

9 February 2006

Reference: EMEA 06004

**PPTA's comments on the proposed Guidelines on  
Validation of immunoassays for the detection of Hepatitis  
B Virus surface antigen (HBsAg)  
(EMEA/CHMP/BWP/298390/2005) and anti-HIV  
(EMEA/CHMP/BWP/298388/2005) in Plasma Pools**

**General observations**

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 or HIV-Ab do not 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 or HIV-Ab positive donations in plasma pools. This depends mainly on the concentration of HBsAg or HIV-Ab 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, and 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.

### **Detailed comments:**

#### **2. Selection of the test kit(s)**

“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.

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.

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.

#### **3. VALIDATION**

##### **3.1 Specificity and determination of a greyzone for pool samples**

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 "greyzone" leaves important questions unanswered.

By definition, the greyzone 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.

However, the draft does not state what the required action would be if a plasma pool result fell into this greyzone. Would a pool with a result within the greyzone have to be destroyed? If no, the question arises why a greyzone should be determined in the first place. If yes, this would be no greyzone but a lowered cut-off.

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 greyzone 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 greyzone needed to be kept available. NAT would not be helpful because the presence of HIV-Ab/HBsAg is not necessarily accompanied by presence of virus.

### **3.2 Robustness**

Neither certified "representative negative pool samples" nor "low positive samples" exist and their definition is missing in the document.

If the inter-assay variability is investigated in 3 independent assays, the term "if available" should be added after "equipment".

### **3.3 Detection limit HIV-Ab**

"... the detection limit has to be determined with a representative panel of positive samples reflecting different subtypes and groups taking into consideration the epidemiological situation in the respective regions where plasma is sourced".

This requirement loads a fairly high burden on the presumptive user of an assay. First, samples of all respective subtypes have to be obtained and skillfully manipulated. Subsequently, all available assays for single donation screening have to be assessed for the given composition of subtypes in order to evaluate the one, which gives the highest dilutional sensitivity under the given conditions (Selection of the test kit(s), Section 2). From this assessment procedure the most suitable kit will be chosen and validation will be conducted.

In the absence of independent reference material the data created in such a sensitivity determination will not allow any direct evaluation of assay sensitivity as the content of HIV-Ab in the samples used is not known. This fact drastically limits the value of a validation based on such sample material.

Consideration of the number of donations in a typical pool may be difficult since pools sizes may vary even for the same manufacturer for different production pathways. Testing samples at pool dilution will only demonstrate what is already known: Some samples may be detected at pool dilution and others not.

However, would non-detection of a positive sample at a certain pool dilution require to reduce pool size in fractionation? If yes, this would have a huge impact on the established, validated and approved production processes that needs to be considered. If not, why would pool size be a matter of concern?

### **3.3. Detection limit HBsAg**

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.

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.

#### **4. QUALITY ASSURANCE**

##### **4.1 Standard operation procedures for plasma pool testing**

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.

##### **4.3 Test kit independent controls**

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.

##### **4.4 Proficiency testing**

Proficiency testing schemes that incorporate the considerations laid out in this document are currently not offered.

#### **SECTION 5. CONFIRMATION STRATEGIES**

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. NAT, as stated above, is of little support because presence of antibodies is not necessarily accompanied by presence of virus.

#### **In conclusion**

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.

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):

"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."

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.

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.

#### References

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? *Infusionsther. Transfusionsmed.* 23:124-130
2. European Pharmacopoeia, Supplement 5.3, valid from 1.1.2006.