STANDARDIZATION OF IMMUNOCHROMATOGRAPHIC TEST FOR DIAGNOSIS OF ENTEROTOXIGENIC *ESCHERICHIA COLI* THROUGH HEAT-LABILE TOXIN DETECTION

Henrique, C. P., Piazza, R. M. F., Caetano, B. A., Polatto, J. M. , Rocha, L. B.

Bacteriology Laboratory, Butantan Institute (Av. Dr. Vital Brasil, 1500 - Butantã, São Paulo - SP, 05503-900)

Abstract:

The immunochromatographic (IC) test, also named lateral flow, is widely used to detect different pathogens and substances such as hormones and pesticides. It is basically composed of two antibodies with distinct binding epitopes presents on the analyzed molecule. Comparing molecular detection methods, the immunoassay test presented as an alternative tool to the traditional methods, with several advantages, fast, easy to perform and it is a low cost test, reliable with the social reality of many developing countries. Considering the described features, the production of polyclonal antibody (pAbs) and monoclonal antibody (mAbs) enabled the development of an IC test for enterotoxigenic *E. coli* (ETEC) detection. ETEC is responsible for 200 million episodes of diarrhea and around 380 thousand deaths per year, known to cause watery diarrhea, which may cause from mild symptoms and be self-limiting, to cholera like diarrhea, dehydrating and fatal episodes. It is also the main agent associated with the called "traveler's diarrhea", annually affecting more than 10 million travelers in developing countries. Among the virulence factors, ETEC produces heat labile toxin (LT), responsible for watery diarrhea. The diagnosis is an important tool for the correct treatment and control of outbreaks. Although the IC handmade test for ETEC detection has been developed, automated tests needed to be standardized. Thus, the objective of this study was the standardization and evaluation of the IC test for ETEC detection. For standardization, it was used LT pAb as the detection antibody conjugated to colloidal gold (pAb-Au) and LT mAb utilized as capture antibody on the nitrocellulose membrane (NM). Different treatments in the sample pad portion, NM with different porosities and different concentrations of capture antibody were employed. The tests demonstrated promising results using a sample pad treated with buffer containing 1% BSA and 1% Tween; NM pore 180; 4 mg/mL of LT mAb on the capture line. This effective standardized combination allowed the positive signal in the presence of LT toxin and non-reactivity without the toxin (only *E. coli* broth). Tests using bacterial isolates are currently been performing to evaluate the sensitivity, specificity and efficiency of this assay.

Key words: immunochromatographic test, enterotoxigenic *Escherichia coli,* heat-labile toxin, standardization.

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