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Views & Comments

Food Safety and Health—Past Problems and Future Solutions

Christopher J. Smith

Department of Clinical Sciences and Nutrition, University of Chester, Chester CH1 4BJ, UK

The dictum of Hippocrates "Let thy food be thy medicine and medicine be thy food" is potentially both more accurate and more dangerous than ever before. This dictum can only be realistically applied if all foods are known to be safe. Nowadays foods are being produced, which are processed in a variety of ways with ingredients sourced from many different countries and processed in a variety of ways. This processing may include the addition of supplements which are intended to improve health and of adulterants which are potentially harmful. Additionally, foods may be contaminated with pesticides and other materials encountered during production and processing. Thus there is a need to identify "unsafe" foods. The question may be better put as: Which foods are safe?

Food safety relies on six fundamental principles: safe food production, a safe food supply chain, safe food processing systems, safe wholesale food supply, safe food retail systems, and safe food handling at home. These principles encompass the entire food supply chain (Fig. 1). Provided that the appropriate precautions are taken at each stage of the food production and food supply chain, the food should be safe. Fig. 1 shows a simplified version of the range of relationships within the food chain.

However, in addition to considering the individual sections of the supply chain, consideration should also be given to the interrelated nature of the food web. Isolation of the individual phases of the food chain may in fact be useless as a form of protection against contamination. In a simple example such as that shown in Fig. 2, factors outside the control of the food producer, processor, wholesaler, manufacturer, and consumer can result in a heavily contaminated final product. Factors such as air pollution and pesticide drift following spraying within the locality mean that, as a result of the activities of others, food can be contaminated "inadvertently." Simply the air pollutants and the pesticides can settle on the grass which is eaten by cows in a local field. The pollutants can also enter watercourses and contaminate fish, edible water plants, and water. The route for the pollution to the final food product, in this case pasta bolognese, is as follows: Cattle eat the grass and are commonly also fed fish meal as well as being exposed directly to the pollutants in the air they breathe. In this example the cattle produce the milk used in cheese production and the meat which form the basis for the final product. The cheese, the meat, and fresh milk all from the contaminated cows are used in the production of the processed food, spaghetti bolognese. This dish may be further contaminated by the water, from the rivers, streams, and lakes, which is abstracted for use in the preparation of the pasta, the cheese sauce, and cooking the meat. It may also be used in the supposedly positive activities of washing equipment and cleaning work areas. Given such a complex interwoven web of relationships it is clear that although the efforts to prevent contamination at each individual stage of the food production process are vital, it is also essential to take a much more holistic view of the production process. This is a very simple example of a route by which contamination of food with environmental pollutants may occur despite all the efforts of the food industry.

This is only a hypothetical example but there is concern about food safety hazards among consumers and also among producers as a result of food poisoning incidents and subsequent recall

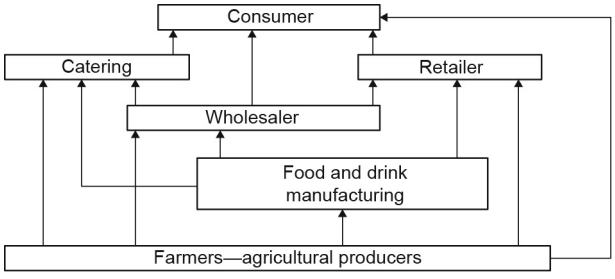

Fig. 1. Conventional food supply chain.

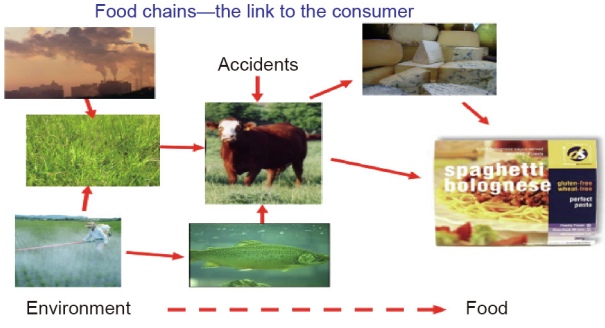

Fig. 2. An example of various routes through the food chain by which contaminants can reach food products.

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actions which have been widely reported in the press. Food comes from all over the world and so the production process is an uncertain factor. Consumers and producers want safe and high quality food in the supermarkets, shops and marketplaces want to ensure continuing successful trade and consumption. It benefits no-one to have contaminated food in the marketplace. It makes consumers wary of purchasing products. Also the consumption of such food may include a risk of ill health which has many consequences such as loss of productivity, use of health services with associated costs, and other social consequences. Governments therefore have a particular interest in ensuring that food is not contaminated.

Consumers and producers want:

(1) Legislation and food safety control;
(2) Quality systems;
(3) A safe food supply chain.

Equally worrying is the problem of adulteration. Contamination is a problem which is accidental but adulteration is the result of the deliberate actions of criminals in the food industry. Adulteration is a major problem which also requires monitoring of food but with different requirements of the analytical and monitoring systems which will be discussed later in this paper.

Food safety is managed via several different routes: ① legislation and monitoring; and ② education and training. Legislation, education, and training are on-going processes which necessarily change as the methods of food preparation and the facilities used change. Legislation has a complex written history of over 10 000 years from the laws of ancient Sumer (8000 BCE), written in cuneiform, which covered food quality to the most modern legislation in China in 2015. Amongst the most famous being the German purity laws (i.e., Reinheitsgebot; 1516 CE) which are still in force. Despite all these many laws, there continue to be violations both accidental contamination and deliberate adulteration. Thus legislation alone is not sufficient to ensure safe, good quality food. Education has also been used for thousands of years but it also has failed to eliminate either contamination or adulteration of food.

This failure of legislation and education to adequately manage the human aspect of food safety in which either indifference or greed results in adulterated or contaminated foods is clear. Thus there needs to be a system of enforcement which attempts to ensure the laws and accepted practices are complied with. This means that there needs to be a system of monitoring procedures which can prove or disprove compliance with legislation and acceptable practice. As it is essential to be able to prove failure to comply with legislation, one of the most important aspects of the work of food scientists is to develop methods of analysis which will function correctly in the complex matrices that food represents.

Methods of food analysis are varied but they all attempt to comply with the constraints imposed by the food industry and consumers. Essentially the requirements are that the assays should be rapid, specific, low cost, and simple. The reasons behind these requirements are easily understood. Food generally has a short shelf-life and hence any delay in testing or waiting for results reduces the overall time during which products are available for sale. Therefore, rapid tests are preferred. Equally importantly is the cost of the tests given that the food industry is reputedly one with very small profit margins and therefore there is little leeway to fund expensive scientific equipment or laboratory facilities. However, the converse argument is that failure to monitor food quality and safety can result in expensive recalls, finds, and possibly completely ruin the company. Therefore, a balance has to be achieved. Add to this the need for the tests to be highly specific yet simple to understand. There are various analytical methods which will fulfil some of these criteria. Mass spectrometry can be highly specific provided the analyte is known and has a unique molecular ion signature but it can require extensive preparation to extract samples from complex matrices hence it is generally only used by public analysts and similar monitoring authorities. Similarly, DNA-based assays are frequently recommended as the most sensitive and specific form of testing, but unfortunately not all food samples contain DNA nor is it possible to detect contaminants such as pesticides, herbicides, and insecticides which have no nucleic acid components. Therefore, these assays have limited use despite their sensitivity and high specificity.

There is one assay format which can fulfil all the requirements: the immunoassay [1,2]. Although there are many different forms of immunoassay, they all have similar features which render them rapid, specific, sensitive, and low cost. In essence, the technology of the immunoassays is built into the molecules. The correct combination of antibody, antigen, and indicator can be used to analyse almost every possible analyte regardless of the complexity of the associated matrix if the assay is in the appropriate format. Now the repertoire of antibodies is supported by an additional range of binding ligands, aptamers [3]. In addition to widening the range of detectable moieties, aptamers allow the analyst to design and synthesise ligands without relying upon expensive and potentially unreliable methods related to production in animals, polyclonal antibodies, or *in vitro*, monoclonal antibodies.

Of course these statements have to be justified. Simply the claim is that immunoassays in various formats with antibodies or aptamers provide all of the assays in all the formats with all the specificities, sensitivities, and simplicity, which the food industry requires, such that there is no need to resort to the use of other expensive, complex, and time-consuming assays.

If each of the properties required of assays is considered individually, it is possible to understand how the claims being made for the immunoassays indicate their superiority over all other assay methodologies. The basis of the immunoassay is the interaction between an antibody and its specific antigen partner. There are many ways to detect the antigen/antibody interaction. The history of immunodiagnostics shows that methods of detection have developed as techniques have improved and assay techniques have developed [4]. The development of enzyme labels has allowed the production of rapid, visual detection systems. Labelling of antibodies with enzymes takes advantage of the structure of antibodies which gives antibody molecules many of the other advantages upon which enzyme-linked immunosorbent assays are dependent. Fig. 3 shows the generalized structure of immunoglobulin G (IgG).

The important features of the IgG molecule are that it consists of two fractions, antigen-binding fractions (Fab) and the crystalisable fraction (Fc). Significantly there are two identical antigen-

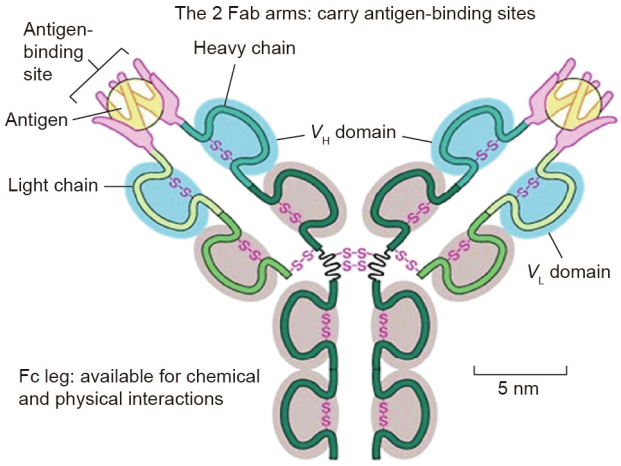


Fig. 3. Generalized structure of IgG. Fab: antigen-binding fragment; Fc: crystalisable fraction; V_H: heavy chain variable; V_L: light chain variable.

binding sites which allows the antibody molecule to interact with two antigens simultaneously. Similarly, the structure means that there is no interference with the binding sites when labels and other molecules are chemically linked to the antibody via the Fc region. These are amongst the many features which have allowed the development of a plethora of assay formats. There are essentially only three assay formats but there are many variations on these three basic formats: non-competitive, competitive, and sandwich with the options of direct or indirect detection of the antibody antigen. Thus the basic concept of the immunosorbent assay is that the antibody is fixed via its Fc region to a solid phase leaving the Fab free to bind antigen. This binding reaction is then detected either by means of a second labelled antibody producing a sandwich assay or labelled antigen giving either competitive, unlabelled antigen competes with the same antigen labelled, or non-competitive, the antigen being measured is labelled prior to assay (Fig. 4).

The development of the appropriate label has been an important factor in immunoassay development. Initially Ekins [5] and Yalow and Berson [6] described immunoassays which used radioactive labels. However, it was the introduction of enzyme labels, thereby removing the problems of handling radioactive material, which allowed the rapid development of immunoassay applications. Horseradish peroxidase is the most commonly used enzyme. It converts hydrogen peroxide to oxygen and water, the free oxygen reacts with an appropriate reagent which changes color when oxidised. There are a number of substrates available which allow the analyst a range of options. However, an alternative method of visualisation used in rapid format assays, lateral flow and transverse flow, is to attach a solid particle such as colloidal gold or colored latex nanoparticles, which effectively reduces the number of steps involved in the assay and also provides a visual indicator in "yes/no" assays. Two examples of which are shown in Fig. 5.

Such assays may seem relatively simple compared with the highly sophisticated assays such as mass spectrometry, surface plasmon resonance, and real-time TaqMan polymerase chain reaction (PCR). However, it is their very simplicity that is the significant advantage because, with all the technology designed within the assay, immunoassays can outperform each of the other systems. For example, the speed of assays, such as surface plasmon resonance, is often cited as an important factor. Many assays require prior extraction of the specific analyte before the analysis can be attempted, but with immunoassays provided the analyte is soluble in an aqueous buffer, all the preparation needed is to dissolve the sample and then the antibody will provide specificity and sensitivity. An example of the development of immunoassays along these lines is the assay for soya. Originally Hitchcock et al. [7] developed an immunoassay which took 5 d because of the time taken to extract the soy protein, however, Rittenburg et al. [8] reduced the time to 30 min by using the 6 $mol \cdot L^{-1}$ urea to extract and denature the protein and then measuring the renatured soy which was produced when the urea was diluted.

Table 1 shows the rapidity of the assays.

Another supposedly important requirement for the analysis of adulterated food is sensitivity but adulteration of food is not something which involves small amounts of material. Indeed, the purpose of adulteration is to make money and therefore the more of the adulterant that can be introduced into the food the larger the profit made. Hence sensitivity is not essential for the detection of adulteration although sensitivity is. A comparison of the reported sensitivities of different assays shows that immunoassays can be more sensitive than the most sensitive alternative formats (Table 2).

As can be seen from Table 2, the enhanced enzyme-linked immunosorbent assay (ELISA) reported in 1989 [9] can be 100 times more sensitive than the most sensitive DNA assay. In fact, commercial meat speciation assays are deliberately lowered in sensitivity.

The other aspect of food analysis is contamination for which sensitivity is required because unlike adulteration, contamination is essentially accidental and can occur in relatively small amounts which can still do considerable harm. (Table 3 shows a comparison between adulteration and contamination.)

Immunoassays are capable of considerable sensitivity an important aspect when considering the detection of toxic pesticides which cause major health problems even at very low concentrations. Similar considerations also apply to the detection of microbial contaminants. However, if assays for these types of materials are considered DNA-based assays are not possible because the pesticides, herbicides, insecticides, and bacterial toxins are not composed of DNA, RNA, or nucleotides.

Currently food scientists have to consider much more when attempting to ensure food quality. Food production has moved to larger scales with more stages in the food chain from producer to consumer. And there are more opportunities for deliberate adulteration and for accidental contamination. In order to deal with this increasingly complex situation there must be continuous vigilance. In order for the monitoring to be successful, the different types of adulterants need to be known. This is only possible if there is constant vigilance and checking of food samples to identify novel adulterants. Despite centuries of legislation, adulteration is a continuing and increasingly complex problem. Some forms of adulteration are harmful to those eating them whilst others are not. These latter types of adulteration usually involve fraud. The replacement of foods with cheaper options without reducing the price results in the purveyor increasing their profits. Simple examples of such fraudulent activities are reported to include the replacement of sheep's milk with cow's milk when making sheep's cheese, this does no harm apart from financial loss, other frauds such as mixing pork or horse meat with beef to increase the bulk of the beef being sold does more harm because it breaks various religious and social taboos leading potentially to psychological harm in addition to the financial damage and finally the adding of poisons such as Sudan Red and melamine to foods to enhance their color or apparent protein content which can lead to death

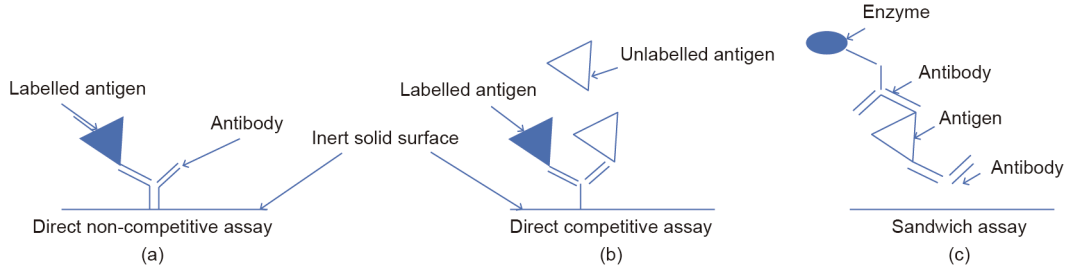
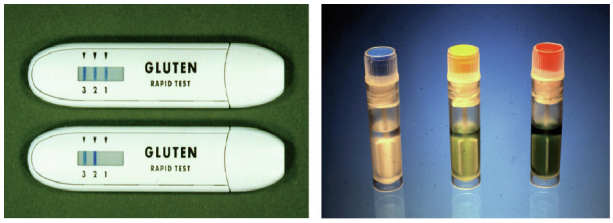


Fig. 4. Basic assay formats of the detection of binding reactions. (a) Direct non-competitive assay; (b) direct competitive assay; (c) sandwich assay.



(a) (b)

Fig. 5. Rapid assay formats. (a) Lateral flow with latex labeling to visualize results; (b) dipstick format with enzyme-moderated visual detection.

Table 1
Comparison of the different assay systems based on time per individual assay.

Type of assay	Number of simultaneous assays	Time per experiment (min)	Time per individual assay (min)
ELISA	20 + controls per 96-well plate	40	2
Dipstick	Single sample	5	5
Transverse flow	Single sample	2	2
Lateral flow	Single sample	2	2
Surface plasmon resonance	Single sample	2	2

ELISA: enzyme-linked immunosorbent assay.

Table 2
Sensitivity of meat species assays used for adulteration testing.

Date	Format	Pork serum albumin detected in beef samples (%)
1989	Direct non-competitive ELISA	1.00
1989	Enhanced ELISA	0.005
2002	Quantitative PCR	1.00
2005	TaqMan real-time PCR	0.5–5.0

Table 3
Comparison between adulteration and contamination.

Adulteration	Contamination
Deliberate	Accidental
Large quantities	Small amounts
Normally does not involve contamination	Includes microbial content
Will be disguised as colorings or flavorings	May be visually obvious
Involves cost	—

of the consumers. Thus it is vital to consider whether the requirement for regular, detailed monitoring of food necessitates the development of high throughput, or multianalyte assays. Once again immunoassays stand out in their potential to be organised into robotically managed high-throughput assays. At a very simple level multi-line lateral flow assays such as that shown in Fig. 6 can be produced to provide rapid on-site preliminary screening assays at low cost for use in the food industry.

It is possible to produce such simple positive/negative assays with considerable specificity for a very wide range of chemical contaminants. One Chinese laboratory (the State Key Laboratory of Food Science and Technology, Jiangnan University) has produced over 100 different immunoassays, examples being the rapid detection of clonidine in pig urine, a non-invasive test which can be applied prior to slaughter [10] and detection of fumonisin B1 [11].

Immunoassays in various formats appear to be able to fulfill all the requirements identified for the ideal assays required by the

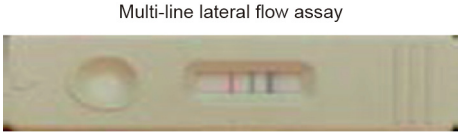


Fig. 6. A multi-line lateral flow assay capable of providing rapid on-site preliminary screening assays at low cost.

food industry. However, there are several areas which merit further efforts. The production of antibodies may raise ethical questions because of the necessity of using animals or cells derived from animals to produce them. However, there is an alternative. Aptamers are short nucleotide sequences which exhibit the same binding specificities and sensitivities as antibodies but they have the advantage that they can be synthesised *de novo*. Selection from naïve libraries of the specific aptamer needed allows the identification of the required aptamers. And once their sequence is known it is possible to synthesise them as needed thus eliminating the ethical issues associated with using animals [12].

The development of novel foods, for example foods developed from insects, provides another opportunity for immunoassay research as the novel food provides more and different opportunities for adulteration and contamination in unusual matrices. It is probable that flours made from alternate grain sources or from insect proteins will be introduced into the diet via mixed grain and mixed protein products.

In conclusion, to ensure food safety and to protect the population from fraudulent activity requires constant vigilance in the form of monitoring and testing at all stages along the food chain. There is already a considerable amount of legislation dating back over a period of some 10 000 years but the existence of laws does not prevent adulteration and contamination. The introduction of block chain methodologies may ensure the security of the food between fixed points at which it is checked but there is still a need for testing at each point where the block chain has a break. There are already a considerable number of assays available but the list is by no means comprehensive leaving the opportunity for researchers to develop new tests. The formats which can provide the food industry with the type of routine, rapid, simple, cheap, on-site assays are already available. It is probably essential to also develop confirmatory testing systems using complex, sophisticated methods but these will not provide routine assays for the industry. Finally, there is a desperate need for food standards which can be used in the quality control of the tests themselves. In many cases the tests are developed in a laboratory setting with little consideration of the matrices in which the analyte might be found or to what processing, heating, drying, pickling, etc., that the analyte might have been subjected. Hence the preparation of standard samples adulterated or contaminated in specific ways and then subjected to conventional processing prior to analysis in order to demonstrate the functionality, accuracy, and precision of the assays is deemed essential. This effort needs to go further as there is probably a need to consider oven-ready/microwave-ready whole meals which may be adulterated or contaminated in a variety of different ways as a result of the considerable variety of components. To achieve this, it is essential to understand how the matrices influence the sensitivity and specificity of the assays.

Thus more tests are needed which have been validated in real foods and quality controlled using reference standards. All of these factors should be considered when agreeing internationally approved standards for food quality and safety.

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