GFORSS LEARNING 

Food Safety Laboratory Capacity Building

Evaluation 1 – Answer Key

1. For each of the following categories of food contaminants (pesticides, mycotoxins, POPs and veterinary drugs), which products are likely to be contaminated among the following products: wheat, cow steak, salmon, water, crustaceans, milk, bread. Include all the potential products.

Example: Phycotoxins: Salmon, Water and Shellfish

Pesticides: Wheat, Cow steak, Water, Milk, Bread

Mycotoxins: Wheat, milk, bread

POPs: Salmon, water, crustaceans

Veterinary drugs: Cow steak, milk and bread

2. Risk management

a. Explain why it is not adequate to use the same Maximal limit for aflatoxin in all food products.

Maximum levels in foods are established in an effort to reduce exposure to a particular food contaminant. Exposure is affected by the concentration of the chemical in food and the amount of the food consumed. This therefore means same maximal limits for aflatoxins can not be set for all foods because:

- The concentrations of the different aflatoxins in different foods are not the same.
- The amounts of different foods consumed are not the same, thus affecting the acceptable daily intakes
- The availability, nutritional value and importance of different foods are not the same.
- The toxicity of the different aflatoxins is not the same.

b. Give example of other risk management options

i. Providing directions on how to reduce contaminant levels These directions may be in the form of Good Manufacturing Practices (GMPs) and Good Agricultural Practices (GAPs).

ii. Providing advice and guidance to consumers on risks and benefits of particular food choices.

iii. Monitoring of levels of hazards in foods through regular surveillance activities by regulatory agencies.

iv. Monitoring both the effectiveness of the control measure and its impact on the risk to the exposed consumer population.

3. Utility of rapid methods

a. Explain why rapid methods like LFD are not reference methods (with few exceptions).

Rapid methods like LFDs, where there is no sample preparation, cannot be used as reference methods because lack of sample preparation leads to poor reproducibility, sensitivity, and accuracy due to matrix effect/interference and volume

limitations, since the analyte cannot be cleaned and concentrated. Buffering may not also be possible to off-set the effect of basicity or acidity on the test. Also, the hook effect may lead to false negatives in samples with high analyte concentrations, in devices without the hook-line. Most of these rapid methods are qualitative, which can not be used as reference methods. Rapid methods are usually used as screening methods because they have good sensitivity, but low specificity and mostly operated by unskilled people, thence not good as reference methods.

b. Explain how rapid analytical techniques could be useful for the monitoring of food contaminants.

In monitoring of levels of contaminants in food and the effectiveness of the control measures, rapid analytical techniques can be useful by;

- By using them as the point-of-care assays like in the field, food processing factories and battle fields
- By making commercial kits that are fairly cheap and largely available.
- Can be operated by non-technical and less skilled personnel
- Most of them do not require sample preparation equipment, refrigeration, and have a long shelf life, thereby making them adaptable in developing countries
- Some rapid techniques have high sensitivity, specificity, accuracy, ruggedness and reproducibility; thence widely accepted by users and regulatory agencies.

Rapid analytical techniques can therefore be used to screen samples and reduce the burden on use of expensive reference methods; only positive samples may be tested for confirmation and quantitation. Qualitative techniques can also be made quantitative by incorporating readers.

All the above reasons therefore explain why rapid analytical techniques are useful in monitoring food contaminants for quick, timely and accurate decision making, in managing risks associated with food safety.

4. ELISA

a. What are the particularities of competitive ELISA tests compared to sandwich ELISA tests?

i. Competitive ELISA is fit for analysis of crude or impure samples as the matrix effect is reduced as compared to the sandwich ELISA.

ii. Competitive ELISA enables the detection of small molecular analytes as compared to the sandwich ELISA.

iii. Competitive ELISA is commonly used when only one antibody is available for the antigen, unlike in the sandwich where two antibodies (direct sandwich) or three antibodies (indirect sandwich) are required to sandwich the antigen and have it detected.

b. Explain the difference between a direct and an indirect format.

In direct ELISA, the coated antigen is detected with an antigen specific primary antibody which is directly conjugated to an enzyme, whereas in indirect ELISA, antigen is detected with a secondary conjugated antibody, usually an anti-species, which binds to the unlabeled antigen specific primary antibody. Also, direct ELISA involves only one antibody (conjugated primary antigen specific antibody), whereas Indirect ELISA involves two antibodies, namely unconjugated antigen specific primary antibody and the conjugated secondary anti-species antibody.

c. Explain why the washing steps are important in ELISA.

The washing steps in ELISA are important because the washing removes unreacted reagents from the wells so as to increase accuracy and also to eliminate cross-contamination and background noise.

d. Explain why the respect of incubation times is essential in ELISA.

This is for reproducibility purposes.

5. Hook effect

a. Explain why the hoof effect is a problem for food regulations purpose.

b. Explain the different options to avoid this risk.

c. Explain why the competitive format is not affected by the hook effect.

d. Do you think that the hook effect can occur in sandwich ELISA assay?

a. Explain why the hoof effect is a problem for food regulations purpose.

The hook effect is the phenomena where high concentrations of the analyte simultaneously competes for the capture and detecting antibodies, thereby preventing the formation of the capture antibody/antigen/detecting antibody complexes. The hook effect is therefore a problem for food regulation purposes as it gives false negatives.

b. Explain the different options to avoid this risk.

The hook effect can be avoided by;

i. Serial dilution of the test sample so that the true analyte concentration will fall within the analytical measurement range.

ii. Incorporating the hook line (competitive test line) to the LFD

iii. Using real-time assay/reaction kinetics monitored by devices that quantify an analyte over a wide range on an LFD.

c. Explain why the competitive format is not affected by the hook effect.

In competitive assays, the detecting antibodies can only attach/bind to existing free or un-competed for sites of the limiting capture antibodies. This therefore means that even if the concentration of the analyte is high, and they attach or form possible complexes with the capture antibodies, the detecting antibodies will still have no sites to attach/bind, and hence no hook effect.

d. Do you think that the hook effect can occur in sandwich ELISA assay?

Very unlikely. The high concentration of the analyte (antigen) will saturate antibody number 1. But the washing step will eliminate the free antigens. So no hook effect.

6. What categories of food contaminant are usually tested by ELISA?

Categories of food contaminants usually tested by ELISA include;

- Mycotoxins
- Phycotoxins
- Food allergens and adulterants
- Veterinary drug residues
- Pesticides
- Antibiotics