

H E A L T H

Technologies for Better Quality of Life

RADIOIMMUNOASSAY

Radioimmunoassay (RIA) is a radioanalytical technique with remarkable sensitivity and a high degree of specificity that is widely used for the estimation of a variety of molecules present in complex matrices. The application of this technique spans over a wide spectra of substances such as hormones, steroids, vitamins, drugs, tumor markers and viral antigens. Radioimmunoassay is an in vitro diagnostic technique and hence does not involve administration of radioactivity to the patient.



A radioimmunoassay in progress

The Department of Atomic Energy had started the RIA programme in the early seventies and the Board of Radiation and Isotope Technology (BRIT) supplies ready to use RIA kits for many hormones. The Bhabha Atomic Research Centre (BARC) also initiated a training programme in the year 1980 in order to make trained manpower for RIA.

Thanks to the early start of the RIA programme by BARC, currently there are over 500 RIA laboratories in India carrying out a few million RIA analysis per year.

Radioimmunoassay is one of the fine examples of the radiotracer technique using a radioisotope. The competition of an analyte with its radioisotopically labeled counterpart for a limited amount of antibody, the specific reagent, is the underlying principle of this technique. Increasing the analyte concentration inhibits the binding of the labeled analyte to the antibody. The concentration of the unknown is thus obtained by comparing its inhibitory effect on the binding of the labeled analyte to that of a known standard.

Immunoradiometric Assay

A more sensitive and specific technique is the immunoradiometric assay (IRMA), which is an improvement of the RIA principle. In IRMA, the analyte is incubated with an excess of radiolabeled antibody. Two-site IRMA technique, a further modification of the IRMA technique, uses two antibodies for sandwiching the analyte of which one of the antibodies is labeled with Iodine-125. Two site IRMAs have better specificity than conventional radioimmunoassays.

The RIA technique has seen several refinements over the years to make it more user friendly. One of the improvements is the introduction of solid phase assays. A separation of the bound analyte and the free analyte after RIA/ IRMA is essential. In solid phase assays, the reagent antibody is immobilized on solid phase such as polystyrene tubes, and the separation is carried out by simply decanting the contents of the reaction tube.

Magnetic particle bound antibodies offer a user-friendly and manufacturer-friendly technique for solid phase assays. In the solid phase assay using magnetic particles, the antibody is coupled to cellulose incorporated in magnetic particles. At the end of the incubation, the separation of bound and free analyte is carried out by placing the test tube rack on a magnet followed by decantation of the free analyte.



A radioimmunoassay quantification using a simple inexpensive counting instrument

The above improvements in RIA technology now enables processing large numbers of patient samples in a batch using automated equipment that are available from commercial sources. Automatic RIA counters that can quantify the radioactivity of an entire batch without intervention as well as processing the data to give the final results have made RIA much more user friendly.



Robotic sample processor for RIA/IRMA that can process samples automatically

Applications of Radioimmunoassay

Radioimmunoassay of thyroid hormones

An important application of radioimmunoassay is in the diagnosis of thyroid disorders. Levels of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) along with that of the thyroid stimulating hormone (TSH), is useful for diagnosis of thyroid disorders. TSH levels before and after thyrotropin releasing hormone (TRH) stimulation helps in the differential diagnosis of primary, secondary and tertiary hypothyroidism. The levels of thyroxine and thyroid stimulating hormones in neonates are used as a routine screening test to diagnose neonatal hypothyroidism.

Radioimmunoassay of Fertility Related Hormones

Estimation of human chorionic gonadotrophin (hCG) is useful for the detection of pregnancy and monitoring progress, to establish the gestational age as well as for proper management of patients with complicated pregnancies such as ectopic pregnancy, threatened abortion, foetal distress etc. RIA of luteinizing hormone (LH) and follicle stimulating hormone (FSH) is used for identifying the causes of infertility and better understanding of the problems associated with child bearing and infertility. Hormonal levels estimated by RIA are of great value for the management of medically aided conception.



A multi well automatic RIA counter

Estradiol determinations have proved to be very useful in many disorders such as investigations of precocious puberty in girls, differential diagnosis of amenorrhea, monitoring ovulation induction and functioning of ovaries. Levels of testosterone are useful in detecting hypogonadism, testicular tumours in males and diagnosis of hirsutism in females.

Radioimmunoassay of Other Hormones

RIA of growth hormone is useful in the early diagnosis of dwarfism in children as well as diagnose of gigantism or acromegaly. Prolactin levels are useful for identifying pituitary adenomas. Cortisol levels are useful in diagnosing Cushings' syndrome and Addison's disease.

Tumour Markers

RIA of tumour markers such as alphafetoprotein (AFP), carcinoembryonic antigen (CEA), b-hCG for chorio-carcinoma, prostate specific antigen (PSA) for prostate cancer, are available for detection and management of cancer. They serve as valuable tools for follow-up of treatment and detection of any recurrence.

RIA of Drugs

RIA of drugs has gained importance owing to the increasing concern for the safety and toxicity of the drugs. It is important to monitor plasma levels of drugs having narrow therapeutic window (therapeutic drug monitoring, TDM). Drugs such as phenytoin, theophylline, cyclosporin, morphine, gentamycin, antidepressants etc., are estimated by RIA. From heroin in drug abusers to steroids in athletes and antibiotics in patients, RIA can estimate the concentration of drugs in blood, urine and saliva.

Non-clinical Applications

Radioimmunoassay due to its versatility has expanded its horizon from clinical to non-clinical applications such as veterinary science, food processing industry, drug industry, forensic science and environmental monitoring. Increasing awareness of the role of toxic metals has placed greater reliance on these techniques.

Aflatoxins are the secondary metabolites of the fungi *Aspergillus* and is a major contaminant in food articles. Aflatoxins are potentially carcinogenic and hence monitoring the levels of aflatoxin contamination is mandatory for exporting food items. Measurement of aflatoxins in food items can be done by the RIA technique for ensuring food safety.

Human Resource Development in RIA

Radioimmunoassay is best utilized in the hands of well-trained personnel. BARC has been conducting since 1980, a training course covering all the aspects of RIA and IRMA. About 400 medical doctors and about 600 technologists have been trained through this course till now. The course is conducted twice a year at BRIT as well as in leading host Institutions in different parts of the country.

The HRD efforts taken by the Department has helped in propagating the RIA technique widely in India. There are over 500 RIA laboratories functioning at various parts of India. While most of these laboratories are catering to the local population, there are a few large RIA laboratories that run a network of collection centres who collect, process and courier the samples to the central laboratories. By using modern communication network, these laboratories make the results available to the referring doctors in 48-72 hours.

Over the years major competitive technologies have emerged which are capable of offering assays, which are of equal clinical use, using non-isotopic labels. However, the radioimmunoassay technique is still the preferred method for many

due to its simplicity, non-interference from matrix effects and cost advantage. It is estimated that about 2-3 millions of RIAs are carried out annually in India.

Technologies Available

BRIT is currently the only manufacturer of radioimmunoassay kits in India. The Board has been providing RIA/IRMA kits for several hormones. Technology for the development of RIA/IRMA of any of the antigens is also now readily available in BRIT. The radioactive component in the RIA technique is very small and the nonradioactive component constitutes the major part. Hence, the RIA technology is aptly suitable for transfer into Indian manufacturer with active input of the radioactive tracer component from the Department.



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