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Impact of pooling on accuracy of hepatitis B virus surface antigen screening of blood donations

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Summary Expenditure on screening blood donations in developing countries can be reduced by testing donations in pools. This study evaluated serological screening in pools for hepatitis B virus (HBV) at the Israeli national blood bank and a hospital blood bank in Gaza, the Palestinian Authority. The accuracy of HBV surface antigen (HBsAg) enzyme immunoassay performed on pools of 3—24 samples was compared with individual tests. Delay in detecting positive samples due to dilution in pools and the possibility of antibody-antigen neutralization were analyzed. The sensitivity of pooled testing for HBsAg was 93—99%, prolonging the window period by 5 days (8.3%). Neutralization of HBsAg by hepatitis B surface antibodies (anti-HBs) could be minimized by testing immediately after pooling. Serological testing for HBsAg in pools may be performed using manually created pools of up to six samples, with 5% loss in sensitivity and a risk of neutralization by anti-HBs present in the donor population. Pooling can therefore be considered as an option only in countries with a low prevalence of HBV.

1. Introduction

Screening for hepatitis B virus (HBV) using enzyme immunoassay (EIA) is mandatory in the blood banks of all developed countries but is not performed or is only

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partially performed in most developing countries due to limited economic resources (Schmunis et al., 1998; Wake and Cutting, 1998). In some of these countries the seroprevalence of HBV among blood donors can be very high, ranging from 1.5% (El-Hazmi, 2004) to 20.3% (Lo et al., 1999).

The EIA screening tests currently used to detect the presence of HBV surface antigen (HBsAg) are characterized by high sensitivity and specificity, approaching 100% for positive verified samples (WHO, 2006). EIA screening for HBsAg in pools could significantly reduce costs and present a feasible solution for countries lacking sufficient resources for a mandatory individual screening program.

Many pooling procedures have been suggested during the last two decades (Litvak et al., 1994a, 1994b; Tu et al., 1994). Promising results were initially obtained for HIV testing, where the assays were not significantly affected by the dilution (Behets et al., 1990; Cahoon-Young et al., 1989; Kline et al., 1989; McMahon et al., 1995; Sanchez et al., 1991). Later, evaluation of EIA pooled screening for hepatitis C virus (HCV) showed no significant loss of accuracy in pools of five or six samples (Garcia et al., 1996; Liu et al., 1997; Novack et al., 2007; Sarov et al., 2007). HBV has been explored by Cunningham et al. (1998), who performed HBsAg screening of an antenatal population in pools of 10 samples, demonstrating how the 10-fold reduction in costs allowed performance of the recommended (but not mandatory) test in that population. Pooling was recommended by the authors in populations with low HBV prevalence. A recent analysis of HBV testing in pools of five samples also showed that the accuracy of testing was not affected by dilution (Fernández et al., 2006).

Despite the positive experience from these two studies, pooling for HBsAg testing has generally been avoided in transfusion medicine practice because of the possibility of reaction between HBsAg and hepatitis B surface antibodies (anti-HBs), leading to neutralization of the antigen in the pool. This may result in under-detection of an HBV-infected donation and is thus a serious obstacle for implementing pooled screening in populations where anti-HBs is frequent due to high prevalence of infection or as a result of immunization programmes against HBV.

The current study, performed in two blood bank settings, aimed to assess the accuracy of pooled screening for HBsAg.

2. Materials and methods

The accuracy and feasibility of pooled screening for HBsAg were analyzed considering four factors that could have an impact on the recommendations for the screening procedures: prolongation of the window period was estimated by testing five HBV seroconversion panels; the sensitivity and specificity of pooled screening were estimated for a cohort of 401 HBV-positive donations from the routine work of the two participating blood banks; possible neutralization of HBsAg in the pool by anti-HBs antibodies was investigated; a cost—benefit analysis was applied to one of the studied blood banks.

2.1. Test sites and blood donor populations

The study was performed on blood donor populations from Shifa Hospital, Gaza, the Palestinian Authority, a community hospital blood bank, for which pooling and testing were performed at the Environmental Protection and Research Institute (EPRI Laboratories) in Gaza, and from the Magen David Adom National Blood Service (MDA) in Israel. Both blood banks perform routine individual HBsAg EIA screening of all blood donations. The prevalence of HBsAg among blood donors in Gaza has been estimated at 3.8% (Yassin et al., 2002) and 4.06% (J. Safi, EPRI, personal communication) and in Israel at 0.22% (E. Shinar, MDA Blood Services, personal communication). The two sites performed the experiments in parallel on random blood donations obtained routinely during a three-year period from 2001 to 2004.

2.2. Proficiency testing of laboratories

To ensure the competence of the participating laboratories in pooling and testing, proficiency testing was performed prior to the initiation of the experiments. The laboratory technicians created and tested four pools of 24; eight pools of 12; 16 pools of six and 32 pools of three samples. Although different equipment was used at the laboratories: AxSYM, (Abbott Diagnostics, Abbott Park, IL, USA) at MDA; AxSYM and IMx (Abbott) at EPRI, both laboratories were in 100% agreement.

2.3. Accuracy and feasibility tests

2.3.1. HBV seroconversion panels

To estimate the influence of pooling on the HBV window period, bleeds from five seroconversion panels (BBI Diagnostics, West Bridgewater, MA, USA: BBI 1999-2001 catalogue #PHM927, #PHM929, #PHM930, #PHM931, #PHM932) were diluted in pool sizes of 6, 12, 24 and 48 with HBsAg- and anti-HBs-negative samples provided from the routine work of MDA Blood Services. For each pool size and seroconversion panel, the delay in detection of the first HBsAg-positive sample was compared to individual testing and was statistically estimated by a log-linear robust regression (Stata version 8; Stata Corp., College Station, TX, USA).

2.3.2. The impact of anti-HBs present in the pool

To determine the optimal pool size, two HBsAg-positive blood units with sample/cutoff (S/CO) values of 222 and 279 (Prism; Abbott) and one blood unit with a high level of anti-HBs (>1000 mIU/ml; AxSYM) were used. HBsAg-positive blood samples were incubated at room temperature with a blood sample containing anti-HBs in concentrations of 100, 200 up to 1000 mIU/ml, in pools of 6—32 samples. Pools were tested for HBsAg (AxSYM) after 1 h or an overnight incubation.

2.3.3. Determination of sensitivity and specificity in pools

At EPRI Laboratories, the number and position of the positive samples in the racks prepared for pooling were not predefined. Pools were created manually in sizes of six, 12 and 24 samples, where the smaller pools were a part of the larger pools. No further verification was performed on the