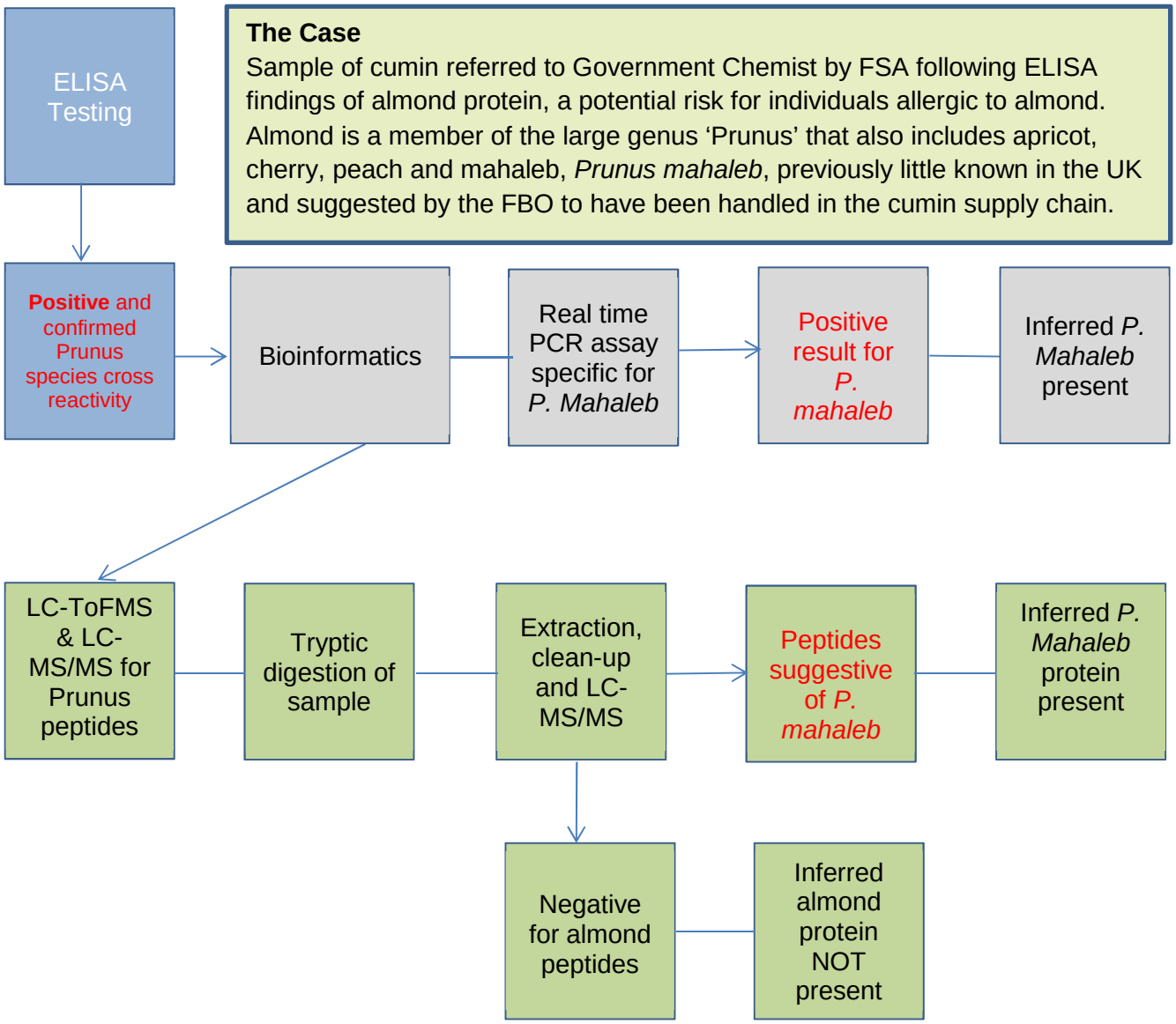
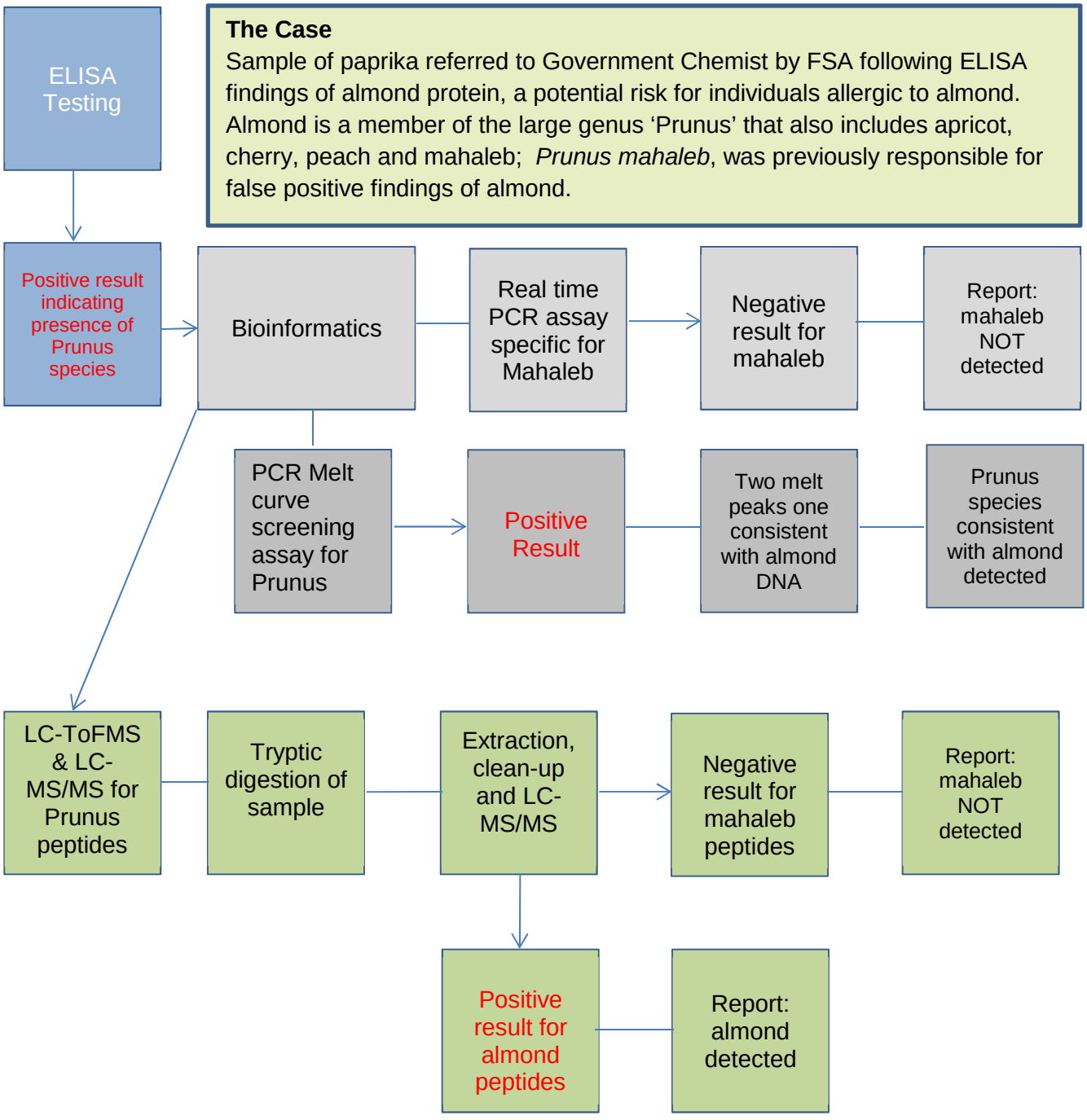
Case Study 1: Alleged presence of almond in cumin



Government Chemist Certificate of Analysis confirmed *Prunus Mahaleb* present
Although no single method proved conclusive, it could be inferred from the combination of ELISA, PCR and LC-MS/MS results that referred sample contained Prunus protein and DNA, the origin of which was consistent with *P. mahaleb* rather than *P. dulcis* (almond). Owing to limitations in the state of the science at the time (e.g. absence of an almond-specific PCR, bioinformatics deficits and lack of validation of the above methods across the Prunus genus, the presence of almond could not completely be ruled out.

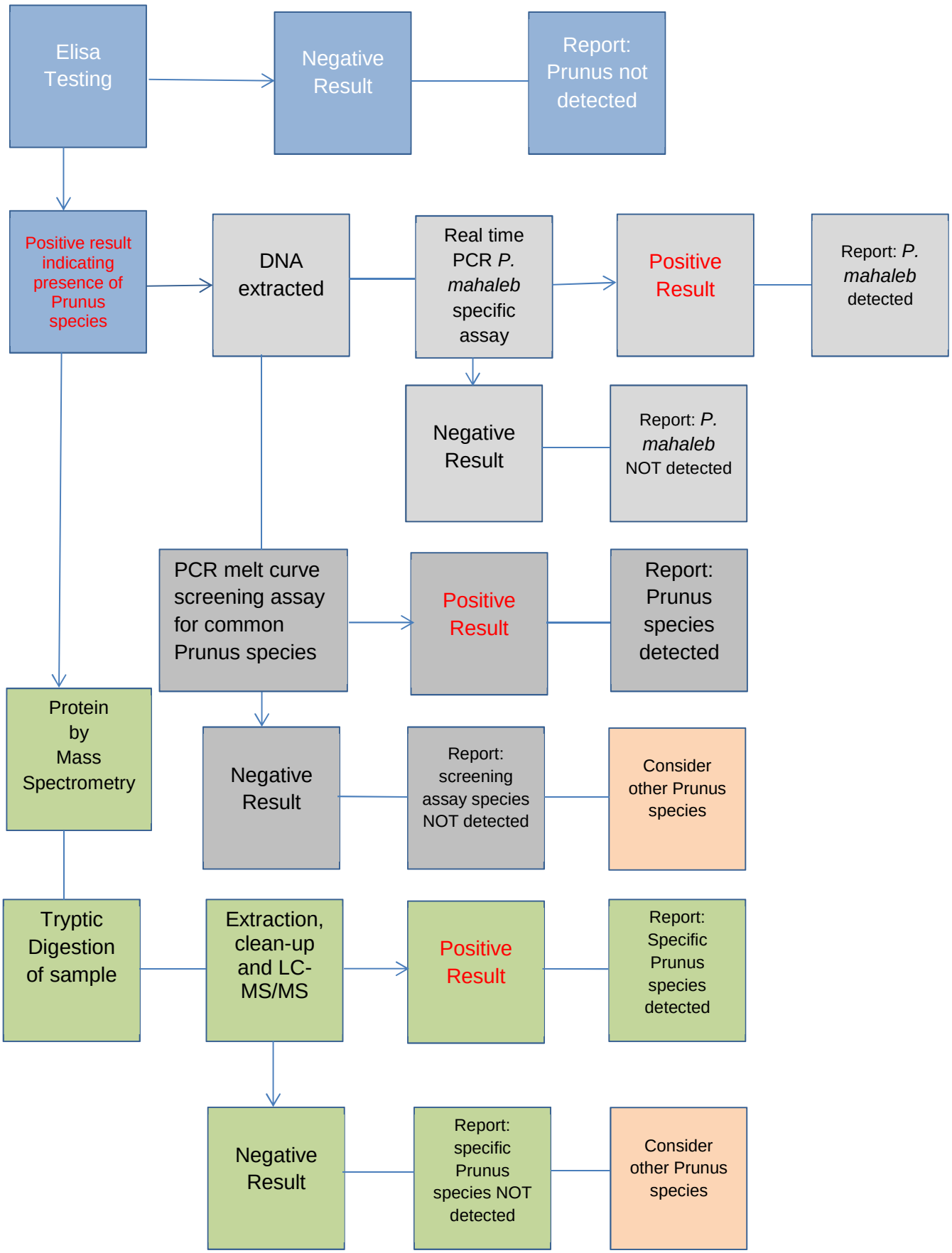
Case Study 2: Alleged presence of almond in paprika



Government Chemist Certificate of Analysis confirmed almond present

The referred sample contains Prunus protein(s) and DNA, the origin of which is consistent with almond rather than mahaleb. The second melt peak curve did not correspond with a common Prunus species and may have been a cultivar different from that from which the standard DNA was extracted. The caveats in case 1 also apply.

Roadmap for the determination of Allergens in Spices



NOTES where the allergen of interest is from the Prunus genus	
1. ELISA	1. Select ELISA for allergen of interest 2. Check cross reactivity stated in ELISA specification, discuss with kit manufacturer, including kit validation. 3. Plan your analyses according to allergen of interest and known cross reactivity of the kit including conducting the necessary quality assurance 4. If ELISA is Prunus negative report all common Prunus species not detected with LoDs for each of the common species from your validation study. 5. If ELISA is Prunus positive, report 'Prunus species detected' with LoDs for each of the common species from your validation study 6. Conduct DNA testing
2. DNA: real-time PCR assay	If it is strongly suspected that Prunus mahaleb is giving the ELISA reaction consider using the LGC real-time PCR assay developed for the specific detection of Prunus mahaleb DNA: Burns, M., Walker, M., Wilkes, T., Hall, L., Gray, K. and Nixon, G. (2016) Development of a Real-Time PCR Approach for the Specific Detection of Prunus mahaleb. Food and Nutrition Sciences, 7, 703-710. http://dx.doi.org/10.4236/fns.2016.78071 *Workshop objective: Delegates should know how to apply this method and estimate order of magnitude concentration for Prunus mahaleb in their sample (if it is found), as ELISA 'almond equivalents' and DNA copy number % (i.e. 'between 0.01 % and 0.1 %' or 'between 0.001 % and 0.01 %'.*
3. DNA: PCR melt curve analysis	If it is not known which Prunus species is present consider using the LGC PCR melt curve approach: Nixon, G., Hall, L., Wilkes, T., Walker, M. and Burns, M. (2016) Novel Approach to the Rapid Differentiation of Common Prunus Allergen Species by PCR Product Melt Analysis. Food and Nutrition Sciences, 7, 920-926. http://dx.doi.org/10.4236/fns.2016.710091 *Workshop objective: Delegates should know how to apply this method and be able to specify to their client which Prunus species is present and a probable concentration range (see above).*
4. Risk assessment	a. If almond is identified as present and other common Prunus species are absent there is a need for a risk assessment. Initially this should be based on a conservative reference dose (if it has become available) failing which the hazelnut reference dose (ED_{01}) of 0.1 mg allergen protein should be used, together with the portion size of the food being investigated. The dilution factor arising from the low level of use of the spice in a finished product should be considered but may be offset by sampling uncertainty and analytical measurement uncertainty. b. If a Prunus species other than almond is identified there is a need for a risk assessment (along the lines of (4a) above. A probable risk may appear evident because of known numbers of people allergic to the Prunus species present, or if amino acid sequence homologies

	suggest a risk to people with almond allergy. Thus it may become important positively to identify the presence of the protein rather than just the species. This can be inferred from the ELISA result but LC-MS/MS confirmation may be required. This can be achieved by following the LGC method described in: Inman, S.E., Groves, K., McCullough, B., Quaglia, M. and Hopley, C., 2018. Development of a LC-MS method for the discrimination between trace level Prunus contaminants of spices. Food Chemistry, 245, pp.289-296 *Workshop objective: Delegates should know how to approach making decisions on the issues in step 4 (*Risk assessment*) and if necessary how to apply (or outsource) the LC-MS/MS method.*

How do I cope with other cases where I suspect my ELISA is cross reacting?

The workshop discussions may produce further strategies but as a start, it is recommended that you do the following:

- Familiarise yourself with common ELISA allergen cross reactivity
- Check that the ELISA validation (both by the kit manufacturer and in-house) follows best practice guidance (e.g. Abbott *et al*., 2010[1])
- Discuss your particular analysis with the ELISA manufacturer
- Use more than one ELISA platform
- Use reference materials (RMs) where available; if RMs are not available then prepare in-house quality control materials.

[1] Abbott, M., Hayward, S., Ross, W., Godefroy, S.B., Ulberth, F., Van Hengel, A.J., Roberts, J., Akiyama, H., Popping, B., Yeung, J.M. and Wehling, P., 2010. Validation procedures for quantitative food allergen ELISA methods: community guidance and best practices. *Journal of AOAC International*, *93*(2), pp.442-450.