Frequently Asked Questions

1) What are the LOD's on Allergen Test Kits?

LOD's have been determined for each test kit (see table), however it is important to note that this can change depending upon the sample matrix. We would always recommend validating using the customer's specific product matrix.

Allergen	AgraStrip® LOD in buffer	AgraQuant® LOD in buffer	AgraQuant® Plus LOD in buffer
Almond	2 ppm	0.2 ppm	0.5 ppm
Beta-Lactoglobulin	0.5 ppm	1.5 ppb	
Brazil nut	5 ppm		
Casein	1 ppm	0.04 ppm	0.2 ppm
Cashew	2 ppm	0.2 ppm	1 ppm
Crustacea	10(wet), 2(dry) ppm	0.9 ppb	
Egg (White)	10 ppm	0.05 ppm	0.5 ppm
Fish		1.4 ppm	
Gluten G12	5-10-20 ppm	2 ppm	
Gluten	4 ppm	0.6 ppm	
Hazelnut	5 ppm	0.3 ppm	1 ppm
Lupin	10 ppm	0.2 ppm	
Lysozyme		2 ppb	
Macadamia nut	2 ppm		1 ppm
Milk	1 ppm	0.05 ppm	0.36 ppm
Mustard	2 ppm	1 ppm	0.5 ppm
Ovalbumin		4 ppb	
Peanut	1 ppm	0.1 ppm	0.5 ppm
Pistachio	2 ppm	0.13 ppm	1 ppm
Sesame	5 ppm	0.2 ppm	1 ppm
Soy	2 ppm	16 ppb	
Walnut	10 ppm	0.35 ppm	

2) "Why should I use an allergen specific kit over ATP or General Protein kits?"

ATP swabs are not allergen specific since they detect ATP from all kind of source (microorganisms …). Furthermore ATP levels vary in food and detection limits are not sensitive enough for allergen monitoring. (US Food and Drug Administration requires protein specific testing).

General Protein kits are very unspecific detecting all proteins and do not differentiate between allergen and non-allergen. Sensitivity is not good enough for allergen monitoring and doesn't correlate well with allergen specific testing.

3) I have multiple allergens in my plant and can't afford to test for all of them. Can I just use one kit? If so, which one?

The technology used in ELISA assays and LFDs is based on antibodies which recognize epitopes assigned to certain allergens. Furthermore the composition of the extraction buffer can be different between different allergens. Thus there is no kit that can detect all allergens.

But what you can do is to focus your allergen validation upon the component with the highest allergenic load. Since allergens are proteins the component with the highest allergenic load will be the one with the highest level of protein. If two or more allergens are part of a formula you don't have to validate the removal of all, you can focus your validation on the allergen in the highest amount. But if allergens are present in different forms (for instance on is present in a paste and the other in a liquid) it may be necessary to validate the removal of both allergens.

4) "When swabbing, how many places should I swab and how often?"

The "critical" places should be swabbed meaning these places (like corners, hidden places,…) where cleaning is more difficult and allergens could be still there. If you can actually see by visual inspection that it is not clean then another cleaning step should be done before swabbing.

Don't only swab flat and smooth areas which are easy to clean. Focus more on the food contact surfaces that are harder to get clean like crevices, joints and so on. Also only use swabs that are provided with every AgraStrip kit or use the AgraQuant Swabbing kit, because these swabs were extensively validated and thus work. With cheaper swabs you may not get allergens as readily from equipment surfaces and allergens might not be released from swab. Sometimes recycled milk cartons are used for the production of cheap swabs which might lead to false positives for milk.

Steve Taylor & Joseph Baumerts article "Best practices with allergen swabbing" in Food Safety Magazine June/July 2013 give further recommendations on that topic http://www.foodsafetymag-digital.com/foodsafetymag/20130607?pg=64#pg22

5) How long can I hold onto to the swabs before testing them using your test kits? Can I freeze or refrigerate these swabs if I can't get to them right away? Can swabs in buffer solution be frozen to be shipped and/or run later?

Yes swabs in buffer can be frozen or stored at 2-8°C for shipments or run later (e.g. next day). Swabs in buffers can be stored at room temperature if analyzed on the same day where swabbing was done.

Due to Romer UK (UKAS accreditation) validated for a week in freezer (keep in fridge overnight, if not possible put it in the freezer)

Due to experience (Romer Inc.Lab) swabs can also be kept 3-4 days at fridge (or immediately freeze and ship the next day to lab

6) "How can you tell if it is a 'true negative' or the 'hook effect'?"

If there is Allergen Management in Place you should be dealing with traces not large amounts. If Allergens are an ingredient or it is known that there is a high contamination obvious then strip tests might not be the best option. Only from the result of the strip test you cannot distinguish between a true negative and hook effect. What you can do is dilute the sample/extract down and run the assay again or test the sample with the respective AgraQuant kit.

7) Positive controls - "How can I see a positive result on a strip to make sure it works?"

It is important not to analyze the pure allergen as this will lead to a hook effect and give a false negative result. Allergens need to be diluted to contain less than 1%.

8) What pH range does our AgraStrip products work well in? Which products should we advise customers that pH may cause an issue?

The strips do not contain additional buffer substances. Acidic samples can lead to false positive results and in the range of pH 6-8 results are reliable. The buffer itself is formulated in a way that most food stuffs can be analyzed. But since it is known that food stuffs with strong acidic pH values can overextend the buffer capacity, it is advised to check the pH of the extract and neutralize if necessary.
In alkaline milieu on the other hand there is a tendency to false negative results.

9) Why is it important to read the strip immediately after the required amount of time? What to do if I want to store the strip for my records?

The method given in the test kits has been rigorously tested and validated. The given instructions are determined to be the optimal conditions for doing the test. The color will continue to develop with time and may give an invalid result if not read at the correct time. If you want to store the strip for your records either take a picture of the result zone immediately after the required amount of time or cut off the filter and wicking pad areas and only store the results zone of the strips.

10) For AS and AQ kits which extraction buffers are generic to each other and can be interchanged? If I extract 1 sample and want to run on many different allergen kits can I or do I have to extract for each kit?
Extraction buffers have been optimized for each test kit. It is highly recommended to use the extraction buffers coming with the respective kit and not the change between kits.
If a high number of samples is to be analyzed, it is recommended to use AgraQuant Plus ELISA test kits. One extract can then be used for the analysis of several allergens.

11) Is there a special protocol for rinse water testing for AQ Allergens?
You can add rinse waters directly to the ELISA plate and treat them as extracted samples, but remember to divide the result by 20 as you have not done an extraction.
Since you don't use extraction buffer here you might want to check the pH of the rinse water.

12) What is the specificity and sensitivity of AgraStrip and AgraQuant kits?
Sensitivity refers to LOD (see 1). Specificity refers to actually detecting (only) the allergen you want to detect meaning no (low) cross-reactivity to other food proteins. You will find all information about cross-reacting materials in the package inserts.

13) Other than beer and soy sauce, what other food product types should be aware of that contain hydrolyzed gluten?
Everything that is highly processed/fermented e.g. sour dough, starch syrup, malt extract, starch, syrups in general, soy sauce, beer

14) Biomedal makes claims that their GlutenTox test kits can detect hydrolyzed gluten. Since we utilize the same antibody and technology, can we make this same claim? This claim is not reliable! The study from Biomedal with beer testing is mainly testing draft beers where gluten is not so much hydrolysed. It is depending on the type of beer how much the gluten is hydrolysed.

15) Is wheat the same as Gluten? And can I use any Gluten ELISA to detect wheat?
We can find Gluten in grains like wheat, rye, barley and oats. Gluten is a composite of proteins – the so called prolamines and glutelins, which differ in nomenclature in the different grains. The prolamins of wheat are called Gliadins, the Glutelins of wheat are called Glutenins. Thus Gluten is a protein composite in wheat as well as in rye, barly and oats.
With Gluten ELISA assays you can detect this composite, but due to the nature of ELISA assays – in particular due to antibodies that are used in these assays – we

cannot distinguish if the Gluten that was detected originated from wheat, rye or barley.
If we for instance compare the 2 most commonly used antibodies in Gluten detection we see that they were raised against different epitopes, but all of them show cross reactivity. The G12 antibody (incorporated in our AgraStrip and AgraQuant) was raised against the 33-mer of wheat gliadin, but also cross reacts to rye and barley. The R5 antibody was raised against rye secalin but also cross reacts to wheat and barley.

16) Why is the extraction of Gliadin so time consuming?
This is due to the nature of the composition of gluten. Prolamins (called Gliadin in wheat) are the alcohol-soluble fraction of gluten. Therefore Prolamins are extracted in alcohol-water mixtures, such as 50% (v/v) aqueous propan-1-ol or 60-70% (v/v) aqueous ethanol. One half of prolamins are present as monomers but the other half of the prolamins are present in polymers that are not soluble in alcohol-water mixtures. To reduce disulfide bonds between the prolamin polymers agents such as 2-mercaptoethanol (2-ME) or dithiothreitol (DTT) are used together with heat treatment

17) Oat question regarding our AS and AQ G12 kits - how do we position ourselves on this?
80-100 pure oats from US and Canada have been tested. Only 10-20% showed positive results for gluten using AQ G12. All positive results below 20 ppm (gluten free claim). Results above 10 ppm also tested with R5 ELISA and gave similar results to G12. So far only one Spanish oat sample that we received from Biomedal (already ground, no individual seeds) was tested >100 ppm. As this is the only sample and we don´t know the exact origin we come to following conclusion: At the moment we can say that we have tested so many oats and can see that there is in some oats a low level of gluten proteins expressed which we can determine with G12. Once we receive more oats to analyse we might find some higher positives. We can also offer to analyze certain pure oats on request for gluten content as well. That’s what the actual status of oats.