

# Sprouted Seeds Good Manufacturing Practices Guidebook



### **S2 PRE-PRODUCTION INSPECTION and TESTING**

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#### **S2.1 Seed Inspection**

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Scientists agree that the likelihood of seed contamination is reduced if Good agricultural Practices (GAPs) have been applied during seed production. However, it is often difficult for sprout growers to know the extent to which GAPs have been applied, or if they have been applied at all unless that information has been supplied by their seed supplier/distributor.

GAPs are just the first of several preventive measures and pathogen screening steps that should be used to ensure the safety of seeds for sprouting. The other steps should include seed sampling, seed inspection, pathogen testing of irrigation water from sprouting seed samples and seed sanitation before sprouting. As a group, these procedures increase the probability of finding or destroying pathogens, if they are present.

There is little point in spending time and money on seed pathogen testing if a visual inspection could result in rejection of the seed lot for other reasons. One major seed supplier reports rejecting more than 50 per cent of seed lots sampled since it started its seed screening program. Nearly all of the rejected lots resulted from its inspection process, not from pathogen testing.

Prior to purchasing or accepting seed, growers should thoroughly inspect, under magnification, a representative sample of the seed for evidence of contamination and damage, as described below.

After receiving a shipment and sampling the seed using the procedures described in Sections S2.3 and S2.4, the seed should be inspected under magnification prior to sprouting for pathogen testing.

After a sample is taken from each bag, the composite sample should be inspected for indications of contamination. Inspection should be done with a magnifying glass, a microscope and the naked eye. Look for mouse droppings, urine, insect excreta, damaged seeds, foreign objects and other types of contamination. Those with access to a “black light” should use it to check for the characteristic fluorescent orange colour produced by rodent urine.

## Sprout Production

### Seed Sampling, Inspection and Testing

International Specialty Supply (ISS), a U.S. seed supplier, reports finding mouse droppings, glass, seed that had been treated with a fungicide, silica, iron filings, mouse parts, insect parts, dirt, organic matter and several other types foreign material in incoming seed lots. The company noted that feces and damaged seed comprise the great majority of rejections resulting from physical inspection. Urine in the seed has been found only four times.

Depending on the type of seed being inspected, a microscope can be used to detect damaged seed. The only way to get an accurate count of damaged seed is to separate out 100 or more seeds. Pick out the damaged ones. An alfalfa seed has two sides. Turn each remaining seed over and inspect it for damage. Count the number of damaged seeds, compare to the number of undamaged seeds and calculate the percentage of damaged seed.

There is a faster, although less accurate, way to determine the percentage of damaged seed. Assume that for each visible damaged seed, there is another that is broken on the other side and not visible. Count the number of seeds that are damaged on the side facing you and double that number. Then count the number of seeds that are broken in two or broken on one or both ends. Add the two numbers together and calculate the percentage of the total.

Mouse droppings can be misidentified as dirt. However, with practice, they are easy to find and are easily identified under a microscope by their fibrous consistency.

ISS reports that the main cause of rejection of seed resulting from pathogen testing was generic *E. coli*.

## **S2.2 Is There Value in Preproduction Microbial Testing?**

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Even if GAPs have been used and the seed has been inspected, pathogens may still be present on seeds intended for sprouting.

Once pathogens are present on seeds, they are able to survive for extended periods of time.

If pathogens are present, they may be difficult to detect for several reasons.

## Sprout Production

### Seed Sampling, Inspection and Testing

- Pathogens may reside inside microscopic cracks on the seed surface, or even inside the seed, making them physically inaccessible.
- Levels of pathogens are usually so low that, from a statistical standpoint, many seeds would have to be tested in order to achieve detection.
- Pathogens may or may not be randomly distributed within the seed lot. Again, microbial testing would need to be applied to a large percentage of seed to have a reasonable assurance of finding pathogens.

Rather than testing the seed itself, scientific literature suggests that a more accurate indication of seed contamination can be achieved by collecting representative seed samples, sprouting them in a commercial setting, then analyzing spent irrigation water collected from the growing sprout sample.

#### Testing Spent Irrigation Water

There may be several reasons that screening spent irrigation water appears to be more accurate. Once sprouting begins, pathogen growth is stimulated and supported by nutrients released from the seed, readily available water and warm temperatures. This increases the pathogen population by 1,000 to one million times so pathogens become easier to detect. As seeds germinate, pathogens residing in seed cracks and crevices expand their presence to areas on the surface of the seed. Irrigation water washes over the surface of the seeds and emerging sprout and, in doing so, washes some pathogens into the spent irrigation water.

The scientific community believes that collecting sprout irrigation water from one irrigation cycle 48 to 96 hours after sprouting begins provides a representative sample from all the seeds. Irrigation water chlorinated at a low concentration level does not affect the pathogen level in the spent irrigation water. However, the seed should not be disinfected before seed sprouting and irrigation water testing begins for the test seed lots. Seeds used for production of sprouts should be sanitized.

Health Canada's Amendment to MFHPB-20 analysis uses a slightly different approach ([www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhp20a-01\\_e.html](http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhp20a-01_e.html)) to test for *Salmonella* in germinating seeds. Seeds (125 g) are kept covered in unchanged sterile water at 30°C for three days. A slurry of the mass is created and enriched with a nutrient broth before analysis using MFHPB-20 methodology ([www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhp20-](http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhp20-)

# Sprout Production

## Seed Sampling, Inspection and Testing

01\_e.html). While not documented, some sprout growers believe this method is better at locating pathogens in lightly contaminated seed.

ISS reports that none of its seed has been implicated in an illness outbreak since the company initiated seed sampling, seed inspection, sprout growing (of the seed sample), spent irrigation water sampling, enrichment of sampled water (to multiply pathogen numbers for easier detection) and pathogen screening in 1999. There was no indication of how many contaminated seed batches were detected and rejected. Using similar testing methods, other seed suppliers should also be able to supply safe seed.

**Testing Sprouts** Sprouts may also be tested for pathogens. There are disadvantages and advantages to this method.

In order to collect a representative sample, a very large number of samples should be taken. Health Canada's *Sample Collection and Testing for Sprouts and Spent Irrigation Water* requires that 5 sample units of approximately 200 g each should be aseptically collected from different locations in the drum or growing trays. Sample units should be collected throughout the entire production lot (e.g., from top to bottom, side to side, and front to back of the drum or trays).

The link for Health Canada's *Sample Collection and Testing for Sprouts and Spent Irrigation Water* is:

[www.hc-sc.gc.ca/fn-an/alt\\_formats/hpfb-dgpsa/pdf/legislation/sprout\\_water\\_testing\\_analyse\\_pousses\\_eau\\_e.pdf](http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/legislation/sprout_water_testing_analyse_pousses_eau_e.pdf).

In some growing configurations, sprout irrigation water may be difficult to collect. Research indicates that levels of *E. coli* detection in irrigation water may be 1 log<sub>10</sub> less<sup>1</sup> than that found on sprouts. At this time, laboratory ability to detect *E. coli* is also lower than its ability to detect *Salmonella* in irrigation water. The FDA recommends irrigation water testing over sprout testing.

Spent irrigation water from soil-grown sprouts cannot be effectively used for testing seeds for pathogens.

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<sup>1</sup> "Log" is the abbreviation of logarithm; each log reduction decreases the pathogen population by 90 per cent—see section S3.1, Why Disinfect Seed, for a more detailed explanation.

## Sprout Production

### Seed Sampling, Inspection and Testing

While microbial testing may identify a portion of those pathogens present in contaminated seed, testing cannot guarantee discovery of all the pathogens. Even a negative test result does not guarantee the absence of pathogens. However, it does provide a level of assurance that a batch of sprouts is probably free of contamination.

### S2.3 Developing a Seed Sampling Plan

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There is substantial evidence that contaminated seed is responsible for the great majority of sprout-related illness outbreaks. Given that there is no method of sanitizing seed that is 100 per cent effective, in order to produce safe sprouts:

**Sprout growers need to ensure that the seed they use is not contaminated before using it in production.**

In order to find pathogens in seed prior to sprouting, the pathogen should be captured and identified. Alternatively, a sprouter may capture and identify *evidence* of contamination. Seed sampling is a method of capturing a pathogen or evidence of contamination for identification.

In order for analysis to be statistically valid, sampling methods should be designed to ensure that samples represent the entire lot of seed. It is known that the greater the number of samples and the larger the size of the samples, the greater the probability of pathogen detection or detection of evidence of contamination. This is especially important at low levels of contamination and when pathogen distribution is irregular.

Seed sampling methods for germination and purity tests are quite different than those trying to determine if the seed is contaminated. Seed sampling methods for germination and purity tests are designed to determine the exact percentage of seed that will germinate and the exact percentage of undesirable seeds. This can be done quite accurately by random sampling and narrowing that sample down to a few hundred seeds for testing.

However, when trying to determine if seed is contaminated, the object is not to find out how many pathogens are present, but to determine if **any pathogens** are present. In this case, sample size becomes the main determining factor in accuracy.

# Sprout Production

## Seed Sampling, Inspection and Testing

**Detection Probability** ISS charted the statistical probability of finding pathogens in seeds based on different sample sizes and different levels of pathogen contamination. *Probability of Finding a Pathogen in a Twenty-Ton Lot of Alfalfa Seed Contaminated with Various Contamination Levels per Kilogram*, may be accessed on the ISS Web site at:  
[www.sproutnet.com/sprouting%5Fseed%5Fsafety.htm](http://www.sproutnet.com/sprouting%5Fseed%5Fsafety.htm)

Selected information from the chart follows.

- In a 20 ton lot of alfalfa seed if the contamination rate is 4 cfu (colony-forming units is a measure of viable bacteria numbers), the probability of locating pathogens (if they are present) in a 20 g sample (10,000 seeds) is only 7.69 per cent. If the contamination rate is 1 cfu, the probability of detection in the same sample decreases to only 1.98 per cent. However, at a contamination rate of 10 cfu, the probability level increases to 18.13 per cent. All are far below acceptable detection levels.
- In a 1 kg seed sample (50 × 20 gram samples or 500,000 seeds) with a contamination rate of 4 cfu, the probability of pathogen detection (if pathogens are present) rises dramatically to 98.17 per cent. At a contamination rate of 1 cfu, the probability of pathogen detection in a 1 kg sample falls to 63.21 per cent. At 10 cfu, pathogens will be detected 99.99 per cent of the time.
- When a 5 kg sample (250 × 20 gram samples or 2,500,000 seeds) is used, the probability of detecting pathogens (if they are present) is 99.99 per cent when pathogen levels are both 4 cfu and 10 cfu. At 1 cfu, the probability is 99.33 per cent.

The chart used assumes uniform distribution of pathogens in the seed. In reality, pathogen distribution will likely be irregular, making detection more difficult. In reality, the overall sample size will depend upon the number of bags sampled and the size of each individual sample taken.

**Small Growers** Small sprout growers and/or buyers of small lots may not have the resources necessary to test all incoming seed for pathogens. Ask for a certificate of sampling and insist on documentation of *E. coli* O157:H7 and *Salmonella* laboratory testing results when purchasing seed. Carefully examine all documentation. Ask for references. Beware of claims by seed suppliers (such as “pathogen free”) that cannot be substantiated. As a

# Sprout Production

## Seed Sampling, Inspection and Testing

further precaution, seed should be carefully examined upon receipt, as described in Section S1.3.

**Effective Sampling** An effective sampling program requires seed samples from every bag of seed. Sampling every bag is not difficult, nor is it significantly more costly than sampling a random portion of the bags. Compared to the time and cost involved in a recall, the extra cost of sampling every bag is insignificant.

Equal-sized samples should be taken from every bag of seed. Suppose your sprout operation has just taken delivery of six pallets of seed. There are 40 × 25-kg bags on each pallet for a total lot size of 240 bags. If a 20 g sample is extracted from each bag, a 4,800 g (4.8 kg) test sample will result. The statistical probability of discovering pathogens (if they're present) during sprouting of this size sample is extremely high (greater than 99.99 per cent).

### **S2.4 Seed Sampling Methodology**

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Samples of seed are usually taken with a probe or trier. The probe should be long enough to reach all areas of the bag or bulk bin. In bagged seed, probe a horizontal bag diagonally from corner to corner (e.g., top right corner to bottom left corner). The probe should have enough slots to sample along the full horizontal distance. Insert the probe into the bag with the slots closed and facing downward. Once fully inserted, rotate the probe slots so they face upward, and then open them. Once seed has filled the probe, close the slots and withdraw the probe. Cover holes in the bags with a sticker that completely seals the hole. Place seed in a clean, sanitary container and probe the next bag. An equal amount of seed should be removed from each bag (minimum 20 g is suggested).

When sampling seed in bulk, far more probes should be made. As with bag sampling, the probe is fully inserted, rotated, opened, and then closed before withdrawal. Unlike bags, in bulk bins or totes, the probes should be inserted from the top only, not the bottom. The probe should be inserted at an angle near the four corners of the bin/tote and in the centre.

It is very important to use aseptic sampling methods when taking any sample. Hand sampling, which carries a higher risk of cross-contamination, is not recommended.