The measurement of hormones and bacterial antigens using rapid particle-based immunoassays

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Abstract Unipath manufacture a range of rapid immunoassays for use in the home and clinical environments. The rapid assay technology used by Unipath and which has been patented, utilise, a chromatographic principle and deeply coloured latex particles. The test consists of two components. A porous sample pad, containing latex particles which nave been sensitised with a monoclonal antibody, and a chromatographic strip which allows sample migration by capillary action through a test zone and a control zone. The test zone consists of an immobilised line of a monoclonal antibody which acts as the partner to the antibody on the latex. The control zone consists of an immobilised line of polyclonal antibodies to mouse immunoglobulin. When sample is applied to the porous sample pad, either directly Of by means of an absorbent wick, the latex participles become resolvated. The sample/latex mixture then migrate up the chromatographic strip and any antigen present in the sample, becomes bound to the surface of the latex. This antigen/latex complex is then captured on the test zone and this turns the line visibly blue, indicating a positive result. If no antigen is present, then the test zone remains invisible. In either case some antibody sensitised latex is carried to the control zone where it is bound by the line of antibodies to mouse immunoglobulin. This control lire gives an indication that the test has been successfully carried out. The technology has been successfully utilised for the determination of hormone levels (e.g. hCG, LH & E-3-G) illustrating both sandwich and competitive assays can be performed and to the determination of microbial antigens such as lipopolysaccharides, polysaccharides and bacterial toxins.

Unipath manufacture a range of rapid immunoassays for use in the home and clinical environments. These include the over-the-counter tests Clearblue Onestep and Clearplan Onestep, for pregnancy and ovulation respectively. As well as the Clearview range of tests for use in the small laboratory. These rapid immunoassays, are now widely available and accepted by the general public and clinical laboratory. However the simplicity and ease of using a rapid immunoassy, often belies the technology which lies within the plastic moulding. This paper will discuss how Unipath has used its knowledge of this technology to develop two new assays.

The rapid assay technology used by Unipath and which has been patented, utilises a chromatographic principle and deeply coloured latex particles. The test consists of two components. A porous sample pad, containing latex particles which have been sensitised with a monoclonal antibody, and a chromatographic strip which allows sample migration by capillary action through a test zone and a control zone. The test zone consists of an immobilised line of a monoclonal antibody which acts as the partner to the antibody on the latex. The control zone consists of an immobilised line of polyclonal antibodies to mouse immunoglobulin. When sample is applied to the porous sample pad, either directly or by means of an absorbent wick, the latex participles become resolvated. The sample/latex mixture then migrate up the chromatographic strip and any antigen present in the sample, becomes bound to the surface of the latex.

This antigen/latex complex is then captured on the test zone and this turns the line visibly blue, indicating a positive result. If no antigen is present, then the test zone remains invisible. In either case, some antibody sensitised latex is carried to the control zone where it is bound by the line of antibodies to mouse immunoglobulin. This control line gives an indication that the test has been successfully carried out.

Although the tests are easy to perform and results are available in 3 to 15 minutes, the underlying technology is sophisticated and ensures that test sensitivity is equal to that of many ELISAs and RIAs, which may take skilled technicians many hours to perform. This level of sensitivity is achieved rapidly for a number of reasons. The latex particles are small and uniform in diameter. They therefore provide a very high parking area onto which monoclonal antibodies can be immobilised. The particles themselves are deeply dyed for maximum visual impact and, by using directly coloured particles, the time needed for enzyme/substrate reactions to occur is eliminated. The nitrocellulose chromatographic strip also provides a very large surface area for the controlled immobilisation of antibodies. In addition, the tortuous flow path along a strip of nitrocellulose ensures that sample, particles and nitrocellulose surface are brought into close proximity. The diffusional distances involved are therefore small and allow binding to take place rapidly. In contrast, the surface area of an ELISA well is small and the diffusional distances involved are large.

In order to manufacture very large numbers of these tests, Unipath has established a sound knowledge of the interactions between the many test components which control the assay. For example, it is important to monitor in real time, the chemistry of the antibody immobilisation process to the latex particles. In addition to the dye content, surface charge and surfactant properties of the latex itself. Similarly, control over the nitrocellulose pore size and surface chemistry also dictates its protein-binding properties. This technology has now been incorporated into two new assays. The first measures the presence of *Clostridium difficile* Toxin A in stool samples. The second, measures two hormones present in female urine and uses a hand-held monitor to provide the user with information about her fertile status.

1) A rapid immunoassay for the identification of Clostridium difficile Toxin A from stool samples.

Clostridium difficile is a Gram-positive anaerobic bacillus which has been identified as a common nosocomial pathogen that causes diarrhoea and pseudomembranous colitis associated with antibiotic therapy. Outbreaks, once established are difficult to control and reinfection of patients is common. *C.difficile* is known to produce at least two toxins, designated A and B. Toxin A causes fluid secretion, mucosal damage and internal inflammation. It is generally accepted that Toxin A plays a more important role in the pathogenesis of the organism.

To run the assay, stool samples (100ul for liquid and a pea-sized portion for solid samples) were mixed with 1ml of buffer to form an homogenous suspension. The samples were centrifuged for 10 minutes to remove particulate matter and 125ul of the supernatant were applied to the sample pad of the test unit. Results were interpreted 15 minutes later. The samples were identified as being toxigenic by cell cytotoxicity using vero cells and neutralisation with C. Sordellii antiserum, or by culture.

A total of 346 samples were tested in association with Barnet and Edgeware General hospitals. Sensitivity was 87.8% (86/98) and specificity was 97.2% (241/248). The positive and negative predictive values were calculated as 92.5% and 95.3% respectively.

2) The Unipath Personal Contraceptive System.

The Unipath Personal Contraceptive System represents a major breakthrough in natural family planning and will provide women with a simple non-invasive method for predicting their fertility status throughout a monthly cycle.

The system incorporates a dipstick, which rapidly and simultaneously measures two hormones in urine. The dipstick is then placed into a hand-held monitor which quantifies the levels of hormone present. A sophisticated algorithm then uses personal data on the woman's cycle, gained from previous months measurement, together with a vast database of female cycle measurement. This algorithm then predicts the woman's fertile status over the next 24 hours and advises her whether it is safe or not safe to have unprotected sex, with regard to contraception.

In the late 1970's and early 80's, a World Health Organisation task force conducted extensive studies on the temporal relationships of the changes in reproductive hormones, to the time of ovulation and to a period of potential fertility. The studies showed that a rise in the level of luteinising hormone is very reliable in predicting ovulation and that 90% of conceptions occur in a 6 day window around ovulation. The studies also indicated that changes in estrogens gave the best warning of the start of the fertile period.

The test stick therefore measures the levels of oestrone-3-glucuronide (E3G) and luteinising hormone (LH), as the predictors of the onset and end of the fertile period, respectively. For the measurement of E3G, blue latex particles are coated with antibody specific for E3G. When the latex is contacted by urine, any E3G in the sample will react with antibody on the surface of the latex. Residual sites on the latex are then free to react with E3G which is immobilised as a line on the membrane. The amount of latex which binds, and the colour generated, is therefore inversely proportional to the amount of E3G in the sample. By contrast, the LH assay is a sandwich assay and increasing amounts of LH in the sample, leads to increasing amounts of latex captured in the test zone.

The monitor compares the colour levels on the test stick with those of previous tests within the cycle, and when the monitor detects a significant change in E3G or LH, it advises the woman of a change in her fertility status.

On the basis of the studies we have conducted on many thousands of cycles, we are currently engaged in prospective clinical trials to confirm the contraceptive efficacy of the system used at home.

In conclusion, we can see that the simplicity and ease of use, which characterise particlebased immunoassays, hides a sophisticated technology. By developing a deep understanding of the way that this technology functions, Unipath is leading the way in producing visual and instrument read assays for the home and clinical markets.