IMMUNOASSAYS, APPLICATIONS

Contents

Clinical

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Introduction

Antibodies were used for the detection and quantitation of clinically important substances long before the development of immunoassays by Rosalyn Yalow and Solomon Berson in the 1960s and 1970s. However, since its first use for the measurement of insulin in blood, the immunoassay has become established as the most popular analytical technique applied in clinical medicine. Immunoassays are the first resort for the detection and measurement of a large number of hormones, blood products, enzymes, drugs, nutritional factors, and disease markers (**Table 1**). The popularity of the technique stems from its simplicity, rapidity, accuracy, and portability, the last allowing the technique to be adapted for 'near-patient testing'. In most cases, immunoassays can be performed directly on untreated samples, such as plasma, serum, urine, saliva, and cerebrospinal fluid. While renowned for its ability to detect and quantify single analytes, more recent developments have seen the emergence of highly multiplexed immunoassays for the detection and quantification of hundreds or even thousands of analytes simultaneously. These novel platforms have permitted a shift in perspective, in which immunoassays are used to generate profiles of clinical samples that can facilitate accurate disease diagnoses and the prediction of drug responses.

Types of Immunoassay Used in Clinical Applications

Immunoassays are analytical methods that achieve the detection and quantitation of analytes in clinical samples through the formation of a stable complex between the analyte and a specific antibody. The first immunoassay techniques were based on the principle of competition between the analyte and a radiolabeled tracer with the same antigenic properties as the analyte. These two components would compete to occupy a limited number of antibody molecules. The amount of analyte present in the sample could be determined when the system reached equilibrium by separating the bound and unbound fractions of the tracer, and measuring the latter. The more analyte present in the sample, the more tracer would be displaced from the antibody and the greater the strength of the radioactive signal from the unbound fraction.

Such assays were described as heterogeneous because they depended on the separation of free and complexed tracer components, a process that was necessary because the label itself was unaffected by antibody binding. This is not necessarily true of non-isotopic labels such as enzymes and fluorophores, whose activities are often modified when the antigen to which they are attached interacts with its cognate antibody. Where antibody binding either inhibits or stimulates the signal generated by such a label, separation into free and complexed components is no longer necessary, and such assays are described as homogeneous. The main advantage of homogeneous assays is the absence of a separation step, which reduces the hands-on time and the likelihood of technical errors.

One of the disadvantages of the competition assays discussed above is that it is necessary to ensure that the amount of tracer added to the sample is not vastly in excess of the amount of analyte, and that the antibody is present at a lower molar concentration than the tracer. This means that very small amounts of reagents are used, which places high demands on the sensitivity of the assay. This problem was addressed by placing the label on the antibody rather than the analyte, as first demonstrated in the case of the immunoradiometric assay. In this format, quantitative data are generated by establishing a stoichiometric interaction between the analyte and the antibody. The signal produced by the antibody is thus directly proportional to the concentration of the analyte, rather than being related to it in a complex manner as is the case for competition analysis.

Table 1 Representative list of immunoassays of major clinical interest

Substance	*Sample*	*Clinical indications*
Polypeptide hormones		
ACTH	Plasma	Distinguishes Cushing syndrome and Addison's disease
AVP	Plasma	Differential diagnosis of diabetes insipidus
Calcitonin	Plasma	Diagnosis of medullary carcinoma of the thyroid
C-peptide	Plasma	Differential diagnosis of hypoglycemia
Erythropoeitin	Plasma	Investigation of anemia/polycythemia
FSH	Plasma	Investigation of infertility
Gastrin	Plasma	Diagnosis of Zollinger–Ellison syndrome
Glucagon	Plasma	Diagnosis of glaucoma
Growth hormone	Plasma	Diagnosis of acromegaly
	Urine	Diagnosis of growth hormone deficiency
hCG	Urine	Confirmation of pregnancy
Insulin	Plasma	Differential diagnosis of hypoglycemia
IGF-I	Plasma	Monitoring acromegaly, response to hCG therapy
IGF-II	Plasma	Differential diagnosis of hypoglycemia
LH	Plasma	Investigation of infertility
Neurotensin	Plasma	Investigation of diarrhea
Pancreatic polypeptide	Plasma	Diagnosis of APUDomas
Parathyroid hormone	Plasma	Differential diagnosis of hypo/hypercalcemia
Proinsulin	Plasma	Differential diagnosis of hypoglycemia
Prolactin	Plasma	Investigation of infertility, galactorrhea
Somatostatin	Plasma	Diagnosis of somatostainoma
TSH	Plasma	Investigation of thyroid disease
VIP	Plasma	Differential diagnosis diarrhea
Steroids		
Aldosterone	Plasma	Investigation of hypertension, hypokalemia
Androstenedione	Plasma	Investigation of hirsutism, virilism, infertility
Bile-salts	Plasma	Investigation of liver disease
Cortisol	Plasma	Diagnosis of Cushing syndrome and Addison's disease
DHAS	Plasma	Investigation of hirsutism, virilism, infertility
11-Deoxycortisol	Plasma	Investigation of virilism
Dihydrotestosterone	Plasma	Investigation of virilism, impotence, feminization
17α-Hydroxyprogeseterone	Plasma	Investigation of congenital adrenal hyperplasia
Estradiol	Plasma	Investigation of infertility
Progesterone	Plasma	Investigation of infertility
Testosterone	Plasma	Investigation of virilism, impotence, infertility
Amino/fatty acid derivatives		
5-HIAA	Urine	Diagnosis of carcinoidosis
Melatonin	Plasma, urine	Investigation of sleep disorders
Reverse T_3	Plasma	Investigation of thyroid disease
Thyroxine	Plasma	Investigation of thyroid disease
Triiodothyroxine	Plasma	Investigation of thyroid disease
Proteins		
Apolipoprotein A1	Plasma	Assessment of coronary heart disease risk
Apolipoprotein B	Plasma	Assessment of coronary heart disease risk
CK-MB	Plasma	Differential diagnosis of chest pain
C-reactive protein	Plasma	Monitoring response to anti-inflammatory therapy
Ferritin	Plasma	Investigation of iron storage capacity
Lipoprotein	Plasma	Assessment of coronary heart disease risk
Sex-hormone-binding globulin	Plasma	Investigation of hirsutism, virilism, infertility
Thyroxine-binding globulin	Plasma	Investigation of thyroid disease
Thyroglobulin	Plasma	Investigation of thyroid disease
Trypsin	Plasma	Investigation of thyroid disease
Disease markers		
α-Fetoprotein	Plasma	Investigation of liver cancer and other cancers
β-2-Microglobulin	Plasma, CSF	Prognosis of myeloma and lymphoma, neronal involvement
CA 15-3	Plasma	Monitoring breast cancer
CA 125	Plasma	Monitoring ovarian cancer

Table 1 Continued

Substance	*Sample*	*Clinical indications*
CA 549	Plasma	Monitoring breast cancer
CEA	Plasma	Monitoring colorectal cancer
HCG	Plasma	Monitoring choriocarcinoma
MCA	Plasma	Monitoring breast cancer
NSE	Plasma	Monitoring neuroectodermal tumors
PSA	Plasma	Monitoring prostate cancer
SCC	Plasma	Monitoring squamous cancer of cervix, lung etc.
Nutritional factors		
Vitamin B_{12}	Plasma	Investigation of anemia, B_{12} status
Folic acid	Plasma	Investigation of anemia, folate status, malabsorbtion
Vitamin D	Plasma	Investigation of metabolic bone disease
25-Hydroxy-vitamin D	Plasma	Investigation of metabolic bone disease
1,25-Dihydroxy-vitamin D	Plasma	Investigation of hypercalcemia and bone disease

ACTH, adrenocorticotrophic hormone; APUD, amine precursor uptake and decarboxylation; AVP, arginine vasopressin; CEA, carcinoembryonic antigen; CK-MB, creatine kinase MB isoform; CSF, cerebrospinal fluid; DHAS, dehydroepiandrostendione sulfate; hCG, human choriogonadotrophin; HIAA, hydroxyindoleacetic acid; FSH, follicle-stimulating hormone; IGF, insulin-like growth factor; LH, lutenizing hormone; NICTH, non-islet-cell tumor hypoglycemia; NSE, neuron-specific enolase; PSA, prostrate-specific antigen; SCC, squamous cell carcinoma; TSH, thyroid-stimulating hormone; VIP, vasoactive internal polypeptide.

The immunometric-type assay has also been adapted for use with nonisotopic labels and is typically carried out in a heterogeneous format in which the antibody is immobilized on a solid support, such as a microtiter dish, membrane, or collection of beads. The canonical clinical immunoassay format in toady's laboratories is the enzyme-linked immunosorbent sandwich assay, which employs two antibodies, one to capture the analyte and the other to detect and quantify it. More details of the principles of these and other immunoassay techniques are given elsewhere in this encyclopedia.

Specific Applications

Detection of Polypeptide Hormones

The first clinical immunoassays were developed for the detection of relatively large polypeptide hormones such as insulin and glucagon, since these are naturally immunogenic and can therefore stimulate the production of antibodies when injected into animals. Antibodies with binding characteristics suitable for competition assays were produced for a range of polypeptides with molecular masses in excess of 3 kDa, but this was not a suitable approach for the production of antibodies against smaller peptides, thyroid hormones, or small-molecule drugs. Solid-phase immunoassays have also been developed for a broad range of polypeptide hormones. The most widely used of these is probably the test for human choriogonadotrophin (hCG) in urine, which is the underlying principle of home pregnancy-test kits.

Detection of Steroid and Amino Acid/Fatty Acid-Derived Hormones

With the rediscovery of Landsteiner's observations that even very small molecules could be rendered immunogenic by linking them covalently to a protein, it became possible to develop immunoassays for almost every molecule of clinical interest. The main medical application of immunoassays remained in endocrinology, producing a revolution in clinical practice by facilitating the detection and quantitation of steroids (such as aldosterone and cortisol) and of hormones derived by the biotransformation of single amino acids and fatty acids (such as thyroxine, melatonin, and 5-hydroxytryptophan). Not only is it possible to measure these hormones at the infinitesimally low concentrations at which they naturally occur in tissues, plasma, and other body fluids, but the assays are sufficiently rapid and economical to be useful in day-to-day patient care.

Detection of Therapeutic Agents and Drugs of Abuse

Once the raising of antibodies to small molecules ceased to be a technical problem, the application of immunoassays to the detection and measurement of drugs in body fluids as part of the process of therapeutic drug monitoring became commonplace. The number of drugs for which immunoassays have been developed is extensive, although comparatively few have found a place in regular clinical practice (**Table 2**). Similar techniques have been employed for the semiquantitative detection of drugs of abuse in urine, blood, saliva, and body tissues. In this context,

Table 2 Clinically useful drug immunoassays

Therapeutic drug monitoring		
Anticonvulsants		
Carbamazepine	Ethosuximide	Phenobarbitone
Phenytoin	Primidone	Valproic acid
Antibiotics		
Amikacin		Chloramphenicol
Gentamicin		
Netilmicin	Tobramycin	Vancomycin
Cardioactive drugs		
Digoxin	Diisopyramide	Lignocaine
Procaineamide	Quinidine	
Psychoactive drugs		
Amitriptyline	Desipramine	Imipramine
Nortriptyline		
Others		
Caffeine	Cyclosporin A	Methotrexate
Theophylline		
Drugs of abuse screening		
Amphetamines	Barbiturates	Benzodiazepines
Canabinoids	Cocaine	LSD
Opiates	Phencyclidine	

immunoassays are advantageous over most of the other available methods for drugs testing because they are sensitive, inexpensive, and simple to perform. This has led to them being used as screening techniques in sport, where drug abuse is common, as well as in more commonplace clinical settings.

Blood Products, Enzymes, and Other Proteins

Increasingly, plasma proteins that were once measured exclusively by their catalytic activities and expressed in terms of enzyme units are now being measured by immunoassay techniques, and expressed in terms of concentration (e.g., micrograms per liter). When the results from these very different forms of analysis are compared, there is often little correlation. In many cases, this reflects the fact that proteins can exist in multiple structural forms, differing in conformational properties, in terms of proteolytic cleavage, or in terms of specific forms of post-translational modification (e.g., phosphorylation). While these forms may retain some conserved epitopes, and thus have common immunological properties, structural differences often have a profound effect on their activities. For example, it has been found that the blood protein factor VIII, which is one of the key proteins in the blood clotting cascade, loses its catalytic activity in hemophilia while retaining many of it immunological properties. Consequently, while factor VIII may appear to be absent from the blood when measured in terms of its ability to convert fibrinogen into fibrin, is found to be present at normal levels when measured by immunoassay.

Disease Markers

In the context of disease, immunoassays were first used for the management of patients suffering from choriocarcinoma, whose tumors produce excessive amounts of hCG. Until the 1970s, this disease was invariably fatal within a few months of diagnosis. However, with the advent of specific chemotherapeutic treatments for tumors of trophoblastic origin and the development of ultrasensitive immunoassays for hCG in plasma as a guide to how long therapy should be continued, the prognosis for complete recovery is now greater than 90%. By ensuring that treatment continues until hCG can no longer be detected, it becomes possible to eliminate every neoplastic cell in the body and avoid recurrence.

The application of immunoassay techniques to the detection and quantification of antigens specifically present or absent in disease has grown enormously since these early successes. Indeed, at the current time, it is estimated that over 1000 recombinant antibodies recognizing disease-specific targets are being developed by biopharmaceutical companies around the world. A large proportion of these antibodies recognize cancer antigens but others have been developed for the diagnosis (and treatment) of infectious diseases, autoimmune disorders, as well as blood, neurological, cardiovascular, skin, respiratory, and eye diseases.

Immunoassay techniques can be used not only for the detection and quantitation of disease-specific markers, but also for their discovery. This has been facilitated by the development of highly multiplexed immunoassay formats, otherwise known as antibody arrays. For example, an array containing 146 antibodies recognizing proteins involved in cell cycle regulation, stress response, and apoptosis has been produced for the screening of tumor samples. VoLo carcinoma cells were irradiated with a cobalt-60 source and cultured for 4 h before protein extracts were obtained and labeled with the fluorogenic molecule Cy3. Protein extracts from parallel cultures of nontreated cells were labeled with Cy5, which is similar to Cy3 but has a different emission wavelength, allowing protein levels in the two samples to be compared on the same array. These experiments identified 11 proteins that were upregulated in colon cancer, six of which were previously not known to be

involved. Most of these proteins had roles in apoptosis, and increased apoptosis of the cells was observed after radiation treatment. Another protein, the carcinoembryonic antigen (which is used in the immunodiagnosis of colorectal cancer), was shown to be downregulated.

Although there have been several reports of novel biomarkers identified using antibody arrays, it is rare to find a single diagnostic marker that is reliable in a clinical setting. However, the ability to determine the levels of hundreds of protein simultaneously allows the overall pattern of protein expression to be used as a diagnostic tool, a method known as pattern profiling (**Figure 1**). Disease diagnosis is achieved by feeding the antibody array into a mass spectrometer and looking at the mass spectra produced by surface-enhanced laser desorbtion/ionization (SELDI). This provides higher sensitivity than the analysis of single biomarkers, which are often expressed in multiple diseases making a precise diagnosis difficult. This is especially true in closely related diseases, such as different forms of cancer or dementia.

A useful example of SELDI pattern profiling is the early diagnosis of ovarian cancer, a disease that is usually detected at the late stage when cancer cells have already spread and the prognosis is poor. In the original study, mass spectra derived from the serum samples of women with ovarian cancer and from unaffected controls were used as a training set for a pattern-matching algorithm. A discriminatory pattern was identified, which was applied to another set of samples. This resulted in the correct diagnosis of all ovarian cancers (including 18 stage I cancers, where the prognosis is favorable because the neoplastic cells are still contained within the ovary) and a false positive rate of only 5%. Similar algorithms have been used to diagnose breast and prostrate cancers. In each case, the sensitivity of the pattern-profiling method has been significantly higher than tests relying on the presence of single biomarkers, and in at least one study the sensitivity of the method has been high enough to achieve 100% correct diagnosis. This new approach to disease detection could revolutionize the clinical laboratory, providing the means to process more samples than before and detect diseases earlier and more accurately than is currently possible using standard immunoassay methods.

Limitations of Immunoassays in Clinical Settings

Specificity

The early claims for the almost absolute specificity of immunoassays for particular antigens were based more upon what was then understood about the specificity of antibody–antigen interactions than on empirical evidence. For example, antibodies raised against insulin did not react against any other proteins known at the time, or against any other known substance. It soon became clear, however, that 'specificity' in the context of an immunoassay is a relative term. Nowhere was that message clearer than in relation to glucagon, which was among the first few polypeptide hormones for which radioimmunoassays were developed.

Antisera raised against pancreatic glucagon, the only form of the hormone known at the time, reacted with substances that could be extracted from the gut, and which were also present in the circulation, but with sufficient differences from native glucagon to suggest their nonidentity. These substances differed from glucagon not only immunologically but also in their biological properties. Portions of the molecule

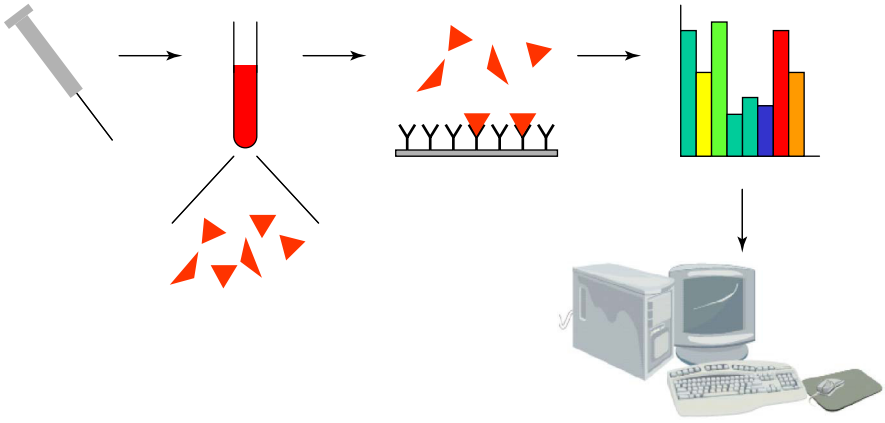

Figure 1 Protein patterns in disease diagnosis. A blood sample contains many proteins, which can be captured and quantified on an antibody array. The relative abundances of the proteins provide a unique signature, or fingerprint, which can be detected by specialized algorithms and used to diagnose disease and classify different forms of tumor. (Reproduced with permission from Twyman RM (2004) *Principles of Proteomics*. Abington, UK: BIOS/Garland Publishers.)

that were essential for biological activity were not necessarily immunogenic, while portions that were extremely immunogenic were seemingly irrelevant in terms of biological activity.

Because many molecular variants of a hormone with shared epitopes but different biological properties may exist in the plasma at the same time, it may be very difficult to measure any one of them accurately and precisely without their prior separation by chromatography or a similar method. Investigations stemming from the problem of immunoassay specificity have undoubtedly led to a greatly increased understanding of the nature of immunological determinants or epitopes. The knowledge so gained has made it possible to develop improved immunoassays with more appropriate specificities. This has been particularly important, for example, in the development of immunoassays for parathyroid hormone in plasma, where an ability to differentiate between the active hormone and its inactive degradation products is essential if the results of the assay are to have any clinical validity. Ironically, while the situation for insulin is similar (in that insulin immunoassays pick up not only mature insulin, but also proinsulin and split insulin fragments that continually circulate in the plasma) the pan-specificity of the immunoassay has been clinically beneficial, as the following example shows.

Despite the enormous contribution that the ability to measure insulin has made to our understanding of disease (especially diabetes), the most reliable clinical indication for abnormally high insulin levels is hypoglycemia (low blood glucose). One of the most important of the uniformly rare diseases causing hypoglycemia is a small benign tumor of the pancreas often referred to as an insulinoma. Left untreated, such tumors can cause potentially fatal hypoglycemia, but they can be cured by surgical excision. The biochemical hallmark of insulinoma is the continued secretion of insulin in the presence of low blood glucose, which normally inhibits it. However, many such tumors produce large amounts of proinsulin, either alone or in addition to insulin. Therefore, the ability of insulin immunoassays to detect these tumors relies upon the antibody recognizing proinsulin as well as the mature form. If the clinical insulin assays were absolutely specific for mature insulin, their usefulness would have been reduced rather than enhanced! Unfortunately, other diseases, including insulin resistance, require accurate measurements of the levels of mature insulin in the blood. Attempts to investigate insulin resistance using comparatively nonspecific immunoassays that also recognize unprocessed forms of the protein are likely to yield unreliable results.

The problem of antibody specificity has also arisen in the context of multiplex immunoassays (antibody arrays). This reflects the fact that most antibody arrays are currently used to detect particular, restricted classes of proteins, often cytokines or other secreted factors that are released into the serum or culture medium. Such antibodies have been developed especially for serum profiling and in many cases have not been checked for broader cross-reactivity, e.g., in cell lysates. In the few studies that have addressed this issue, the data suggest that up to 50% of antibodies used on chips cross-react with nontarget antigens. The proteins in a typical clinical sample cover a broad dynamic range, so antibodies with high affinity for a scarce target analyte and low cross-affinity for an abundant nontarget analyte might bind both equally well. This would provide a completely false indication of the relative abundances of the two analytes in the sample. It is therefore likely that many of the antibodies currently used for single-target immunoassays will be unsuitable for antibody arrays.

Monoclonal, Polyclonal, and Recombinant Antibodies

Although the lack of specificity described above was at first thought to be confined to assays using polyclonal antibodies and would not occur with assays constructed using monoclonal antibodies, it is now quite clear that this was based on a misconception. The problem of cross-reactivity is not so much to do with the multiple antibody types present in polyclonal sera but instead reflects the conservation of epitopes between target and nontarget proteins, such as the processed and unprocessed forms of insulin. The great advantage of monoclonal antibodies is that, because they are produced from an immortal strain of cells rather than a single (mortal) animal, they can be characterized much more thoroughly than a polyclonal antibody. Also, from the point of view of a kit manufacturer, continuity of supply can be guaranteed.

Cross-reactivity can be reduced using the sandwich assay approach, because two noncompeting antibodies (i.e., antibodies recognizing different epitopes of the antigen) are required for each target protein. However, even this does not guarantee specificity. More recent approaches to antibody generation, such as phage display, can be used to circumvent some of the limitations of conventional hybridoma technology and select antibodies that show specificity for particular variants of a given protein. However, maximum specificity and sensitivity requires a combination of immunoassay and physical separation techniques, such as electrophoresis or liquid chromatography.

Table 3 Protein-bound analytes of major clinical significance

Analyte	*Binding protein*	*Factors influencing abundance of binding protein*
Cortisol	Transcortin	Increased by estrogens, pregnancy
IGF-I, IGF-II	IGF-binding proteins (IGF-BPs)	Growth hormone increases IGP-BP 1 and 3 Insulin decreases IGF-BP 1
Insulin	Autoantibodies	Autoimmune disease
Testosterone	Sex hormone binding globulin	Estrogen, thyroxine and pregnancy increase, testosterone decreases
Thyroxine and T_3	Prealbumin	Malnutrition decreases
	Thyroid binding globulin	Estrogens, pregnancy increases Adrogens, malnutrition decreases
Vitamin A	Retinol-binding protein	Zinc deficiency decreases

This approach has been used, for example, to measure tetrahydrocannabinol (the active ingredient of cannabis) in blood plasma in the presence of much higher concentrations of its inactive metabolites, and insulin in the presence of proinsulin and its split products.

Standardization

It is an absolute requirement of all validated immunoassays, whether competitive or immunometric, that for accurate quantitative analysis the standard material used to calibrate the reaction is identical to the analyte. This may be difficult for several reasons, including the chirality of the analyte and its heterogeneity in terms of post-translational modification.

Chirality is generally only a problem in clinical applications when the assay involves a comparatively small molecule, such as a drug or a small synthetic peptide. Antibodies, like most biological reagents, exhibit 'handedness' or chiral specificity. Therefore, where the analyte occurs as a mixture of enantiomeric forms, only one enantiomer may bind to the antibody, or different enantiomers may bind with different affinities. The relevance of the standard depends on whether it is presented as a pure enantiomeric form, or as a mixture of enantiomers that reflects the composition of the analyte.

Where the analyte is a protein, variations in the type and amount of post-translational modification are important. Most proteins produced in mammalian cells are modified either by phosphorylation, glycosylation, or some other form of chemical adduct. In the case of phosphorylation, a protein may have several target sites for the addition of phosphate groups and may exist as a complex mixture of phosphoforms, not all of which will bind to the antibody with equal affinity. The situation with glycoproteins is even more complex, since there may be hundreds of different variants. In many cases, the protein used as a standard in immunoassays is a recombinant protein produced in a heterologous expression system. It should be noted that mammalian proteins synthesized in bacteria are neither phosphorylated (at least not on the typical serine, threonine, and tyrosine residues) nor glycosylated. Additionally, the glycan structures produced in different expression systems are very diverse, and even mouse cells produce subtly different glycans to human ones. A further consequence of this diversity is that the results obtained using one manufacturer's antiserum, label, and standards are generally incompatible with those obtained using another's.

Interference from Binding Proteins

Most clinical immunoassays require neither prior extraction nor purification of the sample containing the analyte before they are added to the reaction containing the appropriate amounts of high-affinity specific antibody and label. This is not the case, however, for analytes such as thyroxin or cortisol, which are tightly bound to highly avid binding proteins that compete with the antibody for both the analyte and the label. Some of the more important protein-bound analytes are shown in **Table 3**.

Similar constraints apply when the patient has developed antibodies of their own to the analyte, either as a result of autoimmune disease (e.g., in the case of thyroxine) or prior immunization (e.g., in the case of insulin or growth hormone). Under these circumstances, extraction of the sample prior to analysis is essential if reliable and accurate results are to be obtained.

See also: **Immunoassays**: Overview; Production of Antibodies. **Immunoassays, Applications**: Forensic. **Immunoassays, Techniques**: Enzyme Immunoassays.

Further Reading

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Food

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Introduction

National and transnational regulatory frames, improved awareness of health- and quality-related issues, and growing occurrence among the general population of adverse reactions toward some food components have increased the need for specific and sensitive methods capable of assessing specific food constituents, as well as additives, adulterants, and contaminants.

In many cases, only immunoassays are able to fulfill these requirements, and thus are gaining increased recognition as regular analytical methods in laboratories involved in food research or inspection. This trend is supported by a growing array of companies that supply the materials – and develop or perfect procedures – for this type of application. The increased diffusion of these techniques has also caused enzyme-based immunoassays to become the most popular methodology, in that it does not bring up all the regulatory issues and does not require the sizable investments associated with the use of radiochemicals. In this frame, enzyme-linked immunosorbent assays (ELISA) offer the advantage of being convenient, of being suitable for automated handling of a large number of samples, and of being relatively cheap.

From the standpoint of sample preparations, foodstuffs are much more complicated systems than clinical samples, which represent the other major area of application for the different techniques used in immunoassays. Food comes in many different physical forms, and very often it has undergone technological processes (physical, chemical, enzymatic, alone or in combination) that may strongly affect the procedures to be developed for detection and/or recovery of a given analyte. This is especially true for proteins, whose structure (including epitopic regions or sequences) is highly sensitive to process-induced changes, which result in a modification of their immunoreactivity. In the case of proteins, changes may stem from process-induced cuts in the primary structure, from the introduction of nonphysiological amino acid through adduct formation, and from alteration in their high-order structures, which may affect their physical properties (such as solubility), as well as destroy conformational epitopes or hide sequential ones. In general terms, the processes used either by the food industry or in the preparation of meals at home may alter fundamental properties of the analytes, such as their solubility in a given extractant or their relationships to other food components.

Furthermore, analytes involved in food immunoassays are of extremely different chemical nature, and are present in a very broad range of concentrations. Whereas macromolecules (such as protein, enzymes, polysaccharides, and microbial cell-wall components) are generally present in the mg per kg range, environmental contaminants, residues from farming practices, antibiotics, toxins, and pathogens are often present at much lower concentration, as low as 0.1 μg per kg. Food analytes for which immunoassays have been developed and are commercially available are listed in **Table 1**.

Identification and Quantification of Selected Proteins

Immunoassays are broadly used in food analysis for detection and quantification of small quantities of proteins in a given food for three main reasons.