Announcement of the National Advisory Committee 'Blood' (Arbeitskreis Blut)
of the German Federal Ministry of Health

Revision to Votum 16 "Minimum requirements for microbiological testing of blood components for transfusion "

The following recommendation (Votum) V 43 was adopted at the 74th session of the National Advisory Committee 'Blood' held on March 05, 2013:

This revision of Votum 16 of the Advisory Committee 'Blood' titled "Minimum requirements for sterility testing of blood components" (V 16) is intended to take into account findings related to microbiological testing of blood components that have been made since 1997. Since these pharmaceuticals cannot be sterilized a final product specification of "sterile" cannot be required so the term "sterility testing" has been replaced by "microbiological testing." It is now possible to use the number of procedures used per month by a production facility as a reference value N to determine the number of random samples required rather than the number of preparations manufactured. In principle it is not possible to obtain relevant predictive information about the microbiological quality of the total population of manufactured preparations if only 1% or 0.4 x $\sqrt{}$ N of the units produced per month are tested. It is therefore appropriate to base the reference value N on the number of procedures used and not on the number of preparations manufactured.
Additional changes pertain to an obligatory second culture in case of a positive result in the first sample as well as empirically adjusted requirements for sampling and storage of material to be tested.

1. Obtaining sample material – Time frame

In the case of destructive testing the sample material is the blood component itself.
If the preparation is to be transfused it is permissible to withdraw aliquots of the material in a functionally closed system (by sterile connecting of sample bags tested for leakage or in a sample bag integrated in the set). The volume of the aliquot must be adequate to allow confirmational testing; the specification of the remaining blood component regarding the volume to weight ratio must be met. The time frame in which the sample is obtained must be chosen to assure a high probability of detecting potential contamination (table 1).

Table 1: Time frames for obtaining testing materials

	Time frame for obtaining test material
Erythrocyte concentrate (EC)	At the earliest 10 days after donation and at the latest 3 days after expiration date.
Thrombocyte concentrate (TC)	At the earliest 24h before expiration of shelf life and at the latest 72h after expiration of shelf life.
Plasma from apheresis for transfusion	After apheresis and subsequent storage at room temperature for at least 96 hours.
Autologous whole blood, Autologous EC	At the latest 10 days after expiration of shelf life from non-transfused units.

2. Obtaining sample material – Frequency with which samples are to be taken

The number of samples N for quality control of random sampling always corresponds to the number of procedures used per month by a given production facility. In order to minimize the losses of rarely manufactured blood products the frequency of sampling should not exceed 1% of N. In cases in which less than 400 procedures are carried out per month 4 preparations are to be tested per month. Manufacturers that carry out more than 1500 procedures per month may set the frequency of sample collection at $0.4 \times \sqrt{N}$. The components to be tested for the respective procedure are summarized in the following table (table 2):

Table 2: Components to be tested per procedure

Procedure to which the reference value N applies	**Component to be tested**
Preparations from whole-blood donations	EC
Production of pool TC	Pool-TC
Erythrocytapheresis Thrombocytapheresis Plasmapheresis	EC TC Plasma for transfusion
Multicomponent donations from apheresis with TC production	TC
Multicomponent donations from apheresis without TC production	EC
Procedure for pre-operative autologous blood preparation	EC or whole blood

Microbiological testing can be omitted for plasma from whole-blood withdrawal since possible contamination will be detected by the microbiological testing of the EC or pool TC and plasma does not undergo any critical production steps.

For EC, TC and plasma from apheresis N is the number of the respective apheresis procedures. For blood components from multicomponent apheresis N is also the number of procedures, and as above for whole-blood withdrawal, microbiological testing is to be carried out on the most sensitive components (TC or EC) that are prepared by this procedure.

For extemporaneous preparations from blood components (e.g. washed EC, reconstituted whole blood) the manufacturer will make a decision concerning additional microbiological testing dependent upon the complexity of the production process and according to their risk analysis. Irradiated blood components as well as blood components that are separated in functionally closed systems do not require any further testing beyond initial procedures.

3. Pre-analysis for microbiological testing

Samples can be stored for up to 3 days at 4-28°C before inoculation in culture flasks. Sample withdrawal for culture is to be performed aseptically after careful mixing of the material to be tested (blood component or aliquot). Both the sample collection area and the septum of the culture flask must be disinfected with a suitable disinfectant, e.g. from the list supplied by the VAH (Verbund für angewandte Hygiene - Association for Applied Hygiene).

Warning: The disinfectant must evaporate before penetration/opening.

The remaining sample material shall be stored for up to 9 days at 2 - 10°C in case a second culture is required.

4. Testing procedures and inoculation volumes

One aerobic and one anaerobic culture shall be established for each sample to be tested. Cultures shall be analysed exclusively using an automatic culture system. Incubation temperature and inoculation volume (4-10 ml per culture medium) shall be chosen according to the recommendations of the manufacturer of the system. An incubation period of 7 days is to be maintained if no microbial growth is observed. No further batch testing is required in the presence of a manufacturer’s release protocol.

5. Test results and evaluation

If microbial growth is absent in the first test the result is negative: no further testing is required.
If microbial growth is detected in the first microbiological test:

- A subculture will be created for differential diagnosis (to the level of species).
- A second culture will be created as replicate test.
- In case of a repeat positive result and identity with the first culture (differentiation of species) the culture is to be considered confirmed positive.

- If the organism is not identical or the second culture is negative it is necessary to clarify whether secondary contamination was caused by a technical error.

If a positive signal is detected in the first culture all related blood components (e.g. other TC from double/triple apheresis, related EC from pool TC) are to be withdrawn and quarantined. Confirmed positive results from the microbiological testing of blood components are to be evaluated as OOS (out of specification).

6. **Microbiological testing of TC as criterion for release**

If rapid assays are used in order to extend the shelf life of TC, random sampling is to be used at the end of shelf life as described in this Votum because of the reduced sensitivity of rapid tests.

This update replaces Votum 16 of the Advisory Group ‚Blood. ‘

In the name of the Advisory Group ‘Blood’

Prof. Dr. R. Burger - Chairman

Dr. R. Offergeld - Managing Director

This Votum is available in German at http://www.rki.de>Kommissionen>Arbeitskreis_Blut>Voten