COMPARISON OF TWO GENERATIONS OF WELLCOZYME KITS

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ABSTRACT: A study to compare the specificity of two generations of Wellcozyme kits was performed using sera obtained from three different groups with different prevalence rates of HIV-1 infection, namely, females with multi-partner sexual contacts (MPSC) with an average HIV prevalence of 20% representing the high risk group, specimens from patients who have been suspected to have been infected with HIV based on clinical diagnosis, and scholarship winners with a prevalence rate of 3.4%. ELISA was performed on 1000 samples from each of the first two groups and 377 samples from the third group using both generation of kits. The percentage of false positivity using the first generation kit was 16.3% (33/202), 5.1% (14/271),36% (9/39) and using the second generation kit, 7.94% (12/151), 0.25% (1/399) and 5.8% (1/19) in MPSC females, suspected AIDS patients and scholarship students respectively. ELISA false positivity was highest in the group with a low HIV sero-prevalence rate. The result of the study, in relation to the specificity of the kits, is presented. Students who have won scholarships to countries that require HIV testing.

INTRODUCTION

Several screening tests have been developed after the advent of acquired immunodeficiency syndrome (AIDS) and the subsequent isolation of its etiologic agent, now termed the human immuno-deficiency virus (HIV). The virus is an RNA virus which belongs to the lentivirus family (6). The ability to cultivate the virus in vitro using T -lymphocyte has made the antigen available for the different tests used for screening. Since the concentration of viral antigens in sera from infected patients varies throughout the infection period, most of the tests are enzyme immuno-assays developed to detect the antibody raised against the virus by the immune system of the body (3).

The two screening methods in widespread use are enzyme linked immunosorbent assay (ELISA) and western blot. ELISA is the most favoured method of testing because it is a relatively simple and rapid method and useful to screen a large number of samples. It is used for screening purposes and has to be combined with a confirmatory test which in most cases is the western blot technique. Although time consuming, labour intensive and expensive, the western blot technique is able to distinguish specific antibodies directed against different viral proteins (5). The fundamental reaction on these immunoassays is antigen -antibody binding which depends on specificity. The occurrence of non- specific reaction of false positivity in HIV screening could be a considerable problem. Hence specificity and sensitivity are the major requirements for a good screening kit.

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This is a report on the results obtained from a comparison of two different generations of Wellcozyme kits commonly used for screening purposes.

MATERIALS AND METHODS

Sera

Serum samples from subjects representing the following three groups were taken: females practicing multi-partner sexual contact representing the high risk group (HRG), suspected AIDS patients from hospitals who have been diagnosed clinically (HP) and scholarship winners(SW).

Kits

The kits compared were Wellcozyme anti-HTL V -Ill (lst generation) and Wellcozyme recombinant (2nd generation), both from the same manufacturer (Wellcome diagnostics, Dartford, England). Each kit includes antigen coated microwells, conjugate, conjugate diluent, substrate tablets, substrate diluent, washing solution and also positive, negative, and cut-off control sera.

The test was performed according to the instructions of the manufacturer. A test sample of 50µ .1 (including one positive, two negative and three cut-off control samples) and 75µ.1 of conjugate were added to each well. After an incubation period of one hour at 45°C, the wells were washed three times, with a soak time of 60 seconds using a titertek plate washer (Flow laboratories, England).

The plates were incubated for 20 minutes in the dark after the addition of l00µ 1 substrate solution to each well. The reaction was stopped by the addition of 50µ1, 2M sulphuric acid and the absorbance value was determined at 450 Dm.

The confirmatory test was performed using a BIORAD western blot kit (Bio-rad NOVAPATH Immunoblot Assay) according to the instructions of the manufacturer. Thirty p.1 of each serum, including positive and negative controls, was incubated with a pre-washed western blot strip for 30 minutes at room temperature. Then the strips were washed three times with ten minute's incubation on a shaker at room temperature of each washing step. Conjugate (3 ml) was added to each strip and the strips were incubated for another 30 minutes after which the same washing procedure was applied. Lastly, the strips were incubated for10 minutes after the addition of 3ml substrate solution to each of them. The reaction was terminated by washing the strips using distilled water. Conjugate, washing solution and substrate together with the strips were provided by the manufacturer .

Samples which were found to be positive by double ELISA and negative by the western blot technique were taken as false positives.

RESULTS

The degree of false positivity for Wellcozyme anti-HTLV-m and Wellcozyme recombinant, with the tested population group, 1 is shown in figures 1 and 2, respectively. 'I The false positivity rates using the first t generation assay is 36% in scholarship winners, 16.3% in females with multi-partner sexual : contact and 5.1 % in suspected AIDS patients from hospitals. On the other hand, the false positivity rate using he second generation assay is 7.9% in females with multi-partner sexual contact, 5.8% in scholarship winners and 0.25% in the suspected AIDS patients from

hospitals. A substantial decrease in false positivity rate is observed in all population groups. The lowest false positivity rate was observed. in the specimens from suspected AIDS patients In both generation of kits. This is indicative of the fact that the false positivity rate is lower when the prevalence of the disease is higher.

DISCUSSION

In this study, the western blot assay was used as a confirmatory test for both generation of kits. In general a substantial reduction in the percentage of false positivity was obtain for all three groups of specimens when the second generation of Wellcozyme was used.

It has been shown that false positivity rates are inversely proportional to the prevalence of HIV-I infection (Zewdie et al 1991). The highest false positivity rate and the lowest false positivity rates were observed in the scholarship winners and the patients from hospitals that had signs and symptoms of HIV / AIDS infection respectively using both generation of kits. The false positivity rate in the specimens from MPSC females falls in between the scholarship students (low seroprevalence rate) and the suspected AIDS patients who already have been clinically diagnosed as AIDS cases, in both generation assays. This is indicative of the inverse relationship between the prevalence of the disease and the false positivity rate in each group. The fact that the false positivity rate decreased when using the second generation kit shows the improvement brought about by the use of recombinant antigens.

In order to have a reliable HIV diagnosis, tests used in HIV serology must be specific and sensitive. The occurrence of false positivity, during HIV antibody screening, could be a considerable problem. Hence each new generation of kit should be evaluated for its specificity and sensitivity.

Several factors have been associated with non-specific reactivity. Presence of immuno-complexes due to past and present parasitic infection malarial infection and anti-HLA antibodies have been indicated to be among the major factors in several studies (1,2,4). Since malarial infection is highly prevalent in some parts of Ethiopia, it may contribute to the occurrence of false positivity in this countries. However, the performance of the second generation kit, with the tested population, showed a significant improvement over the first generation kit. This could be because of the fact that the second generation kit uses recombinant antigen and the antigen could be free of HLA antigens which could otherwise be present in the whole virus antigen preparation used in the first generation kit.

Generally application of the second generation kit avoids the Use of more western blot strips (for confirmatory purpose) which are expensive, labour intensive and time consuming suspected AIDS patients who already have been clinically diagnosed as AIDS cases, in both generation assays. This is indicative of the inverse relationship between the prevalence of the disease and the false positivity rate in each group. The fact that the false positivity rate decreased when using the second generation kit shows the improvement brought about by the use of recombinant antigens.

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FIGURE 1. RELATIONSHIP BETWEEN HIV PREVALENCE AND ELISA FALSE POSITIVITY RATES (FPR) IN 6234 MPSC FEMALES, 1988

FIGURE 2. RELATIONSHIP BETWEEN HlV PREVALENCE AND ELISA FALSE POSITIVITY RATES IN MPSC FEMALES ADDIS ABABA, 1989.

FIGURE 3. THE DIFFERENCE IN FALSE POSITIVITY RATE FOR THE TWO GENERATION OF WELLCOZYME KITS USED.

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