DETECTION OF ROTAVIRUS IN FAECES USING A SIMPLE DIPSTICK SYSTEM WITH MONOCLONAL ANTIBODIES AND COLLOIDAL GOLD

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INTRODUCTION

Rotavirus (RV) is the most common cause of severe diarrhea in infants and young children, each year accounting for 3.5 million cases of illness in the United States alone, and leading to an estimated 800 000-900 000 deaths in the World (1). We have developed a simple 40 minutes, visual dipstick to detect human RV in faeces, using nitrocellulose (NC) as solid phase, two monoclonal antibodies (Mabs), colloidal gold (ColAu) as marker, and silver-ion enhancement.

EXPERIMENTAL PROCEDURES

RV strains were supplied by the Center for Biological Research (Havana). Mabs (2) against different epitopes of the specie-specific antigen VP6 were purified by Protein-A Sepharose CL-4B. Gold conjugates were prepared according to Faulk and Taylor (3). NC 0.2 μm strips spotted with Mab CB-R.1, and blocked with 1% BSA. The samples (filtrate of faeces diluted in PBS) were mixed with the ColAu conjugated Mab CB-R.2, and incubated for 30 minutes on the strips. After washing, the strips were treated with the silver ion enhancer for 10 minutes. All incubation steps were performed at room temperature. Comparisons of this dipstick system with reference kits or techniques (ELISA (2) and RNA-PAGE (4) were made using 142 samples from infants and children with acute gastroenteritis. Discordant samples were analyzed by transmission electron microscopy (TEM; negative staining) with 2% PTA.

RESULTS AND DISCUSSION

This dipstick improved version (4) uses only 100 ng of Mab CB-R.1 per spot. We had previously reported a RV detection system that employed colloidal selenium as marker, where optimal coating conditions utilized 10 times more protein than this one (5). The present dipstick for RV has two basic steps after coating: sample and conjugate, and enhancer. The total time of the assay is 40 min, and the test can be evaluated by visual observation of strips, with permanent records of results. The positive samples can be identified by the development of two brown spots, and negative by only one. The sensitivity of the system was determined as being 1 ng/mL with triplicate dilutions of a virus standard (figure 1). The sensitivity of the test was 100% and the specificity 99.16%. Samples negative by ELISA and RNA-PAGE were found positive by TEM. The stability for all components is still under study but has been established to this point as being one year at 4°C.

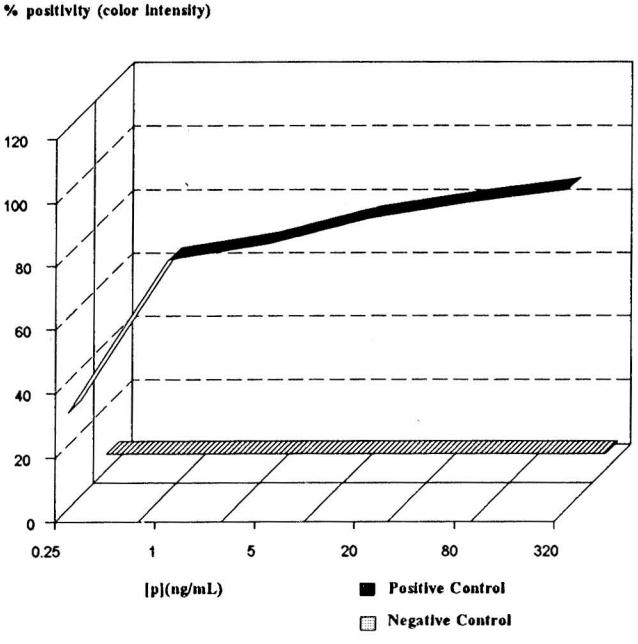

Fig. 1.- Determination of dipstick sensitivity limit

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