplying Sprout Companies Throughout the World



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## ISS Seed Screening for Human Pathogen Testing

Over the last decade commercially produced sprouts have been implicated in outbreaks involving thousands of people.

*"In almost all cases, contaminated seed was the source of the contaminated sprouts both for Salmonella sp. and E. coli O157:H7."* Lester M. Crawford, D.V.M., Ph.D. Acting Commissioner of the FDA.

International Specialty Supply developed a method of screening seed for human pathogens that has been confirmed, referenced or recommended as necessary by:

- The World Health Organization (WHO)
- United States Food and Drug Administration (FDA)
- United States Department of Agriculture (USDA)
- California Department of Health
- New South Wales Food Authority
- South Australian Research and Development Institute
- Ontario Ministry of Agriculture
- The Center for Science in the Public Interest
- Resources for the Future
- Food Standards Agency (UK)
- The Campden Research Group
- Food Safety Authority of Ireland,
- National Center for Food Safety and Technology
- Illinois Institute of Technology
- Center for Food Safety and Applied Nutrition
- Dozens of researchers and food safety experts.

The ISS Seed Screening Program has already prevented several lots of contaminated seed from being used in commercial sprout production. It is literally a life saver.

According to the Center for Science in the Public Interest, in a paper called "Outbreak Alert! 2002, "Closing the Gaps in Our Federal Food-Safety Net", sprouts made up 7% of the outbreaks from fruit and vegetables during the period of 1990-2002 (September).

Produce Outbreaks  
1990-2002

Vegetables	
Potatoes	3%
Mushrooms	4%
Home-canned vegetables	5%
Sprouts	7%
Produce Dishes	9%
Other Vegetables	14%
Lettuce	11%
Salad	28%
Fruit	
Melon	2%
Berries	4%
Other Fruits	13%
<b>Total</b>	<b>100%</b>

Table 1. Sprouts were suspected in 7% of the produce related outbreaks from 1990 to 2002

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En español

## Sprout Related Outbreaks

Although there have been documented outbreaks in sprouts since 1973, federal regulators, public health officials, and the sprouting industry did not wake up to dangers associated with sprouting until about 1996. All parties concerned, including ISS, were bent on coming up with a way to sanitize the seed. Between 1998 and June 2006, there have been 36 reported outbreaks in the world that were attributed to sprouts. Twenty-seven of which involved US and/or Canadian sprout growers.

### US Sprout Related Outbreaks 1996 - 2004

(FDA figures)

Year	Alfalfa sprouts & mixes with alfalfa sprouts	Clover sprouts	Mung bean sprouts	Total Outbreaks	Cases
1996	1	1		2	650
1997	3			3	277
1998	3			3	48
1999	5	1		6	389
2000			1	1	75
2001	1		2	3	88
2002	1		1	2	21
2003	5			5	52
2004	2			2	36
Total	21	2	4	27	1633

Table 2. Sprouts were suspected in 27 outbreaks and 1633 cases from 1996 to May 2005  
Update: There has been one additional US sprout related outbreak reported as of this writing in September, 2008.

### US Outbreaks

- Average outbreaks for 1996-1999 is 3.5 per year
- Average outbreaks for 2000-2004 is 2.6 per year. This is a decline of 26%
- Average outbreaks for 2005-July 2008 is 0 per year.

### US Cases

- 1996-1999 averaged 341cases/yr.
- 2000-2004 averaged 51cases/yr. That is down 84%.
- 2002-2004 averaged 36 cases/year. That is down nearly 90% from 1996-1999.
- 2005-July 2008 averaged 0 cases/year.

### Several of the Outbreaks Appear to be Related

- Three separate bean sprout outbreaks of Salmonella were reported to be the *SAME* phage type. (18, 24, 52, 89) According to the Canada Communicable Disease Report, *“This outbreak represents the first known occurrence of SE PT 913 infection in humans.”* (18)
- Three separate alfalfa sprout outbreaks of E.coli O157:H7 were reported to have the *SAME* PFGE pattern. (31, 101) According to the investigative report, *“These Pulse patterns had never been previously identified in the United States.”* (31)
- One outbreak reportedly involved *FOUR* strains of salmonella in *ONE* seed lot. From the report: *“Six opened packages of alfalfa sprouts were taken from cases' homes... Two packages were positive for S. paratyphi B var java, PT "Worksop", PFGE pattern A1. One package was positive for S. litchfield, one for S. thompson and one for S. newport; one package was negative.”* (33)
- A 1996 outbreak appears to have matched the seed from a 1995 outbreak. From the report on the 1996 outbreak: *“PFGE testing on four isolates from University A students and two from University B students showed them to be identical to the 1995 outbreak strain.”* (117)

### The Source of Contaminated Sprouts is Contaminated Seed

The suspected scenario is that the seed is contaminated in the field by manure used as fertilizer, or by grazing animals, or in silos or bins by bird or mouse droppings and urine. The process of sprouting allows nearly ideal

conditions of food, moisture, and warmth in which a single pathogen cell can proliferate into over one hundred thousand pathogen cells.

Most healthy adults can handle a certain amount of pathogens in their daily lives. It is those who are young, old, ill or in some other manner have relatively weak immune systems that high levels of contamination really take its toll on.

Since 1988, with one exception, seed have been the likely source of contamination in every outbreak in which the source was determined.

### World Sprout Related Outbreaks 1988 - Early 2005 (Food Standards Agency, UK.)

Year	Pathogen	Cases	Location(s)	Type of sprout	Likely source of contamination
1988	S. Saint-Paul, S. Havana, S. Muenchen	148	Sweden	Mung	ND
1988	S. Saint Paul	143	United Kingdom	Mung	Seed
1988	S. Virchow	7	United Kingdom	Mung	ND
1989	S. Gold-Coast	31	United Kingdom	Cress	Seed and/or sprout grower
1990	S. Anatum	15	Washington	Alfalfa	ND
1992	S. enterica	272	Finland	Alfalfa	ND
1994	S. Bovis mordificans	492	Finland, Sweden	Alfalfa	Seed
1995	S. Stanley	242	Finland, 6 US States	Alfalfa	Seed
1995	S. Newport	154	Denmark (Probably USA and Canada)	Alfalfa	Seed
1995	S. Newport	133	7 US States, Canada, Denmark	Alfalfa	Seed
1996	E. coli O157:H7	5,727	Japan	Radish	Seed
1996	E. coli O157:H7	126	Japan	Radish	Sprout growers
1996	S. Montevideo and S. Meleagridis	492	California, Nevada	Alfalfa	Seed and/or sprout grower
1997	S. Anatum & S. Infantis	109	Kansas, Missouri	Alfalfa	Seed
1997	E. coli O157:H7	79	4 US States	Alfalfa	Seed
1997	E. coli O157:H7	108	Michigan, Virginia	Alfalfa	Seed
1997	S. Meleagridis	78	Canada	Alfalfa	Seed (organic)
1997	S. Senftenberg	60	California, Nevada	Alfalfa	Seed and/or sprout drum
1998	S. Havana	18	2 US States	Alfalfa	Seed
1998	S. Cubana	22	5 US States	Alfalfa	Seed
1998	E. coli O157:H7	8	California, Nevada	Alfalfa, Clover	Seed and/or sprout grower
1999	S. Mbandaka	75	4 US states	Alfalfa	Seed
1999	S. Muenchen	>157	Multistate, USA	Alfalfa	Seed
1999	S. Saint-Paul,	36	California, USA	Clover	ND
1999	S. paratyphi var Java	51	Canada	Alfalfa	Seed
1999	S. typhimurium	120	Colorado	Alfalfa	Seed
2000	Salmonella spp.	22	California, USA	Alfalfa	ND
2000	S. Enteritidis PT 4b	27	The Netherlands	Mung	Seed
2000	S. Enteritidis	75	4 US states	Mung	ND
2001	S. Enteritidis PT 913	84	Canada	Mung	Seed
2001	S. Kottbus	31	4 US states	Alfalfa	Seed
2002	S. Enteritidis	n/a	Maine	Mung	ND
2003	S. Saint-Paul	>9	Oregon, Washington	Alfalfa	ND

ND = Non Determined

Table 3. Seed was suspected in all but one of the outbreak in which a likely source was determined.

### So Why Not Just Decontaminate the Seed?

A great majority of the [research](#) on alleviating sprout related outbreaks has focused on decontaminating the seed. And although the FDA went so far as to recommend that all commercially grown sprouts be produced from seed that has been sanitized with 20,000 ppm chlorine, [no sanitizer has yet been found to be reliable](#) at eliminating pathogens from seed without destroying germination.

If the pathogens were sitting on the seed coat, it would not be a problem, 200 ppm chlorine would do the trick. But the bacteria that are trapped in cracks and crevices in the seed coat appear to be protected from disinfectants, and there is evidence that some bacteria can be [internalized](#) within the seed itself. When a single pathogen cell remains alive, it will multiply back to levels as though no sanitation had ever been done.

To date no sanitation technique has been shown to completely eradicate pathogenic micro-organisms from either the seed or the [sprouts](#).

### Avoiding Outbreaks is Not Difficult if the Seed Is Not Contaminated in the First Place

In 2000, Bob Sanderson of Jonathan Sprouts came up with a simple, yet brilliant, idea. ["If the problem is pathogens in the seed, why not just make sure there aren't any pathogens in the seed in the first place?"](#)

In a collaborative effort between International Specialty Supply and Jonathan's Sprouts, a method was developed in which sprout growers and seed suppliers could substantially reduce the odds of there being pathogens in seed.

## Seed is Generally Tested for Quality, Not Safety:

Seed sampling for seed borne *plant* pathogens has been practiced for many years and the statistical probabilities are well documented.

For over a hundred years seed has been tested for germination, purity, hard seed and other properties that relate to the quality of the seed. These are addressed in terms of percentage. For example, a seed lot might have a quick germination of 90% with 3% hard seed and 1% abnormal seeds, for a total germination of 94%. The statistical probabilities used to determine these percentages are extremely accurate.

## Testing Seed for Human Pathogens can be Far More Accurate

When testing for pathogens, one is not looking for a percentage, but for any at all. If a *single* pathogen is detected, the lot is contaminated and cannot be used for sprouting purposes.

The entire sample, which in a truckload of seed can be over 40 lbs (thousands of times larger than a normal lab sample), is sprouted, which increases the bacteria level approximately 5 logs (100,000 times) in 48 hours. Then the runoff water is sampled, enriched, and tested for Salmonella, E.coli O157:H7, and generic E.coli.

## ISS Seed Screening Procedures

The seed screening procedures, developed by International Specialty Supply for ISS Screened Sprouting Seed, can substantially reduce the risk of food-borne illness related to commercial sprout production. The process involves: seed sampling, seed inspection, sprout growing (enrichment), spent water sampling, enrichment of sampled water, and pathogen testing.

Every step is critical, must be precisely done, and accurately documented. No seed which has been sampled, enriched, and tested in this way, prior to use in production, has ever been implicated in an outbreak.

### ✓ Seed Sampling

In order to detect a pathogen in a lot of seed, you first have to “capture” it in the seed sample you intend to test. The probability of picking up at least one contaminated seed in a 40 lb seed sample is 99.999999% at a contamination level of 4 CS/kg (4 contaminated seeds per 2.2 pounds of seed), which is extremely light contamination.

Seed Sampled		Pathogen Level of Contamination (Contaminated Seeds/kg)							
		0.1	1	2	3	4	5	10	100
0.00002	1	0.0000%	0.0002%	0.0004%	0.0006%	0.0008%	0.0010%	0.0020%	0.0200%
0.0002	10	0.0002%	0.0020%	0.0040%	0.0060%	0.0080%	0.0100%	0.0200%	0.1998%
0.002	100	0.0020%	0.0200%	0.0400%	0.0600%	0.0800%	0.1000%	0.1998%	1.9803%
0.02	1,000	0.0200%	0.1998%	0.3992%	0.5982%	0.7968%	0.9950%	1.9802%	18.1286%
0.2	10,000	0.1998%	1.9801%	3.9211%	5.8236%	7.6884%	9.5163%	18.1271%	86.4692%
2	100,000	1.9801%	18.1269%	32.9680%	45.1189%	55.0672%	63.2122%	86.4667%	99.9999%
1	500,000	9.5163%	63.2121%	86.4665%	95.0213%	98.1685%	99.3262%	99.9955%	99.9999%
2	1,000,000	18.1269%	86.4665%	98.1685%	99.7521%	99.9665%	99.9955%	99.9999%	99.9999%
3	1,500,000	25.9182%	95.0213%	99.7521%	99.9877%	99.9994%	99.9999%	99.9999%	99.9999%
5	2,500,000	39.3469%	99.3262%	99.9955%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%
10	5,000,000	63.2121%	99.9955%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%
12	6,000,000	69.8806%	99.9994%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%
25	12,500,000	91.7915%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%
50	25,000,000	99.3262%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%
100	50,000,000	99.9955%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%	99.9999%

Probability (P) = 1-(C/T)^N, where (C/T) = assumed ratio of Clean seeds to Total, and N = Number of seeds sampled.

Table 4. Probability of Capturing a Contaminated Seed in a Twenty-Ton Lot of Alfalfa Seed Contaminated with Various Contamination Levels Per Kilogram.

The number of contaminated *seeds* in an outbreak has not been investigated. But the FDA White Paper gives the level of pathogens found in contaminated seed to be as low as 4 cfu per kilo. Any level of contamination above this estimate will raise the probability of detection. Lower levels are harder to detect.

### ✓ Seed Inspection



After a representative sample is taken, the composite sample of seed is inspected for indicators of contamination. This includes inspecting the bags for mouse urine or dropping, holes in the bags, insect larva, bird droppings, etc. The seed is then carefully inspected with both a magnifying glass and microscope to determine its fitness for human consumption. Again, we look for traces of visitation by animals and insects. In the process we have also found glass, seed that was blended with seed that had been treated with a fungicide, and other things.

#### ✓ Sprouting (Enrichment)

The entire sample is sprouted. The seed is not sanitized prior to sprouting. In 48 hours the pathogens, if present, should have increased about 1,000,000 times, substantially increasing the probability of detection.

#### ✓ Water Sampling and Testing

At about 48 hours, a sample of the runoff water is collected using FDA procedures recommended for commercial sprout producers. The water is then enriched to make the pathogens multiply about 5 log (100,000 times) and the water is tested for salmonella, E.coli, and E.coli 0157:H7. The tests are run in duplicate.

#### ✓ Documentation

Each step is thoroughly and accurately documented and signed by the person taking responsibility for completing each step of the process. The adage is, "It if isn't documented, it wasn't done."

#### ✓ Pre-Screening

We add an extra step as well. Before we receive a shipment, we bring in a sample and inspect it, sprout it, and test it. We stop at the point of rejection. That is, if the seed does not pass visual inspection, we reject it without taking the time to sprout it out and test it for pathogens.

Generally if we receive seed with mouse dropping in it, that same processor will send bad samples time after time, and year after year. After a while, one learns who to stay away from. We are not only screening seed, we are screening seed processors.

## ISS Seed Screening Discussion

### Ground Rules

1. When it comes to seed sampling for human pathogens, sample size and total contamination *per lot* are the two factors that determine the probability of capturing a pathogen.
2. There's a possibility that two or more pathogens could be lodged on one seed. So we base probabilities on *contaminated seeds* (CS) rather than CFU. Seed, while being processed, does get mixed well enough for very accurate seed sampling estimates.
3. For ease of explanation, throughout the rest of this discussion all samples will be 25 grams, and all bags will be 25 kilogram bags. So the sample size is always 1/1000<sup>th</sup> of a bag or one seed per thousand seeds.

Some Question the Reliability of Seed Sampling Because There is a Possibility that the Contaminated Seeds Could be in a Corner of One Bag or in a Clump and Never be Detected.

If the seed is poorly distributed, such as in the corner of the bag, the probability of capture is significantly diminished. But this scenario may not occur often. The seed is harvested, transported, and dumped into a silo or bins.



Figure 1. Seed is mixed during harvest.

It is then poured or augured into the seed cleaning equipment, processed, and poured into a bag. The cleaning and grading process does not allow even two seeds to clump together or they won't fit through the screens. Seed with pathogens are not likely to stay next to each other throughout this process. They will be somewhat, if not thoroughly distributed.

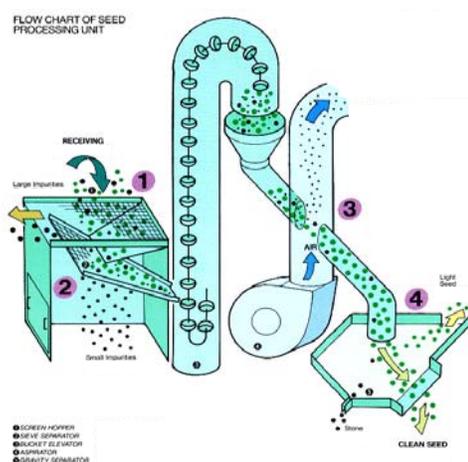


Figure 2. Seed is mixed during processing.

When Trying to Detect Plant Pathogens, Similar Sample Sizes are Used and the Probabilities are Extremely High as Well.

When looking for *plant* pathogens, you are looking for *frequency* rather than *any at all*. In order to determine the percentage of pathogens in wheat, 8 kg is sampled for each 100 tons of seed. Then 300 seeds are pulled from that 8 kg for testing. Distribution of the plant pathogens is good enough that this method is very accurate. In the same lot size, we would sample and inspect the lot, and then 25 *million* seeds would be tested in order to find a single human pathogen!

Larger Samples Increase the Probability of Capture at a Given Contamination Rate.

Considering that we sample at least 1/1000 of the seed, the larger the lot, the larger the sample.

If you have seed that is contaminated at 1 seed per kilogram, pulling one sample from one bag would only give you a 2.5% chance of finding it. But if you didn't find it in the first bag, you have another chance, with identical odds in the second bag. And the more bags there are, the more 2.5% chances you get at capturing a pathogen. You get a 2.5% chance enough times, say 400 times, and you have a 99.99% chance of capturing at least one of those contaminated seeds.

Contaminated Seeds per kg	Bags & Samples (Same #)	Kg of Seed Sampled	Contaminated Seeds per Lot	Probability of Capture
1	1	0.025	25	2.50%
1	10	0.25	250	22.10%
1	40	1	1,000	63.20%
1	100	2.5	2,500	91.80%
1	400	10	10,000	100.00%

Table 5. Different size lots in which all lots are contaminated at the rate of one contaminated seed per kilogram. The probability of capturing a pathogen increase as the contaminated seed per lot and the amount of seed sampled increase.

OK, the Seed *Should* be Reasonably Distributed, but What if it Isn't, and Pathogens are in Just a Few Bags?

Uneven distribution is a problem if you are trying to find a particular *level* of contamination, but is not a problem if you are trying to find *any at all*. What matters is the total *number* of contaminated seeds in the lot, not how many *bags* the contaminated seeds are in.

The chart below shows what happens if you sample various size lots, in which all lots have 2000 contaminated seeds, evenly distributed. Notice that if you have one contaminated bag you have 2000 contaminated seeds,

and one pull will capture one or more of those seeds 86.5% of the time. If you divided those 2000 contaminated seeds among 2 bags, you would have 1000 seeds per bag, but you have doubled the number of pulls, and the odds even out.

Bags in Lot	Bags Sampled	Contaminated Seed per Bag	KG Sampled	Odds of Detection
1	1	2000	0.025	86.50%
2	2	1000	0.05	86.50%
10	10	200	0.25	86.50%
100	100	20	2.5	86.50%
800	800	2.5	20	86.50%

Table 6. Even Distribution among five different lots with the same (2000) total number of Contaminated Seeds (CS/lot, not CS/kg or CS/bag)

Our charts are actually based on the odds of capturing *clean* seed. Then we reverse the numbers in order to predict the number of contaminated seed. If you try this with small numbers, such as a few marbles in a cup, the odds will change as you move the marbles from cup to cup. However, there are about 12 ½ million alfalfa seeds in a bag. In this example, each bag in the lot that contains 800 bags has only 1997.5 more *clean* seeds than the lot with just one bag. That is a difference of only 0.00016.

So the number of *clean* seeds remains virtually unchanged, and the probabilities, for all practical purposes, remain the same.

This next chart shows a large lot which, except in the bottom row, is *unevenly* distributed among 800 bags. The first row has all 2,000 contaminated seeds in one bag. The odds, as in the previous chart are 86.5%. But it does not matter that you pulled seed from the 799 bags that are clean. You pulled one pull, from the one bag, that was so contaminated, that it gave you an 86.5% chance of finding one contaminated seed.

Bags Not Contaminated	Bags Contaminated	Number of Contaminated Seeds Each Bag	Samples Taken in the Lot	Odds of Detection
799	1	2000	800	86.50%
798	2	1000	800	86.50%
790	10	200	800	86.50%
700	100	20	800	86.50%
0	800	2.5	800	86.50%

Table 7. Uneven Distribution in an 800 bag lot. If only a few bags are contaminated, they need to be *very* contaminated in order to have a good probability of capturing a pathogen. If many bags are contaminated, likelihood of finding a contaminated seed is strong even if contamination is low.

It should be noted that if you take those 1, 2, 10, 100, or 800 contaminated bags and put *different* levels of contamination in each bag you will still have an 86.50% chance of capturing a pathogen as long as there are 2000 contaminated seeds in the lot.

#### Does Blending Make ISS Seed Sampling Less Reliable?

Yes and no. In the following example, two bags of seed, contaminated at the rate of 4 Contaminated Seeds/kg contain 200 contaminated seeds. If these two bags are blended in with 800 bags of non-contaminated seed, the new lot still has 200 contaminated seeds. The probability of detection, by pulling 802 samples is 18.5%. These are the same odds as if you had pulled one sample from each of the two contaminated bags before they were blended in.

	Large Lot	Small Lot	Combined
Bags	800	2	802
CS/kg	0	4	0.01
CS/lot	0	200	200
Samples	800	2	802
Seed Sampled	1/1000	1/1000	1/1000

Probability	N/A	18.5%	18.5%
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Table 8. If a contaminated and non-lot is combined, the odds of capture will remain the same if the percentage (1/1000 in this example) remains the same.

However, using the ISS Seed Screening Protocol on the two bags, 3 kg would have been pulled for testing (instead of just two 25 gm samples). This increases your sample size from 1/1000 to 1/17th. The odds of capture go to 99.9994%.

		<b>Small Lot</b>
Bags		2
CS/kg		4
CS/lot		200
Samples	(3 kg Total)	120
Seed Sampled		1/17
Probability		99.9994%

Table 9. Increasing the sample size from 2 pulls to 120 pulls, taken from the 2 contaminated bags prior to blending, substantially increases the probability of capturing a contaminated seed.

So it does not make any difference if the lot is blended or not, as long as the protocol is followed prior to blending. Or, if there is more than 120 bags (25gm x 120 = 3kg) in each lot that made up the blended lot.

**Why is it Hit and Miss When Health Officials Try to Find Contamination in Seed That They Are Certain Caused an Outbreak?**

Sample size and the total number of contaminated seeds in the lot determine probability of capture. Seed companies can screen the seed when the entire lot is in tact. This is when the sample size will be greatest and the total number of contaminated seeds is at its highest.

By the time the epidemiologists determine that sprouts are most probably the cause of an outbreak, a good portion of that lot is gone, and a portion, if not all of the contaminated seed was used up in the outbreak. The outbreak could have easily consumed the entire number of contaminated seeds needed to have a high probability of capture.

**What Happens to the Odds When the Minimum Sample Size Requirement is in Play?**

The protocol requires drawing at least 3 kg for testing. But let's suppose it didn't. If there is light contamination, say 4 contaminated seeds per kilogram, and you sample one bag, the odds of finding it in those 25 grams are only 9.5%. Sampling 7 bags increases the odds of capture to over 50%. It would take sampling 47 bags (4,700 contaminated seeds and 1175 gm sample) to increase the probability of capture to 99%. So when you are testing a full truckload of seed (800 bags), contamination only needs to be in 47 (6%) of the bags, at very low levels, to get a 99% probability of capture.

But because the protocol is to draw at least 3 kg, sampling one bag, 120 times, would give you a 99.99+% chance of capture. Sampling 7 bags with 17.15 pulls each (120 total) will also give you 99.99+%, or pulling 2 pulls from each of sixty bags would give you the 99.99+% chance.

Bags Per Lot	1	1	7	7	47	47	800
CS/kg	4	4	4	4	4	4	4
CS/lot	100	100	700	700	4700	4700	32000
Samples	1	120	7	120	120	120	800
Seed Sampled	25g	3kg	175g	3kg	3kg	3kg	20kg
Probability	9%	99.99+%	50%	99.99+	99.99+	99.99+	99.99+

Table 10. Even small lots can be sampled with a high probability of capture if the seed is contaminated.

**The Effectiveness of ISS Seed Screening is Inversely Related the Effectiveness of Chlorine.**

The more contaminated the seed is, the less effective seed sanitizing is. Yet, the more contaminated the seed is, the easier it is to detect a pathogen using ISS' Seed Screening Procedures.

A truckload of seed, contaminated at the rate of 4 cfu/kg has roughly 80,000 individual pathogen cells. The US

FDA recommends a chlorine soak of 20,000-ppm calcium hypochlorite and states that it can give a 2.5 - 3 log reduction of pathogens in contaminated seed. A three-log reduction would theoretically lower the number to 80 viable pathogen cells in the truckload, which will multiply during sprouting. In other words, unless the pathogens are detected in post testing, an outbreak is still likely to occur. Not every batch of sprouts will be contaminated. Theoretically, only 80 batches could be. But if the lot is contaminated at even ½ of one CS/kg, there are 10,000 contaminated seeds in the lot and the probability of capture, using the ISS Seed Sampling Protocol is well over 99.99%. And even if a cell were not captured, evidence of contamination would likely have appeared during the inspection process.

On the other hand, a 20 ton lot that is very lightly contaminated, for instance at the rate of 0.01 cfu/kg (that's 1 cell every 4 bags or 200 cells per truckload), has a probability of detection of <18.5%. Not very good. But the chlorine soak that sprout growers use would theoretically reduce the cells to 1/5 of one cell, for an 80% chance of eliminating every single cell.

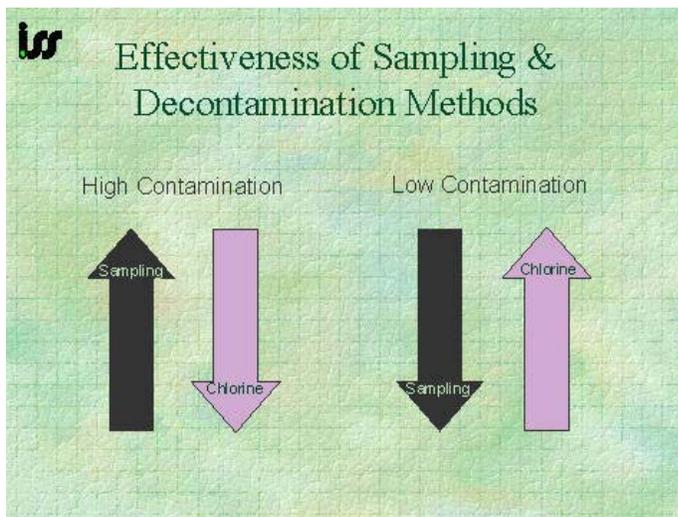


Figure 3. ISS Seed Screening is most effective when the seed is heavily contaminated, which is when sanitation is least effective.

Another way to look at it is to compare two lots of seed; one with 1,000 contaminated seeds and one with 10,000 contaminated seeds. In order to have a complete kill on the first lot, you have to kill the pathogens from 1,000 seeds. In the second lot, you have to kill those same the pathogens from 1,000 seeds, and kill the next 1,000 contaminated seeds, and the next and next until you have killed 10 times as many pathogens as the first group. So chlorine has better odds of effectively sanitizing the 1,000 contaminated seeds, than the 10,000 contaminated seeds.

On the other hand, seed sampling has a 63.2% chance of capturing a contaminated seed in the first lot, and a 99.99% chance in the second lot.

Seed screening along with decontamination compliment each other to reduce the risk of salmonella or E.coli 0157:H7 in sprouted seed.

### Don't All Seed Companies Follow These Procedures?

ISS developed this program, and appears to be the only company in the world who does this extensive testing.

Some seed companies will take a handful of seed from a bag and send it to a lab for pathogen testing. The lab will take 20-grams (less than an ounce) from the sample and test it for pathogens. Yet in previous outbreaks in which contaminated seed was recovered, the contamination level have been as low as 2 pathogens per *pound* of seed (4cfu/kg).

What do you think the likelihood of finding those two pathogens is when testing less than an ounce of seed? It is less than 8%.

So why do labs only test 20 grams? They do not have commercial sprout equipment! And what are they going to do with the 360 lbs (163 kg.) of sprouts produced from a 36 pound sample of seed?

### So is ISS Proposing That Sprout Growers Don't Need to Sanitize their Seed or Post-Test for Pathogens?

Not at this point, but if seed companies had been selling seed that was not contaminated, the sprout industry would not be in the situation it is. The FDA would not be alarmed at all, and sprouts might actually be considered one of the safest products in in the produce department.

That is water over the dam, and it will take many years of no outbreaks before sprouts are considered a safe product again. But because pathogens can be internalized in seed, the sprout industry may never be able to

count on sanitization. The best solution is to start with seed that is well screened and to verify through post testing that the seed screening was effective.

### What are the Weaknesses of Seed Screening?

The probability chart above is based on the probability of capturing a pathogen. But once captured, the pathogen needs to be detected. As of this date, no [test for salmonella](#) or [E.coli O157:H7](#) is perfect. According to the papers highlighted in the previous sentence, the best one can expect from a test kit is detecting contamination 97% of the time it is present in a sample. To overcome this shortcoming, the water samples need to be tested in duplicate, which brings the probability of detection closer to the probability of capture.

Similar to the ISS' Seed Sampling, the probability that at least one of the duplicates is positive is greater than the probability of a single test being positive (given that the pathogen/organism is present). So, if a pathogen test has a sensitivity of 97% (i.e. probability of a test +ve given the sample is +ve) then a testing scheme utilizing duplicates should have an overall sensitivity of  $1 - (1-0.97)^2 = 0.9991$  - which, is virtually 100%.

The main weakness of the ISS Seed Screening Program is the possibility of garbage in, garbage out. The weakness is that you are forced to rely on seed lot information provided by other people. A seed lot is supposed to be one uniform lot. I don't know that this ever happens, but suppose someone along the line, farmer, seed processor, seed supplier, etc, retags seed. The reliability of the information provided by the test would be lower than the probability charts. For instance, if you purchase 800 bags of lot A, and a seed supplier decided they could get rid of the last 30 bags of lot B, the last 18 bags of lot C, and the last 2 bags of lot D by retagging them all as lot A. Your test would still be quite accurate for the 750 bags that originally made up lot A, but not very reliable for the fifty bags that made up lots B, C, and D. Although this marginalizes seed screening, it would have still been effective on 750 bags, which is 94% of the lot and therefore still a useful risk reduction step.

### ISS Seed Screening Does Not do the Following Things:

- ❑ It does not cost much.
- ❑ It does not produce hazardous wastes.
- ❑ It does not put production workers at risk.
- ❑ It does not effect germination, vigor, yield, or the quality of the sprouts.
- ❑ It does not reduce background flora.
- ❑ It does not introduce the possibility of resistance.
- ❑ It does not disenfranchise organic sprout growers.
- ❑ It does not negatively affect or take away from other food safety procedures, such as decontamination.

### What ISS Seed Screening Does:

- ❑ It helps the seed industry identify practices that introduce pathogens into foods.
- ❑ It can prevent contaminated blended lots from entering the market if sampling is done prior to blending.
- ❑ It can prevent some seed with visible contamination from entering the market. This includes glass and other things that would not show up on a pathogen test.
- ❑ It is most effective when sanitizers are least effective.
- ❑ It helps identify farms and seed processors lacking Good Agricultural Practices.
- ❑ It is used identically on all types of seed.
- ❑ It affects commercial sprout growers of all sizes, with all levels of sophistication and using all types of equipment. It can be applied evenly throughout the sprout industry.
- ❑ It can be used on seed destined for home sprouters, and may be the only realistic defense that home sprouters have. Chlorine is not a very good option for home sprouters.
- ❑ It shifts some of the responsibility of providing safe seed to the seed industry itself.
- ❑ It has substantially reduce the number of food borne illnesses from sprouts. (*Currently* at zero for all sprouts produced from seed screened using this protocol. It is only a risk reduction step though. Seat belts reduce the risk of injury in traffic accidents, but people still get hurt. So just

because this seed screening program has a perfect record, it is just a method of reducing risk, not eliminating it. We screen millions of pounds of seed a year. At some time, contaminated seed will get through undetected, and an outbreak will occur. Just not yet. )

### Who Should Do These Procedures?

It is the growers who are ultimately responsible for producing safe sprouted products. They can do these procedures themselves, they can get screened sprouting seed from International Specialty Supply, or they can do both. The more the seed is sampled and tested, the less likely it is to be contaminated. No matter who does it, the grower needs to have good documentation of exactly what was done, when it was done, and who did it. Without this documentation, the grower (and health inspectors) need to consider the seed unscreened and unsafe.

These procedures do not eliminate or reduce pathogens in the seed. If seed is contaminated before it is screened, it will still be contaminated after it is screened. The procedures reduce the risk that the seed may contain the human pathogens that they were screened for. If International Specialty Supply does these procedures, it does not guarantee that there are no pathogens in the seed. It does guarantee that ISS did everything it signed off on in writing for a particular lot of seed. Once the seed is properly sampled, inspected and tested, a document is signed by ISS making it "[ISS Screened Sprouting Seed](#)".

These tests are extensive and vary from seed to seed and lot-to-lot. ISS cannot practically test every lot in every instance. Drop shipments, for example, are almost never tested. With rare exception, all of the seed that comes into ISS' warehouse is tested. But the only way to know for sure is to receive the documentation from us for the particular lot of seed you are interested in purchasing.

ISS strongly encourages growers to get the safety documentation regarding each lot of seed they purchase. A copy of ISS' documentation is attached. A copy of the ISS Seed Sampling and Testing Flow Chart and its Seed HACCP plan are available upon request. A copy of the [ISS Seed Screening Documentation](#) is available by pressing the navigation bar at the top of this page.

### Who Else Believes in the ISS Seed Screening?

- [The FDA invited ISS](#) to present their seed screening procedures at the five year meeting in May, 2005. The FDA will very likely adopt ISS' seed screening procedures as recommendations for all seed sold as sprouting seed. The FDA has advocated the "Six-step procedure developed by ISS" in numerous reports and [presentations](#).
- [The New South Wales Food Authority](#) implemented seed screening in their [Plant Products Safety Manual](#) and noted that "The seed pre-screening sampling process is based on the method developed by International Specialty Supply of Cookeville, TN, USA and Jonathon Sprouts of Marion, MA USA. [www.sproutnet.com/sprouting\\_seed\\_safety.htm](http://www.sproutnet.com/sprouting_seed_safety.htm). Its applicability to Australian conditions was reviewed and confirmed by the South Australian Research and Development Institute." (Pages 28, 31 and 33)
- [The Ontario Ministry of Agriculture, Food and Rural Affairs \(OMAFRA\)](#), adopted ISS' seed screening program as recommendations for seed companies and sprouters in its "[Sprouted Seeds Good Manufactures Guidebook](#)." 2007.
  - *"Using similar testing methods, other seed suppliers should also be able to supply safe seed."*
- [The World Health Organization](#)
  - *"Outbreak investigations have indicated that microorganisms found on sprouts most likely originate from the seeds"*
  - *"There is currently no treatment available that can guarantee pathogen free seeds."*
  - *"Seed producers, distributors, and sprout producers should test lots of seeds for microbial pathogens"*
  - *"Sprouting seeds before testing increases the possibility of finding pathogens that may be present. If lots of seeds are found to be contaminated, they should not be sold or used for the production of sprouts for human consumption."*
  - *"failure to find contamination does not guarantee that the seeds are pathogen free. However, if contamination is found at this stage, it allows seeds to be diverted or destroyed before entering sprout production for human consumption."*
- [Codex Alimentarius](#)
  - [5.2.3.1 Testing of seed lots before entering production](#)
    - The seed sample selected for testing should be sprouted prior to analysis to increase the potential to detect pathogens if present. Analysis may be performed on the sprouted seeds or the water used to sprout the sample.
    - Seed samples for microbial analysis should not be subject to any microbiological decontamination treatment at the sprouting facility.

- The Center for Science in the Public Interest [advised the FDA](#):
  - [ISS] *"...has demonstrated that seed screening has many benefits and is very inexpensive. FDA should mandate that seed distributors conduct this kind of screening and certify their seeds as safe [Actually, ISS certifies them as being screened], in order to [help] prevent contaminated seed lots from entering the market. This could significantly reduce the number of outbreaks associated with sprouts."*
  
- Resources For the Future, a Washington DC based think-tank, in its seminar titled ["Achieving A Safe Food Supply in Increasingly Global Markets"](#) wrote:
  - [ISS] *"developed a program of seed screening that has allowed it to avoid all sprout-related outbreaks or recalls in North America since 2000, causing health organizations worldwide to start recommending ISS screening procedures be used on any seed destined for sprouting."*
  
- FSA (Food Standards Agency, UK)
  - *"Outbreak data indicate that the seed used for sprouting is the most significant source of the pathogens implicated. ...bacteria that are trapped in cracks and crevices in the seed coat appear to be protected from disinfectants and there is evidence that some bacteria can be internalized within the seed itself.....to date no technique has been shown to completely eradicate pathogenic micro-organisms from either the raw or sprouted seed...Given the difficulty of decontaminating seed another important aspect ...is the detection and isolation of pathogens from the seeds used to produce sprouts."*
  
- The Campden Research Group in England in their 2004 report titled "Review of microbiological risks associated with sprouted seeds" concluded that
  - *"Absence of pathogens in seeds is critical and, consequently, microbiological testing of seeds prior to use for production of sprouts is essential."*
  
- Seed sampling is also suggested in the [Codes of Practice for Food Safety in Ireland](#).
  - *"As seeds are thought to be the most likely source of contamination...The dried seed should be sampled and tested microbiologically upon arrival. Seed contamination is thought to be sporadic, at low levels or unequally distributed throughout seed lots. Therefore, a negative result does not guarantee the absence of pathogens, however a positive result allows a producer to avoid using contaminated seed lots."*
  
- Leading researchers for the USDA and FDA, William F. Fett, Tong-Jen Fu, and Mary Lou Tortorello in "Seed Sprouts, the State of Microbiological Safety" Chapter 6 of Microbiology of Fresh Produce, Edited by Karl R. Mathews, 2006 pp202-207.
  - *"Proper sampling inspection and testing of seeds for pathogens can substantially reduce the chance of using contaminated seeds for sprout production and therefore help prevent foodborne illness. However, improper seed screening protocols may still result in outbreaks."*
  - *"A comprehensive seed sampling and testing procedure has been developed [ISS]." Then they explain ISS' process described above and close with: "The adoption of these seed-screening procedures has already prevented at least four potential sprout-related outbreaks do to Salmonella and E.coli O157:H7."*
  
- Food Safety Researcher and Professor Keith Warriner ["Interventions to Improve Food Safety of Sprouted Seeds"](#) in his PowerPoint Presentation for British Columbia's Premier Food Safety Conference - October 18, 2007.
  
- The California Department of Public Health highly recommends sampling in their [Advisory to Commercial Sprout Producers](#), May 1, 2008 (Comment #2). Their advice is based on ISS' seed screening program.
  
- Trevor Suslow, Plant Sciences and Postharvest Technologist, UC Davis, In his presentation titled ["Evaluating Seed Borne Pathogens as a Risk Factor in Produce Outbreaks"](#) for the Plant Disease Seminar, Nov, 13, 2007 concluded his presentation with *"Recommendations for Seed Industry GAPs & BMP's - "Most critically, before engaging in any seed testing program, a uniform and standardized test procedure must be determined. Statistically sound sampling protocols need to be established...The experience of the sprout seed industry with pathogen-free certification programs can provide insight into approaches and problems (SproutNet has valuable and practical information on testing programs and access to abstracts of published seed food safety research)."* Trever Suslow
  
- In the study ["Factors Influencing the Growth of Salmonella during Sprouting of Naturally Contaminated Alfalfa Seeds"](#) FDA and National Center for Food Safety and Technology researchers referenced the ISS Seed Screening Program when they point out that *"To prevent the consumption of contaminated sprouts, the absence of pathogens in seeds must be assured. Screening of seeds by seed suppliers for the presence of pathogens can help to reduce the number of contaminated seeds entering the marketplace."*

NOTHING ELIMINATES RISK: ISS Seed Screening or the use of ISS Sprouting Seed is one of several methods that should be employed by commercial sprout producers to *reduce* the risk of human pathogens in sprouts. Seed should also be properly sanitized prior to production and the sprout run-off water should be tested for human pathogens between 24 and 48 hours of production.

For more information on seed sampling and testing see "[Seed Sampling and Testing: A risk-reduction strategy for sprouts.](#)"