Viral Safety of Plasma-Derived Products

SLIDE 1

This presentation will cover viral validation studies for plasma-derived products. FDA requires that the manufacturing process for biopharmaceutical products be validated for its capacity to inactivate and remove viruses. This presentation will give an overview of validation studies, and FDA's approach in assessing such validation data.

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The presentation will outline current approaches for ensuring viral safety of blood and blood products. The emphasis will be on manufactured products, and how viral validation and viral clearance play a major role in ensuring their viral safety.

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Various layers of safety are in place to ensure the safety of both transfused components and manufactured products. These include screening donors for health status, and the risk factors for transmissible diseases. In addition, donor deferral registries are maintained, to eliminate blood and plasma collections from unsuitable donors. The next precautionary step is to test donations for certain viral markers, which you may be familiar with. You will see the list of these viral markers in the next slide. Donations that are collected will be quarantined until the test results become available. Finally, there is also a monitoring system to investigate any type of adverse event reporting related to blood and blood products, to ensure that deficiencies are corrected.

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This chart shows the viral marker tests required or recommended for blood intended for transfusion and for plasma intended for use to manufacture other blood products. Testing requirements for donations of blood are slightly different from donations of plasma. Blood donations are tested for Hepititis B Surface Antigen, called HBSAG, the surface antigen for the Hepatitis B virus, called HBV, and the anti-HBc, the antibody to HBV core antigen. The anti-HBc test is a required test for blood, but not required for plasma. Testing for hepatitis C, called HCV, is done both by testing for anti-HCV antibody and for nucleic acid, HCV-NAT. These tests are required for "blood for transfusion" and for "plasma for further manufacture."

The same is true for HIV. We test using both serology and NAT. P24 antigen testing has been superseded by HIV-NAT. For HTLV-1 and 2, serology testing is done for "blood for transfusion" but not "plasma for further manufacture." West

Nile nucleic acid testing is required for blood, but it is not required or recommended for "plasma for further manufacture."

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With regard to plasma-derived products, which is the focus of this talk, in addition to the five layers of safety outlined earlier, there is another major step to ensure viral safety of these products -- the inclusion of a viral clearance step in the manufacturing process of these products. For any manufactured product, the sponsor needs to show the capacity of the manufacturing process to clear the viruses that are of concern for that particular product. In addition to that, of course, current good manufacturing practice, or cGMP, is also enforced with regard to manufactured product. In relation to viral clearance, FDA makes sure that the product that is virally inactivated is segregated from non-inactivated product.

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The aim of the viral clearance, and why FDA requires viral validation studies, is to provide evidence that a manufacturing process has the capacity to effectively inactivate and remove the viruses of concern -- the pathogenic viruses that potentially could be found in donors. These are viruses that donors are currently screened for. Clearance steps are intended to essentially clear the residual virus that could remain in the manufacturing pool. For example, when a positive donation is inadvertently pooled, or a positive donation tests negative, because it was in both the sirological and NAT "window period," viral clearance ensures that viruses do not end up in the final product.

In addition to clearance of known pathogenic viruses, the viral validation studies should also show that the viral clearance steps are robust, in that they remove or inactivate viruses that are unknown, or for which donors have not been tested. This is demonstrated by validation studies showing that the manufacturing process could clear a wide variety of viruses with different physiochemical characteristics.

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Note that viral clearance refers to both viral inactivation and/or removal. The viral clearance step in a given manufacturing process could be deliberate -- that is, it has been included in the manufacturing process for the purpose of clearing viruses. These, for example, could be a chemical inactivation like solvent detergent treatment, which is a step taken for the purpose of clearing the virus. Another example is filtration, like nanofiltration, that is intended to remove viruses. Another is terminal heat treatment, which is used in a number of products.

However, some viral clearance steps in manufacturing processes may not be undertaken with the explicit purpose of clearing viruses, but, instead, are part of the overall protein purification steps that are shown to contribute to clearance of

viruses as well. For example, some of the precipitation and separation steps in the manufacturing process of plasma-derived products and recombinant products have viral clearance capacity as well. Another example is low pH treatment, which is shown to contribute to virus clearance.

In estimating the overall viral clearance capacity of a given manufacturing process, each clearance step will be assessed separately. The total log reduction for a given process is the sum of the individual clearance steps in the process. These multiple steps should function with different, independent mechanisms in order to be additive, and should not overestimate the clearance capacity of the manufacturing process.

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In the next couple of slides, the current strategy FDA expects for doing viral clearance in a manufacturing process will be covered, with an emphasis on plasma-derived products.

The first step is the selection of the viruses to be studied. Obviously, one wants viruses that are relevant to the product at issue, and relevant to the starting material. Then, one needs to ensure that validated assays are available, to allow the detection and determination of the titer of viruses under study.

Viral validation studies are not done in the context of the actual manufacturing process, but are done on a laboratory scale outside the manufacturing setting, in order to prevent introduction of the virus into the manufacture area. One of the important validation components that FDA looks for is how relevant the small scale or lab scale model is to the actual manufacturing process. Once the relevancy of this small scale to the actual manufacturing process step is established, then a particular clearance step will be spiked with a high titer of the test virus. The test virus could be a relevant virus or it could be a model virus. The final stage will be to determine the virus log reduction for the step that is being validated.

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As mentioned earlier, different steps are validated separately. The total virus log reduction for the whole manufacturing process is determined by summing up log reduction contributed by individual step. In evaluating viral clearance studies, FDA wants to make sure that the critical parameters for a given clearance step at the small scale are the same as the actual manufacturing process. For this, the manufacturer is asked to provide a head-to-head comparison of the critical parameters between the two scales. As mentioned earlier, this is done to ensure the relevancy of the small scale to the actual process. If the small scale does not mimic the actual process, then the whole exercise will be futile in terms of providing assurance with regard to viral clearance.

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The validation package submitted to FDA should include the choice of viruses that were used in the study as well as a justification for their selection. Obviously, the test viruses should be relevant to the starting material. If the product is cell culture-based -- that is, a recombinant product -- then viruses that are likely found in tissue culture should be used for validation studies. And, if the product is human-derived, one would be concerned with the pathogenic viruses that potentially could be present in the blood and plasma, particularly those viruses that are tested at the donor level. This approach may not be feasible in all situations. For example, some of the pathogenic viruses may not grow in cell culture. Therefore, the availability of a suitable culture could limit the choice of viruses used in validation studies.

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Some viruses may grow poorly in culture; that is, they never reach a high titer needed for validation studies. In addition, for some viruses, a reliable assay to accurately determine the virus titer may not be available.

Overall, the approach in choosing viruses for a validation study is to use the relevant virus, if feasible. The relevant virus will be as close to what we expect in a real case of contamination. If use of relevant virus is not feasible, then model viruses will be used as substitute for the actual viruses of concern.

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The viruses that are adapted for validation studies may behave differently than the wild type virus of concern. If you have to use a model virus, the expectation is to use a model virus that has been shown to be more resistant to inactivation, if the inactivation step is being validated. And, if removal is to be validated, the choice of model viruses should be based on size and physicochemical similarities with the relevant virus. There is a tendency to use a model virus that gives a better log reduction. As mentioned, if different model viruses are available for a virus of concern, one should use the more resistant virus.

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For plasma-derived product, these are the viruses that FDA expects and asks the manufacturers to use when they do their viral validation studies:

HIV. Use of this virus is a requirement, and it should be included in any viral validation study that concerns human derived products.

With regard to HBV, the virus does not grow in culture, nor is there a specific model for HBV. But, there is sufficient data indicating that a few of the large DNA viruses can represent HBV. In the past, chimps were used to study HBV clearance. However, chimp studies are not currently required or necessary to study HBV clearance validation. It should be noted that the effectiveness of established viral inactivation and removal methodologies is fairly well established for certain viruses. In performing viral validation studies, the FDA wants to ensure

that manufacturers can successfully adopt established methodologies to their manufacturing process, and achieve an expected level of clearance for known viruses.

HCV. This virus also does not grow in culture, so there are a number of acceptable model viruses that are used for clearance validation.

HAV does grow in cell culture, so the expectation is that the actual virus would be used in its validation studies.

For parvovirus B19, which is a non-enveloped virus, there are also a number of small non-enveloped viruses that are used as models for B19.

In short, a virus panel for viral validation studies should include HIV and HAV using the actual virus, and use model viruses for HBV, HCV, and B19. With regard to B19, you are probably aware that there are some culture media for B19 that are still considered experimental. Therefore, we expect the manufacturer to rely on model viruses for B19. Model viruses for B19 usually have high resistance to inactivation, as compared to cultured B19, and are therefore more suited for inactivation studies.

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Small scale or laboratory scale validation is another major component of overall viral validation studies that needs to be evaluated. The small scale should include all the critical parameters that are present in the actual process. For example, in chromatography relative values for the volume, size and the geometry will be considered. And in filtration, one expects pressure, the volume to the surface area of the filter, and the flow rate to be the same for the small scale study and the actual manufacturing process. Absolute values, such as temperature, and incubation time should be identical in lab scale and manufacturing scale.

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As the small scale study is being designed, it needs to be validated by looking at certain product characteristics, like recovery of the product. Overall, the small scale production method should reflect the full scale process as much as possible. Also, multiple runs should be performed without the virus, to make sure that the small scale actually represents the actual step under consideration. There should also be some statistical comparison with the actual process. If there are some unavoidable differences between small scale and the actual scale, which are expected, then some validation studies should be provided, to show that this kind of variation does not impact the overall virus clearance capacity, and is not overestimating the virus kill or virus removal. Again, as mentioned, one needs to make sure that what is done in the laboratory represents the actual manufacturing process step.

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In the case of inactivation, validation should provide the kinetics and the extent of the inactivation. Kinetic data will tell us how fast and efficient the inactivation was achieved. For example, take heat inactivation of PRV at 60 degrees for 10 hours, done at two different places for two different products, achieving the same level of virus reduction. Kinetic studies may show that for one product, the inactivation occurred in the first 30 minutes, whereas for the other product, inactivation may have been achieved only after 10 hours. The slower inactivation may happen when one manufacturer uses a higher concentration of a stabilizer, which also stabilizes the virus. Here the kinetics data would show that inactivation was more robust and effective for one product, and was less so for the other. Therefore, the data received from the kinetic studies are very important in establishing whether the step is effective or not.

If the step is removal, FDA wants to see where the removed virus is.

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Overall, for a plasma-derived product, FDA expects the manufacturing process to have at least two clearance steps. Removal steps are difficult to scale down and validate. They are also inherently less reliable. Therefore, in a manufacturing process, a complete reliance on removal steps for virus clearance is not acceptable. At least one of the clearance steps must be inactivation.

Because of the difficulty of removing non-enveloped viruses, like B19 and HAV, and because these viruses are not inactivated, especially in the case of B19, at least one removal step is expected to be included in the manufacturing process. These, of course, will depend on the type of products. Some products are considered at higher risk of contamination from non-enveloped viruses. For others, the risk is very small. Therefore, how much of removal capacity is needed, will depend on the manufacturing process and the product.

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The other thing that should be considered is assessing the effect of manufacturing change in the viral clearance capacity of the process. When FDA gets a supplement reporting a change or changes in a process, the first thing that is considered is the potential impact of the change on the overall viral clearance. Therefore, if changes in production and purification are made, the effect of those changes on viral clearances should be determined, and that may require new viral validation studies.

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Overall criteria for an effective virus clearance step are outlined in this slide. Significant viral clearance is needed. The steps that are included for viral clearances are considered more effective if they are reproducible and controllable. There is a higher assurance with regard to such steps, especially if it can easily be scaled down, and validated. Most of the inactivation steps fall in

this category. Examples are solvent detergent treatment and heat treatment. Another criteria for demonstrating that a clearance methodology is effective is showing there is a minimum impact on the product quality and also on the potency of the product. Essentially, the virus needs to be cleared, without product damaged, or the product becoming less potent. Also, the clearance step should not generate new antigens, which may cause immunogenicity.

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Validation data provide an estimate of the capacity of the manufacturing process to clear viruses. Validation does not make the product safer, but provides the assurance of safety. There are some limitations inherent in validation studies that one needs to be mindful of. For example, the virus strains that are used in validation studies may be different than the wild type. This is demonstrated by a number of studies which show even different strains of the same virus may generate different levels of clearance. The scaled-down model, or the lab model that is used in the validation studies, may not fully represent the actual step in the actual manufacturing process. There is also a tendency to overestimate the viral kill and viral clearance.

The other limitation that should be taken into account is that of removal by filtration, which may decline as the filtration process proceeds. An example would be a nano-filtration step. In that step, the capacity to clear the virus for the initial volume of filtered product may be different than the subsequent volume of the product. This phenomenon has been demonstrated in a number of studies. These are the limitations that need to taken into account when one assesses viral validation studies.

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Despite the limitations just outlined, overall viral clearance steps have provided excellent viral safety with regard to enveloped viruses. The available inactivation methodologies have proven to be very effective in clearing enveloped viruses.

However, there are still limitations with regard to clearance of non-enveloped viruses, such as HAV and B19. The reason is that most of the well-established inactivation steps have no or little effect on the non-enveloped viruses. For example, heat treatment has some effect on the Hepatitis A virus, especially if heat in solution or pasteurization is used. However, heat inactivation is not effective against B19, as shown by the use of model virus for B19.

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The reason B19 is considered a problem is because it is a small non-enveloped virus, resistant to inactivation, and, because of its small size, is difficult to remove. Also, the virus could be present at very high titer in the plasma manufacturing pools.

Nanofilters with small pore size could remove B19 to a great extent. However, using a small pore size filter may not be practical for most of the products that we deal with, because of their large molecular size, and also because it is not practical to filter a very large volume of the product through filters with small pore size in a reasonable length of time.

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With regard to B19, FDA recommends that the source plasma units that contain a high level of B19 DNA should be detected and excluded from the manufacturing pool. This is done by mini-pool testing. B19 NAT testing is done only if the plasma is intended for further manufacturing. Currently plasma donations that are intended for further manufacturing will be tested for B19 in a mini-pool format, and any unit that contains a certain level of B19 DNA will be excluded from the manufacturing pool. The overall limit of B19 DNA for the manufacturing pool is 4 logs per milliliter. The reason for screening for B19 DNA is the high titer of virus in an infected unit, and the fact that the virus is difficult to remove or inactivate, because of its small size and its resistance to inactivation. Limited levels of B19 DNA in the manufacturing pool, combined with the presence of anti-B19 antibody in a manufacturing pool, and the contribution of other viral clearance steps in the manufacturing process, is expected to eliminate or greatly reduce the residual virus, and with it reduces the risk of infection from B19 virus.

FDA has issued a guidance that outlines FDA's recommendation concerning B19 in manufactured product.

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In the next few slides, you will find a list of references, guidances, and contact information you may find useful.

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And this slide lists more reference materials.

SLIDE 26
For more information, please use the contact information listed here.

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You can find additional information at these websites.

Slide 28
This concludes the presentation, "Viral Safety of Plasma-Derived Products".

We would like to acknowledge those who contributed to its development. Thank you.