Guide to Best Practices for Sampling and Testing and Risk Management for Gluten May 15 2018

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Disclaimer: This guide is intended to be descriptive and not prescriptive in any way and should be adapted as needed in a risk-based management system approach. It is not intended to replace the services of professional consultants.

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CONTEXT

Products labelled gluten-free have appeared on store shelves at an exponential rate in the last decade due to growing demand. This greater availability of products benefits people affected by gluten-related disorders such as celiac disease, dermatitis herpetiformis, gluten ataxia, non-celiac gluten sensitivity, wheat or barley allergy, etc.

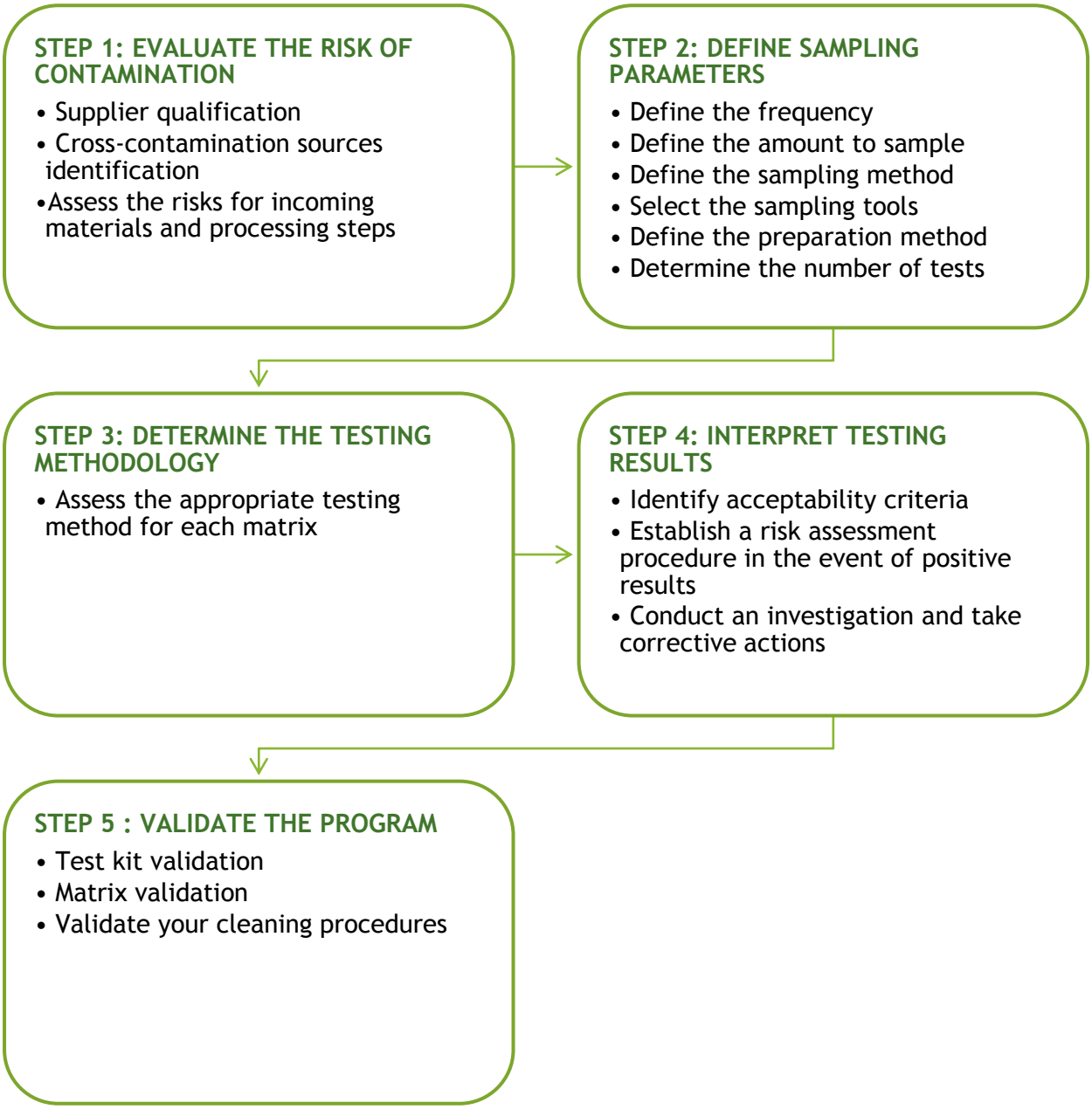
In people with celiac disease, the ingestion of gluten triggers an autoimmune response that causes damage to the villi in the small intestine, leading to a wide range of symptoms. These individuals need to follow a strict gluten-free diet for life to avoid complications, as even small amounts of gluten can cause serious health complications (nutritional deficiencies, neurological complications, cancers, etc.). Consequently, it is important for individuals who must avoid gluten to be able to find safe and reliable gluten-free products in stores.

Gluten is the protein fraction of wheat, rye, barley, oats or their cross-bred varieties to which some people are intolerant (1). Oats are a topic of debate around the world. It is generally acknowledged that oats can be tolerated by most people who are celiac or gluten intolerant. However, this grain is often cultivated, transported or processed in the same environment as gluten-containing grains, which makes it very vulnerable to cross contamination. Therefore, in Canada, only gluten-free oats that have been especially produced are authorized to bear the "gluten-free" label. Many other countries have a lesser concern and therefore assume a lower level of protection to the public.

INTRODUCTION

This sampling guide is intended to help manufacturers committed to produce gluten-free products, whether they are manufacturing their products in a dedicated gluten-free facility or not. It provides an overview of best practices for developing sampling plans and testing protocols to detect gluten in a wide variety of foods and on environmental surfaces in order to support gluten-free claims.

This approach will be explained in five simple steps:



Each step is accompanied by figures and tables to facilitate understanding of the important points. In addition, technical or scientific terms used in this document are defined in the glossary at the beginning of this guide. Refer to it as needed.

After reading this guide, you should be able to develop your own sampling plan based on risk assessment. Your sampling program will detail how to test your incoming materials, environmental surfaces and finished products to ensure that your products are safe. To help you, a case study is presented at the end of this guide. This example will show you how to develop an effective sampling plan according to each step explained in this guide.

DEFINITIONS

Acid: A sour-tasting material that produces positive ions (H^+) in solution. Acid matrices have a pH of 0 to 7 (the opposite of alkali).

ACSP: Accredited Container Sampler Program administrated by the Canadian Grain Commission (CGC).

Alkali: A basic solution that is a bitter-tasting material and that forms hydroxide ions (OH^-) when dissolved in water. Alkali matrices have a pH of 7 to 14 (the opposite of acid).

Antibody: An antibody is a large Y-shaped protein produced by B cells after stimulation by an agent called antigen. Antibodies are used in ELISA test kits to detect the presence of a specific substance.

CCSP: Certified Container Sampling Program administrated by the Canadian Grain Commission (CGC).

CGSP: Canadian Grain Sampling Program administrated by the Canadian Food Inspection Agency (CFIA) Grains and Oilseeds Section.

Composite: Sample obtained by mixing multiple individual subsamples.

Distillation: Process of separating volatile substances from a liquid by evaporation and condensation.

Extraction: Extraction is the action of removing a substance from another substance with the help of a solvent. Extracting gluten from food is the first step in detecting gluten.

Extrusion: Extrusion is a process used to produce food by which ingredients are forced through an opening in a perforated plate of a specific design and then cut. It includes the production of pasta, croutons, bread sticks, breakfast cereals, snacks, baby foods, pet foods, etc.

Fermentation: Fermentation is a metabolic process that converts sugar into acids, gases and alcohol. This process is widely used to produce foods and beverages (e.g. pickles, kimchi, cheese, beer, yoghurt, etc.). If gluten proteins are present during fermentation, they are broken down into smaller peptide fragments.

Hydrolyzation: Hydrolyzation is a chemical process in which a molecule of water is added to a substance, causing the cleavage of chemical bonds and the degradation of a substance. The hydrolysis of proteins results in smaller molecules such as amino acids.

Limit of detection (LOD): The limit of detection is typically defined as the lowest concentration or quantity of a substance that can be reliably distinguished with a specific analytical method.

Limit of Quantification (LOQ): The limit of quantification is the lowest concentration at which the analyte can be reliably detected but at which some predefined goals for bias and imprecision are met. The LOQ may be equivalent to the LOD or it may be a much higher concentration.

LLSP: Licence Seed Sampler Program administrated by the Canadian Food Inspection Agency (CFIA).

Lot: A group of product that is processed under uniform conditions.

Mass spectrometry: A proteomic method that enables identification of individual proteins from the amino acid sequence of peptides. Can be used to identify the source of gluten.

Matrix: Matrix refers to all components of a sample, with the exception of the analyte of interest (in this case, gluten) that can affect the testing result.

PCR: A DNA-based method that relies on amplification of specific small fragments of the target deoxyribonucleic acid (DNA) until a sufficient number of copies are obtained for visualization or quantification. Can be used to identify the source of gluten.

Peptide: Peptides are short chains of amino acid molecules linked by peptide bonds that form proteins.

Recovery: The process of finding the accurate quantity of substance in a sample.

Sample: A unit that is drawn from a lot.

Spiking: Adding a known amount of an analyte to a sample to confirm the performance of an analytical procedure.

Subsample: A unit that is drawn from a lot and combined with other units to form a composite sample.

Validation: The collection and evaluation of scientific, technical and observational information to determine whether control measures are capable of controlling hazards. A validation study therefore makes it possible to measure the performance of a process.

Verification: The application of methods, procedures, tests and other evaluations in addition to monitoring to determine whether a control measure is or has been operating as intended.

STEP 1 – EVALUATE THE RISK OF CONTAMINATION

All manufacturers should evaluate their risk of contamination with gluten before they start developing a sampling program for all **incoming materials** and for each **processing step**. The objective is to ensure that the finished product meets regulatory requirements (i.e., not more than 20 ppm of gluten) or a lower limit imposed by the establishment (e.g., <5 ppm or undetectable).

1.1 INCOMING MATERIALS

1.1.1 SUPPLIER QUALIFICATION

Since gluten can be hidden in all types of products, the gluten-free status of your incoming materials and the capacity of your suppliers to produce and deliver gluten-free raw materials should be evaluated. This qualification should be performed for all incoming materials that may come in contact with your gluten-free products (e.g. ingredients, packaging materials, non-food chemicals, processing aids, etc.).

At this stage, you should ask your suppliers to provide all the appropriate documents, such as:

- Ingredient formulations
- Technical specifications
- Certificates of analysis
- Letter of guarantee of gluten-free status
- Supplier evaluation questionnaire (see Appendix 1 and 2)
- Allergen checklist for food suppliers and manufacturers (see Appendix 3)
- Food safety accreditation certificate (e.g. SQF, BRC, HACCP, GFCP, etc.)

Requesting that your suppliers fill in questionnaires may help you better understand the risk of contamination associated with your incoming materials. These questionnaires should be specifically adapted to your internal requirements and the kind of material you are buying (grains, ingredients, packaging materials, etc.). They may also be designed to reflect other requirements that you may have (e.g. good manufacturing practices, genetically modified organisms, organic status or other allergens).

Suppliers' qualification should be reviewed on a regular basis (e.g. every year) taking into account testing results, non-compliances issued and other food safety problems. This assessment will allow you to confirm if the measures in place are sufficient or if, conversely, monitoring needs to be increased by other means (e.g., increased number of gluten tests performed internally, supplier audits, testing certificates for each lot, etc.). You must make sure to renew the documentation regularly and require your suppliers to inform you of any changes. It is essential that you establish a relationship of trust with your suppliers.

1.1.2 CONDUCT A RISK ASSESSMENT

Risk assessment can be conducted using several tools. For example, the scoring system presented in Appendix 4 can help manufacturers to classify incoming materials into three risk categories (i.e. Low, Medium and High) by considering the ease of detection of gluten and the likelihood of occurrence.

Ease of detection

Ease of detection evaluates the sampling and testing methodology's ability to accurately detect gluten in food samples. This measure can be influenced by some interfering food components and processing steps, and some products are more difficult to analyze than others, such as:

- food containing high amounts of polyphenols or tannins (e.g. tea, hops, cocoa products, coffee, spices, chestnut flour, buckwheat, millet, etc.),
- highly processed products (e.g. bread, pasta, baby food, snacks, etc.), and
- fermented and hydrolyzed foods (e.g. beer, soy sauce, sourdough, vinegar, etc.).

In the case of the above food items, it may be necessary to use methods that are adapted to the specific challenges of these products (see Step 3 for more details).

A sample's ease of detection may also vary according to its physical form, as gluten might not be homogenously distributed in all samples. Solid samples (e.g. bakery products, prepared meat) are usually more difficult to homogenize than liquids.

Gluten is usually extremely difficult to detect in grains because it is generally not well distributed within samples. Indeed, when a sample is contaminated by a gluten containing kernel, gluten-containing particles commonly coagulate into small masses during the milling process. This is called pilling of proteins in the scientific literature. Some particles may also stick to grinding equipment surfaces during sample preparation. These phenomena will contribute to a heterogeneous distribution of gluten in the sample and therefore to misleading analysis results.

Ease of access for sampling is another important factor to consider, since it influences collection of a representative sample. For example, sampling of large lots, such as bulk, can sometimes be difficult.

Likelihood of occurrence

The likelihood of occurrence is the probability that an ingredient contains gluten at a higher level than the regulatory limit (e.g. 20 ppm) or a limit that is self-imposed by the facility (e.g. 5 ppm or none detectable). Likelihood of occurrence mainly depends on the nature of the ingredient and the risk of cross-contamination by other gluten-containing grains.

For example, **grains, seeds and legumes** that are considered inherently free of gluten may still be at a high risk of cross-contamination by other gluten-containing grains. The cross-contamination risk depends on the diversity and region of production. Separation techniques are used to clean the

grains, but the effectiveness of these techniques depends on the physical properties of the grains (size, colour, density, etc.). Ideally, products that are guaranteed or certified as gluten-free should be procured for an added level of assurance of lower risk.

Spices and herbs do not usually contain gluten. In rare cases, spices can be contaminated with gluten-containing grains or cross-contaminated during harvesting, transportation or processing. However, typically very low quantities of spices are added to product formulations (e.g. 1–2% by weight). It is important to distinguish spices from seasonings. Seasonings often comprise a blend of spices, herbs, salt, sugar, modified milk ingredients, starch, flour and/or an anti-caking agent. Since seasonings sometimes contain wheat flour, wheat starch or hydrolyzed wheat protein, the risk is higher for such ingredients. It is therefore essential to carefully check the list of ingredients.

Appendix 5 explains the guidelines to help you better assess the occurrence in several categories of products that contain or may contain gluten. This tool can be used to support your assessment in addition to the other information gathered when qualifying suppliers such as the history of testing results and supplier control practices. For example, products that are certified as gluten-free by a third-party organization can be considered lower risk than products not certified. When buying products that are not certified or guaranteed to be gluten-free, the buyer carries full responsibility and accountability for the acceptance of high-risk products. This can be very costly without evidence of adequate upstream management by the seller.

1.2 PROCESSING STEPS

1.2.1 CROSS-CONTAMINATION SOURCES IDENTIFICATION

Potential cross-contamination risks should also be evaluated at the facility level. The extent of this work depends on the type of production and whether manufacturers process any gluten-containing products in the same facility. In a non-dedicated gluten-free facility where both gluten-containing and non-gluten-containing ingredients are present, assessment of risk factors should cover the entire processing operation. However, in a dedicated gluten-free facility where no gluten enters the production or storage areas, some elements may not be applicable or necessary, as shown in Table 1.

Table 1: Some elements to cover in a risk assessment for the manufacturing of gluten-free products

Elements	Examples of cross-contamination with gluten	Non-dedicated gluten-free facility	Dedicated gluten-free facility
Receiving	Spillage during distribution and transportation	✓	✓
Storage	Spillage	✓	
Production	Air flow, shared equipment and utensils, improper production sequencing	✓	
Cleaning and sanitation	Cleaning tools, shared equipment, inadequate cleaning, poor equipment design	✓	
Maintenance	Maintenance tools, contractors	✓	✓
Personnel	Contamination by employees, visitors, contractors and service providers (e.g. contaminated clothing or hands, job rotation practices)	✓	✓

1.2.2 CONDUCT A RISK ASSESSMENT

Each processing step for which a cross-contamination risk exists should be assessed with an HACCP-based food safety system or equivalent. The hazard assessment should consider the likelihood of occurrence and severity as presented in the evaluation tool in Appendix 6.

Likelihood of occurrence is the probability that an undesirable outcome could occur. It depends on whether gluten is present in the same manufacturing environment. Measures of control in place (e.g. segregation, training, cleaning procedures, traffic patterns, air flow, etc.) should also be considered.

Severity measures the possible consequences of a hazard. For gluten, severity is highly influenced by the quantities involved if contamination occurs. For example, direct addition of wheat flour to a recipe represents a high risk. However, the cross contamination of a packaging bag after a possible spill during transport is a low risk because it is packaged in a cardboard box.

STEP 2 – DEFINE SAMPLING PARAMETERS

The parameters of a sampling plan greatly depend on the outcome of the risk analysis. A good strategy is to test ingredients and products in their earliest, less complex forms and always within the context of a gluten-free management system which focuses on a « start clean, stay clean » approach.

Incoming materials and processing steps that are at higher risk should be tested more intensively. Similarly, finished products manufactured in a facility that produces both gluten-free and non-gluten-free products should be tested regularly, especially if products are made on shared equipment or in a common room.

When designing sampling plans, many elements should be considered, such as the nature of the product, lot size, lot homogeneity, testing result history, risk of cross-contamination, etc. Sampling procedures generally consist of three essential steps: sample collection, sample preparation and analysis. The effectiveness of each step can be maximized by making good choices when designing sampling plans (Table 2).

Table 2: Key elements to include in a sampling protocol

Steps	Key elements
Sample collection	• Determine sampling frequency • Determine quantities to be collected to obtain a representative sample (number of samples, time interval, sample size) • Choose the right sampling tool
Sample preparation	• Assess uniformity of subsamples • Determine the preparation method to homogenize samples • Clean equipment
Analysis	• Choose the proper testing method according to the sample type • Determine the number of analytical measurements

2.1 SAMPLE COLLECTION

First, it is important to ensure that samples are randomly collected from a lot in a manner that gives each sample an equal chance of being chosen. Selecting a representative sample is critical, since only a very small amount of the sample is usually analyzed.

Commodities contained in bins, boxes, bags, totes, trucks, rail cars or other static containers should be sampled from different locations randomly dispersed throughout the lot. The number of samples per container and the sample size should be based on the lot size and risk assessment to ensure a good representation of the entire lot.

When samples are collected in dynamic lots where the product is moving (e.g., when unloading bulk grains), small quantities should be collected at frequent and uniform intervals at a specific sampling point. Samples can then be combined to form a composite.

When sampling, it is also important to use appropriate sampling devices depending on the type of product sampled, the type of container and the sampling method. For example, probes like the Nobbe trier and the double sleeve trier (Figure 1) can be used to sample grains in static containers. Probes are available in different lengths, and triers should be selected according to crop type (2).

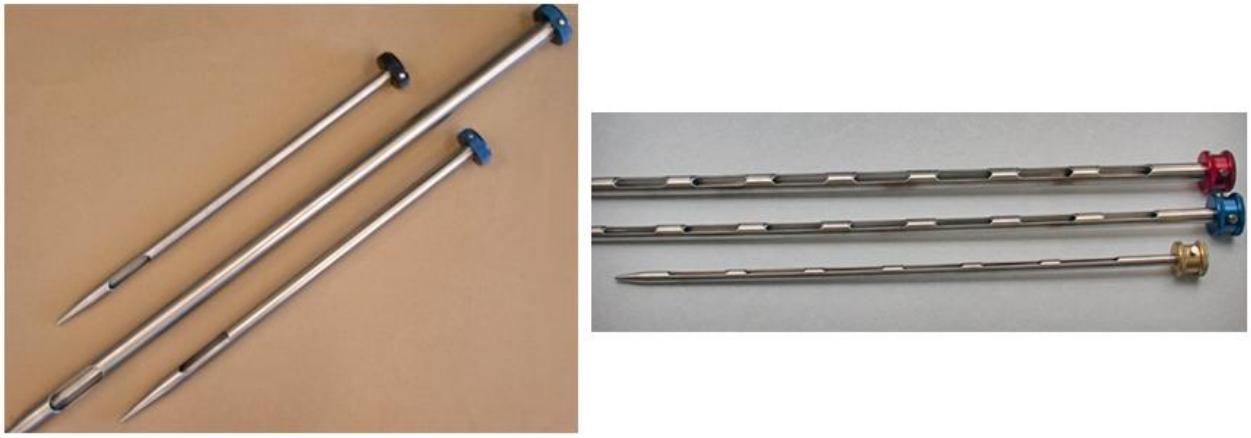


Figure 1: Different sizes of Nobbe triers (left) and double sleeve triers (right)

It is also possible to use manual tools such as hand scoops, buckets or automatic sampling devices, more specifically when the product is sampled from a dynamic lot. On the other hand, when the product is transferred in a completely closed circuit, it is important to designate a place where an adequate sample can be collected.

Incoming materials should be visually inspected upon their arrival at the plant and analyzed using a rapid ELISA test kit. Testing frequency should be determined according to the **risk assessment** results, since it might not be necessary to test all incoming materials at each delivery. Risk level, lot size and frequency of delivery should also be considered. Table 3 gives some guidelines for assigning testing frequency to incoming materials.

Table 3: Guidelines for determining testing frequency of incoming materials

Risk level	Minimum testing frequency
Low	Once a year
Medium	Every 2 to 5 receiving lots
High	At each delivery

The sample size and the number of samples to collect depend on the homogeneity of the product from which the sample is drawn. To do this, it is important to consider the statistical validation. For incoming materials, five subsamples of a minimum of 100 grams from different locations throughout the same lot should be collected and combined to reduce variability. However, smaller quantities could be collected for ingredients received in very small quantities (e.g. spices).

Sampling of incoming materials and testing must be conducted as quickly as possible after raw materials are received. Ideally, incoming materials should not be unloaded in the company's facilities until testing results are available to avoid introducing a source of gluten. This is particularly important for bulk raw materials unloaded directly in storage facilities (e.g., silo, tank).

The development of sampling plans to determine gluten concentration of finished products is complex. Currently, no standardized sampling method is acceptable for all types of food. Testing frequency of finished products should also be determined based on the risk assessment results. Analyzing all finished products at least once a year is recommended, but the frequency of analysis may be higher. For example, each lot of products considered high risk should be tested.

Finished product testing is the final step in verifying a gluten control program. Although testing is essential, finished products should never be deemed compliant based on gluten testing results alone, since a production's true gluten concentration can never be determined with 100% certainty. In addition, for some foods that are more likely to be contaminated, significant variability in sample analysis results is often noted. Thus, it is best to use different approaches to check the absence of gluten in your product throughout its processing. For example:

- Check the cleanliness of direct food contact surfaces with swabs once the production line has been cleaned or after maintenance. Target shared equipment, areas known to be difficult to clean and dead spots.
- Check the first products coming off the production line after changeovers. When wet cleaning is impossible or restricted (e.g., flour, chocolate), be sure to check the purge product at reasonable intervals after leaving the production line. The product can be

considered safe when two consecutive samples (e.g., after three and five minutes) meet the limits established in your cleaning validation procedure.

- Check the gluten concentration of rinse water after the cleaning cycle when a cleaning-in-place system (CIP) is used. Some strip tests are specifically designed for this type of analysis.
- Check the packaging of incoming materials visually and using swabs.
- Check for gluten on employees' hands and clothing with a swab.
- Check the production environment and the ventilation grids with a swab to confirm the absence of gluten, especially when using volatile raw materials (e.g. flour).
- Collect and analyze air samples using an air sampler.
- Check tools used when servicing equipment, especially of subcontractors.

When collecting in-process samples, taking samples of 100 grams to 1 kilogram throughout the production run is recommended. Subsamples may be combined to form a composite sample. Composite sampling can help companies reduce costs and increase the probability of finding gluten. However, it may also dilute low amounts of gluten to an undetectable level. For this reason, composite samples should not be used for **difficult-to-homogenize** samples such as oat products and other grain-based products. Individually analyzing multiple units of these products is highly recommended. The specific case of oats will be discussed in Appendix 7.

It is essential that companies implement rigorous sampling protocols. Thus, the people performing the sampling should always wash their hands and sampling equipment. Furthermore, before collecting a sample using a probe, the packaging exterior must be cleaned to avoid contaminating the sample. Then, the holes must be securely sealed with a label or adhesive tape. Lastly, each lot analyzed must be placed in quarantine until the results are obtained and meet specifications. Sub-samples must be kept in a secure place so they can be retested as needed.

2.2 SAMPLE PREPARATION

Samplers should assess the uniformity of each subsample by verifying its colour, size, shape and the presence of visible impurities before adding it to a composite sample. **Composite** samples should be mixed thoroughly to make them as homogenous as possible.

Different techniques can be used depending on the type of sample and the sample size.

Granular and solid samples are usually reduced to powder with a coffee grinder, mill or food processor. The equipment should reduce the particle size of the test sample to the smallest size possible. The sample should then be measured (e.g. sieve size) and recorded. This will increase the homogeneity of the test sample and reduce variability. Gluten contaminants are known to be difficult to disperse within a ground sample due to kernel hardness and pilling of proteins, resulting in highly concentrated pockets of gluten even after grinding (3).

Liquids, pastes and fine powders are usually more homogenous and can be blended with a food processor or mixed manually.

Equipment used to prepare samples should be cleaned between each sample to avoid contamination and preserve the integrity of the next sample. Rinsing equipment with an ethanol solution is highly recommended, as gluten is soluble in ethanol but not in water.

2.3 ANALYSIS

The gluten of a sample can be detected or quantified using various analytical procedures. Selecting the right testing methodology is essential and depends on many factors. More details will be given in Step 3 to help you choose the proper method for your samples.

Typically, a very small portion of the sample is withdrawn for analysis. For example, ELISA tests are conducted on a sample of 0.25 to 1 gram. It is important to follow the instructions of the test manufacturer. Some manufacturers suggest modified testing protocols to users to allow for the testing of a larger sample (see the case of oats in Appendix 7). Analyzing a larger sample size improves confidence in testing results but may make testing significantly more expensive.

Analytical errors and the variability of the testing results can also be reduced by increasing the number of measurements for the same subsample. Duplicates or triplicates are usually recommended, especially for high-risk products.

To avoid contamination during testing, performing the analysis outside production rooms and wearing gloves during the assay is recommended. Materials should be cleaned and stored properly. Personnel should also be trained to ensure compliance with good manufacturing practices. Split samples should also be stored for use at a later date, if needed for comparison.

STEP 3 – DETERMINE THE TESTING METHODOLOGY

There is currently no general agreement on a single "best" analytical method that should be used to measure the gluten content of all food products. The choice of testing method depends on the product. However, several other considerations must be taken into account, as each testing method has its own strengths and weaknesses.

3.1 GENERAL OVERVIEW OF TESTING METHODS AVAILABLE

Enzyme-linked immunosorbent assays (ELISA) are among the best analytical methods to detect and quantify gluten in a wide range of food product matrices. They are easy to use, sensitive and specific. They also require relatively inexpensive equipment. Complementary methods such as PCR and mass spectrometry are sometimes used to confirm the source of contamination, which cannot be ascertained using ELISA methods. However, these methods are more labour-intensive than ELISA and require greater expertise and expensive laboratory equipment (4). Their use is therefore currently limited in food facilities.

ELISA methods are based on antibodies raised against specific amino acid protein sequences, and the reaction is made visible by a colour reaction. ELISA tests are available in three different formats, as outlined in Table 4.

Quantitative ELISA tests are extremely sensitive. They are available in sandwich and competitive formats. Sandwich ELISA-based methods are appropriate when gluten proteins are intact or relatively intact, while competitive ELISA assays are more suitable for fermented and hydrolyzed foods. Quantitative test kits can analyze multiple samples at the same time, and results can be obtained after only a few hours. However, since these tests require a high degree of expertise, they are mostly conducted in external laboratories. It usually takes more than 24 hours to receive analytical results because of the time required to transport samples to the laboratory and process results.

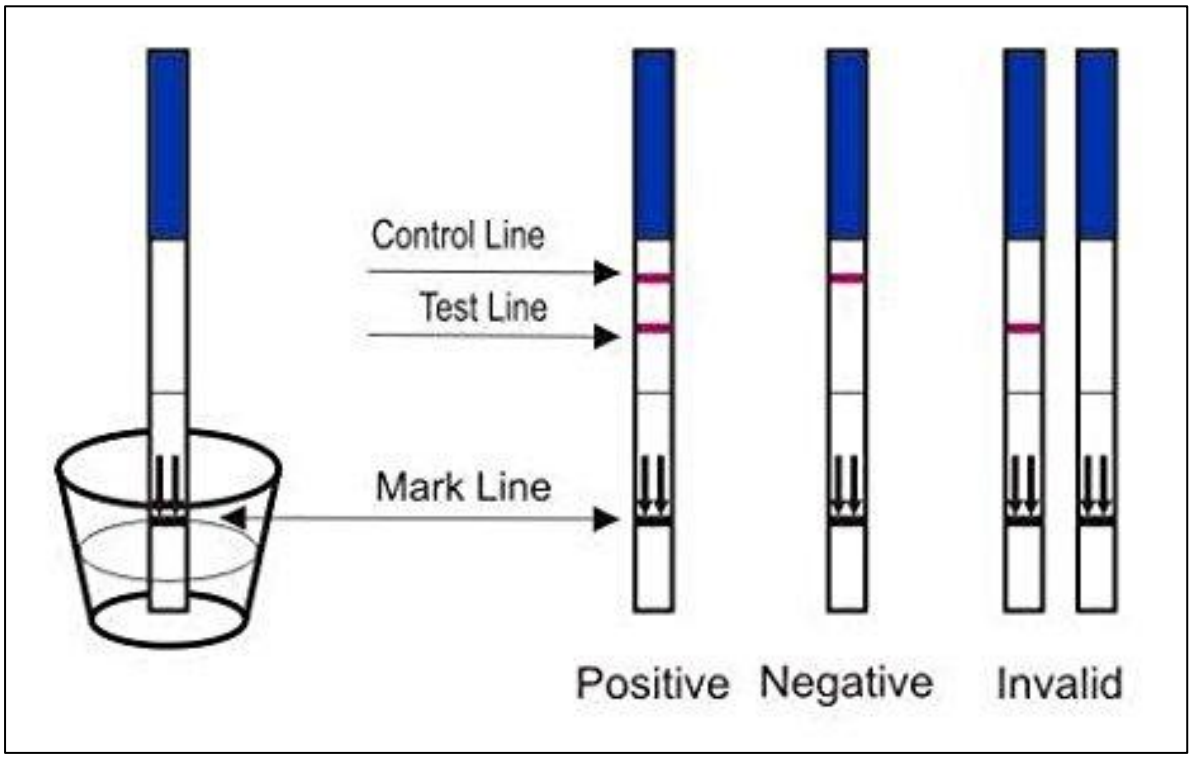
Qualitative ELISA test kits such as lateral flow devices are also available and can be used by food manufacturers to quickly screen food products and to check the cleanliness of surfaces. However, each type of food matrix should be validated internally before using this type of test, as explained in Step 5. Lateral flow devices offer a faster, cheaper, easy-to-use solution for food manufacturers to use in their own premises. Test results can be obtained in less than 15 minutes without using any sophisticated laboratory equipment. However, these test kits are usually less sensitive than quantitative methods.

Table 4: Main characteristics of ELISA analysis methods

Elements	Quantitative Method		Qualitative Method
	Sandwich	**Competitive**	**Sandwich lateral flow devices**
Time to get results	More than 24 hours (ask your external laboratory)	More than 24 hours (ask your external laboratory)	About 15 minutes
Expertise	Requires laboratory equipment and trained personnel Mostly conducted in external laboratories	Requires laboratory equipment and trained personnel Mostly conducted in external laboratories	Easy to use, tests can be conducted internally with little equipment Results are sometimes difficult to read
Sensitivity	Generally very sensitive (≤ 5 ppm)	Depends on the food matrix to evaluate, generally ≤ 10 ppm	+ Less sensitive, varies according to the test kit (≤ 5 à 20 ppm)
Cost	More expensive	More expensive	Less expensive
Application	Generally used for finished products and raw materials Can be used for surfaces, but qualitative results only Not appropriate for assessing fermented and hydrolyzed products	Finished products and fermented and/or hydrolyzed products	Fast screening of raw materials, in-process samples, surfaces and rinse water Finished products can be analyzed but validation is needed

When using an ELISA lateral flow device, a small quantity of the sample is first weighed with an analytical scale and mixed with an extraction solution. A test strip is then inserted into the solution. The strip absorbs the gluten extraction solution, allowing the antibodies to bind with the sample if it contains gluten. The antibodies and the sample migrate together across the surface of the strip and the reaction becomes visible, as illustrated in Figure 2. The sample is considered positive if two coloured lines are visible and negative if only the control line is visible. Moreover, in all cases, if the control line is not visible the result is invalid and the test needs to be redone. The intensity of the colored test lines may vary making it difficult to read the results.

Figure 2: Analysis with an ELISA lateral flow device



When choosing a testing methodology, it is important to consider:

- time to get the results
- expertise required
- cost
- the matrix (e.g. state of food, formulation)
- expected results (quantitative, qualitative, identification of gluten sources)
- sensitivity and performance (limit of detection, limit of quantification and other technical specifications).

3.2 ASSESS APPROPRIATE TESTING METHODOLOGY

Companies should select the testing method most appropriate for their needs according to the type of food they are analyzing and the type of contamination possible. ELISA is the most widely used analytical method in the food industry and by regulatory agencies to determine compliance. However, several ELISA test kits are available on the market. Appendix 8 presents the main characteristics of the tests most used by the industry. Unfortunately, none of them is considered universally acceptable for all food matrices.

Health Canada and the Food and Drug Administration (FDA) in the United States both utilize R-Biopharm's R5 RIDASCREEN® Gliadin, which was endorsed by the Codex Alimentarius Commission. The FDA also uses the Moringa Wheat/Gluten (Gliadin) test kit when necessary to conduct tandem analysis for special matrices.

ELISA test kits often give different results when compared to each other because different antibodies (R5, G12, Skerritt, etc.) target different peptides, and different standards are used to calibrate the assays. Extraction protocols may differ as well. Ethanol is widely used to extract gluten in food because gluten is not soluble in water. Ethanol has been found to be less effective in samples of products that are highly processed. Cocktail solutions have been developed and should be used by food product manufacturers to improve the extraction of gluten in samples.

It is important to understand that some test kits and extraction protocols work best with some matrices and not with others. Special attention should thus be given to the products presented in this section to choose the optimal testing methodology (Table 5).

Table 5: Guidelines for determining the appropriate method of analysis

Product	Potential problems	Recommended method of analysis
Heated and extruded products	The protein structure is modified, which makes proteins difficult to extract with ethanol.	Use cocktail extraction with a quantitative ELISA test kit (external laboratory) or lateral flow device.
Foods containing polyphenols and tannins	Gluten content is underestimated due to polyphenol and tannin interactions with proteins.	Test with a quantitative ELISA method (external laboratory) or lateral flow device. Add additives to the extraction solution according to test kit protocols.
Extreme conditions (e.g. strong acid/alkali, high salt, high fat,…) presence of food gum	Various interactions are possible, influencing gluten extraction.	Test with a quantitative ELISA method (external laboratory) or lateral flow device.
Hydrolyzed or fermented products	Gluten content is underestimated with sandwich methods, since proteins are broken down into smaller fragments.	Opt for a competitive ELISA method (external laboratory).
Food containing both intact and hydrolyzed/fermented proteins	Gluten content is underestimated in the presence of both types of proteins.	Use both sandwich and competitive ELISA methods (external laboratory).
Distilled products	The removal of gluten depends on the effectiveness of the distillation process. Most products are fermented.	Verify the effectiveness of distillation for finished products by measuring the total protein content of the distillated product. For raw materials, chose a competitive ELISA method if the product has been fermented (external laboratory) in combination with a sandwich method.
Enzymes	Contamination by fermented proteins and false positives are possible.	Opt for a competitive ELISA method (external laboratory).
Packaging materials	Surfaces should be swabbed.	Choose a compatible ELISA method for swabbing.
Surfaces and production environment	Surfaces should be swabbed.	Choose a compatible ELISA method for swabbing.
Inherently gluten-free grains, seeds and legumes	Very heterogeneous matrices.	Conduct a visual inspection before performing any processing steps. Test with a quantitative ELISA method (external laboratory) or lateral flow device with a limit of detection of ≤ 5 ppm. Take special care when grinding prior to testing.
Gluten-free oats	Certain oat varieties are detected by the G12 antibody, resulting in false positive results. The Skerritt antibody has a weak response to barley.	Conduct a visual inspection of raw materials before performing any processing steps. Use an ELISA test kit based on the R5 antibody with a limit of detection of ≤ 5 ppm. Take special care when grinding prior to testing.

3.2.1 HEATED AND EXTRUDED PRODUCTS

When proteins are heated, baked or extruded, their structure is modified. Gluten proteins aggregate when they are baked, cooked or extruded. These changes result in a lower solubility of proteins in ethanol, which is the main solution used to extract gluten. Cocktail solution developed by test kit manufacturers should be used rather than ethanol to improve recovery of gluten for samples that have been heated or extruded.

Assessing the gluten content of finished products with a quantitative sandwich ELISA test that has been fully validated with the proper matrix is highly recommended. Lateral flow devices may also be used if matrices are validated, as explained in Step 5.

3.2.2 FOODS CONTAINING POLYPHENOLS AND TANNINS

When foods contain large amounts of polyphenols or tannins (e.g. tea, hops, cocoa products, coffee, spices, chestnut flour, buckwheat, millet, etc.), detecting and quantifying gluten is more difficult. These constituents create interactions with proteins, which affect the detection and quantification of gluten. The accuracy of test results can therefore be reduced. Underestimation of the gluten content can be avoided by adding extraction additives such as fish gelatine, skim milk powder, polyvinylpyrrolidone (PVP) or urea to the extraction solution. These additives disrupt gluten protein–polyphenol interactions, thereby rendering the gluten "detectable".

The gluten content of these types of foods can be assessed with a quantitative ELISA test method or with rapid ELISA lateral flow devices. Special instructions provided by the test kit manufacturer should be followed. The method should always be validated for foods containing high amounts of tannins and polyphenols (see Step 5). Test kit manufacturers can assist you in developing special extraction protocols when problems are encountered.

3.2.3 EXTREME CONDITIONS (STRONG ACID/ALKALI, HIGH SALT, ETC.)

Foods are complex matrices made of various components, and the interaction between ingredients can have an impact on gluten detection. ELISA test kit performance might also be affected in extreme conditions such as the presence of strong acid or alkali or high amounts of salt, fat, sugar, food gums, food additives, artificial colouring, flavours, etc. (5)

Many of these extremes are controlled by buffer solutions during the extraction process. However, the gluten recovery rate should be validated for each matrix.

3.2.4 HYDROLYZED AND FERMENTED PRODUCTS

During fermentation and hydrolyzation, gluten proteins are broken into smaller fragments, which make them difficult to detect. Hydrolyzed and fermented peptides are found in many manufactured products such as beer, distilled alcohol, hydrolyzed proteins, vinegar, malt extract, sourdough, soy sauce, glucose syrup, guar gum, xanthan gum and starter cultures.

Sandwich ELISA-based test methods are not appropriate for assessing the gluten content of these kinds of foods because proteins are broken down into smaller fragments to such an extent that gluten proteins become too small or are chemically altered for the two antibodies to attach to different binding sites. The competitive method is the only suitable method, as only one binding site is necessary for detection.

It is also important to consider that foods may contain proteins with different types and degrees of hydrolyzation and/or fermentation (e.g. wheat starch, wheat starch hydrolysates, brewer's yeast, yeast extract, etc.). Food containing both intact and hydrolyzed proteins and food that could be contaminated after fermentation or hydrolyzation should be analyzed using both sandwich and competitive tests. This applies also to food that could be contaminated after fermentation or hydrolyzation.

3.2.5 DISTILLED PRODUCTS

During distillation, a liquid is heated and volatile components like alcohol and flavours are separated from non-volatile materials like proteins and sugars. Generally, distilling alcohols and vinegars eliminates residual proteins (6) and is thus considered a process for removing gluten, resulting in an inherently gluten-free product. However, the effectiveness of the distillation process can have an impact on the purity of the final product. It is also important to know that not all vinegars are processed by distillation (e.g. malt vinegar). In addition, cross-contamination could occur during processing or after, especially in a gluten-rich environment.

As the production of vinegars begin with the fermentation of grains or fruits into alcohol that is further fermented by acetic acid bacteria, products should be analyzed by an ELISA competitive assay in combination with a sandwich test. However, the FDA recommends that manufacturers verify the total protein content of distilled products. No detectable level of protein indicates an absence of gluten. (7)

3.2.6 ENZYMES

Some enzymes have been shown to react with ELISA assays, causing alarmingly inconsistent results (8). When enzymes are tested for gluten, they should be first deactivated to prevent false positives.

As enzymes used by the food and dietary supplement industry are typically produced on a wheat fermentation media (9), they should be tested with competitive ELISA methods. External laboratories should develop an appropriate testing protocol in collaboration with their test kit manufacturer.

3.2.7 PACKAGING MATERIALS (DIRECT CONTACT)

Most packaging materials are naturally free of gluten. However, in rare cases, packaging materials and wax paper may be coated with gluten-containing materials (10). For example, gluten can be found in some cardboard box adhesives. Wheat gluten can also be used as an additive in plastic formulations to enhance mechanical performance, especially for renewable and biodegradable products (11). A risk of cross-contamination also exists, since many packaging companies also handle products that contain gluten.

The gluten-free status of packaging materials cannot be verified in the same way as other raw materials. However, it is possible to directly swab your packaging material, as with environmental surfaces. It is important to swab the areas that are the most likely to be contaminated (e.g. contact surfaces, joints and corners of boxes, wax coating).

3.2.8 SURFACES AND PRODUCTION ENVIRONMENT

The absence of gluten on surfaces and in the production environment should be checked using strip tests or a quantitative ELISA method. Strip tests are a specific method that has the advantage of giving results in just a few minutes, which makes it possible to act quickly in the event of positive results.

3.2.9 INHERENTLY GLUTEN-FREE WHOLE GRAINS, SEEDS AND LEGUMES

A visual inspection should be carried out on a representative sample of cereals, seeds and legumes prior to unloading due to the heterogeneous nature of these products. To do this, take several subsamples of about 500 grams to one kilogram depending on the size of the batch that will form a composite. Then, perform a visual inspection of the sample on a white surface to find other cereals that contain gluten. Note, this inspection should be done by a person trained to recognize the different types of cereals and seeds.

The gluten-free status of grains, seeds and legumes can also be confirmed by testing the product with an ELISA method with a detection limit of ≤ 5 ppm. Special care should be taken when preparing the samples to ensure their homogeneity.

The Government of Canada has published a guide reflecting current international methods and procedures for sampling seeds and grains (12). This document is intended to assist manufacturers involved in one or more of the Canadian sampling programs (CGSP, LSSP, CCSP or ACSP) in designing their own sampling procedures. The guide describes general sampling principles and the approved sampling equipment and procedures for obtaining a representative sample. The Canadian Grain Commission, in collaboration with the Allergen Control Group, has also participated in the Gluten-Free Grain Project to identify best practices for visual inspections of such products.

3.2.10 GLUTEN-FREE OATS

Oats are recognized as structurally different from other gluten-containing cereals, making oat proteins generally not detected by ELISA test kits. Most clinical studies have shown that the majority of persons affected by celiac disease can consume oats in moderate amounts without any adverse effect on their health if the oats are not contaminated with wheat, rye or barley (13) (14). However, oats pose a high risk of contamination with gluten without special on-farm, transport, storage, handling and cleaning procedures. Moreover, gluten is often unevenly distributed in products, making oats difficult to sample and test.

The gluten-free status of oats should always be confirmed with a fully validated test kit based on the R5 antibody with a limit of detection of ≤ 5 ppm. Certain oat varieties can be detected by the G12 antibody, which can result in false positives. The Skerritt antibody was found to give a weak response to barley, one of the principal contaminants of oats. As with other grains, special care should be taken when preparing the samples to ensure sample homogeneity.

STEP 4 – INTERPRET TESTING RESULTS

Regulations provide a standard for manufacturers across the food industry, and adherence to these standards helps consumers to be confident that products labelled as gluten-free are safe. In Canada and the USA, the 20-ppm threshold recommended by the Codex Alimentarius has been adopted. Appendix 9 provides additional information about the various national regulations on gluten-free claims. (Note: Due to the statistical range of recovery of gluten in test kits, "not more than 20 ppm" for Canada and < 20 ppm for the USA are virtually the same).

When analyzing gluten-free products, testing results should be interpreted with care. Gluten concentration should be compared to the regulatory limits of the country of destination in order to determine the product's safeness. Figure 3 illustrates a recommended method for determining the acceptability of a lot based on testing results for a product sold in Canada or the USA and analyzed with a quantitative ELISA test method.

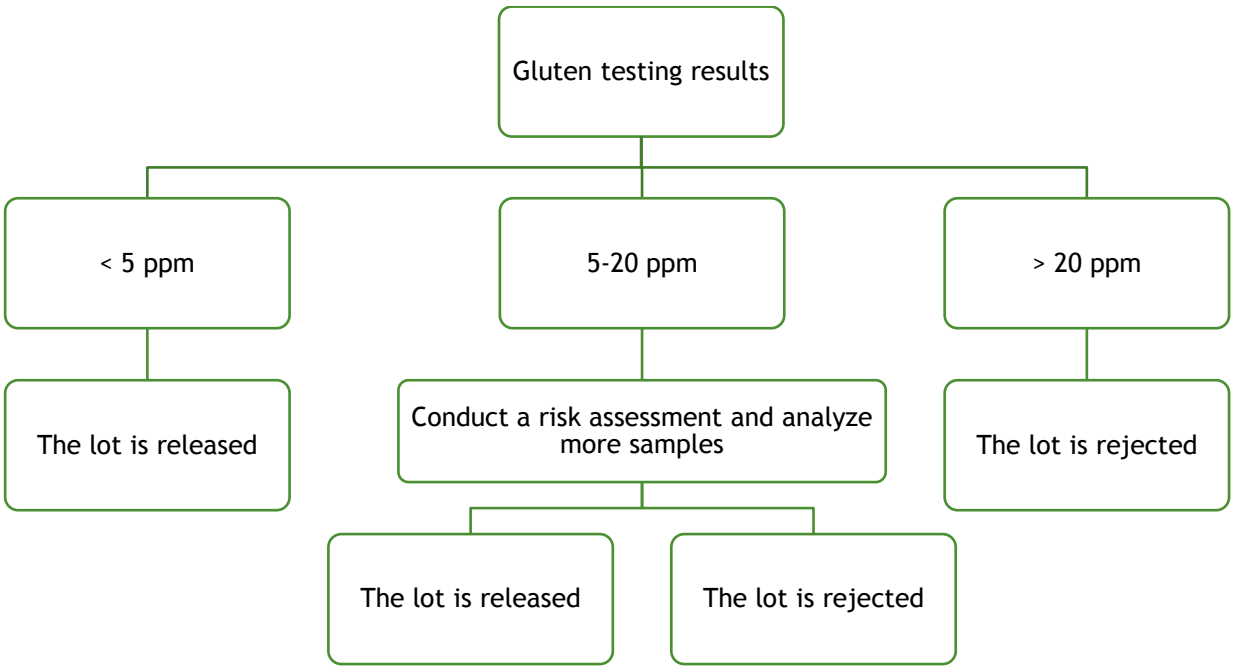


Figure 3: Decision tree for determining lot acceptability according to testing results

It is important to understand that a negative result (i.e. none detected) does not necessarily indicate the absence of gluten in a lot, as gluten may not be homogenously distributed or the level of gluten in the product might be below the limit of detection. Acceptance of the lot after a result of less than 5 ppm greatly depends on the effectiveness of your sampling plan.

When positive yet compliant results are obtained (typically between 5 and 20 ppm), particularly in the case of a first reading, a risk assessment should be performed to determine if the lot can be accepted. Testing more samples is generally recommended, since higher levels of gluten could be found in other samples from the same lot (3). The number of subsamples to retest depends on the type of product tested and its homogeneity. Retesting a minimum of 5 to 10 additional samples is generally recommended. If composites are tested, each of the subsamples should be retested at a minimum. When interpreting results, consideration should be given to the type of product tested, the source of contamination as well as the homogeneity of the analytical results. It is important to consider the risk for gluten at a level of 20 ppm but also for allergens (e.g. wheat) that are known to cause severe adverse reactions even in small doses.

Finally, testing results greater than 20 ppm of gluten should always lead to the rejection of the lot. Appropriate corrective actions should be taken and documented. For example, raw materials could be returned to the supplier and a re-evaluation of the risk category and the number of samples to analyze should be conducted. For finished products, the batch should be destroyed, sold as a non-food (e.g. animal feed) or directed to the food supply with appropriate labelling.

STEP 5 – VALIDATE THE PROGRAM

5.1 TEST KIT VALIDATION

Test kit manufacturers should conduct a pre-market validation study. Most test kit manufacturers publish the results of their validation on their websites. It is important to keep in mind that validation studies are usually conducted under optimal conditions and for a limited number of food matrices.

External laboratories should also validate manufacturers' methods. Before sending your samples to an external laboratory, you should verify if the laboratory has obtained ISO 17025 accreditation from a competent authority (e.g. ANAB, Standards Council of Canada, etc.). You should also verify that the laboratory's methods fall within the scope of their accreditation and that the methods have been fully validated at their site for your matrix.

Testing validation involves adding a known amount of gluten to a sample (i.e. spiking) in order to attempt to recover the same quantity. Ideally, a recovery of between 80% and 120% should be achieved. However, a recovery of 50% to 150% is usually considered acceptable for allergens, including gluten, as long as the results can be shown to be consistent (15).

5.2 MATRIX VALIDATION

Before using a strip test, conducting an internal validation of each type of food matrix is strongly recommended. Indeed, some matrices may not be suitable for certain types of analysis, thus causing false positives or false negatives. Validation demonstrates the compatibility of a matrix with the analytical method, verifies the ability of the analytical method to detect known amounts of gluten and avoids misinterpretation of the analytical results.

It is important to validate the effects of the matrix you want to analyze to account for possible interference. For example, high levels of tannins, polyphenols, fat, sugar, salt, gelling agent or acidity may interact with the proteins and mask the presence of gluten in the sample. Other constituents could cause false positives because of their very similar structure to gluten.

Each food matrix that you analyze should be validated. If you cannot validate all your matrices, the risk assessment will allow you to target complex matrices. Validation results published by test kit manufacturers can help you chose products for which validations are required**.**

5.2.1 VALIDATION PROCEDURE FOR RAPID ELISA LATERAL FLOW DEVICES

Step 1: Prepare your spiking material

First, you prepare the spiking material you will use in Step 3. You can prepare a spiking material yourself or opt for a commercial standard. The spiking material should mimic sample contamination. You should therefore understand what type of proteins could contaminate your sample. In most cases, non-processed wheat gliadin is used. However, in specific cases, it could be appropriate to use processed proteins, barley hordeins or another mixture.

To prepare the reference material yourself, you should add a known amount of wheat flour into a gluten-free flour (e.g., rice flour). Then, you must validate the concentration of your mixture with a quantitative analysis by using an external laboratory. The accuracy of the result will depend on the homogeneity of your spiking mixture.

Preparing a reference material can be a puzzle for many manufacturers. It requires specific laboratory equipment and considerable expertise. Therefore, it is possible to use a commercial standard with a known concentration (e.g. MP Biomedicals Gliadin from Wheat, Roquette® Vital Wheat Gluten or Sigma-Aldrich gliadin) that you will add directly to the extraction solution of your testing kit. It is also possible to buy ready-to-use spiking material such as the Trilogy® Reference Material or to use the matrix validation services offered by some test kit manufacturers or other competent service providers.

STEP 2: Test the Unspiked Matrix

The next step is to test your sample with your rapid ELISA test kit for gluten to confirm that your matrix will not produce a false positive. Follow your test kit instructions and verify that you get a negative testing result.

If you get an unintended positive result, send your sample to an external laboratory to verify the gluten content with a fully validated quantitative ELISA test. If you get a positive test result, it means that your sample was contaminated (true positive result). In contrast, if you get a negative result, it means that your matrix is cross-reacting with your rapid test kit (false positive result). In this case, you should contact your test kit manufacturer or use another test kit and repeat the validation process.

Step 3: Test the spiked Matrix

At this step, you should spike your sample with a standard gluten material at the desired concentration and verify that your matrix will not interfere with your test kit.

For example, if you want to verify that you can detect gluten at a concentration of 20 ppm, here is how you could process the sample.

1. Prepare a standard material of 200 ppm or use the Trilogy® Reference Material.
2. Add 1.0 g of the standard material to 9.0 g of ground and homogenized samples. The dilution ratio is now 1:10.
3. Add 100 mL of your extraction solution (ethanol at 60%) to your spiked sample.
4. Mix your sample thoroughly and follow your test kit instruction for analysis.
5. Record the result.

If you get a positive result, your test kit can detect gluten in your matrix at a concentration of 20 ppm. On the other hand, if you get a negative result, contact your test kit manufacturer or use another test kit and repeat the validation process.

Note that this method can also be used to verify the specific limit of detection (LOD) for your matrix (i.e. the lowest quantity that gives a positive result). To do so, spike your sample at a concentration a little above the test kit LOD. For example, for a verification at 5 ppm, dilute the 20 ppm solution 1:4. For some matrices, the LOD could be different than specified in the test kit. If you get a negative result, try to increase the spiking level by appropriate increments until a positive result is achieved. This will give you the kit's detection limit for this type of matrix.

5.3 VALIDATE YOUR CLEANING PROCEDURES (NON-DEDICATED FACILITIES ONLY)

Cleaning procedures should be validated to confirm that changeover practices are consistently effective in removing gluten, especially in non-dedicated facilities. It is important to remember that cleaning procedures that are satisfactory for microbiological safety may not be adequate for removing gluten. Furthermore, only degreasers can eliminate gluten, sanitizers are not effective.

A validation study implies a combination of visual inspection of food contact surfaces, swabs taken before and after cleaning, and analysis of samples taken from the subsequent production run (i.e. beginning, middle and end of production). Testing should always be conducted using specific and validated methods (typically ELISA). General protein testing methods alone cannot be used for validation. The number of swabs and locations to be sampled depends on your cleaning and production practices but should cover all aspects of cleaning (e.g. type of residue, type of surfaces, cleaning methods). When validating your cleaning methods, you should always swab the most difficult places to clean since they are at higher risk.

Validation should be carried out on three consecutive production runs and reviewed when necessary (e.g. when introducing changes to product formulation or manufacturing equipment and tools) and ideally annually. All results should comply for the validation to be considered successful. If the validation fails, then the risk assessment and cleaning procedures need to be reviewed.

CONCLUSION

For people suffering from celiac disease, accurate labelling of gluten-free foods is essential, as it enables them to make safer choices and avoid further physiological complications. Because gluten is sometimes hidden in unexpected sources, gluten-free claims provide critical information that allows consumers to identify safe and reliable gluten-free food products.

This guide has demonstrated that sampling procedures and testing methods have a significant impact on gluten testing results. An appropriate sampling plan should be developed based on **risk assessment.** Risk assessment helps manufacturers to determine adequate sampling parameters when building sampling plans needed to support a gluten-free management system.

In a dedicated facility where no gluten ever enters the production or storage areas, manufacturers should:

- Confirm the gluten-free status of their incoming materials (ingredients, packaging materials, processing aids, non-food chemicals, etc.).
- Confirm that incoming materials have not been cross-contaminated during distribution, storage or transportation (e.g. spillage).
- Control the risk associated with personnel, contractors and visitors.
- Validate the gluten-free status of their processes and finished products.

In a non-dedicated gluten-free facility where both gluten-containing and non-gluten-containing ingredients are present, manufacturers should also control the risk of contamination at each processing step, from receiving to shipping. Routine check procedures (e.g. swabbing surfaces, testing rinse water, etc.) should be put in place during the "at-risk" steps.

Food manufacturers should select the testing methodology most appropriate for their needs according to the type of product being analyzed and the type of contamination suspected. Even though most regulations do not specify how manufacturers should verify the absence of gluten in their products, government agencies recognize that testing is an essential tool for good manufacturing practices. Manufacturers should interpret testing results with care if they want to ensure that gluten-free claims are truthful and not misleading and that their claims comply with all regulatory requirements or their corporate internal limit which may be more restrictive (e.g. <5 ppm, 10 ppm or other <20 ppm).

Since food matrices can have a significant impact on analytical results, each matrix should always be validated. When samples are sent to an external laboratory, be sure to select a laboratory that has fully validated their methods at their site and has achieved ISO 17025 accreditation from a competent authority. Validation ensures that the results obtained are specific and accurate.

It is important to understand that gluten-free compliance should not rely solely on finished product testing. Manufacturers should focus on incoming materials and processing steps in addition to the

finished product to better manage the risks of contamination. A preventive gluten-control system supported by validated gluten testing is the best way to ensure product compliance.

CASE STUDY

Molly & Holly bakery must design a sampling plan to support their gluten-control system. This case study describes the risk assessment and decision-making process followed by this company. It will allow you to apply the theory described in each of the five steps previously explained in this guide as well as the tools available in the appendices. You can read the case study, step by step, in parallel with the guide or once you have finished reading.

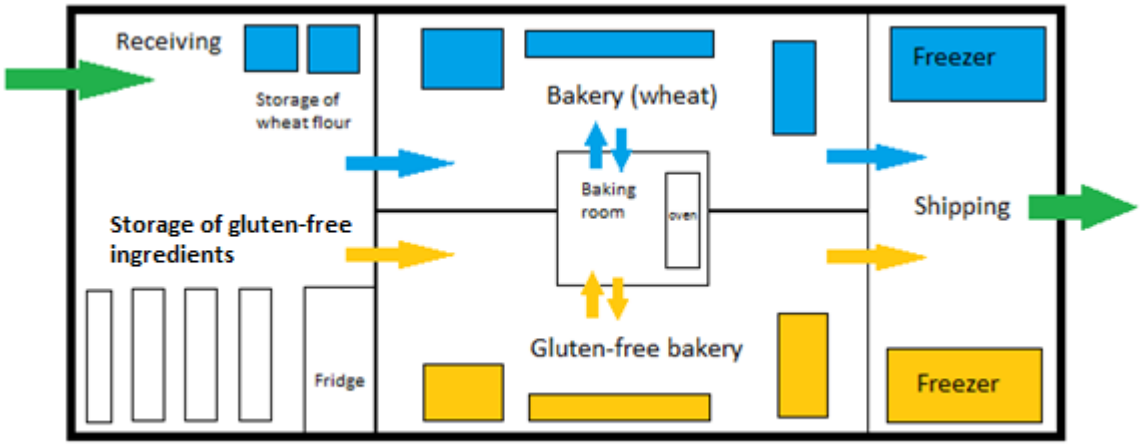
Company's information

Molly & Holly bakery is a food processing plant that produces baked goods containing gluten. The company would like to start producing three gluten-free products to meet the demand of its customers. The company will sell its gluten-free (GF) products in yellow bags, while products containing gluten will be sold in blue bags.

Gluten-free loaves and breads containing gluten will never be produced on the same day. Products containing gluten and gluten-free products will be produced in distinct separate production rooms (see the diagram of the traffic plan). Each room will have its own positive pressure air supply. Gluten-free loaves will be baked in moulds dedicated to gluten-free production, but the baking will take place in shared ovens located in the common baking room. All other equipment (e.g., mixers, utensils, moulds, proofer, slicers, packaging equipment, etc.) will be dedicated to the production of either gluten-free or gluten containing products and will be clearly identified.

The only ingredient that contains gluten is wheat flour. It will be received in bags of 25 kg and will be stored in a dedicated section of the shared storage area. Wheat flour bags will be carefully identified in receiving with blue labels.

Traffic plan



Step 1: Evaluate the risk of contamination

INCOMING MATERIALS

Molly & Holly bakery conducted a risk assessment of all incoming materials. To do so, the company asked all its suppliers to complete questionnaires (see Appendices 1 to 3) and to provide the documents requested. The risk assessment for gluten was performed for all incoming material using the risk assessment matrix presented in Appendix 4 and described in Step 1 (see 1.1).

Part of this assessment:

General information				Risk assessment for gluten		
Incoming material	**Supplier**	**Product code**	**Certification**	**Ease of detection**	**Likelihood of occurrence**	**Level of risk**
White rice flour	Rice 1,2,3	363	SQF certificate	4 Gluten is usually extremely difficult to detect in grains because it is generally not well distributed.	2 Grain products are likely to contain gluten; however, the supplier guarantees its gluten-free status.	8
Gluten-free oat flour	Baker Farm	6884	HACCP certificate	4 Gluten is usually extremely difficult to detect in grains because it is generally not well distributed.	3 Oat products are extremely likely to contain gluten, but the supplier is using a purity protocol and cleaning equipment.	12
Quinoa flour	Best Quinoa Inc.	95542	Gluten-free certification program	4 Gluten is usually extremely difficult to detect in grains because it is generally not well distributed.	1 Grain products are likely to contain gluten; however, the supplier is gluten-free certified by a recognized third-party organization. Gluten testing results for each lot are sent with each delivery.	4
Cinnamon	H&H	333	BRC certificate	3 Gluten is difficult to detect in the presence of polyphenols and tannins.	2 Spices are likely to contain gluten; however, very low quantities are used in the product.	6

General information				Risk assessment for gluten		
Incoming material	**Supplier**	**Product code**	**Certification**	**Ease of detection**	**Likelihood of occurrence**	**Level of risk**
White vinegar	Vinegrium	568	Gluten-free certification program	3 Gluten is more difficult to detect in fermented products, but it is easy to get a homogeneous sample for liquids.	1 The supplier does produce malt vinegar in the same facility, but production occurs in a different room. The supplier is gluten-free certified.	3
Bags	Kittbags	7894	BRC certificate	3 Gluten can be difficult to detect since it cannot be extracted as with other raw materials.	1 It is extremely unlikely that the bags contain gluten, since they are made from petroleum products and there is no gluten in the same facility.	3
Salt	Landers Inc.	24456	SQF certificate	2 Gluten is easy to detect since it is relatively homogenous. However, salt can interfere.	1 Salt is generally extremely unlikely to contain gluten. The supplier guarantees the gluten-free status of this product.	2

PROCESSING STEPS

Molly & Holly bakery has conducted a gluten risk assessment for each manufacturing step. The evaluation was conducted with the hazard assessment matrix presented in Appendix 6 and described in Step 1 (see 1.2). Here is a part of what was done:

Processing steps	Hazard	Measures of control	Occurrence	Severity	Level of risk
Storage of raw materials	Contamination due to broken bags and improper storage of wheat flour.	Wheat flour is stored in an identified and dedicated zone. The storage area is located near the wheat bread production zone to limit cross-traffic flow.	2 Spillage could happen, but the wheat flour storage area is away from the gluten-free bakery and storage area for other raw materials.	2 If spilling occurred, the gluten-free production will not be directly contaminated.	4
Preparation of ingredients	Contamination due to the incorporation of wheat flour during the preparation of a gluten-free formulation	Wheat flour bags are identified with blue labels and stored away from the production zone for gluten-free loaves. Employees are trained.	1 Wheat flour is clearly identified and stored away from other raw materials.	4 If contamination occurred, wheat would be directly incorporated into the formulation and the product would contain high level of gluten.	4
	Cross-contamination due to wheat flour dust in the air	Gluten-free and gluten containing products are produced in separate rooms with positive air pressure. Gluten-free products are scheduled on different days than gluten-containing products.	2 Flour dust can hang in the air. However, gluten-free products are manufactured in separate room and on different production days. Separate ventilation system for 2 rooms.	3 Low quantities of dust could settle directly on finished products.	6

Processing steps	Hazard	Measures of control	Occurrence	Severity	Level of risk
Dough mixing	Cross-contamination during mixing	Equipment and utensils are dedicated (bowls, utensils, mixers, paddles) and clearly identified for gluten-free use.	1 Equipment and utensils are dedicated and identified. They are located in different rooms.	2 Indirect contamination is possible; all equipment should be washed between batches.	2
	Cross-contamination due to wheat flour dust in the air	Gluten-free products are produced in a separate room with positive air pressure. Gluten-free products are scheduled on different days than gluten-containing products.	2 Flour dust can hang in the air. However, gluten-free products are manufactured in separate room and on different production days. Separate ventilation system for 2 rooms.	3 Low quantities of dust could settle directly on finished products.	6
Baking	Cross-contamination during baking due to gluten particles blown by the oven fan	Gluten-free products are scheduled on different days than gluten products. The oven is thoroughly cleaned at the end of each day of production. Dedicated trays and racks are used.	4 The oven is shared.	3 Gluten particles could directly contaminate the products if the oven is not well cleaned.	12
Packaging	Undeclared gluten due to mislabelling or misidentification	Gluten-free bags are yellow and other bags are blue. Final packaging labels are verified.	1 Colour coding makes it easy to prevent mix-ups.	4 High level of contamination in case of error.	4
	Cross-contamination due to wheat flour dust in the air	Gluten-free products are packaged in a separate room on dedicated gluten-free equipment with positive air pressure. Gluten-free products are scheduled on different days than gluten-containing products.	2 Flour dust can hang in the air. However, gluten-free products are manufactured in separate room and on different production days. Separate ventilation system for 2 rooms.	3 Low quantities of dust could settle directly on finished products.	6

Step 2: Define sampling parameters

INCOMING MATERIALS

Sampling frequency has been defined based on the risk assessment.

Risk category	Frequency
Low	Once a year
Medium	One lot out of three
High	Every lot

Analyses will be performed on composite samples when possible according to the following quantities:

Categories	Number of containers or bags to sample per lot	Minimal quantity to sample per container	Sampling tool	Number of analytical measurements
Gluten-free flour, starch and grains (bags of ≈ 25 kg)	5	100 g	Nobbe trier	1
Oats (bags of ≈ 25 kg)	3	200 g	Nobbe trier	3
Dry ingredients in small containers	3	50 g	Scoop	1
Liquids	1	200 mL	Cup	1
Packaging materials	1	Swab one area of plastic film (10 cm^2)	Swab	1

Five 100-g subsamples of gluten-free flour, starch or grains from different bags will be combined and the composite sample will be analyzed. Since oats are more likely to be contaminated with gluten than other grains, three individual analytical measurements will be taken for this product and the minimal quantity to sample per bag will be 200 g as recommended by the test kit manufacturer. Since some ingredients are received in very small quantities (e.g. spices), three 50-g subsamples will be necessary for testing. When composite samples are analyzed, split samples will be kept for further analysis in the event of a positive testing result. For liquids, only one 200-mL sample will be required for analysis since liquid samples are already homogenous. For packaging materials, one area of the plastic film will be swabbed for gluten analysis as per the test kit instructions.

Samples will be prepared according to the test kit instructions. A coffee grinder will be used to reduce solid samples to a powder. Samples in a fine powder will be mixed vigorously with a food processor before analysis.

The testing frequency will be reviewed each year according to the results.

PROCESSING STEPS

Environmental testing for medium- and high-risk areas will be conducted for verification purposes with a gluten-specific test.

All equipment rated as high risk (e.g. oven) will be swabbed after each cleaning and again before starting gluten-free production to allow time for gluten particles to be deposited on the equipment after cleaning.

Testing will also be conducted at least once a month on medium-risk equipment and zones to verify the absence of wheat flour dust. Surfaces to be sampled will vary each year according to the cleaning validation schedule.

Lastly, air testing will be performed in the baking room and the gluten-free bakery.

FINISHED PRODUCTS

Given the significant risk of contamination due to sharing the premises and the volatility of wheat flour, Molly & Holly bakery decided to analyze its products in an external laboratory each day of production. Thus, three separate finished products (at the beginning, middle and end of each production) will be tested. The finished products will be placed on hold in the freezer until the results are received.

Step 3: Determine the optimal testing methodology

The testing methodologies have been selected and validated according to the nature of the products.

INCOMING MATERIALS

Incoming raw materials will be analyzed internally in-house. Molly & Holly bakery has decided to use a rapid ELISA lateral flow device with the R5 antibody since the company will also need to analyze oat samples. Moreover, the company chose to use a test kit with a limit of detection of 5 ppm to better identify cross-contamination trends and risk in grains and flours more likely to contain gluten. Packaging materials will be swabbed with the same test kit.

Incoming materials that have been fermented or hydrolyzed (e.g. vinegar) will be sent to an external laboratory and analyzed with a competitive ELISA test method. However, split samples will also be analyzed internally with the rapid lateral flow device, since the sample could also contain non-fermented contaminants (e.g. from ambient sources).

PROCESSING STEPS

Equipment surfaces will be swabbed and analyzed with the same rapid ELISA lateral flow device.

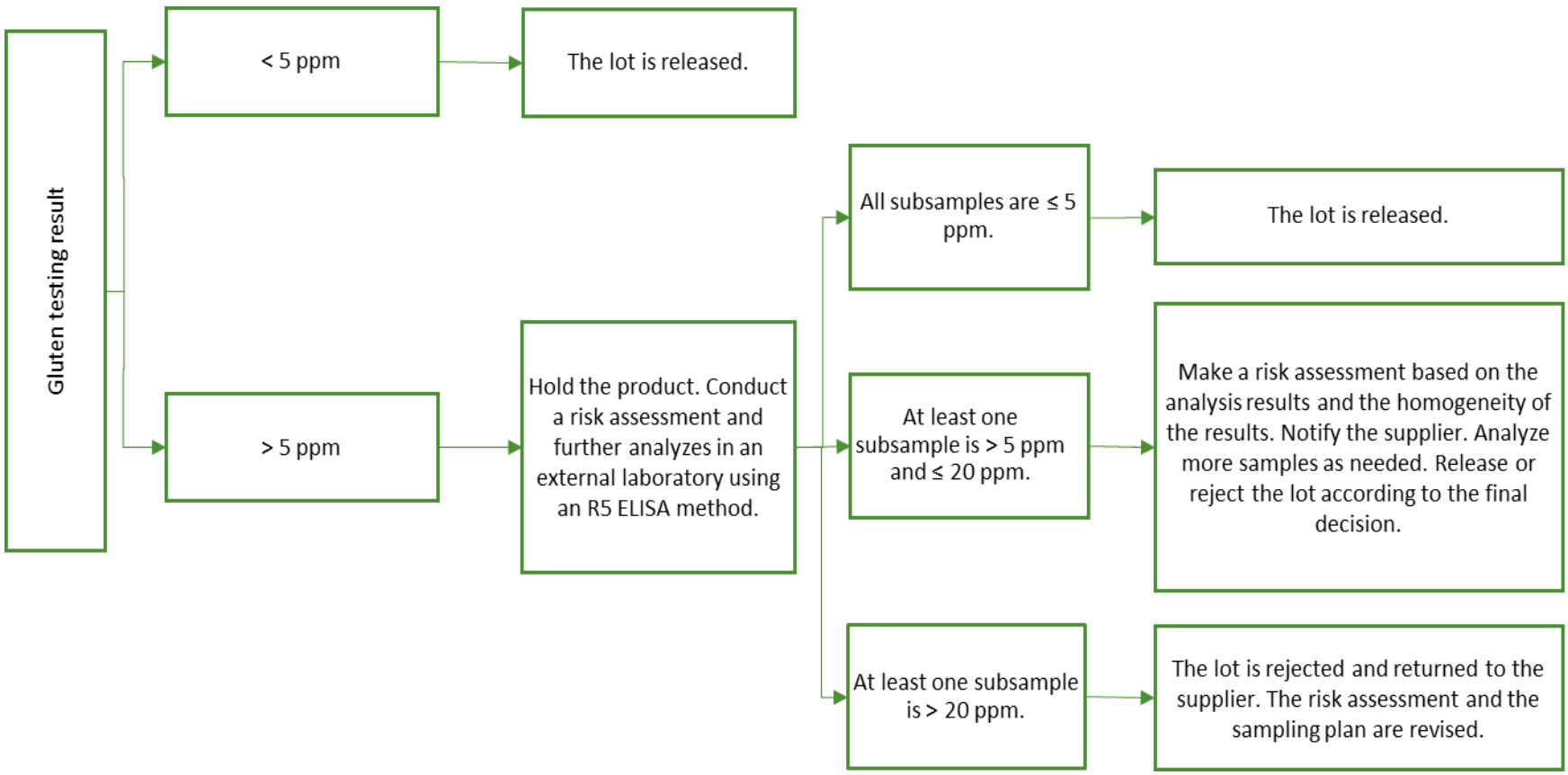
FINISHED PRODUCTS

Gluten-free products will be analyzed by an external laboratory with a quantitative R5 ELISA test kit.

Step 4: INTERPRET TESTING RESULTS

Since all products could be sold in Canada and the USA, they need to comply with the 20-ppm threshold for gluten-free products. At this step, it is important to anticipate the different possible scenarios based on test results to make the best decisions. The following decision-making process will be used for incoming materials and finished products.

INCOMING MATERIALS

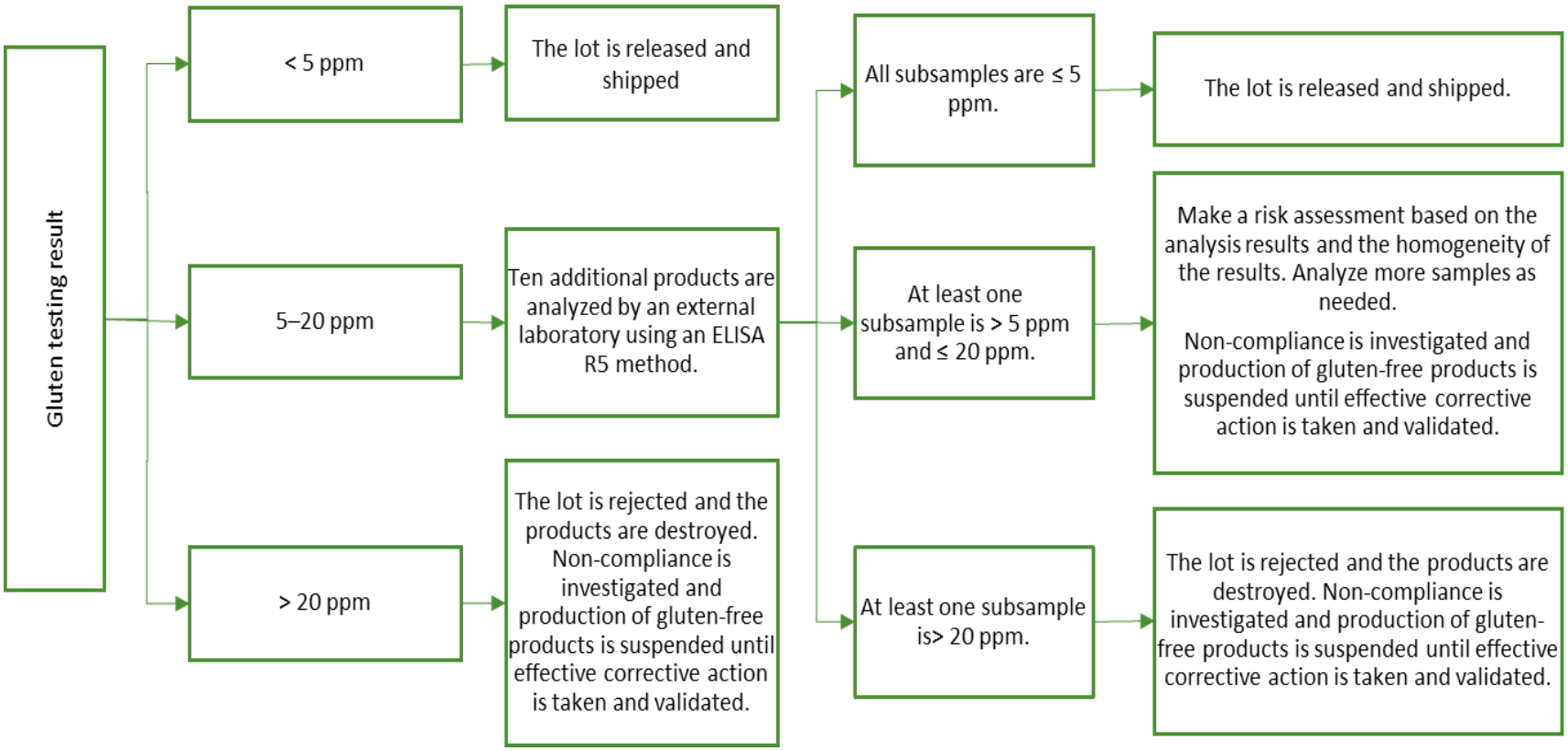


PROCESSING STEPS

Any positive test result of surface or environmental testing should be assessed seriously as it would be an indicator of a deviation in the procedures. First, the equipment should be cleaned again and a risk assessment should be performed to determine if other equipment need to be cleaned. Surface analyses should be repeated after cleaning. An investigation should also be conducted to determine the root

cause and corrective action must be taken to avoid recurrence (e.g., check airflow, change and validate cleaning practices, review procedures, employee training, etc.).

FINISHED PRODUCTS



Step 5: VALIDATE THE PROGRAM

All external laboratory analyses will be conducted using validated and certified methodologies. Molly & Holly bakery has confirmed that the laboratory has been accredited to ISO 17025 by a competent authority. An internal validation has been conducted to demonstrate

the compatibility of certain matrices with the chosen testing methodology. The risk assessment described below was used to determine the matrices for which an internal validation was performed.

Product	Risk analysis	Internal validation
White rice flour	It is an unprocessed material. Rigorous validation was performed by the test kit manufacturer for this matrix (AOAC).	No
Gluten-free oat flour	It is an unprocessed material. Validation was performed by the test kit manufacturer for this matrix. Validation required due to possible cross-reactions of some oat varieties with some test kits.	Yes
Quinoa flour	It is an unprocessed material. Validation was performed by the test kit manufacturer for this matrix (see cross-reactivity and interference studies).	No
Cinnamon	It is an unprocessed material. Cinnamon contains polyphenols and/or tannins that can cause interference.	Yes
Bag	The only method is swabbing.	Yes (a packaging material that is known to contain gluten should be tested and used as a positive control)
Salt	It is an unprocessed material. High salt content can cause interference when gluten is detected.	Yes
Gluten-free loaf	Complex matrix because the dough is fermented and cooked (e.g. processed). Validation required in collaboration with the external laboratory.	Yes

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APPENDIX 1

GENERIC SUPPLIERS EVALUATION QUESTIONNAIRE

Please send the completed questionnaire by email to Holly & Molly at quality@hollymolly.com.

For each ingredient sold to Holly & Molly, please provide a copy of the technical specification sheet, the allergen questionnaire, a signed guarantee letter indicating that your ingredients are gluten-free and a copy of accreditation certificates.

SUPPLIER IDENTIFICATION

COMPANY NAME	
ADDRESS	
PHONE NUMBER	
MAIN CONTACT	
EXTENSION	
EMAIL	
QUALITY MANAGER	
EXTENSION	
EMAIL	
Type of facility (Manufacturer, Wholesaler, etc.)	
PRODUCTS/PROCESSES	

CERTIFICATIONS

Please indicate which certifications you have.

CERTIFICATIONS		CERTIFICATION BODY
HACCP	☐	
FSSC 22000	☐	
SQF	☐	
BRC	☐	
IFS	☐	
C-TPAT	☐	
GLUTEN-FREE CERTIFICATION PROGRAM	☐	
OTHER (SPECIFY): _____	☐	

If applicable, please provide a copy of your certificates or audit reports.

QUESTIONNAIRE (OPTIONAL FOR SUPPLIERS THAT ARE HACCP/GFSI/GFCP CERTIFIED OR THAT CAN PROVIDE A RECENT THIRD-PARTY AUDIT REALIZED IN THE LAST YEAR)

1. BUILDING AND EQUIPMENT

	YES	NO	N/A
1.1 - The building is designed and constructed so that walls, ceilings and floors are easy to clean and prevent the contamination of the products.	☐	☐	☐
1.2 - The building is designed to prevent cross-contamination caused by employee traffic patterns, food product flow and equipment.	☐	☐	☐
1.3 - Interior and exterior inspection programs have been established and documented.	☐	☐	☐
1.4 - The establishment is supplied with potable water and a water quality program has been implemented.	☐	☐	☐
1.5 - Equipment in direct contact with food has smooth surfaces, is easy to clean and is rust-resistant.	☐	☐	☐
1.6 - The plant has a documented preventive maintenance program for equipment that presents a food safety issue.	☐	☐	☐
1.7 - When food safety issues arise, a calibration program exists for measuring devices such as thermometers and scales and for control devices such as metal detectors.	☐	☐	☐
1.8 - The temperature of refrigerated processing areas, coolers and freezers meets regulatory requirements, and temperatures are monitored.	☐	☐	☐
1.9 - Non-food chemicals are identified and stored away from food and packaging materials and access to these products is controlled.	☐	☐	☐

2. PURCHASING, RECEIVING AND SHIPPING

	YES	NO	N/A
2.1 - A purchasing and supplier approval program has been implemented.	☐	☐	☐
2.2 - Non-food chemicals such as processing aids, water treatment chemicals, cleaners and sanitizers are approved by a competent regulatory authority or deemed acceptable as food grade (e.g. CFIA, FDA, USDA).	☐	☐	☐

	YES	NO	N/A
2.3 - Packaging materials in contact with food are food grade.	☐	☐	☐
2.4 - There is an inspection program in place for monitoring inbound deliveries.	☐	☐	☐
2.5 - Finished goods are shipped in conditions that prevent product contamination and deterioration (i.e. adequate temperature, no incompatible chemicals, clean and undamaged trucks).	☐	☐	☐
2.6 - Trucks are inspected before loading.	☐	☐	☐
2.7 – An stock rotation system is in place.	☐	☐	☐
2.8 - There is a system in place to manage non-conforming and returned products.	☐	☐	☐

3. TRACEABILITY AND RECALL

	YES	NO	N/A
3.1 – The system allows the traceability of raw materials, packaging materials and finished goods. All finished goods have a lot number.	☐	☐	☐
3.2 - A recall program has been implemented.	☐	☐	☐
3.3 - Traceability exercises and mock recalls are conducted at least once a year to verify that the system is effective.	☐	☐	☐
3.4 - A complaint management program has been established.	☐	☐	☐

4. CLEANING AND SANITATION

	YES	NO	N/A
4.1 - A cleaning program that includes cleaning instructions and a master cleaning schedule has been implemented.	☐	☐	☐
4.2 - Preoperational sanitation inspection has been established for all production-related areas prior to start production.	☐	☐	☐
4.3 - A validation program for cleaning activities has been implemented.	☐	☐	☐
4.4 - An environmental monitoring program for detecting pathogens, allergens including gluten has been implemented.	☐	☐	☐

5. PERSONNEL

	YES	NO	N/A
5.1 - A documented GMP policy that includes rules on personal hygiene, dress code, diseases and wounds has been documented and implemented for employees, contractors and visitors.	☐	☐	☐
5.2 – A training on good manufacturing practices is given to employees upon hiring and at least once a year.	☐	☐	☐
5.3 - A technical training program has been implemented (i.e. CCP monitoring training, sanitation training, etc.).	☐	☐	☐

6. PEST CONTROL

	YES	NO	N/A
6.1 - A pest control program including at least monthly visits by a qualified pest control company has been established.	☐	☐	☐
6.2 – The pest control program includes inspection records, location of pest control devices, chemicals required, pesticide log, corrective actions to be taken and pest control operator licences.	☐	☐	☐

7. ALLERGENS, GLUTEN AND SULPHITES

	YES	NO	N/A
7.1 – Food allergen ingredients and their derivatives are declared on labels (and/or on the specification sheets of finished products).	☐	☐	☐
7.2 - An allergen control program has been implemented, including:			
Allergen containing product identification	☐	☐	☐
Dedicated or segregated storage of ingredients containing allergens	☐	☐	☐
Employee training	☐	☐	☐
Production schedule	☐	☐	☐
Finished product labelling procedures	☐	☐	☐

8. SECURITY AND FOOD DEFENCE

	YES	NO	N/A
8.1 – Access to the building is controlled and secured.	☐	☐	☐
8.2 - A visitor policy has been implemented for visitors, contractors and service suppliers.	☐	☐	☐
8.3 - Trucks are secured and locked during transportation.	☐	☐	☐

9. HACCP PLAN

	YES	NO	N/A
9.1 - An HACCP plan has been developed for all processes and products.	☐	☐	☐
9.2 - Critical control points (CCPs) have been identified and validated as needed. Critical limits and corrective actions have been established for each CCP.	☐	☐	☐

Comments / justification of all N/A responses

SUPPLIER AGREEMENT

As a supplier, I agree to provide ingredients that meet all established specifications as well as the Canadian requirements of the *Food and Drugs Act* and any other applicable regulations.

I agree to inform HOLLY & MOLLY in case of any change in a product (e.g. product recalls, technical specifications, allergens, formulation or processes) and to send the required supporting documentation. I agree to inform HOLLY & MOLLY of any changes concerning a certification status (e.g. GFCP, HACCP, GFSI).

NAME	
TITLE	
DATE	
SIGNATURE	

APPENDIX 2

GENERIC SUPPLIER EVALUATION QUESTIONNAIRE SPECIFIC TO GRAIN SUPPLIERS

Please email the completed questionnaire to Holly & Molly at quality@hollymolly.com.

For each product sold and delivered to Holly & Molly, please provide a copy of the technical specification sheet, the allergen questionnaire, a signed guarantee letter indicating that your ingredients are gluten-free and a copy of accreditation certificates.

SUPPLIER IDENTIFICATION

COMPANY NAME	
ADDRESS	
PHONE NUMBER	
MAIN CONTACT	
EXTENSION	
EMAIL	
QUALITY MANAGER	
EXTENSION	
EMAIL	

CERTIFICATIONS OR THIRD-PARTY AUDIT

Please indicate which certifications you have.

CERTIFICATIONS		CERTIFICATION BODY
GLOBAL G.A.P.	☐	
PRIMUS GFS	☐	
HACCP	☐	
SQF	☐	
OTHER (SPECIFY): _____	☐	

THIRD-PARTY AUDIT	YES	NO	CERTIFICATION BODY	DATE OF AUDIT
Have you been audited by a third-party organization in the last two years?	☐	☐		

If applicable, please provide a copy of your certificates or audit reports.

GLUTEN QUESTIONNAIRE

Indicate whether these grains are grown on your land or present in your facility (e.g. stored grain).

Type of grains containing gluten	Cultivated on your land		Present in your facilities	
	Yes	No	Yes	No
Wheat, Kamut, spelt or other varieties of wheat	☐	☐	☐	☐
Rye	☐	☐	☐	☐
Barley	☐	☐	☐	☐
Triticale	☐	☐	☐	☐
Oats	☐	☐	☐	☐

Is your company dedicated to the production of gluten-free grains?

Yes ☐ No ☐

Indicate if control measures are in place to prevent cross-contamination of grain by gluten. If so, please describe these measures.

STEPS	CONTROL MEASURES IN PLACE? YES	NO	N/A	SUMMARY OF THE CONTROL MEASURES
Planting and cultivation	☐	☐	☐	
Harvest	☐	☐	☐	
Storage	☐	☐	☐	
Transport	☐	☐	☐	

SOME EXAMPLES OF CONTROL MEASURES TO AVOID CONTAMINATION WITH GLUTEN

PLANTING AND CULTIVATION

- Use certified pure gluten-free seeds (e.g. Canada Foundation No. 1 or equivalent)
- Use land that has not been cultivated with grains containing gluten for at least two years.
- Clean seeding equipment if it was used to sow grains containing gluten.
- Inspect and clear fields of undesirable plants (e.g., wheat, barley, etc.).
- Delineate a perimeter between fields.
- Identify and segregate (store separately) seeds during storage and seeding to avoid contamination between grain types.

HARVESTING AND CLEANING

- Clean and inspect harvesting and cleaning equipment to prevent contamination by previous grains.
- Sample and inspect harvested grains for presence of gluten-containing grains.
- Clean the grains.

STORAGE

- Use dedicated silos to store gluten-free grains.
- Clean and inspect silos used to store grains.
- Identify storage areas and stored grains.

TRANSPORT

- Clean and inspect shipping vehicles before loading.

GOOD PRACTICES QUESTIONNAIRE

	YES	NO	N/A
A pest control program has been implemented in your production and storage facilities.	☐	☐	☐
Where applicable, pesticides are applied according to the directions for use (e.g. concentrations, pre-harvest intervals).	☐	☐	☐
Where appropriate, pesticides are authorized by regulatory authorities and applicators are licensed as required.	☐	☐	☐
Grain storage and cleaning facilities are maintained in good condition and kept clean.	☐	☐	☐
Good practices for production, harvest, storage and transport are applied to prevent contamination and mycotoxin development in grains.	☐	☐	☐
Grain containers are identified with the name of the grain, name of the producer and a lot number.	☐	☐	☐
Bulk grains are transported in clean vehicles designed for contact with food products.	☐	☐	☐
Vehicles carrying bulk grains are not used for the transport of incompatible products (e.g. fertilizers, chemicals, pesticides, etc.).	☐	☐	☐
Vehicles are inspected before loading the grain. Transport vehicles are in good condition.	☐	☐	☐

Please comment on or explain all N/A responses to the above questions.

COMMITMENT AND GUARANTEE OF THE GRAIN HANDLER

I agree to ensure compliance comply with good agricultural practices and applicable legal requirements and to provide gluten-free grains (≤ 20 ppm for gluten) to bakery HOLLY & MOLLY.

I agree to inform bakery HOLLY & MOLLY of any changes that may affect the product specifications (e.g. product recalls, technical specifications, allergens, formulation or processes) or the status of a certification.

NAME	
TITLE	
DATE	
SIGNATURE	

APPENDIX 3

ALLERGEN CHECKLIST FOR FOOD SUPPLIERS AND MANUFACTURERS

SUPPLIER IDENTIFICATION	
NAME	
ADDRESS	

PRODUCT IDENTIFICATION	
CODE	
NAME	

ALLERGEN
Please fill in the following table **for each product**. Column I indicates the allergens that may be found in the product, either by means of addition or cross-contamination. Column II indicates the allergens present in other products that are processed on the **same** equipment at a different time. Column III indicates any allergens that are present in the facility. Please fill in each cell of the table with a **YES** or a **NO** and, when applicable, include the name of the ingredient. **Do not leave any cells empty.**

COMPONENT	**COLUMN I** Present in the product	**COLUMN II** Present in other products manufactured on the same line	**COLUMN III** Present in the same manufacturing plant
Peanuts or their derivatives, such as peanut pieces, protein, oil, butter, flour, or mandelona nuts (an almond-flavoured peanut product), etc.			
Tree nuts (almonds, Brazil nuts, cashews, hazelnuts [filberts], macadamia nuts, pecans, pine nuts [pinyon, pinon], pistachios and walnuts or their derivatives, such as nuts, butters and oils, etc.			
Sesame or its derivatives, e.g. paste, oil, etc.			
Milk or its derivatives, e.g. milk caseinate, whey, yoghurt powder, etc.			
Eggs or their derivatives, e.g. frozen yolk, egg white powder, egg protein isolates, etc.			

Fish or its derivatives, e.g. fish protein, oil, extracts, etc.			
Crustaceans (including crab, crayfish, lobster, prawn and shrimp) and **shellfish** (including snails, clams, mussels, oysters, cockle and scallops) or their derivatives, e.g. extracts, etc.			
Soy or its derivatives, e.g. lecithin, oil, tofu, protein isolates, etc.			
Wheat, triticale, oats, barley, rye or their derivatives, e.g. flour, starches, brans, etc. Includes other wheat varieties such as spelt, durum, Kamut and emmer or any source of **gluten**.			
Mustard or its derivatives, e.g. mustard seeds, mustard flour, ground mustard, prepared mustard, etc.			
Sulphites, e.g. sulphur dioxide and sodium metabisulphite, etc.			

ALLERGEN PROGRAM
Have you implemented an allergen control program to avoid cross-contamination of the product with the allergens not present in the product but noted in columns II and III? Yes ☐ No ☐

NOTIFICATION
In case of modification (allergen present in a product, on a production line or in your facility) you must notify us immediately.

SIGNATURE	
COMPLETED BY (NAME, TITLE)	
DATE	

APPENDIX 4

HAZARD ASSESSEMENT MATRIX FOR INCOMING MATERIALS

		EASE OF DETECTION			
		1	2	3	4
LIKELIHOOD OF OCCURRENCE	1	1	2	3	4
	2	2	4	6	8
	3	3	6	9	12
	4	4	8	12	16

LEVEL OF RISK	LOW RISK	MEDIUM RISK	HIGH RISK
SCORE	1 – 4	6 – 9	12 – 16
COLOUR CHART			

SCORE	EASE OF DETECTION	LIKELIHOOD OF OCCURRENCE
1	Gluten is extremely easy to detect	Extremely unlikely to contain gluten
2	Gluten is easy to detect	Likely to contain gluten
3	Gluten is difficult to detect	Somewhat likely to contain gluten
4	Gluten is extremely difficult to detect	Very likely to contain gluten

	EASE OF DETECTION	LIKELIHOOD OF OCCURRENCE
EVALUATION FACTORS	**Presence of interfering food components** **Processing steps** **Physical form of the sample (solid, liquid, powder)** **Ease of sampling**	**Nature of the ingredient** **Risk of cross-contamination** **Testing result history** **Supplier gluten control practices**

APPENDIX 5

EVALUATION OF LIKELIHOOD OF OCCURRENCE PER PRODUCT CATEGORY

This tool will help you evaluate the occurrence, i.e., the probability that an ingredient contains gluten, of various ingredients used in the food industry. Please note that the following lists are not exhaustive and are provided for information purposes only. During the risk assessment, you must always check the list of ingredients. Furthermore, your risk assessment must be performed in conjunction with a review of the test results and your supplier's evaluation.

VERY LIKELY TO CONTAIN GLUTEN

The following ingredients are prepared from a gluten-containing cereal and should not be used for the production of "gluten-free" products.

- Barley bran
- Barley or malt flour
- Barley or wheat flakes
- Beer, ale, porter, stout
- Brewer's yeast
- Bulgur
- Couscous
- Graham flour
- Hydrolyzed wheat/barley protein
- Malted barley, malted barley flour
- Malted beverages or milk
- Malt extract
- Malt flavouring
- Malt syrup
- Malt vinegar
- Orzo
- Rye flour
- Tabbouleh
- Toasted wheat crumbs
- Triticale
- Wheat bran, wheat bran hydrolysate
- Wheat flours and starches (e.g. atta, dinkel, durum, einkorn, emmer, farina, farro, fu, Kamut, spelt)
- Wheat germ
- Wheat germ oil
- Wheat grass

Gluten is highly likely to be found in ingredients manufactured from oats because of the elevated risk of cross-contamination with other cereals. Use only ingredients manufactured from specially produced "gluten-free" oats. Check that the company uses processing steps that make it possible to prevent contamination and eliminate gluten (e.g., purity protocol, cleaning/sorting). Ingredients manufactured from oats should contain <20 ppm of gluten and be tested using an appropriate method.

- β-Glucan
- Oat bran
- Oat extract
- Oat fibre/oat hull fibre
- Oat flakes/Rolled oats
- Oat flour
- Oat groats
- Oatmeal

SOMEWHAT LIKELY TO CONTAIN GLUTEN

Some ingredients can be made from a gluten-containing cereal or from a gluten-free source. The source of these ingredients must be confirmed with the processor. Ingredients manufactured from a cereal containing gluten should not be used in a gluten-free formulation unless the manufacturer is able to show that the ingredient was processed to eliminate the gluten. However, some private gluten-free certification schemes (e.g. Gluten-Free Certification Program (GFCP)) may not authorize them if derived from gluten sources because alternative gluten-free sources are available. Moreover, test results are usually difficult to interpret or may be deceptive. These ingredients should contain <20 ppm of gluten and be tested using an appropriate method. For example:

- Autolyzed yeast
- Dextrin
- Food starch and modified food starch
- Fermented beverages
- Hydrolyzed proteins
- Vitamin E/tocopherol
- Yeast extract
- Yeast

Agricultural products that are inherently gluten-free may be contaminated by cereals containing gluten during planting, cultivation, harvesting, transport, processing, etc. For these ingredients, you must ask your suppliers what controls are in place to prevent cross-contamination. These ingredients must contain <20 ppm of gluten and be tested using an appropriate method. For example, products (flours, grains, starch, flakes, etc.) derived from the following agricultural products:

- Acorn
- Amaranth
- Arrowroot
- Buckwheat (not in the wheat family)
- Cassava
- Chestnut
- Corn
- Manioc
- Millet
- Potato
- Pulses (beans, chickpeas, fava beans, lentils, peas, etc.)
- Quinoa
- Rice (glutinous, wild, etc.)
- Seeds (chia, flax, hemp, pumpkin, sesame, sunflower, etc.)
- Sorghum
- Soy
- Spices and herbs
- Sweet potato
- Tapioca
- Teff

LIKELY TO CONTAIN GLUTEN

Some ingredients are considered gluten-free by most regulatory organizations regardless of the source, because processing normally eliminates the proteins. However, some private gluten-free certification programs (e.g., GFCP) may not authorize them if derived from gluten sources because other alternative gluten-free sources are available and testing results are difficult to interpret and prone to false negatives. For these ingredients, it is recommended to check the source with the manufacturer as well as the measures in place to control the risk of contamination. These ingredients should contain <20 ppm of gluten and be tested using an appropriate method. For example:

- Caramel colouring
- Citric acid
- Dextrose, glucose and other oses
- Distilled alcoholic beverages (e.g. gin, vodka, whisky)
- Ethanol
- Glucose syrup
- Maltodextrin
- Sugar alcohol (e.g. sorbitol, isomalt, lactitol, maltitol, mannitol, xylitol)

Some product categories are likely to contain gluten. Reading the list of ingredients of these products attentively as well as the measures put in place by the supplier to control the risks of contamination is advised:

- Baby food
- Bacterial culture[1]
- Baked beans
- Baked goods
- Baking powder[2]
- Breakfast cereals
- Bouillon
- Candy and chocolate candies (e.g. licorice)
- Cheeses[3]
- Colouring agents (e.g. caramel)
- Condiments (e.g. barbecue sauce, curry paste, dressing, ketchup, mirin, miso, pickles, prepared mustard, soy sauce, tamari sauce, teriyaki sauce, Worcestershire sauce, etc.)
- Flavoured ice cream
- Flour mixes
- French fries
- Gravy
- Icing, frosting
- Imitation fish (e.g. surimi)
- Instant coffee
- Koji
- Mustard flour
- Rice mixes
- Rice syrup, rice vinegar
- Sauces
- Seasonings
- Smoke flavouring (e.g. liquid smoke, smoke seasoning, smoke powder)
- Snack foods
- Soups
- Sprinkles
- Pasta, noodles
- Prepared meat (cold cuts, lunch meats, sausage, etc.)
- Pudding
- Yeast (e.g. active dry, baker's, nutritional, torula)[4]

[1] Check the substrate used because bacterial cultures are sometimes grown on rye grains, malt or wheat starch.
[2] Check for wheat starch
[3] Some cheeses might contain gluten (e.g. blue cheese, flavoured cheeses, shredded cheeses, cheese spreads) and cross-contamination is possible when handled or packaged in stores.
[4] Sometimes made on a gluten-containing medium.

EXTREMELY UNLIKELY TO CONTAIN GLUTEN

The following ingredients are generally free from gluten. Nonetheless, verifying the list of ingredients as well as the measures put in place by the supplier to control the risk of cross-contamination is recommended. For example:

- Additives
- Egg products
- Baking soda
- Chocolate and cocoa
- Coffee
- Coconut by-products
- Cream of tartar
- Food gums (agar-agar, carrageenan, cellulose, guar, locust bean, pectin, xanthan, etc.)
- Fish and seafood
- Fruits and vegetables (fresh, frozen, canned, juice)
- Meat and poultry
- Milk products (butter, buttermilk, cream, sour cream, unflavored yoghurt)
- Nuts
- Oil and fats (e.g. lard, margarine, shortening, vegetable oils)
- Water
- Gelatin
- Inulin
- Lecithin
- Mono and diglycerides
- Pure and artificial flavours (e.g. monosodium glutamate, vanilla)
- Salt
- Sugars and sweeteners (e.g. acesulfame potassium, agave syrup, aspartame, brown sugar, cane sugar, corn syrup, fructose, honey, icing sugar, maple syrup, molasses, stevia, etc.)
- Tea
- Tofu (plain)
- Wine[5]
- Vinegar (e.g. apple cider, balsamic, distilled white, red wine, white wine)

[5] Check the type of fining agent used.

APPENDIX 6

HAZARD ASSESSMENT MATRIX FOR PROCESSING STEPS

		SEVERITY			
		1 – LOW	2 – MEDIUM	3 – HIGH	4 – VERY HIGH
LIKELIHOOD OF OCCURRENCE	1 – RARE	1	2	3	4
	2 – OCCASIONAL	2	4	6	8
	3 – COMMON	3	6	9	12
	4 – VERY COMMON	4	8	12	16

LEVEL OF RISK	SCORE	COLOUR CHART
LOW RISK	1 – 4	
MEDIUM RISK	6 – 9	
HIGH RISK	12 –16	

APPENDIX 7

THE SPECIFIC CASE OF OATS

COMPOSITION

Oats are recognized as structurally different from other gluten-containing cereals such as wheat, rye, barley and their hybrids. Oat proteins are primarily composed of globulins (70 to 80%). Oat prolamins, known as avenins, are present in an extremely low concentration, i.e., 4 to 14%, which may in part explain their low toxicity for people with celiac disease. It is generally recognized that specially prepared "gluten-free" oats do not represent a risk for gluten-sensitive individuals, although health professionals and regulatory organizations recommend gradually integrating oats into a gluten-free diet under medical supervision.

The amino acid sequences of avenins contain less proline (P) and glutamine (Q) than wheat gliadins, barley hordeins and rye secalins. This has the positive effect of making them almost undetectable by most ELISA test kits.

CROSS-CONTAMINATION RISK

Regular oats are frequently contaminated by other grains containing gluten because they are often grown in the same fields or in proximity to wheat, rye and barley crops. The purity of the seeds used by producers can also be an issue. Lastly, suppliers sometimes use the same equipment to sow, harvest, transport, store, process and package these cereals.

Producers of "gluten-free" oats must ensure that their product meets regulatory requirements at all times (i.e., 20 ppm or less gluten). To do so, producers have several options available to them.

First, producers can use a purity protocol. A purity protocol is composed of documented control measures with the objective of reducing the risk of gluten contamination in all processing steps. It involves several procedures including the use of pure seeds, field management, cleaning and inspecting equipment, grain sampling, etc. Examples of control measures recommended for avoiding gluten contamination are provided in Appendix 2.

Gluten-free oats can also be obtained after mechanically and optically sorting grains based on their size, shape, colour and density. However, because of the elevated risk of contamination, these practices must be carefully validated before confirming that the product is gluten-free and safe for people with celiac disease. The combination of a purity protocol and optical sorting techniques considerably increase the likelihood of obtaining a gluten-free product.

Thus, when you want to buy gluten-free oats, it is essential to ask suppliers on their control measures, testing frequency, the tests used for analysis, testing results, etc.

CASE STUDY

A recent study published by Fritz R.D. et al. in 2016 demonstrated the difficulty of interpreting the testing results of cereals. In this study, oat samples were voluntarily contaminated with wheat grains, then tested with an ELISA kit. Then the samples that obtained a gluten reading of 5 to 20 ppm were retested 10 times. Testing results varied between < 5 ppm and > 160 ppm, even when the test was conducted on ground samples of 50 grams. This shows wide variability in the testing results and an irregular distribution of gluten in the samples. It was suggested that grinding samples would contribute to an inadequate dispersal of gluten. Thus, a relatively low gluten content (i.e., a testing result of 5 to 20 ppm) may indicate a higher level of contamination in the rest of the sample.

Based on these results, it was determined that approximately 10 testing results of 0.25 g samples with an average of 11.4 ppm or less were necessary to obtain 95% assurance that the average does not exceed 20 ppm. Otherwise, the potential for an incorrect interpretation would be higher. It was also established that when a sample had real gluten content of 20 ppm, the probability of obtaining a false negative with a single reading of a 0.25 g sample is approximately 63%.

RECOMMENDATIONS

The main problem with assessing the gluten content of oats is that gluten is often unevenly distributed in the lot. Consequently, the testing results vary significantly when analyzing different samples of the same lot. A sufficient number of samples should be collected at different places to avoid an incorrect interpretation of the test results. Furthermore, each sample must be tested individually, because combining samples could result in a dilution of the testing results.

To increase the accuracy of testing results, R-Biopharm developed a specific protocol to extract gluten from oats samples. In its updated instructions, R-Biopharm recommends that companies homogenize a sample of 200 g (rather than 50 g) and perform the extraction using a 1 g sample (rather than 0.25 g).

APPENDIX 8

COMPARISON OF COMMERCIALLY AVAILABLE ELISA TEST KITS

Test kit	Aller-Tek Gluten ELISA	Wheat/Gluten (Gliadin)	Veratox® for gliadin	Veratox® for Gliadin R5	RIDASCREEN® Gliadin
Manufacturer	**ELISA Technologies**	**Morinaga Institute**	**Neogen**	**Neogen**	**R-Biopharm**
Method	Sandwich ELISA	Sandwich ELISA	Sandwich ELISA	Sandwich ELISA	Sandwich ELISA
Type of results	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative
Reference material	Wheat gluten and barley standard available	NIST SRM 1567a - Wheat Flour	Gliadin G3375 (Sigma-Aldrich)	Gliadin G3375 (Sigma-Aldrich)	PWG gliadin
Antibody	Skerritt monoclonal (401.21)	Anti-gliadin polyclonal antibody	Skerritt monoclonal (401.21)	R5 monoclonal	R5 monoclonal
Extraction protocol	40% ethanol with extraction mix	Specific extraction solution	40% ethanol or cocktail solution	60% ethanol or cocktail solution	60% ethanol or cocktail solution
Interval of quantification (ppm of gluten)	5–80 ppm	0.26–68 ppm	10–100 ppm	5–80 ppm	5–80 ppm
Limit of detection (LOD) (ppm of gluten)	5 ppm	0.26 ppm	10 ppm	2.2 ppm	1 ppm
Validation	AOAC-RI 081202	Interlaboratory study supported by the Japanese Ministry of Health, Labour and Welfare	Not available	AOAC-RI 061201	AAOAC-OMA 2012.01 AOAC-RI 120601 AACCI 38.50.01 Codex Alimentarius Reference Method (Type I)
Usage	Processed and unprocessed food products, environmental surfaces	Processed and unprocessed food products, environmental surfaces	Processed and unprocessed food products, environmental surfaces	Processed and unprocessed food products, environmental surfaces	Processed and unprocessed food products, environmental surfaces
Other specifications	- It underestimates barley.	- High recovery of gluten for processed foods. - Used by the FDA for gluten-free products (in tandem with RIDASCREEN® Gliadin kit). - It underestimates barley and rye.	- It is generally no longer in use, since it has been replaced by the R5 test kit.	- It overestimates barley and rye.	- It overestimates barley. - Used by the FDA for gluten-free products (in tandem with Morinaga Wheat/Gluten [Gliadin] kit).

Test kit	AgraQuant®	RIDASCREEN® Gliadin competitive	GlutenTox® Pro	GlutenTox® Sticks Plus	EZ Gluten®
Manufacturer	**Romer Labs®**	**R-Biopharm**	**Biomedal Diagnostics**	**Biomedal Diagnostics**	**ELISA Technologies**
Method	Sandwich ELISA	Competitive ELISA	Lateral flow device	Lateral flow device	Lateral flow device
Type of results	Quantitative	Quantitative	Semi-qualitative	Semi-quantitative or quantitative	Qualitative
Reference material	VWG gliadin	Hydrolysate mixture of wheat, rye and barley	N/A	N/A	N/A
Antibody	G12 monoclonal	R5 monoclonal	G12 monoclonal	G12 monoclonal	Skerritt monoclonal (401.21)
Extraction protocol	60% ethanol or cocktail solution	60% ethanol	Universal Gluten Extraction Solution (UGES)	Universal Gluten Extraction Solution (UGES)	Extraction buffer
Interval of quantification (ppm of gluten)	4–200 ppm	10–150 ppm	N/A	8–85 ppm	N/A
Limit of detection (LOD) (ppm of gluten)	2 ppm	2.7 ppm	Samples: 5, 10, 20 or 40 ppm Swabs: 16 ng/16 cm^2	Samples: 3, 10, 20, 30 or 100 ppm Swabs: 16 ng/16 cm^2	Samples: 10 ppm Swabs: 1 µg/25 cm^2
Validation	AACCI 38.52.01 AOAC-OMA 2014.03	AACCI 38.55.01 AOAC-OMA 2015.05	AOAC-RI 061502	Validated by FAPAS and AESAN only (Spain)	AOAC-RI 051101
Usage	Processed and unprocessed food products, environmental surfaces	Hydrolyzed and fermented food products	Lightly processed and unprocessed food products, environmental surfaces	Lightly processed and unprocessed food products, environmental surfaces	Lightly processed and unprocessed food products, environmental surfaces
Other specifications	- It detects some oat varieties suspected to trigger a response in people with celiac disease.	- It can also be used to detect intact and unprocessed proteins, but it will be less specific. - It cannot be used with the cocktail solution.	- It should not be used for matrices with a high content of polyphenols and tannins. - It detects some oat varieties (suspected to trigger a response in people with celiac disease).	- It detects some oat varieties (suspected to trigger a response in people with celiac disease). - Quantitative results can be obtained in combination with the GlutenTox® Reader.	- It underestimates barley.

Test kit	AllerFlow Gluten	Reveal® 3-D	Alert® for Gliadin R5	RIDA®QUICK Gliadin	AgraStrip®
Manufacturer	**Hygiena**	**Neogen**	**Neogen**	**R-Biopharm**	**Romer Labs®**
Method	Lateral flow device	Lateral flow device	Lateral flow device	Lateral flow device	Lateral flow device
Type of results	Qualitative	Qualitative	Qualitative	Qualitative	Semi-qualitative
Reference material	N/A	N/A	N/A	N/A	N/A
Antibody	G12 monoclonal	R5 monoclonal	R5 monoclonal	R5 monoclonal	G12 monoclonal
Extraction protocol	Extraction solution with reducing and dissociating agents	Extraction solution	80% ethanol or cocktail solution	60% ethanol or cocktail solution	60% ethanol
Interval of quantification (ppm of gluten)	N/A	N/A	N/A	N/A	N/A
Limit of detection (LOD) (ppm of gluten)	Swabs: 5 µg/100 cm^2	Samples: 5 ppm Swabs: 80 µg/100 cm^2	Sample: 20 ppm Swabs: 1–2 µg/100 cm^2	Samples: 5 ppm Swabs: 2–4 µg/100 cm^2	Samples: 5, 10 or 20 ppm Rinse water: 35 ppb Swabs: 4 µg/25cm^2
Validation	Internal validation	Internal validation	Internal validation	AOAC-OMA 2015.16 (AACCI in process)	AOAC-RI 061403
Usage	Environmental surfaces and rinse water	Environmental surfaces and rinse water	Lightly processed and unprocessed food products, environmental surfaces and rinse water	Lightly processed and unprocessed food products, environmental surfaces	Lightly processed and unprocessed food products, environmental surfaces and rinse water
Other specifications	- Similar to Biomedal's GlutenTox®. - It detects some oat varieties (suspected to trigger a response in people with celiac disease).				- It detects some oat varieties (suspected to trigger a response in people with celiac disease).

APPENDIX 9

REGULATIONS RELATED TO "GLUTEN-FREE CLAIMS"

INTERNATIONAL STANDARDS

At the international level, food standards, guidelines and codes of practice are collected in the **Codex Alimentarius.** Many countries throughout the world use these standards to establish their own standards and regulations. The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) has been responsible for developing labelling standards for gluten-free products for several years. Gluten-free foods are currently defined in CODEX STAN 118-1979 as follows: "dietary foods consisting of or made only from one or more ingredients which do not contain wheat (i.e. all *Triticum* species, such as durum wheat, spelt and khorasan wheat, which is also marketed under different trademarks such as KAMUT), rye, barley, oats or their crossbred varieties, and the gluten level does not exceed 20 mg/kg in total, based on food as sold or distributed to the consumer" and/or "dietary foods consisting of one or more ingredients from wheat (i.e. all *Triticum* species, such as durum wheat, spelt, and khorasan wheat, which is also marketed under different trademarks such as KAMUT), rye, barley, oats or their crossbred varieties, which have been specially processed to remove gluten, and the gluten level does not exceed 20 mg/kg in total, based on food as sold or distributed to the consumer".

According to the Codex Alimentarius Commission, the authorization of oats that have not been contaminated with wheat, rye or barley may be determined at the national level. Recent studies have shown that limited amounts of oats can be considered safe for most people with celiac disease who are under the supervision of a qualified healthcare professional.

A review of available information on tolerable amount of gluten for people with celiac disease published in 2008 concluded that "a daily gluten intake of less than 10 mg is unlikely to cause significant histological abnormalities". In other words, most people with celiac disease are unlikely to be affected if they limit their gluten intake to less than 10 mg per day. Regulators around the world have conducted gluten exposure estimates from foods containing gluten, taking into consideration the gender and age of consumers and various rates of food consumption. Several of these organizations concluded that if gluten was present in food at levels not exceeding 20 ppm, exposure would remain below 10 mg per day for all age groups studied.

Most international regulators have adopted the 20-ppm threshold recommended by the Codex Alimentarius for voluntary labeling of gluten-free foods. However, there are some differences, as summarized in Table 5. Since regulations can change quickly, it is important to consult the latest version of the applicable legislation in the country where the products are sold.

Table 5: Authorized Gluten-Free Claims and Conditions

Type of product	Canada	USA	European Union	Australia/ New Zealand
Foods that inherently do not contain gluten (e.g. fresh vegetables, milk, eggs)	Gluten-free claims generally not authorized (check with CFIA and Health Canada)	**Gluten-free** if < 20 ppm gluten	**Gluten-free** if < 20 ppm gluten	**Gluten-free** if no detectable gluten
Foods with gluten-containing grains that have been processed to remove gluten[1] (e.g. wheat starch, glucose syrup, maltodextrin)	**Gluten-free** if ≤ 20 ppm gluten (check with CFIA and Health Canada)	**Gluten-free** if < 20 ppm gluten	**Gluten-free** if ≤ 20 ppm gluten **Very low gluten** if ≤ 100 ppm gluten	**Low gluten** if < 200 ppm gluten
Gluten-free foods that might contain gluten as a result of cross-contamination	**Gluten-free** if ≤ 20 ppm gluten	**Gluten-free** if < 20 ppm gluten	**Gluten-free** if ≤ 20 ppm gluten	**Gluten-free** if no detectable gluten **Low gluten** if < 200 ppm gluten
Oats	**Gluten-free** if ≤ 20 ppm gluten[2]	**Gluten-free** if < 20 ppm gluten[3]	**Gluten-free** if ≤ 20 ppm gluten[2] **Very low gluten** if ≤ 100 ppm gluten	Claims not authorized

1. Processing steps should be validated to demonstrate their effectiveness in removing gluten.
2. "Gluten-free" claims are allowed only for specially produced gluten-free oats if they can be verified as gluten-free.
3. Oats are treated as a gluten-free grain but may contain gluten as a result of cross-contamination.

CANADA

In Canada, gluten-free foods are regulated under Division 24 of the *Food and Drug Regulations*, "Foods for Special Dietary Use", which includes foods that have been specially processed or formulated to meet the particular dietary requirements of a person in whom a physical or physiological condition exists as a result of a disease, disorder or injury; or for whom a particular effect, including but not limited to weight loss, is to be obtained by a controlled intake of foods. Section B.24.018 of the *Food and Drug Regulations* states that:

> *It is prohibited to label, package, sell or advertise a food in a manner likely to create an impression that it is a gluten-free food if the food contains any gluten protein or modified gluten protein, including any gluten protein fraction, referred to in the definition "gluten" in subsection B.01.010.1(1).*

Gluten is defined as any gluten protein from the grain of any of the following cereals or the grain of a hybridized strain created from at least one of the following cereals: barley, oats, rye, triticale, wheat or any modified gluten protein, including any gluten protein fraction that is derived from any of these cereals.

To meet Canadian regulations, foods labelled "gluten-free" must contain no more than 20 ppm of gluten as a result of cross-contamination and no intentionally added gluten sources. However, products made from a gluten-containing grain ingredient can also bear a gluten-free claim if the manufacturer can demonstrate that the food was processed to reduce gluten to a level of less than 20 ppm (e.g., wheat starch, wheat maltodextrin, wheat glucose syrup).

The "gluten-free" claims for specially produced oats that contain no more than 20 ppm of gluten from wheat, rye, barley, or their hybrid strains have also been allowed since May 2015. The objective is helping people with celiac disease gain access to a wider selection of nutritious products.

Fermented and hydrolyzed foods or foods containing fermented and hydrolyzed ingredients are also authorized to bear "gluten-free" claims as long as they are clearly labelled. However, beers are not authorized to carry "gluten-free" claims as they must meet the standards of composition in the *Food and Drug Regulations*, which means that they are always made from barley and/or wheat. However, Health Canada and the CFIA do not object to the use of the following precautionary statement: "This product is fermented from grains containing gluten and [processed or treated or crafted] to remove gluten. The gluten content of this product cannot be verified, and this product may contain gluten". In this case, the manufacturer must be prepared to provide evidence to substantiate their claim, including a detailed description of the method used to remove gluten from the product, appropriate gluten assay results for the finished product, and the name and the manufacturer of the assay.

It is important to note that most certification schemes (e.g., GFCP) do not allow fermented products to which a gluten-containing ingredient has intentionally been added, nor the precautionary statements permitted under regulations. In contrast, similar products made from cereals that do not contain gluten may use a "gluten-free" claim if they meet all other requirements.

Products that inherently do not contain gluten (e.g., fresh vegetables, milk, and eggs) are not authorized to bear a "gluten-free" claim because they do not meet the intent of Division 24 of the *Food and Drug Regulation*s on Foods for Special Dietary Use.

UNITED STATES

In the United States, the FDA's Final Rule concerning gluten-free food labelling (21 CFR Part 101.91) has been in force since August 2014. Gluten-free foods were defined by the FDA as food and dietary supplements that does not contain any of the following:

1) *An ingredient that is a gluten-containing grain (e.g., spelt wheat);*
   - *An ingredient that is derived from a gluten-containing grain and that has not been processed to remove gluten (e.g., wheat flour); or*
   - *An ingredient that is derived from a gluten-containing grain and that has been processed to remove gluten (e.g., wheat starch), if the use of that ingredient results in*

the presence of 20 parts per million (ppm) or more gluten in the food (i.e., 20 milligrams (mg) or more gluten per kilogram (kg) of food);

- Inherently does not contain gluten;

or

2) *Any unavoidable presence of gluten in the food bearing the claim in its labeling is below 20 ppm gluten (i.e., below 20 mg of gluten per kg of food).*

Other claims such as "no gluten," "free of gluten" and/or "without gluten" are also authorized on food packaging and must meet the intent of the regulation.

Unlike Canada, voluntary gluten-free food labelling in the United States applies to any food meeting the requirements, including inherently gluten-free foods and oats. However, since these products could be cross-contaminated by gluten, they must also respect the 20-ppm threshold.

American gluten-free labelling regulations also exclude drugs, cosmetics and products that are regulated by the U.S. Department of Agriculture (USDA) and the Alcohol and Tobacco Tax and Trade Bureau (TTB), which includes meats, poultry, certain egg products and most alcoholic beverages.

Most fermented beverages are regulated by the TTB under the *Federal Alcohol Administration Act* (FAA Act). Malt beverages are defined as beverages made with both malted barley and hops and cannot be labelled as gluten-free. However, the TTB may accept the use of one of the following specific statements: "Product fermented from grains containing gluten and [processed or treated or crafted] to remove gluten. The gluten content of this product cannot be verified, and this product may contain gluten" or "This product was distilled from grains containing gluten, which removed some or all of the gluten. The gluten content of this product cannot be verified, and this product may contain gluten". However, most private certification schemes (e.g. GFCP) do not allow any intentional addition of gluten even with a disclaimer permitted under regulations. Other alcoholic beverages produced without any ingredients that contains gluten (e.g. wine fermented from grapes, vodka) may use a gluten-free claim.

Lastly, beers that do not meet the definition of a malt beverage under the FAA Act are subject to the same labelling requirements as foods administered by the FDA. On November 18, 2015, the FDA published a proposed rule in the Federal Register to establish requirements for fermented, hydrolyzed and distilled foods or ingredients that are labelled as gluten-free. Under the proposed rule, these products were permitted to bear a gluten-free claim if the manufacturer keeps records showing that the ingredients are gluten-free before fermentation and that the risk of cross-contamination is under control. Records to demonstrate conformity should be kept.

EUROPEAN UNION

As of July 20, 2016, *Regulation (EU) No. 828/2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food* came into force in

the European Union. The labelling requirements apply to all prepackaged and non-prepackaged foods (including meals served in cafes, restaurants, schools and hospitals).

European foodstuffs can bear a gluten-free claim if they contain no more than 20 ppm of gluten. Additionally, food containing one or more ingredients made from wheat, rye, barley, oats or their cross-bred varieties that have been specially processed to reduce the gluten content to a level of no more than 100 ppm of gluten can bear a "very low gluten" claim. These statements may be accompanied by the following affirmations: "suitable for people intolerant to gluten," "suitable for coeliacs," "specifically formulated for people intolerant to gluten" or "specifically formulated for coeliacs," under certain conditions. Other statements used to provide information to consumers on the absence or reduced presence of gluten in food are no longer authorized.

AUSTRALIA/NEW ZEALAND

The *Australia New Zealand Food Standards Code* (Standard 1.2.7) defines gluten-free foods as foods that have no detectable gluten, no oats and no cereals containing gluten that have been malted. The threshold is based on the best available testing method, which is currently represented by 3 ppm. The "low gluten" label can also be used if food contains a detectable gluten content of less than 200 ppm.