

## COLLOIDAL GOLD PRODUCTION FOR ANTIBODY LABELING IN IMMUNOCHROMATOGRAPHIC TEST FOR DETECTION OF BACTERIAL ENTEROPATHOGENS

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The uses of colloidal gold-labeled antibodies have drawn attention to the potential of antibody-antigen interactions, which can be used either for drug delivery or antigen detection in several pathologies. The production of colloids with correct size and shape allows the interchangeability of the commercial gold to the in house, contributing to autonomy and reduction of test costs. Foremost, colloidal gold particles are obtained through the reduction reaction of tetrachloroauric acid (HAuCl<sub>4</sub>) at boiling temperature. One of the most widespread processes is the Turkevich method, which consists of HAuCl<sub>4</sub> reduction with 1% sodium citrate. All glassware used was properly washed with distilled water and neutral detergent, stirred until boiling and rinsed with deionized water. In a flask containing 47.5 mL of deionized water 2.5 ml of HAuCl<sub>4</sub> 5 mM were added and stirred until boiling, the acid was then reduced with 1.5 or 2 mL of 1% sodium citrate solution and immediately transferred to stir at 24 °C, approximately 25 sec after the solution turned into a ruby red color. The characterization of colloids occurred by reading absorbance in spectrophotometer at wavelength of visible light, and to assess the formation of spherical gold particles of 20 nm in diameter in a Zeiss transmission electron microscope (TEM) (LEO 906E). The in house colloidal gold showed the same absorbance profile and spherical size as the commercial one. Thus, in order to develop an immunochromatographic (IC) test for detection of enteropathogenic *Escherichia coli* and enterohemorrhagic *E. coli* using secreted protein B (EspB) as target antigen, the colloidal gold was employed to label EspB monoclonal antibody (mAb 4D9). When conjugated to EspB mAb either the commercial one or the in house colloidal gold were able to stain the IC control and test lines, with the same intensity. The IC test detects the EspB protein up to 1 µg/mL. Since in low-income countries diarrheagenic *E. coli* is responsible for 30-40% of the diarrhea cases affecting mainly children under 5 years and the elderly, early diagnosis is essential to minimize sequelae, thereby immunodiagnostic tests represents a rapid, simple and low cost alternatives.

**Key words:** Colloidal gold, immunochromatographic, EPEC, EHEC, monoclonal antibody.

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