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Introduction of Nucleic Acid Amplification Testing of Plasma Pools – Implications for an Integrated Blood Service

Key Words
Plasma pools · Fractionation · HCV RNA · Minipool testing · Virus safety

Summary
Most fractionated plasma products are manufactured by processes that include at least one process step capable of inactivating 6 or more logs of HCV. The requirement to test fractionation pools for HCV RNA is being developed on the back of experience of HCV transmission by a product that did not include such an inactivation step. Application of nucleic acid amplification testing (NAT) will contribute little to the safety of fractionated products; in time it will provide an opportunity to measurably enhance the safety of cellular products. The National Blood Service (NBS) will implement NAT in two distinct phases. In the first phase NAT will be applied to minipools of about 500 donations in order to release plasma for fractionation to allow compliance with such regulatory requirements as are defined for fractionation pools. In a second phase NBS will look to secure the more obvious benefits of application of NAT to increase the assurance of safety of cellular products, probably once again by minipool testing. Testing is likely to be extended to genomes other than HCV as experience develops.

Schlüsselwörter
Plasma-Pools · Fraktionierung · HCV-RNA · Minipool-Testung · Virussicherheit

Zusammenfassung
Die meisten fraktionierten Plasmaprodukte werden mit Verfahren hergestellt, die wenigstens einen Schritt enthalten, der die HCV-Viruslast um 6 oder mehr log-Stufen reduzieren kann. Die Forderung, Plasma-Pools zur Fraktionierung auf HCV-RNA zu testen, wurde durch die Erfahrung der Virusübertragung durch ein Produkt hervorgerufen, das einen solchen Inaktivierungsschritt nicht enthielt. Die Anwendung der Nukleinsäure-Amplifikationstestung (NAT) wird wenig zur Sicherheit fraktionierter Produkte beitragen, sie bietet jedoch die Möglichkeit, die Sicherheit zellulärer Produkte meßbar zu verbessern. Der National Blood Service (NBS) wird die NAT in zwei Phasen einführen. In der ersten Phase werden Minipools von ungefähr 500 Spenden getestet, um Plasma zur Fraktionierung entsprechend den regulatorischen Anforderungen freigeben zu können. Die zweite Phase wird dazu dienen, die offensichtlicheren Vorteile der NAT bei der Verbesserung der Virussicherheit zellulärer Produkte zu nutzen, wahrscheinlich ebenfalls durch Minipool-Testung. Mit zunehmender Erfahrung wird die Testung voraussichtlich auf andere Viren ausgedehnt.

Introduction

Assurance of virus safety has been a dominant consideration in the manufacture of fractionated plasma products over the last 15 years. It is accepted that one of several factors contributing to the safety of such products is the virus burden in the pooled plasma used in their manufacture. Such virus burden is minimised by attention to donor selection (for low risk), by exclusion of plasma from donors found to be marker-positive in serological testing for viruses subject to mandatory exclusion (HBV, HCV and HIV-1 and -2) and by tests for the same markers in plasma pools (though the contribution to product safety of this last step might be debated). In Europe at least, the definition of viruses subject to mandatory exclusion has been made in the context of blood (and derived cellular components), with the same standards attaching to individual plasma donations by default.

On February 23, 1994, Baxter Healthcare Corporation re-

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moved its intravenous immunoglobulin preparation 'Gammagard' from the world-wide market, following reports of acute hepatitis C infection in patients treated with the product [1]. Concluding a relationship between infectivity and the introduction of multi-antigen screening for HCV antibodies of source plasma donations used in the manufacture of an intravenous immunoglobulin without a specific virus inactivation step [2], the Center of Biologics Evaluation and Research (CBER) in the USA established a requirement for testing such finished preparations for HCV RNA as a requirement for release [3, 4].

The response in Europe to the same circumstances was different. The Paul-Ehrlich-Institut established a requirement (for the German market) that only HCV RNA-negative plasma pools should be used in the manufacture of intravenous immunoglobulin preparations without a validated virus inactivation procedure [5]. This same requirement was adopted for community-wide implementation by the European Committee for Proprietary Medicinal Products (CPMP) [6], applicable to intramuscular immunoglobulins. These actions were seen as a first urgent response to the evidence of HCV transmission by Gammagard. The establishment of a similar test requirement for all fractionation pools, irrespective of derived products, was a logical extension. CPMP has indicated its intention to implement such a requirement although at this stage the date for and conditions of introduction of this extended testing have yet to be determined.

The National Blood Service in England

The National Blood Service (NBS) in England combines blood supply management (in three 'zones') and manufacture of plasma products (at Bio Products Laboratory (BPL), Elstree, UK) under a single managing authority, the National Blood Authority (NBA). It is in that respect a truly *national* blood service, plasma from the three zones being transferred to Elstree for fractionation, allowing national demand for fractionated products from UK voluntary, unpaid donors to be met in full. More than 95% of the plasma fractionated by BPL is fresh frozen plasma recovered from whole blood, with approximately 2 million of the annual total of 2.4 million blood donations being handled in this way. Testing of donor samples for mandatory virus markers is undertaken at blood centres in each zone, before shipment of released plasma to Elstree for fractionation.

The Basis of Testing

The outcome of testing plasma pools for HCV RNA will be determined by, *inter alia*, the incidence of HCV RNA-reactive donations in the donor population, the fractionation pool size and the sensitivity of the applied test for HCV RNA. Testing will be undertaken both by the manufacturer and by the Official Medicines Control Laboratory (OMCL) of the member state with responsibility for control authority batch release. It will be understood that a typical HCV window phase donation, containing (say) 2.5×10^6 genome equivalents (geq) per ml and diluted in a fractionation pool with (say) 25,000 other plasma donations, will result in a pool content of HCV RNA of approximately 100 geq/ml. Since nucleic acid amplification testing (NAT) methodology is applied in different testing laboratories with sensitivities ranging from 20–1,000 geq/ml (with an agreed control sample dilution specified at approximately 400 geq/ml), it is clear that the outcome of testing will be critically dependent on the sensitivity of the applied test.

It is argued (most often by regulators) that, notwithstanding in-process virus inactivation, products will be safer as a result of the introduction of a test which gives confidence that no donation included in the pool contained significant levels of HCV RNA. Fractionators have sought to establish the reasonableness of using NAT as a limit test (rather than on the basis of reactivity or non-reactivity), typically arguing a limit for HCV of 1,000 geq/ml in the plasma pool as being reasonable maximum virus burden, consistent with secure removal by in-process virus inactivation. This approach is not universally acceptable to regulators.

Experience of NAT Testing of Fractionation Pools in England

Utilising epidemiological data on the UK donor population within a model based on that reported by Schreiber and colleagues [7], an estimate of the likely level of HCV RNA-positive but antibody-negative donations can be made. This analysis suggests that a figure of 1:250,000 should be used as the basis for projection of HCV RNA reactivity in donor samples (unpublished data). Considering 2 million donations per annum fractionated as (say) 100 plasma pools, it may be projected that 8 HCV RNA-reactive donations will be fractionated, probably in 8 separate pools – an unacceptable rate of loss. Since the tests applied by both BPL (ref) and by the OMCL (National Institute for Biological Standards and Control, NIBSC) operate in the sensitivity range of 20–40 geq/ml, it must be assumed that most HCV RNA-positive donations will be detected, even at the fractionation pool dilution.

First experience of testing confirms this, with 4 of 40 pools tested found to be reactive for HCV RNA (unpublished data). In order to allow compliance with the anticipated regulatory requirements without excessive loss of plasma and, potentially, product, the NBS in England has determined to establish a system for testing minipools. This should permit HCV RNA-reactive donations to be excluded prior to the generation of the fractionation start pool.

Proposed Arrangements for Minipool Testing

The primary objective in establishing an initial system for minipool testing within the NBS has been to ensure that testing of plasma fractionation start pools, both by BPL and NIBSC, will give negative results when tested for evidence of HCV RNA by PCR.

A number of options were evaluated before a final strategy was determined. This strategy will involve an additional dedicated sample being taken from donors, plasma from the samples being utilised to generate the minipool for testing, thus reducing any risk of cross-contamination. A standard EDTA (sequestrine) tube will be utilised.

Preliminary studies confirm the stability of HCV RNA in stored, anticoagulated blood and plasma under operational conditions. When samples are cooled within 30 min. of collection, acceptable preservation of HCV RNA is seen at 72 h. Further studies are planned to determine whether storage conditions can be further relaxed without further loss of HCV RNA.

The final specification for production and resolution of reactive minipools has yet to be determined. A number of principles have however been established.

Firstly, it has been decided that minipools should be prepared only from donations shown to be negative by HCV EIA testing. This approach is expected to reduce problems arising from cross-contamination during minipool preparation and also reduce the requirement for resolution testing.

All donations which might be suitable for preparation of components for clinical use will be subjected to minipool testing, including donations not associated with plasma destined for fractionation. This approach will permit the development of uniform sampling procedures for all blood donations, thus, minimising the impact on blood collection procedures.

Minipools will be prepared using a Tecan Genesis robotic sample processor (RSP). Minipools will be produced within a limited number of blood centres, each minipool being created from approximately 500 donations. Two approaches to pool preparation are being considered, both will allow resolution of positive results to single donation level. Multi-dimensional pooling algorithms permit rapid resolution of positive results [8]. A sequential resolution approach is however the approach favoured within the NBS. This involves the production of a number of subpools which are combined to produce a primary test pool. For example, a minipool of 500 donations could be produced from 10 pools each comprising 50 donations each of which has been constructed from 5 pools of 10 donations. Resolution testing of a positive primary pool will involve testing of the 10 secondary pools followed by testing of the 5 tertiary pools involved in the production of the positive secondary pool. This approach is less elegant than the multidimensional approach and resolution will take longer. It is, however, likely to be more robust and easier to implement.

A central testing facility will be established on a single site within BPL, this will permit standardisation of testing protocols and consistent sensitivity. Testing will be undertaken utilising commercial reagents (Qiagen nucleic acid extraction techniques with a Roche Amplicor detection method) to achieve consistent detection of at least 400 geq/ml. Plasma start pools, created from donations cleared by minipool testing, will be tested by a more sensitive 'in-house' PCR technique with a sensitivity detection level of 40 geq/ml. The increased sensitivity of the 'in-house' assay must be balanced against the increased dilution effect in start pools when compared to minipools.

The Impact of Minipool Testing Arrangements on the National Blood Service

Within Europe the regulatory requirements will not refer to the use of NAT testing in the context of labile blood components. However, the implementation of minipool testing will raise a number of questions for those blood services which in addition to taking responsibility for the production of blood components for local use also act as providers of plasma for fractionation. Testing undertaken on minipools will require to be traceable to individual donation level, indeed the FDA have identified this as an absolute requirement for the implementation of minipool testing within the USA [9].

Irrespective of which approach is used for resolution of initially reactive minipool results, procedures will require to be developed to confirm results, analogous to the confirmatory algorithms utilised in serological testing of blood donations. As a minimum this should include repeat testing being undertaken on a second sample derived from the implicated minipool. When an individual donation is identified as HCV RNA positive, serological testing on the original sample should be repeated and PCR testing should be undertaken on a sample obtained from the frozen plasma component (if available). The procedures should clearly identify the requirements that must be fulfilled before donors and, ultimately, patients are contacted.

The response to a positive minipool test will need to be carefully considered. Two approaches are theoretically possible. Firstly, the blood service can decide that local components will not be issued until results of minipool testing are available. This is likely to impact on the availability of red cells and particularly platelets.

The alternative approach is to decide that, at least in the initial phase of testing, the results of minipool testing will not be used to influence the release of labile blood components. This pragmatic approach recognises the limitations of currently available technology. If this approach is pursued, then a situation will inevitably arise when individual donations will be implicated by minipool testing when components derived from the donation have already been transfused. This will raise issues around notification of patients and donors which will re-

quire careful consideration, including possible medicolegal implications. This approach will be adopted within the NBS with respect to red cells and platelets. Frozen components, fresh frozen plasma and cryoprecipitate, will be held pending the result of HCV RNA testing and, if operationally feasible, such components will only be issued once negative results have been obtained. These decisions will be kept under review and reassessed in the light of increasing experience and results of testing.

A number of reasons have contributed to the development of the conservative approach identified above. Firstly, there is a recognition that NAT represents a new challenge to blood services and that it is important that its implementation is carefully undertaken to ensure that it does not adversely impact on the quality systems currently in place within blood centres or on our ability to maintain an adequate and secure supply of components. Secondly, it is considered important to demonstrate that the systems which will be developed to support NAT testing are effective and that testing is not associated with a significant level of aberrant minipool results. Aberrant results refer to the situation where a reactive minipool result cannot be resolved to individual donation level. Such results have been identified in at least one centre [10].

Options for Further Development of NAT

Whilst there may be doubt as to the benefit of NAT for the safety of fractionated products, there can be little doubt that the establishment of NAT for HCV RNA, even based on minipool testing, would result in avoidance of HCV transmission in a predictable number of recipients of cellular blood components, if the timing of result declaration could be made to serve release of red cells and platelets. These benefits would be further enhanced by extending NAT to other virus genomes, including HIV-1 and -2 and HBV. The overall safety gain associated with implementation of this type of testing will not be great, there is, however, increasing recognition that the requirement for HCV RNA testing of plasma start pools will significantly influence future strategies for blood donation testing. The Paul-Ehrlich-Institut has already issued a consultation paper which identifies a desire to utilise PCR technology as a release criterion for red cell components [11]. The NBS will aim to undertake a parallel development of systems which might support extension of NAT testing into the blood centre environment. This will require the availability of operationally robust testing systems, ideally based on commercially developed package solutions.

Non-enveloped viruses, in particular HAV and parvovirus B-19, are of special interest to the fractionator in that they are not reliably addressed by inactivation processes such as solvent-detergent incubation and have been reported to have been transmitted by coagulation factor concentrates. Therefore, it is likely, that these will also be targeted for minipool screening by NAT.

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