



London, 21 September 2006
Doc. Ref. EMEA/CHMP/BWP/94181/2006

**OVERVIEW OF COMMENTS RECEIVED ON DRAFT GUIDELINE
ON VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF
HEPATITIS B VIRUS SURFACE ANTIGEN (HBSAG) IN PLASMA
POOLS**

Table 1: Organisations that commented on the draft Guideline as released for consultation

	Name of Organisation or individual	Country
1	Amgen Dated 21.12.05	UK
2	BAXTER Dated 21.01.06	AU
3	International Plasma Fractionation Association (IPFA) – Dated 19.12.05	NL
4	International Plasma Fractionation Association (IPFA) – HBsAg – Dated 22.12.05	NL
5	Plasma Protein Therapeutics Association (PPTA) – Dated 9.02.06	BE
6	A. Portela - Agencia Española de Medicamentos y Productos Sanitarios – Dated 19.03.2006	ES
7	Dr. E. Rusvai, rusvaie@oek.antsz.hu Dr. M. Takács, Head of Hepatitis and Molecular Virology Department, takacsm@oek.antsz.hu – Dated 13.12.06	HU
8	Dr. B. de Vries - National Institute for Public Health and the Environment - RIVM-BMT – Dated 28.11.06	NL

Table 2: Discussion of comments

GENERAL COMMENTS - OVERVIEW	
Comment and Rationale	Outcome
<p>The guidance is felt to be very general. Specific examples where further detail would be advantageous have been provided below.</p> <p>It is felt that there is not enough specific information around the very important topics of sensitivity, robustness and specificity.</p> <p>For example, the assay criteria do not specify that all classes of antibodies should be detected. There is no mention of discriminating IgM from IgG antibodies.</p> <p>Greater clarity on the kind of examples which should be evaluated as negative controls would be valuable.</p> <p>Further information regarding the required level of sensitivity would be useful.</p>	<p>As specified by the NfG on plasma derived medicinal products, pool serology was introduced as a measure to detect errors in testing or pooling. In the early phase of infection, the technical limitations of tests available exclude 100% sensitivity, as low affinity antibodies (and IgM) are rapidly diluted below the detection limit. There are no kits specifically for the detection of anti-HIV IgM.</p> <p>Due to the nature of pool serology, pools contaminated with HBsAg or anti-HIV should be a rather rare event. For the purposes of this guideline, selection of representative pool samples found negative by a manufacturer and by an OMCL is considered adequate.</p> <p>The requirement for sensitivity has to be deduced from the size of the pools to be tested, see NfG on plasma derived medicinal products: "... in relation to pool size ...". Detection limit is addressed in the document.</p>
<p>Both documents describe the potential use of a 'user-defined' grey-zone below the cut-off for reactive/non reactive as presented in the 'Instruction for Use'. The argument for the lower value is based on the dilution of non-specific factors potentially present in the single donations and diluted in the pool. We observe in practice also the opposite, mixing of different plasmas can induce non-specific factors in the pooled material, leading to a higher cut-off value in comparison to negative samples of blood donors. We prefer that the diagnostic kits used for 'in process' control should be used according to the 'Instruction for Use' for the diagnostic device, except in cases where this is not feasible because of the type of material tested or other typical aspects of pooled plasma for manufacturing. Compliance with the 'Instruction for Use' is necessary because the manufacturer can only be made accountable for the claims in these instructions. The S/CO ratio for non reactive/reactive is controlled by the diagnostic manufacturer as part of the batch release for the qualitative test. It is possible that the 'user-defined' grey zone S/CO level is not controlled in the batch release of the diagnostic device. The grey-zone value can fluctuate between lots of kits that are released within specification. The Notified Body responsible for the batch release of these kits under the CE-market authorization will only control the claims presented in the 'Instruction for Use'.</p>	<p>The evaluation criteria (cut-off, grey-zone) established for single donations must not be applied to pool samples without critical (re)evaluation/validation. The cut-off calculation laid down in the Instructions for Use (IFU) is validated for the intended use (testing of single donations) only. When the test kits are used for plasma pool samples, the user is fully accountable for the evaluation criteria (including cut-off calculation and retesting strategy) applied and the final results obtained, as the use for pool samples is not covered by the Common Technical Specifications (CTS), the validation of the IVD manufacturer or the evaluation by notified bodies in the CE marking process. If validation of sensitivity and specificity according to this guideline demonstrates adequate performance on negative and spiked pool samples, the interpretation criteria proposed by the IFU may of course be used, but experimental results demonstrate that significant (and in some cases crucial) increase of dilutional sensitivity without loss of specificity may be achieved by definition of a lower cut-off.</p> <p>The argument of unspecific results after pooling might hold true for some assays or pools although the experts involved in the drafting of the guideline do not really experience this phenomenon. The definition of a new (lower) cut-off is just a potential</p>

<p>When the manufacturer of the diagnostic device claims a grey zone in the 'Instruction for Use', this cut-off value can be included in the procedure for plasma pool testing, when further guidance for the resolution to a final result by repeat testing is given for the diagnostic device.</p>	<p>decision one might take, of course based upon respective results and experience. Most important is the identification of an assay with high dilutional sensitivity.</p>
<p>If it comes to that a 'user-defined' grey-zone is allowed in the guidelines EMEA/CHMP/298388/2005 and EMEA/CHMP/298390/2005, the documents should contain guidance how to solve a sample after an initial grey-zone result. This repeat testing (e.g. testing in duplicate) should use the cut-off ratio for reactive/nonreactive as presented in the 'Instruction for Use'. The concept guideline indicates insufficiently the status of a grey-zone result. Should grey-zones be considered as reactive samples or as potential reactive sample for which the non-reactivity should be proven? We propose that a grey-zone value will never be used to formulate a final result.</p>	<p>For all practical applications in the context of this Guideline, if a grey-zone limit is used as the cut-off value for pool samples, this limit is used to identify initially and repeatedly reactive plasma pools.</p> <p>A negative result on repeat testing in duplicate (HBsAg from a fresh sample) is accepted as a negative.</p> <p>Repeatedly reactive samples should be considered positive unless proven otherwise by adequately validated assays.</p> <p>HBsAg: For HBsAg testing a neutralization test should always be used on repeat reactive samples. The neutralization test has to be validated for pool plasma samples, considering the neutralization effect of antibodies already present in the pool (self-neutralization) in comparison to the neutralized sample (incubated with additional anti-HBs).</p>
<p>What is the status of the guidelines EMEA/CHMP/298388/2005 and EMEA/CHMP/298390/2005 with regard to the tests in place in the documentation of products with market authorization at the date for coming into effect of the guidelines? Should the validation be repeated when the validation report or SOP in place does not comply with the new guidelines or are the guidelines applicable to validation reports and/or SOPs to be filed for a new market authorization or a renewal procedure.</p>	<p>This guideline has been developed to respond to inadequacies in the validation of plasma pool testing for anti-HIV and HBsAg observed during evaluation of dossiers. Marketing Authorisation Holders and Plasma Master File Holders should review the validation of their pool testing methods in the light of this guidance. If the key aspects described in the guideline have already been covered by existing validation, no further validation is needed. If this is not the case, pool testing should be validated in accordance with this guideline and reported in the next annual update of the documentation on the plasma starting material.</p>
<p>Both documents do not clearly indicate in the § 5 Confirmation Strategies' whether a reactive results of manufacturing pool with the initially used serological assay can be overruled by the confirmation assay based on another test principle. Can NAT-tests or immunoblot assays be used to demonstrate that the EIA result is 'false'-positive?</p>	<p>The initially reactive test result may be overruled by retesting in duplicate with the same assay (note!: for HBsAg: on a fresh sample). Repeat reactive samples have to be confirmed with a properly validated serological method (HBsAg: alternative assay, neutralization with neutralization reagent, "self-neutralization" by signal loss kinetics over time in comparison to appropriate controls; anti-HIV: alternative assay, Immunoblot (see general comments in the HIV consolidated overview of comments. Doc. Ref. EMEA/CHMP/BWP/94181/2006). For HBsAg testing, a neutralization test should always be used on repeat reactive samples. NAT should be used in addition to but not instead of a neutralization test.</p> <p>Negative NAT results do not invalidate positive serological results, as HBsAg and anti-HIV may be present in patients with low or undetectable nucleic acid plasma levels. On the other hand, positive NAT results do confirm serological results.</p>
<p>The use and status of the grey-zone is not clear:</p>	<p>See comment above: Grey-zone is an option and it is up to the test lab, which has to</p>

<p>The difference between the cut-off for pool samples and the grey-zone should be explained.</p> <p>The grey-zone is introduced as an example in par. 3.1. However, the title of par. 3.1 and the wording in par. 5 suggest that the grey-zone is more than just an example. The status of the grey-zone should be clarified.</p> <p>The robustness study described in par. 3.2.1 seems to cover precision instead of robustness. In this context, the use of the word 'robustness' is confusing.</p> <p>Furthermore, for a reliable calculation of the %CV of the inter- and intra-assay variability at least 6 determinations are needed.</p>	<p>validate its approach and this approach has to be scientifically defensible. In any case, the interpretation criteria applied have to be defined on the basis of relevant data (produced with negative and spiked pool samples).</p> <p>A clarification on the status of grey-zone reactive samples was introduced in both guidelines.</p> <p>3.2.1. covers robustness and precision.</p> <p>Text changed to recommend 6 determinations.</p>
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GENERAL COMMENTS – OVERVIEW – HBsAg	
<p>The assays commercially available are not suitable for testing of pooled specimens, because a pooled sample may contain anti-HBs antibodies which form a complex with the HBsAg. The immune-complex can not be determined by an assay using ELISA technique. The samples should be checked by PCR method.</p>	<p>At least 3 confirmed HBsAg positive pools were detected during OCABR in the last 4 years and communicated as rapid information in the OMCL network. The sample detected by BifA (AT) in 2002 was DNA negative by 2 routine PCR methods and borderline reactive after ultracentrifugation in a high sensitive realtime PCR method. Even on single donation level, HBsAg positive/NAT negative samples are a well known published fact (e.g., see: Ikeda H, Sato S, Ihara H, Sakaya S, Kato T: A serological HBV testing for the detection of low viremic carriers in donor screening, Vox Sang Vol. 79, Supplement 1, 2000, P364). Complex formation has to be accounted for by control of time and temperature.</p>

The guidelines summarize requirements in order to validate commercially available serological test kits for the testing of plasma pools to ensure that single donations being positive for HBsAg do not escape testing or might reach plasma pools for production otherwise.

Concern is expressed that single donations being positive for HBsAg might escape testing or might reach plasma pools for production otherwise.

Commercially available serological test kits are validated for single donation testing only. No test is commercially available that has been released by kit manufacturers for plasma pool testing. Test kits validated for single donation screening might not detect all HBsAg positive donations in plasma pools. This depends mainly on the concentration of HBsAg in the donation, the size of the pool (i.e. dilution factor) and may partly be influenced by the necessary compromise between specificity and sensitivity in single donation screening assays.

The drafted guideline proposes validation procedures for commercially available single donation test kits including improvement of sensitivity and to use these altered tests for screening plasma pools.

In presence of a regulatory requirement for plasma pool testing and in absence of suitable test systems, the fractionators are complying by using test kits designed for single donation testing. Such test kits are validated by the fractionators for use in pool testing within justifiable limits.

The drafted guideline suggests, that each fractionator qualifies commercially available single donation kits for the use in pools by adjusting (i.e. lowering) their sensitivity threshold, following certain procedures as outlined in the drafted guideline. This, however, will result in the fact that each fractionator will validate a given commercially available single donation screening assay with individually characterized positive samples. In addition, each fractionator will establish individual evaluation criteria based on many different approaches. This redundant approach (various assays, different positive samples, different plasma pool compositions) will lead to highly diverse results.

To avoid this situation, it would generally be highly preferable if the test kit manufacturer validate their single donation kits for use in plasma pool testing or produced test kits specifically designed for this purpose. The kit producers know their assays best and, by following the outline of the new validation guideline, could produce generally available assays for plasma pool testing. Those assays would have the same characteristics for all users. This approach not only would lead to a controlled improvement of the kits but also support a more harmonized and uniform approach to this testing issue.

Kit manufacturers cannot be required to develop kits for pool testing. Until now, kit manufacturers have not validated their assay kits for use on pooled plasma presumably because there is not a large enough market to make this worthwhile.

<p>In conclusion</p> <p>A series of measures are implemented to avoid positive donors from donating and to intercept positive donations once received. In addition, state-of-the-art data management and sample/donation logistics make the escape of a positive donation (once identified) highly unlikely. Should a donation not be detectable at single unit level by a commercially available test kit, no other assay will react positive on pool level, regardless of additional measures taken to improve its sensitivity.</p> <p>Already 10 years ago Dr. Rabenau (Institute of Medical Virology, J.W.Goethe University, Frankfurt) concluded in his work on serological testing of plasma pools (1):</p> <p>“If a contamination with donations originating from individuals with acute HBV, HIV, or HCV infection or with a poor humoral immune response exists, serologic testing for HBsAg, anti-HIV, and anti-HCV may fail to detect potentially infectious plasma pools. Serologic testing of plasma pools in addition to single blood donation screening may not necessarily increase the security of blood products. However, when screening of blood donations or inactivation procedures are not adequate, it might be of (limited) value for the detection of potentially infectious plasma pools.”</p> <p>In light of this statement, dated from 1996, when wide-spread NAT testing was not yet established, and taking into consideration the improvements in screening assays and inactivation methodology that were made since then - it might be questioned whether ten years later serology-based virus marker testing of plasma pools has retained any value at all for the viral safety of plasma derived products.</p> <p>This view is corroborated by the recent changes in the European Pharmacopoeia (2), concerning HCV viral marker screening. From 2006 on, serological screening for anti-HCV antibodies in production pools is no longer required.</p> <p>References</p> <p>1. Rabenau, H., R. Schutz, A. Berger, H. W. Doerr, and B. Weber. 1996. How accurate is serologic testing of plasma pools for hepatitis B virus surface antigen, anti-human immunodeficiency virus 1 and 2, and anti-hepatitis C virus? <i>Infusionsther. Transfusionsmed.</i> 23:124-130</p> <p>European Pharmacopoeia, Supplement 5.3, valid from 1.1.2006.</p>	<p>The purpose of pool testing for anti-HIV and HBsAg is a safeguard against errors in testing of individual donations or errors in pooling of donations. It is a measure to detect GMP failures. As anti-HIV and HBsAg may be present in donations with low or undetectable viral nucleic acid levels, a pool positive for anti-HIV or HBsAg because of a GMP failure may not have detectable levels of nucleic acid.</p> <p>3 confirmed HBsAg positive pools have been detected during OCABR in the last 4 years and communicated as rapid information. Also see:</p> <p>Ferguson M, Minor PD, Garrett PD, Page M, Thorpe R, Barrowcliffe T: Testing Plasma Pools for markers of viral infection: the UK experience, <i>Vox Sang</i> 1996;71:21-26.</p> <p>Linden JV: Error contributes to the risk of transmittable disease, <i>Transfusion</i> 1994;34:1016.</p> <p>This topic was also addressed in the report of the EMEA Workshop on the Plasma Master File 2001 (EMEA/CPMP/BWP/1737/02, December 2002) published on the EMEA website.</p> <p>In contrast to state-of-the-art HBsAg and anti-HIV test kits, dilutional sensitivity of currently available anti-HCV kits is insufficient to detect single donation contaminations in manufacturing pools. This is the reason why pool testing for anti-HCV is no longer required.</p> <p>It should be noted that it is possible to detect anti-HCV in pools from unscreened donations (multiple positive donations in a pool, as could be demonstrated with samples from 1994 and 1995 evaluated retrospectively).</p>
<p>Currently, as it is stated in both guidelines, there is not a numerical limit regarding detection of HBsAg and antibodies against HIV in plasma pools. Moreover, there is neither a limit for the minimum sensitivity of the assays that are used in detection of the viral markers. This information is well known for both manufacturers and assessors and it is also clear that it is not straightforward to introduce such limits. For example, there is not an international standard for anti-HIV antibodies, which is a very early requirement if a limit were to be established the presence of anti-HIV antibodies in plasma pools.</p> <p>In my opinion, the lack of a limit of detection makes the guideline very general and vague in many aspects. It is not a matter of poor writing of the guideline (in fact the guidelines are very well written and cover all aspects of validation). Just one example, it is stated in the guideline: “Selection of a test kit for plasma pool testing should be based</p>	<p>The approach of the validation guidelines is not to give straightforward requirements for analytical sensitivity, but to give guidance to select, optimize and validate commercial single donation kits for the purpose of pool testing. As stated in the guidelines, plasma pool serology is not capable of detecting all contaminated single donations that may have escaped single donation screening. However, as was seen in the context of PMF certification, some MAHs used (or still use) methods that do not stand a chance to detect even a high titre sample, or employed sampling and retesting strategies that endangered the detection/confirmation of HBsAg.</p>

on a high analytical sensitivity.”. This sentence is so general and obvious that does not help much neither manufacturers nor assessors on how to select the test kit.

On the other hand it is clear (as it is very well explained in the validation guideline) that some validation of the assays is needed since detection of serological viral markers is currently done using commercial kits developed for testing individual donations and not for testing pools of many donations. In addition, it is generally useful (for both manufacturers and assessors) to have some guidance on the aspects to be analyzed when validating an analytical test.

Based on the above considerations, I just wonder whether it would be better to include a short text in the “Note for guidance on plasma derived medicinal products” (CPMP/BWP/269/95, 3.2.2)” rather than implementing these two guidelines on validation. The text to be introduced: i) it would mention the requirement of using kits for single donations subject to the Common Technical Specifications (CTS, 2002/364/EC) (as indicated in the validation guidelines) (in my opinion this ensures the use of kits of adequate sensitivity and specificity); ii) it would mention the need of validating the assays for testing plasma pools; and iii) it would list and describe in short the characteristics to be validated.

Due to the complex nature of pool serology validation, introduction of a short paragraph in the NfG on Plasma Derived Medicinal Products seems insufficient guidance.

SPECIFIC COMMENTS ON TEXT		
1. SCOPE		
Line no. + paragr. no.	Comment and Rationale	Outcome
<u>HbsAg</u> <u>Comments specifically on document EMEA/CHMP/BWP/29839/0/2005:</u> § 1.	Scope page 4 line 4. Is the wording 'commercial pool' correct? We prefer the wording 'Manufacturing pool'..	Guidelines reworded
2 SELECTION OF THE TEST KIT(S)		
Line no. + paragr. no.	Comment and Rationale	Outcome
Par. 1	<p>“Selection of test kits should be based on a high analytical sensitivity.” This requirement results in a very large evaluation study in order to determine the most sensitive kit on the market. Authorities could be of much help in this respect. Since all assays are either CE marked or otherwise controlled it would be easy for the authorities to recommend/approve certain assays that fulfill this requirement.</p> <p>Alternatively, the authorities could define a lower limit of detection as done e.g. for the HCV NAT test (100 IU/ml), which automatically would narrow the selection of suitable tests.</p> <p>The guideline suggests selecting only kits that show high dilutional sensitivity. As a selection criterion, it is suggested that "relative endpoint dilution titers of well-characterized positive samples" should be compared. However, such "well-characterized positive samples" with a few exceptions do not exist and the guideline lacks further detail on this topic.</p>	<p>Authorities cannot recommend the use of specific assays. Analytical sensitivity may change when assay design changes and the users must be aware of this e.g. several of the HIV antibody/antigen combination assays have a lower analytical sensitivity than the antibody only version. CE marking of anti-HIV assays does not include an evaluation of analytical sensitivity although there is a limit given for HBsAg.</p> <p>Working standards, which are dilutions of positive donations or pools of positive donations, which can be used to monitor the analytical sensitivity of assays are available.</p>

3. Validation		
3.1 Specificity and determination of a grey-zone for pool samples		
Line no. + paragr. no.	Comment and Rationale	Outcome
Par. 1-4	<p>In theory, it appears feasible to increase sensitivity of an assay by lowering specificity provided that non-specific factors are sufficiently diluted in pools as assumed by the authors. However, the introduction of such a "grey-zone" leaves important questions unanswered.</p> <p>By definition, the grey-zone would be positioned between the cut-off value validated by the kit manufacturer for single donation testing and the average result of the plasma manufacturer obtained from pool testing with the suggested mean cut-off of negative pools plus 3x standard deviation.</p> <p>The draft does however not state what the required action would be, if a plasma pool result fell into this grey-zone. Would a pool with a result within the grey-zone have to be destroyed? If no, the question arises why a grey-zone should be determined in the first place. If yes, this would be no grey-zone but a lowered cut-off.</p> <p>Lowering the cut-off value to a limit below the one validated for single donation testing based on a small set of pool test data is not without danger, because this measure may lead to an increased rate of false-positive results. It is difficult to predict the response of a given commercial test to changes in the plasma pool composition. Fractionating plasma from many different suppliers with a high degree of variability in the composition of plasma pools it will be very difficult to come up with a reliable cut-off applicable for all pools testing. If new plasma suppliers are added, the influence of their plasma on the previously established system will be an important unknown. An additional factor to be considered is pool size. Since pool sizes vary between manufacturers (and often within manufacturers for different pathways) it is important to keep in mind that such a suggested grey zone would have to be validated for each pool size individually, since non-specific factors present in single donations would be diluted to a different degree in fractionation pools of different size. In addition, how could such a grey-zone result be verified? Using a different standard kit for single donation screening would not be sufficient, thus, a second validated assay system with an identical grey-zone needed to be kept available. NAT would not be helpful because the presence of HBsAg is not necessarily accompanied by presence of virus.</p>	<p>Section 3: Use of grey zone was clarified in the guidelines.</p> <p>Experience of OMCLs who test a wide range of manufacturers' plasma pools has not shown this theoretical concern about the influence of plasma pool composition to be a practical problem.</p>

3.2 Robustness		
3.2.1 Inter-assay and intra-assay		
Line no. + paragr. no.	Comment and Rationale	Outcome
	<p>Neither certified "representative negative pool samples" nor "low positive samples" exist and their definition is missing in the document</p> <p>If the inter-assay variability is investigated in 3 independent assays, the term "if available" should be added after "equipment".</p>	<p>3.2: Manufacturers can use a panel of their own negative pools. These will have been 'validated' through their own assays and those of the OMCL undertaking plasma pool testing on their behalf. As an alternative, the panel of pools set up by EDQM for the validation of HCV NAT (H1005000) could be used if available. However, it is recommended to use the former.</p> <p>The definition of a low positive control is given in Section 4.3.</p> <p>Editorial change to add "if available" has been made.</p>
§. 3.2.2	<p>The remark on complex formation between HBsAg and anti-HBs is not relevant for large plasma pools. These pools are mixed for some time for the preparation of the first homogeneous pool. Antigen-antibody complexes have already been formed at the moment of the preparation of the test sample from this homogeneous material. It is not clear why always a fresh sample should be thawed for the test, because HBsAg is a stable antigen, that can be stored for several days to weeks in plasma or plasma without losing immune-reactivity according to several 'Instructions for Use' of diagnostic devices for HBsAg.</p>	<p>The comments on stability of HBsAg refer to single donations in which antibody is negative so not entirely applicable. The stability of HBsAg referred to in IFU of single donation test kits can only be demonstrated in single donations, where high levels of anti-HBs do not occur simultaneously with HBsAg (or, if both analytes should be present, immunocomplex formation did already take place before the donation was taken).</p> <p>Formation of immunocomplexes in pooled plasma at fractionation temperature is a rather slow process (approx. 3-4 days for 50% signal loss), so efforts should be taken to reduce time from sampling to freezing, and thawing to testing, and the use of fresh samples for retesting/confirmation to avoid avoidable loss of detectable analyte.</p>
3.3 Detection limit HBsAg		
Line no. + paragr. no.	Comment and Rationale	Outcome
Par. 1,2	<p>The establishment of the detection limit for pool cut-off is to be carried out by using anti-HBs-free matrix, representative of a plasma pool. Such, the limit is expected to be "considerably" lower but it remains undefined what an acceptable limit would be.</p> <p>However, the real production pool may contain various amounts of anti-HBs antibodies and this might affect the result. This is addressed in the document by the requirement that the impact of the anti-HBs-containing matrix is to be assessed. No guidance is given what is expected and how to handle the outcome.</p>	<p>Acceptability should be decided on basis of risk assessment, involving the size of the pool (number of donations). As HBsAg titres in donor plasma are subject to broad variability, it is not possible to give a clear definition except "as good as technically possible". This should be demonstrated by a proper validation study, which includes assessment of the "worst case scenario" (in respect to time, anti-HBs concentration, temperature and agitation) allowed by pooling, sampling and testing SOPs that might result in signal loss by complex formation.</p>
4.1 Standard operation procedures for plasma pool testing		

Line no. + paragr. no.	Comment and Rationale	Outcome
	Pertaining to bullet point four we would like to point out that for some automatic instruments the operative parameters of the test are not disclosed in detail by the manufacturer	Bullet point 4 was adapted: Incubation procedures (including tolerance limits for time and temperature, e.g. according to the test kit manufacturer's specifications/instrument settings)
4.3 Test kit independent controls		
Line no. + paragr. no.	Comment and Rationale	Outcome
Par. 1	The "low level of reactivity likely to be found in contaminated pool samples" is not specified. Also, the requested "low-level positive control" remains undefined. Qualification of each new lot of test kits, using an "off-the-shelf" qualification panel, is suggested as an alternative to inclusion of kit independent controls on every test run.	<p>The low level of reactivity depends on the dilution factor of an individual positive donation in the plasma pool.</p> <p>For preparation of the control, any confirmed HBsAg positive sample may be used as spiking material, anti-HBs-free plasma or serum may be used as diluent. The signal of the low-level positive control should be near the cut-off and in the dynamic range of the assay, e.g. 2-3 times the single donation cut-off.</p> <p>The qualification of every test kit lot with an "off-the-shelf" qualification panel is not considered an adequate alternative to low level controls in every test run, as day-to-day performance is not monitored.</p>
4.4 Proficiency testing		
Line no. + paragr. no.	Comment and Rationale	Outcome
Par. 1	Proficiency testing schemes that incorporate the considerations laid out in this document are currently not offered.	Proficiency schemes aimed at diagnostic laboratories are not necessarily appropriate for laboratories testing only plasma pools as labs testing pools are interested in analytical and not clinical sensitivity.
5. CONFIRMATION STRATEGIES		
Line no. + paragr. no.	Comment and Rationale	Outcome
Par. 1	Alternative tests are suggested in the document. Since the standard assay has been diligently and individually optimized for the given purpose it is highly unlikely to find positivity in a low level positive pool with non-optimized assays or with assays optimized for another system. Therefore, two independent assays need to be maintained one for routine screening and one for verification.	HBsAg results may be confirmed by alternative validated assays (using different (monoclonal) antibodies), neutralization with commercial reagents or "self-neutralization" by anti-HBs in the pool sample (signal loss kinetics in comparison to spiked samples).