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EDITORIAL

Immunodiagnosis of Bacterial Enteric Infections : Where do We Stand ?

Ensuring the rationale treatment and appropriate management of patients with infectious diseases requires the rapid and reliable detection of the etiologic agent. Diarrhoeal diseases comprises one of the major component of infectious diseases especially in the developing areas of the world where unhygienic sanitary conditions and poverty perpetuate the disease. Over the years, antibodies against human enteric pathogens or against signature antigens of the etiologic agent have provided the means of developing a number of highly specific and reproducible immunological assays for rapid and accurate diagnosis of bacterial enteric infections. From a modest beginning several decades ago, immunodiagnostic has now prevailed in almost all conceivable areas of diagnosis and includes an impressive array of formats ranging from simple coagglutination tests to highly complex immunoassays directed towards precise quantitative estimations. Historically some of the major developments of recent years have stemmed from the efforts of pioneering scholars like Widal and Wassermann, at least a century back, on whose efforts some of the first immunological assays emerged.[1,2] Not known to many, the discovery of the etiology of shigellosis by Kiyoshi Shiga was influenced by the principles of the Widal test.[3]

Among the variety of immunodiagnostic techniques currently in vogue for the detection of enteric infections, the immunoenzymatic methods (enzyme-linked immunosorbent assay [ELISA]) are undoubtedly the most extensively utilized. The ELISA, particularly the sandwich type because of its enhanced sensitivity and specificity, has become widely popular in research and reference laboratories. The first immunoenzymatic assays developed for detection of enterotoxins used readily available purified cholera toxin (CT) and antitoxin in the indirect ELISA format[4] to detect heat-labile enterotoxin (LT) of enterotoxigenic *Escherichia coli* (ETEC), an important agent of diarrhoeal disease which affects both humans and agricultural animals. Using antitoxin against LT, a receptor based ELISA followed wherein GM_1 ganglioside was utilized as a capture molecule for LT, resulting in an assay comparable in sensitivity to tissue culture methods.[5] Substitution of anti-LT with anti-CT for detection of LT resulted in an unacceptable decline in the sensitivity of the assay.[6] By improving the growth medium and by using lincomycin to enhance LT production, Ristaino *et al*[7] significantly improved the sensitivity of the GM_1 ganglioside ELISA despite using anti-CT. Another major innovation by these investigators involved the direct culture of *E. coli* strains in GM_1 ganglioside coated wells of microtitre plates followed by ELISA. The ELISA to detect Shiga toxin and its related toxins was also developed.[8] More recently, using the natural binding receptor, globotriosyl ceramide (Gb_3), of Shiga toxin and a family of verocytotoxins comprising of Verotoxin I (or Shiga-like toxin I) and Verotoxin 2 (or Shiga-like toxin II), a receptor based ELISA has been developed for detection of these cytotoxins in nanogram quantities.[9]

Much excitement has been generated in the medical diagnostic industry with the advent of the hybridoma technique and the generation of high affinity monoclonal antibodies. In the area of enterotoxins, monoclonal antibodies have found good use in the detection of the family of heat-stable enterotoxins (STs).

High affinity monoclonal antibodies against *E. coli* STs,[10,11] *Vibrio cholerae* non-O1 ST[12] and *Yersinia enterocolitica* ST[12] have been used for detection of these toxins in a competitive ELISA format. However, the need for pure antigen to perform the assay restricts the utility of this ELISA and most laboratories still depend on the conventional cumbersome suckling mouse assay for detection of STs. Monoclonal antibody against Shiga toxin has been employed very effectively for the specific detection of the toxin.[13] For detection of prototype CT and not related CT-like enterotoxins, a polyclonal-monoclonal antibody based sensitive sandwich ELISA has recently been reported.[14] Other immunological tests like the passive immune haemolysis[15,16] and radioimmunoassay[17] have been concurrently developed for detection of bacterial enterotoxins but have proved to be too complex for wide-spread adoption particularly by poorly equipped laboratories.

Despite such imposing progress in the immunodiagnosis of enteric infections, one of the less acclaimed drawbacks of several immunodiagnostic techniques which have been proposed or which are in use are their limited applicability in developing countries due to constraints pertaining to the need for expensive equipment, reagents and trained personnel. This is an ironic situation because the incidence and mortality of diarrhoeal disease especially among infants are highest in the less-developed countries. The need, therefore, for quick and specific diagnosis of enteric infections is perhaps the most compelling in such areas of the world. A simple, inexpensive assay using robust reagents and no instrumentation could have many diagnostic application.

Immunoprecipitation on agar plates which combines the principles of the Elek test[18] and Ouchterlony double diffusion test[19] resulting in the formation of precipitin bands was exploited by Honda and his coworkers for the detection of thermostable direct haemolysin of *Vibrio parahaemolyticus*[20] and LT (Biken test).[21] The usefulness of these tests were subsequently confirmed by several independent evaluations.[22-24] While this test offered one of the most simple alternatives, the main drawback related to the time taken (about 4 days) for a positive diagnosis thereby relegating the test to epidemiological significance rather than of clinical utility. The staphylococcal coagglutination test for detection of LT was subsequently developed as a simple and rapid alternative to other immunoassay.[25] To further augment simplicity, an alternative highly sensitive ELISA which used 6-mm diameter polystyrene beads as the solid phase (bead ELISA) and which can be performed in 3.5 hours in standard laboratory test tubes for detection of CT and other bacterial protein toxins has been recently developed.[26,27]

Direct detection of enterotoxins in stool specimens of patients with acute diarrhoea represents a greatly simplified approach for the rapid diagnosis of toxin-mediated diarrhoeas. Attempts have been aimed to detect the causal agent directly in stools of diarrhoeal patients in order to obviate bacterial culture and thereby expedite laboratory diagnosis. Mixed success has been achieved using this approach. The low sensitivity of oligonucleotide DNA probes for detection of enteric pathogens from stool blots[28] and the need for sterile stool filtrates for tissue culture techniques[29] limited their utility. Immunoenzymatic detection of enteric pathogens or enterotoxins directly in stools seem to be more easily applied. A recent evaluation of the CT-bead ELISA for detection of CT showed that this assay could successfully detect the toxin directly in 84.7% of the culture-positive cholera stool samples[30] thereby enabling a potential diagnosis of cholera to be made within four hours of receipt of the sample at the laboratory. Using the CT-bead ELISA an outbreak of cholera which occurred in a passenger ship was established despite being unable to isolate *V. cholerae* O1 from the stool samples of the outbreak by demonstrating the presence of anti-CT IgG absorbable faecal CT in the stools by the bead ELISA.[31]

Where do we currently stand as far as immunodiagnosis is concerned for diagnosis of bacterial enteric infections is an unreasonable but relevant question. While there has been excellent headway in the area of developing an assortment of immunoassay for detection of variety of antigens, there has been lesser accent on the feasibility of utilizing these immunoassays in the less-developed world. The future must therefore aim at developing more pragmatic assays which should withstand the innumerable harsh variables of the developing world rather than the simulated laboratory conditions of the developed countries Simplified test formats are the important need on which will rely the quality of life of millions of distressed humans.

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