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BLOOD PRODUCTS LABORATORY RADIOIMMUNOASSAY FOR DETECTION OF HEPATITIS B SURFACE ANTIGEN (HBsAg) USING ANTIBODY-COATED BEADS (BPL-BEAD-RIA) : COMPARATIVE EVALUATION FOR BLOOD DONOR SCREENING

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SUMMARY

An antibody-coated-bead solid-phase sandwich radioimmunoassay (BPL-Bead-RIA) produced and supplied by the Blood Products Laboratory, Elstree, Hertfordshire, England, and designed for routine screening of blood donations, was compared with AUSRIA-II and a modified commercial assay (MOD-RIA)[1] during the screening of over 7,000 donor sera.

BPL-Bead-RIA and AUSRIA-II were of comparable sensitivity and specificity, readily detecting 250pg/ml. HBsAg (approximately 0.4 B.S.U./ml. HBsAg) in a 3-hour assay, compared with 1ng/ml. (approximately 1.5 B.S.U./ml.) for MOD-RIA.

BPL-Bead-RIA was easily introduced into routine screening as existing protocols for bead washing and handling were retained. Unreacted label from the HBsAg assay could be recovered and subsequently used to screen for anti-HBs using an inhibition assay[2].

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INTRODUCTION

Contained in the third report of the Advisory Group on Testing for the Presence of Hepatitis B Surface Antigen and its Antibody is a recommendation that U.K. Blood Transfusion Centres should screen donations for HBsAg using a test system capable of detecting at least 2 B.S.U.*/ml. (approximately 1.3ng/ml.) HBsAg. In practise this means that many Centres will be required to discontinue current haemagglutination tests in favour of more expensive R.I.A. or E.I.A. (enzyme immunoassay) systems. Previous attempts to incorporate both sensitivity and economy led the Edinburgh Transfusion Centre to adopt a modified form of commercial RIA[1] (MOD-RIA), which has been in routine use for over two years.

During a recent (unpublished) evaluation of the BPL removawell[3] test for detection of HBsAg, it became apparent, in the Edinburgh Centre at least, that the latter system was less easily handled than coated beads. A BPL-Bead-RIA was, therefore, made available for comparison with AUSRIA-II and MOD-RIA under routine donor screening conditions. The findings of this comparative evaluation are presented.

* British Standard Units based upon the HBsAg Standard BS 80/549.

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MATERIALS AND METHODS

Mod-RIA was performed as previously reported[1], while AUSRIA-II was performed according to the manufacturers' (Abbott Laboratories, Queensborough, Kent, England) instructions. The BPL-Bead-RIA was performed as previously recommended for the removawell system (2 x 90 minute incubations at 50°C and counting for 60 seconds) except that the 150ul of label per test, supplied with each kit, was supplemented with 50ul of 0.02M TRIS/Hcl pH7.2 containing 50 percent normal (HBsAg/anti-HBs negative) human serum to circumvent the possibility of beads drying out during incubation. This led to an isotope input per test of approximately 60,000cpm.

6.5mm. dia. polystyrene beads (Northumbria Biologicals Limited, South Nelson Industrial Estate, Cramlington, Northumberland, England), coated with ammonium sulphate-precipitated horse anti-HBs, were supplied in stabilising solution. These were stored at 4°C until required, then dried as previously described[1]. Incubation and washing steps were performed in either washed AUSRIA-II trays or in 25-well interlocking trays (Northumbria Biologicals Limited, and Precision Plastic Ball Company, 3000 North Cicero Avenue, Chicago, Illinois 60641, U.S.A.) suitable for use with the Pentawash gun (Abbott Laboratories).

In the Edinburgh Centre (comparing BPL-Bead-RIA with MOD-RIA) c.p.m. data from an NE 1600 gamma counter (Nuclear Enterprises, Sighthill, Edinburgh, Scotland) was processed on-line by a Commodore P.E.T. 32K mini computer programmed, among other things, to calculate the "cutoff" value as 2.0 times the negative control mean from which it had already subtracted individual backgrounds[4]. In the Dundee Centre (comparing BPL-Bead-RIA with AUSRIA-II) the cutoff count-rate (1.5 times the negative control mean including average background for BPL-Bead-RIA, and 2.1 times the negative control mean including average background for AUSRIA-II) was calculated and screening results determined by direct observation of the gamma counter print-out.

Serum from 6,424 fresh blood donations were tested using MOD-RIA and BPL-Bead-RIA, while a further 1,000 donations were tested by BPL-Bead-RIA and AUSRIA-II. Sensitivity was determined for each run by titration of a 1ng/ml. (approximately 1.5 B.S.U./ml.) HBsAg control. Wellcome HBsAg panel No. 3 was tested by BPL-Bead-RIA and AUSRIA-II at the Dundee Centre.

During the course of this evaluation four different BPL-Bead-RIA batches were studied, the kits being transported from London via British Rail Red Star service in appropriately labelled Blood Boxes.

The relationship between ng/ml. HBsAg and B.S.U./ml. HBsAg had been previously determined by calibration of an in-house standard (mixture of ad and ay subtype) against BS 80/549, and subsequent comparison of the standard with a 1ng/ml. control supplied by the Blood Products Laboratory. 1ng/ml, was found to be equivalent to 1.53 B.S.U./ml., and 1 B.S.U./ml. equivalent to 0.66ng/ml.

RESULTS

At the Dundee Blood Transfusion Centre both BPL-Bead-RIA and AUSRIA-II detected at least 250pg/ml. (approximately 0.4 B.S.U./ml.) HBsAg, while at the Edinburgh Centre the BPL-Bead-RIA detected 50-250pg/ml. (approximately 0.1 - 0.4 B.S.U./ml.) HBsAg compared with 1ng/ml. (approximately 1.5 B.S.U./ml.) HBsAg for MOD-RIA (table 1).

Of 6,424 donations tested at Edinburgh, 40 (0.62 percent) gave count rates on or above the cutoff by BPL-Bead-RIA, compared with 121 (1.9 percent) for MOD-RIA. However, all were negative after a further wash cycle. Repeated washing did not affect the count-rate of positive controls.

The 1,000 donations tested in Dundee by BPL-Bead-RIA and AUSRIA-II produced initial false positive rates of 0.9 percent and 0.4 percent respectively.

Both BPL-Bead-RIA and AUSRIA-II detected the same samples in the Wellcome HBsAg panel No. 3 (Table 2).

DISCUSSION

The BPL-RIA system for detection of HBsAg is a solid phase sandwich assay in which ammonium sulphate-precipitated horse anti-HBs is passively absorbed to the solid phase. HBsAg, trapped during subsequent incubation with the test serum, is signalled by the binding of radiolabelled goat anti-HBs. It is the result of a successful collaboration between the Virology Department of the Middlesex Hospital Medical School in London, the North London Blood Transfusion Centre at Edgware, and the Blood Products Laboratory at Elstree, evolving from the tube-based RIA first reported by Dane and colleagues as a personal communication in the Hepatitis Scientific Memoranda, and subsequently published by Cameron and Dane[5] (1974) and Cameron et.al.[6] (1980). In its initial format, the BPL-RIA favoured an antibody-coated removawell as the solid-phase. While this form of RIA represents a significant advance for those Centres currently screening with less sensitive haemagglutination assays, a number of the Scottish Regional Transfusion Centres, who already possess considerable experience of routine donor screening by RIA, consider the removawells less practical to handle en masse than the more convenient polystyrene beads. A BPL-Bead-RIA was, therefore, made available for evaluation at the Edinburgh and Dundee Regional Transfusion Centres. Prior to this evaluation, the Dundee Centre was routinely screening blood donations for HBsAg with AUSRIA-II, while the Edinburgh Centre was using a more economical modification (MOD-RIA)[1].

The BPL-Bead-RIA proved easy to handle as existing bead washing equipment could be utilised without modification. A slight difference in sensitivity was noticed between the two Centres, Edinburgh experiencing a sensitivity ranging from 50pg/ml. (in accordance with BPL quality control data) to 250pg/ml., dependent upon incubation conditions. Dundee, on the other hand, reported a sensitivity consistantly in the region of 250pg/ml. HBsAg for both BPL-Bead-RIA and AUSRIA-II. This inter-laboratory difference may be due, in part, to the

manner in which the cutoff count-rate was calculated and underlines the necessity for adoption of a standardised/optimised system of data processing. In Edinburgh the background count-rate was automatically subtracted at source prior to determination of negative control mean, a feature which, in view of the lower BPL negative control count-rate may lead to a high ratio (particularly among weak positives) and marginally greater sensitivity. In Dundee the cutoff included an "average" background. At Edinburgh the greatest sensitivity (50-100pg/ml) was achieved during incubation in a 50°C waterbath or a 55°C incubator, while a 45°C incubation reduced sensitivity to 250pg/ml. HBsAg. However, whichever form of incubation and calculation is employed, the BPL-Bead-RIA has a sensitivity well beyond the level of 2 B.S.U. (1.3ng/ml.) recently recommended by the Advisory Group on Testing for the Presence of Hepatitis B Surface Antigen, a feature confirmed by results obtained from testing the Wellcome Sensitivity Panel, and in agreement with previously reported evaluation of the BPL-RIA reagents[3].

No significant variation in sensitivity or specificity was noticed between the different BPL-Bead-RIA batches used during this evaluation, indicating the existence of an effective quality control programme at the point of production. Furthermore, sufficient reagents exist for an estimated 150 million tests, a feature lending assurance to supply continuity.

At a cost of 21 pence per test, the BPL-Bead-RIA is the cheapest HBsAg kit commercially available, and would appear to represent relatively good value as the suppliers are prepared to confirm "problem" positives, and deliver free of charge (in England and Wales). A further bonus is the finding that unreacted labelled anti-HBs, recovered from the antigen assay, may be re-used in a solid phase inhibition assay (SPI-RIA) for screening blood donors for anti-HBs to a sensitivity level of 0.1 I.U./ml.[2].

It is the authors' opinion that for reasons of economy, test uniformity and reagent standardisation, Transfusion Centres in the United Kingdom should give serioufconsideration to adopting a uniform system of HBsAg screening.

TABLE 1 Comparative evaluation of BPL-Bead-RIA, MOD-RIA and AUSRIA-II for sensitivity and specificity in Blood Donor Screening.

ASSAY	No. OF DONATIONS TESTED	No. OF TRUE POSITIVE DONATIONS	No. OF INITIAL FALSE POSITIVE DONATIONS (%)	SENSITIVITY* (pg/ml.)
BPL-Bead-RIA[a]	6, 424	0	40 (0.62)	50-250 (0.1-0.4BSU)
v MOD-RIA[a]		0	121 (1.9)	1,000 (1.5 BSU)
BPL-Bead-RIA[b]	1,000	1	9 (0.9)	250 (0.4 BSU)
AUSRIA-II[c]		1	4 (0.4)	250 (0.4 BSU)

* Based upon daily titration of 1ng/ml. standard.

(a) cutoff = T/N ratio of 2.0 with background count-rate subtracted.

(b) cutoff = T/N ratio of 1.5 without background count-rate subtracted.

(c) cutoff = T/N ratio of 2.1 without background count-rate subtracted.

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TABLE 2 Comparative evaluation of BPL-Bead-RIA and AUSRIA-II using Wellcome HBsAg Panel No. 3.

Sample Code	AUSRIA-II(P/N)	BPL-Bead-RIA(P/N)
301	-	-
302	+ (4.4)	+ (3.1)
303	+ (3.5)	+ (2.1)
304	-	-
305	+ (166)	+ (75.3)
306	+ (12)	+ (5)
307	+ (17.4)	+ (11.7)
308	-	-
309	+ (154)	+ (76.1)
310	+ (146)	+ (64)
311	-	-
312	+ (8.5)	+ (3.5)
313	+ (4.7)	+ (2.4)
314	+ (108.7)	+ (72.7)
315	+ (4)	+ (3.5)
316	+ (3.9)	+ (2.8)
317	+ (5)	+ (3.1)
318	-	-
319	+ (102)	+ (100.4)
320	-	-

P/N = ratio of cpm of test divided by negative control mean cpm.

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