

National Institute for Research in Reproductive Health (NIRRH), Mumbai

Fertility Assessment Kits

Product/Process: Fertility assessment kits and their assays-

- Indulsa - a kit for estimation of estrone glucuronide in urine (E_1G) by ELISA.
- Corpulisa - a kit for estimation of pregnanediol glucuronide (PdG) in urine.
- Luteolisa - a kit for estimation of leutinizing hormone (LH) in urine by ELISA.
- Follilisa - a kit for estimation of follicle stimulating hormone (FSH) in urine by ELISA.

Application/Uses:

- Indulsa - E_1G assay is used in conjunction with either ovarian ultrasonography and / or cervical mucus score in:**
 - Monitoring induction of ovulation therapy.
 - For timing the retrieval of pre-ovulatory oocyte *in vitro* fertilization and Assisted Reproductive Technology (ART) programmes.
- Corpulisa - PdG assays is used for:**
 - Detection for occurrence of ovulation.
 - Assessment for corpus luteum function.
- Luteolisa - LH assay is used in conjunction with FSH and / or sex steroids hormone in-**
 - Investigation in delayed and precocious puberty.
 - In determining the cause of subfertility such as gonadial failure, polycystic ovarian disease and evaluation of hypothalamic pituitary gonadial axis.



- d. **Follilisa - FSH assay is used for estimation of FSH in urine. It is used in conjunction with LH assay for the above disorders.**

Salient Technical Features:

- a. **Indulsa** - The ovarian follicular development can be properly assessed by serial ultrasonographic scanning of ovarian follicle and serum 17β oestradiol measurements. Assessment by oestradiol assay requires collection of a series of blood samples at an appropriate time during the menstrual cycle. Collection of a series of blood sample is stressful and causes discomfort to patients. It has been reported that the pattern of excretion of E_1G , principal metabolite of oestradiol in urine, closely resembles that of its parent oestradiol 17β in circulation. Therefore, estimation of E_1G in urine is more advantageous as sampling is non- invasive.
- b. **Corpulisa** - The plasma progesterone concentration rises rapidly following ovulation and raised level of progesterone during luteal phase as an indicator of occurrence of ovulation. After achieving ovulation, a properly functional corpus luteum is used for implantation of ovum. Pregnanediol glucuronide is the principal metabolite of progesterone in urine and the levels of which reflect those of progesterone in circulation.
- c. **Follilisa** - FSH and LH are intimately involved in the control of the growth and reproduction activities of the gonadal tissue which synthesize and secrete male and female sex hormones. The level of FSH and LH are controlled by the sex hormones negative feedback relationship. FS promotes the proliferation of follicular cells, development of the graafian follicle and maturation of the ovary and appear to act with LH to stimulate testosterone production.
- d. **Luteolisa** - Leutinising hormone (LH) is produced in both men and women from the anterior pituitary gland in response to gonadotropin releasing hormone (GnRH) which is released by the hypothalamus. LH is responsible for cyclical ovarian changes during the female menstrual cycle, including maturation of graafian follicle, ovulation and steroid production. LH in conjugation with FSH is required for maturation of spermatozoa in the seminiferous tubules and has the primary function of stimulating the interstitial cells to produce testosterone.

Scale of Development:

(a) The technology has been developed as follows -

- (i) Bulk quantities of well-characterized and high titre antisera (about 60-70 ml for each analyte) have been raised.

- (ii) Methods for preparation for enzyme analyte conjugation have been standardized.
- (iii) Presentation of antisera coated plates, enzyme labeled analytes, standard analytes, QC polls and substrate color indicator solution to suit kit format have been identified and their stability at defined conditions for defined period of time achieved.
- (iv) Presentation of individual reagents in the kit format is achieved.

Therefore, products can be up-graded to any scale.

(b) Technique used for mass production of antigen/antibody and outline of the processes involved are:

- (i) Raising of polyclonal antisera to analyte by immunizing rabbits.
- (ii) Purification and characterization of antisera.
- (iii) Preparation of enzyme labeled analyte: Mix-anhydride reaction in case of E₁G and PdG conjugations. One-step glutaraldehyde method for protein hormones.
- (iv) Selection of appropriate dilutions of labels and antisera.
- (v) Stability studies of reagents and other components of the kits.
- (vi) Selection of appropriate containers and packaging materials.

(c) Annual production of the kit: Approximate 1000 kits can be produced per annum.

Status of Commercialization: The technology has been transferred to M/s. Zydus Cadila Healthcare, Ahmedabad, through Biotech Consortium India Ltd. (BCIL), New Delhi, for development, promotion, licensing and commercial exploitation of technology know-how. Presently, all serum hormone assays are being imported. A certificate mentioning satisfactory absorption of technology has been received from M/s. Zydus Cadila Health Care, Ahmedabad.

Pregnancy Detection Kit - Pregstrip

Product/Process: Pregstrip - Pregnancy detection test based on excretion of human chorionic gonadotropin (hCG) in urine.

Application/Uses: Pregstrip is a dipstick ELISA test for pregnancy detection. Pregnancy detection is very simple and convenient due to the concentrated coating of high affinity antibodies on the nitrocellulose membrane pad for precise detection. Its sensitivity, specificity, and accuracy is 99.2%, 99.6% and 99.84% respectively, and detects pregnancy just after period is missed. Easily interpretable results can be achieved by colour differentiation.

Salient Technical Features: At present, the card tests available for pregnancy detection are not manufactured in India. hCG is glycoprotein hormone secreted by the developing placenta in doubling quantities shortly after fertilization. The appearance and rapid rise in concentration of hCG in the maternal serum and urine, makes it an excellent marker for detection of pregnancy. The test utilizes β specific antibodies to hCG, immobilized on nitrocellulose membrane (0.45 μ) attached to plastic strip. These tests are based on immunochromatographic principle and are sensitive (20 mIU/ml). However, in India reasonably sensitive (50 mIU/ml) and simple tests are required as most Indian women approach clinicians in 1-2 weeks after missing periods. Keeping in view the needs of Indian women, the pregstrip is user-friendly, rapid and more sensitive. It can strengthen MTP programme of our country. Presently pregnancy detection kits are based on imported technologies and there is a great scope of detection test based on indigenous technology.

Scale of Development:

1. The technology has been developed up to laboratory scale (500 strips).
2. Methods of preparation of enzyme analyte concentration have been standardized.
3. Presentation of strips in dried format, enzyme label, control and substrate color indicator solution in kit format have been identified and stability at defined conditions for defined period of time have been achieved. Therefore, product can be upgraded to any scale.

Status of Commercialization: Technology commercialization is being explored.

Iron Deficiency Detection - Ferritinst

Product/Process: Ferritinst—a kit for the measurement of ferritin in serum to detect iron deficiency.

Application/Uses: Ferritinst provides a reliable immunoassay with minimum detection of limit 1.7 ng/ml. The antibody developed is specific and shows negligible cross reactivity with other serum component such as human serum albumin, alpha foetoprotein, haemoglobin, transferrin and ferric chloride. The test is cost effective, more sensitive and stable at refrigerator temperature. It is regularly required for screening general population, blood donors and patients undergoing renal dialysis.

Salient Technical Features: The system comprises of one polyclonal antiferritin antibody coated on microtitre well and another polyclonal anti ferritin antibody from a different source, which is conjugated to the enzyme horse radish peroxidase. The concentration of ferritin is directly proportional to the colour intensity of the test.

Scale of Development: The present technology is developed up to laboratory scale only and can be upgraded to industrial scale.

Status of Commercialization: Technology commercialization is being explored.

Biochemical Marker for Osteoporosis

Product/Process: Osteocalcin is a biochemical marker for diagnosis of osteoporosis and assessment of bone pathophysiology.

Application/Uses: Weakening of bone is an age related phenomenon and more so in geriatric population. Early diagnosis by using osteocalcine can reduce the incidence of future risk of fracture.

Salient Technical Features: Osteocalcine is a low molecular weight peptide and is secreted by osteoblasts. The synthesis of osteocalcine increases with enhancement in demineralization of bone. Elevated level of osteocalcine in serum and its appearance in the circulation is an early and acceptable indicator of bone pathology. Since it is cost effective, more sensitive and stable at refrigerator temperature, it is regularly required for screening general population and renal dialysis patient and therefore, has commercial potential.

Scale of Development: Pilot experiments are completed in which osteocalcine isolated was checked for purity through SDS-PAGE. Immunoreactivity of this preparation was also studied through a standard ELISA using DSL kits.

Status of Commercialization: Technology commercialization is being explored.

Biochemical Marker for Reproductive Tract Infections

Product/Process: Elastase (hydrolase) enzyme is used as a biochemical marker for reproductive tract infection (RTIs) including sexually transmitted infection (STIs).

Application/Uses: Elastase one of the proteins released during the inflammation due to the infection is used as biochemical marker for RTIs.

Salient Technical Features: Inflammation leads at the time of RTIs /STIs which liberates different types of proteins including elastase. A simple colorimetric assay was standardized to measure elastase in different biological samples like semen, urine, blood and cervical samples. The sensitivity and specificity of the assay is 87% and 98%, respectively.

Scale of Development: Technique is standardized and available in the laboratory for screening of RTIs and STIs.

Status of Commercialization: No indigenous technique is commercially available in India.

Nisin Peptide - A Contraceptive

Product/Process: A novel composition useful as a contraceptive comprising nisin peptide.

Application/Uses: It is used as non-vaccine preventive measure for RTIs/HIV infection and possess spermicidal properties.

Salient Technical Features: This composition comprising a therapeutically effective amount of biologically sufficient to cause spermicidal activity together with non-toxic vehicle. The vehicle used in the composition is saline and compound may be formulated as a lotion or a solution or any other form available for topical administration.

Scale of Development: Technique is developed up to laboratory scale. An Indian patent (no. 218373) has been granted.

Status of Commercialization: Technology commercialization is being explored.