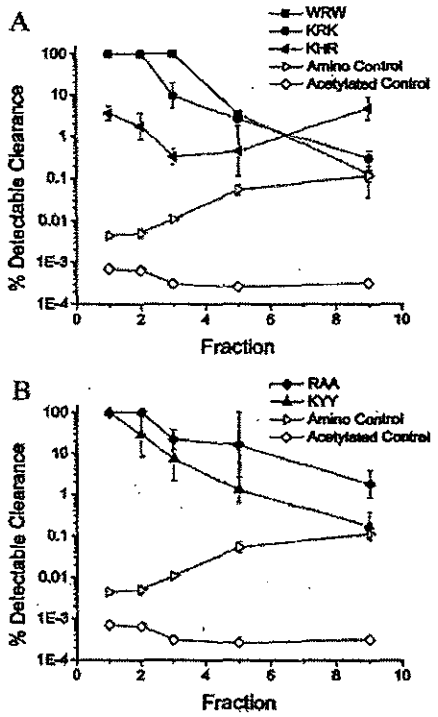

Figure 2. Binding of trimeric peptides to PPV in 7.5% human blood plasma. WRW was able to bind 100% of the detectable PPV in the first 3 column volumes. After 9 column volumes, WRW was still able to bind as much as the amino control. The amino control and acetylated control are the same in each figure as a reference point for comparison of the different peptide resins. Columns were run in duplicate, and the error bars represent the detectable clearance of each column.

a possibility, as this was observed in the purification of tumor necrosis factor-α using an ion exchange column (*37*). The longer these host cell proteins are retained on the column, the greater the potential for protein denaturation, and this may provide different binding sites for the virus. Virus binding to host cell proteins is confirmed by the fact that when highly purified virus suspensions containing less than 100 μg/mL of total protein are used in resin challenge, the amino control resin binds no more than 1 log of PPV even after 10 column volumes (data not shown). Viruses are also known to easily aggregate (*40*), and so the presence of denatured protein could become a new binding surface for the virus.

The trimer KHR was able to achieve 4 logs clearance in the first column volume but still left in solution 3 log (MTT/mL) of virus. This peptide column showed the same decrease in viral clearance in the first three column volumes seen with the other peptide resins, but in subsequent column volumes, it exhibited an increase in detectable clearance. This latter behavior was only seen in the amino control resin and not the other peptide resins. It is suspected that this resin may be causing denaturation of proteins in solution as discussed above, but this issue was not examined further. This resin was just discarded as one of the lead candidates, as its performance as a viral clearance ligand was unacceptable.

The trimers WRW, KRK, RAA, and KYY all exhibited breakthrough of PPV in the presence of plasma proteins after the first three column volumes (Figure 2). There was no detectable cooperative binding observed for these resins in the flowthrough fractions tested. Of these resins, only WRW was able to completely clear all detectable PPV in the first three column volumes from 7.5% human blood plasma. With optimization of the peptide density and spacer length, this resin may be able to clear PPV in all nine column volumes of challenge solution containing 7.5% human blood plasma. Human blood plasma also contains many different proteins, and only one or two may be interfering with the binding of PPV. For example, if albumin is the predominant protein binding to the resin, then the peptide may be able to clear PPV very well from a solution that contains other proteins but not albumin. In this case, WRW has the potential to be used effectively for final purification of a pure protein with excellent removal efficiency.

Chromatographic beads are not the most efficient way to remove large particles from process streams. The viruses, having a diameter on the same order of magnitude as the pore diameter, have small diffusion coefficients in the pores of the beads, and viruses quickly clog the pores. Consequently, the accessible surface area of the beads is mainly associated with the outside surface of the bead, and the inner pore surface is not available for binding. Membranes have a better geometry for binding of particles such as viruses, as there are not any diffusional limitations. However, the screening of a combinatorial library of peptides is difficult to do on a membrane surface. The SPOT method, developed by Ronald Frank (*41*), is used to produce peptide libraries on a cellulose membrane surface, but if done manually, only several hundreds of peptides can be created in 2−3 days (*42*). This is a small library compared to the thousands of peptides that can be screened on chromatographic beads. In addition, the binding to a peptide on cellulose fibers may be quite different from that observed on other membrane materials. There are currently no large ligand libraries on any membrane surface that is likely to be used for large-scale virus removal. This study provides proof-of-concept that peptides have the ability to remove viruses specifically. In the future, it may be beneficial to change the geometry of the support for improved access of all of the ligands to the viral particle, but currently the bead geometry offers a better screening platform.

4. Conclusions

Small trimeric ligands that specifically bind to porcine parvovirus were isolated from a solid-phase peptide library. In PBS, 100% of detectable infectious virus was removed from solution for every fraction that was tested, up to nine column volumes. This demonstrates the potential of these peptides for use in virus removal from samples of relatively simple composition, such as for water purification applications. In more complex mixtures, such as 7.5% human blood plasma, peptide WRW was able to remove all detectable infectious viruses in the first three column volumes. This is impressive for a ligand that contains only three amino acids, as most peptide ligands are a minimum of six amino acids in length. Enhanced specificity and binding affinity may be found using an increased number of amino acids in the ligand, and this is currently being examined. Tethering one or more of the ligands to a membrane with more suitable geometry may improve virus removal efficiency from complex mixtures. The ligands could also be optimized for application to specific process streams, so that a single ligand must only compete with one therapeutic protein, thereby overcoming competitive binding and facilitating use as an efficient virus absorbent.

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医薬品　研究報告 調査報告書

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一般的名称	－		研究報告の公表状況	http://www.fda.gov/cber/gdlns/natparo.pdf	公表国 米国	
販売名（企業名）	－					

研究報告の概要

パルボウイルス B19 DNA を検出するための実施手順に関するガイダンス案が示された。
・全ての血漿由来製剤について、製造プール中のパルボウイルス B19 DNA のウイルス負荷を確実に 10^4IU/mL 以下とするため、製造過程の品質管理検査としてパルボウイルス B19 の NAT を実施すべきである。
・ミニプールサンプルのパルボウイルス B19 の NAT 検査は 10^6IU/mL 以上の個別ユニットを検出できる感度とすべきである。
・個別ユニットのパルボウイルス B19 DNA の力価が 10^6IU/mL 以上であることがわかった場合、又は、製造用プール血漿で 10^4IU/mL のパルボウイルス B19 DNA を上回る可能性がある場合、その後の製造に使用すべきではない。

原料血漿及び回収血漿において、パルボウイルス B19 DNA を検出するため、ならびに製造プールにおけるパルボウイルス B19 DNA のウイルス負荷が 10^4IU/mL 以下であることを示すために用いるパルボウイルス B19 の NAT 検査の精度、感度、特異度、再現性及びその他の性能特性を示すバリデーションデータを維持管理すべきである。

報告企業の意見	今後の対応
血漿由来製剤製造用原料血漿に関するパルボウイルス B19 の NAT 検査に関するガイダンス案の情報である。 当社血漿分画製剤は最終製品において NAT 検査を行い、パルボウイルス B19DNA 陰性であることを確認している。	今後ともパルボウイルス B19 に関する血漿分画製剤の安全性に関する情報に留意していく。

使用上の注意記載状況・その他参考事項等

慎重投与（次の患者には慎重に投与すること）
・溶血性・失血性貧血の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］
・免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］

重要な基本的注意
(1)本剤の原材料となる‥［スクリーニング項目、不活化・除去工程］‥投与に際しては、次の点に十分注意すること。
1）血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。

妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Parvovirus B19 Transmission by Plasma-Derived Products

DRAFT GUIDANCE

This guidance document is for comment purposes only.

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to either http://www.fda.gov/dockets/ecomments or http://www.regulations.gov. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this draft guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact Mahmood Farshid, Ph.D., at 301-496-0952, or by Fax at 301-402-2780.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
July 2008

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Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Parvovirus B19 Transmission by Plasma-Derived Products

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, FDA, are issuing this guidance to provide you, manufacturers of plasma-derived products, with recommendations for performing parvovirus B19 nucleic acid testing (NAT) as an in-process test for Source Plasma and recovered plasma used in the further manufacturing of plasma-derived products. Such testing will identify and help to prevent the use of plasma units containing high levels of parvovirus B19. This guidance also recommends how to report to the FDA implementation of parvovirus B19 NAT.

We recognize that in the current business practice for parvovirus B19 NAT in-process testing, several weeks can elapse between collection of the units of Source Plasma or recovered plasma and identification of B19 NAT-positive pools or units. We encourage manufacturers of plasma-derived products to employ practices that will reduce the time between product collection and in-process testing to allow for the meaningful notification of blood and plasma collection establishments of positive test results within the dating period of components.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Parvovirus B19 is a small, non-enveloped single strand DNA virus. This virus is highly resistant to all commonly used inactivation methods, including heat and solvent/detergent (S/D) treatment, and is also difficult to remove because of its small size. The parvovirus B19 can be

transmitted by blood components and certain plasma derivatives, and may cause morbidity to susceptible recipients such as pregnant women (and their fetuses exposed in utero), persons with underlying hemolytic disorders, and immune compromised individuals (Refs. 1 and 2). The disease transmission by transfusion of blood components is rare; however, extremely high levels of parvovirus B19, up to 10^{12} IU/mL, in plasma of acutely infected but asymptomatic donors may present a greater risk in plasma derivatives due to pooling of large numbers of plasma units in the manufacture of these products. The virus can be detected by NAT in plasma pools when there are high levels of parvovirus B19 DNA in viremic donations. For example, the parvovirus B19 DNA can be detected in various plasma-derived products, particularly in coagulation factors (Refs. 3 and 4). There have been a few reports of parvovirus B19 infection associated with the administration of coagulation factors (Refs. 5 and 6) and S/D Treated Pooled Plasma (Refs. 1 and 7). Parvovirus B19 DNA is less frequently detected in albumin and immunoglobulin products and, when detected, the levels are usually low. There are no confirmed reports that albumin and immunoglobulin products have transmitted parvovirus B19 infection.

We have held or participated in several meetings to discuss the potential risk of parvovirus B19 infection by plasma-derived products, and the strategy for reducing such risk. The meetings included FDA-sponsored NAT workshops in 1999 and 2001 (Refs. 8 and 9), Blood Products Advisory Committee (BPAC) meetings in 1999, and 2002 (Refs. 10, 11, and 12), the National Heart, Lung, and Blood Institute-sponsored Parvovirus B19 workshop in 1999 (Ref. 1), and an ad hoc Public Health Service (PHS) panel in 2002 (discussed at the 2002 BPAC meeting (Ref. 12)). In these meetings, it was recognized that the scientific data indicate that parvovirus B19 is highly resistant to the available viral inactivation methodologies, and is difficult to remove because of its small size. The viral inactivation/removal steps routinely used in the manufacturing process of plasma-derived products do not alone appear to be sufficient to completely clear the virus if high viral load is present in the starting material. Therefore, in these meetings, a common recommendation for mitigating the risk of parvovirus B19 transmission by plasma derivatives has been to limit the virus load in the manufacturing plasma pool by testing the plasma donations for high titer parvovirus B19 DNA, using a minipool format. This viral load reduction strategy combined with the ability of the manufacturing process to clear the residual virus could greatly reduce the risk of parvovirus B19 infection by plasma-derived products.

The recommended limit in this guidance for viral load of parvovirus B19 DNA in the manufacturing plasma pool (i.e., not to exceed 10^4 IU/mL) was primarily derived from studies that were conducted on the transmission of parvovirus B19 associated with S/D Treated Pooled Plasma (Refs. 1, 7, and 10). In principle, testing in a minipool format to measure the viral load for parvovirus B19 DNA in a manufacturing plasma pool is acceptable in order to exclude only the high-titer plasma donations, thereby avoiding too great a loss of plasma for further manufacturing. Furthermore, during the viremic period for parvovirus B19 infected donors, which can be very lengthy, low levels of parvovirus B19 coexist with parvovirus B19 antibodies (potentially complexing with and neutralizing the virus). Therefore, it is undesirable to remove plasma units with low levels of B19 DNA, because it would diminish the parvovirus B19 antibody levels in plasma pools and in some of the resulting plasma-derived products (Refs. 13 and 14).

III. RECOMMENDATIONS

We recommend that you implement the following procedures to detect the presence of parvovirus B19 DNA:

- For all plasma-derived products, you should perform parvovirus B19 NAT as an in-process quality control test to ensure that the viral load of parvovirus B19 DNA in the manufacturing pools does not exceed 10^4 IU/mL.
- Use parvovirus B19 NAT on minipool samples to screen plasma units intended for further manufacturing into plasma-derived products. The sensitivity of the NAT assay, in any size minipool, should be at least 10^6 IU/mL for detection of any single donation when tested in the minipool (i.e., if the titer of an individual unit is 10^6 IU/mL or higher, the test result on the minipool will be positive). Primers and probes selected for parvovirus B19 NAT should detect all known genotypes of the virus (Ref. 15).
- When identified, you should not use individual plasma units intended for further manufacturing into plasma-derived products, when such units are found to have a titer of parvovirus B19 DNA at or above 10^6 IU/mL, or when use of a positive unit might result in plasma manufacturing pools exceeding a parvovirus B19 DNA titer of 10^4 IU/mL.

You should maintain validation data demonstrating the accuracy, sensitivity, specificity, reproducibility, and other performance characteristics of the parvovirus B19 NAT assay used for the detection of parvovirus B19 DNA in the Source Plasma and recovered plasma, and for demonstrating that the viral load of parvovirus B19 DNA in the manufacturing pool does not exceed 10^4 IU/mL.

If the recommendations are implemented, you must notify FDA of the changes to an approved application under 21 CFR 601.12(c)(5) ("Supplement-Changes Being Effected"), and submit the information required in 21 CFR 601.12(b)(3)(i) through (vii).

IV. REFERENCES

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