Guidelines for the Blood Transfusion Services

Chapter 9: Microbiology tests for donors and donations: general specifications for laboratory test procedures


Chapter 9: Microbiology tests for donors and donations: general specifications for laboratory test procedures

Blood donations make up the majority of donations collected and processed by the UK transfusion services, but tissue and stem cell donations are a growing part of their portfolio. While the screening requirements for blood, tissues and stem cells largely overlap, there are some important differences that should be acknowledged and incorporated into any guidelines.

For the purpose of these guidelines, tissue donations include all of the types of tissue normally retrieved from living or deceased donors, and stem cell donations include haemopoietic progenitor cells (HPC) and therapeutic cells (TP). These guidelines therefore specify the screening requirements for blood, tissue and stem cell donations managed by the UK transfusion services.

9.1: General requirements

Update notice: Table 9.1 - Technical information has been updated following the issue of Change Notification 12 - 2016.

All screening must be performed within Blood Safety and Quality Regulations (BSQR)¹ compliant laboratories and meet any other appropriate regulatory requirements.

Secure and effective procedures must be in place to ensure that:

- all donations, any subsequent components/products and their laboratory samples are correctly identified by barcoded and eye-readable numbers
- donations can be linked to their donor
- information about previous test results which would preclude issue of a subsequent donation cannot be automatically overridden by a subsequent negative test result
- donor samples are suitably stored under appropriate conditions of temperature and time to preserve the targets for which they will be screened
- the screening assays used are properly evaluated and validated
- tests are appropriately performed and controlled, and the results properly and accurately recorded, using validated procedures

- test results and other relevant test information are retained for the appropriate period, as set out in the BSQR\(^1\) or equivalent

- appropriate confirmatory testing is available to investigate screen reactivity

- relevant data relating to screening and confirmatory test results are reported to a centralised surveillance system, allowing the monitoring of trends in screening test reactivity and confirmed positive results.

### 9.1.1: Test reagents, kits and equipment

All assays used must be CE marked and must have been assessed (in respect of sensitivity and specificity) and deemed suitable by the UK Blood Transfusion Services kit evaluation groups (NHSBT KEG or SNBTS/NIBTS MTEG) for the detection of the required markers in the donation types being screened. Unless specifically validated for alternative use/performance, test kits and reagents must be stored and used according to the manufacturer’s instructions.

Each new manufacturer’s lot of each assay should be assessed prior to being accepted and put into use.

Additionally, all testing laboratories must ensure that the expected standard of performance of the assays used is being achieved, by using appropriate assay batch pre-acceptance testing, delivery acceptance testing and statistical monitoring of test results on defined quality control samples.

All test procedures must be documented and an inventory maintained of kits and reagents in stock, including supplier, batch number, expiry date, date of receipt, version number of product insert and record of pre-acceptance testing.

Procedures must ensure the traceability of the batch number and manufacturer of kits and reagents and the serial number of equipment used to test every donation.

Equipment must be validated, calibrated and maintained. Appropriate records for these activities must be made and retained as defined in extant regulations (currently 30 years).

Appropriate reactivity with manufacturers’ and any external control samples must be demonstrated with every series of tests.

A series/batch of tests is defined as the number of tests set up at the same time, under the same conditions and processed in a similar manner:

- Where the microplate format is used each plate constitutes a series of tests even if only a few wells are used.

- Where a closed system is used the size of a series/batch of tests must be determined by each individual Service through an appropriate risk assessment.

### 9.1.2: Recording and reporting of results

The laboratory final output should indicate the result of every test performed, using a system that provides positive sample identification. Each test result should be recorded by a system that does not require transcription. If manual completion of screening is performed it must be thoroughly documented and controlled and the results handled electronically following the same basic principles applied to fully automated testing.

### 9.1.3: Release of tested components/products
Standard procedures must ensure that no donations, or components/products prepared from them, can be released for issue until all the required laboratory tests (mandatory and additional) have been completed, documented and approved within a validated system of work. Compliance with this requirement can only be achieved by the use of a validated computerised system that requires the input of valid and acceptable test results for all the mandatory and required laboratory tests to permit the release of each individual donation.

**Table 9.1 Screening required for blood donations**

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Minimum requirement</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV 1+2</td>
<td>anti-HIV 1+2 or HIV 1+2 Ag/Ab (M)</td>
<td>RNA screening in pools of a maximum of 48 donations**</td>
</tr>
<tr>
<td></td>
<td>HIV RNA*</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>anti-HCV (M)</td>
<td>RNA screening in pools of a maximum of 48 donations**</td>
</tr>
<tr>
<td></td>
<td>HCV RNA (M)</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>HBsAg (M)</td>
<td>DNA screening in pools of a maximum of 48 donations**</td>
</tr>
<tr>
<td></td>
<td>HBV DNA*</td>
<td>Donations that are anti-HBc reactive and have anti-HBs &gt;100 mIU/mL are considered suitable for release</td>
</tr>
<tr>
<td></td>
<td>anti-HBc [+ anti-HBs] (A)</td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td>anti-treponemal Ab (M)</td>
<td></td>
</tr>
<tr>
<td>HTLV I/II</td>
<td>anti-HTLV I/II (A)**</td>
<td>ID or screening in pools of a maximum of 48 donations**</td>
</tr>
<tr>
<td>HEV</td>
<td>HEV RNA (A)</td>
<td>Screening in pools of a maximum of 24 donations**</td>
</tr>
<tr>
<td>HCMV</td>
<td>anti-HCMV (A)</td>
<td>Ideally both IgG and IgM, but IgG alone is considered sufficient</td>
</tr>
<tr>
<td>Plasmodium sp.</td>
<td>anti-P. falciparum/vivax (A)</td>
<td></td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>anti-T. cruzi (A)</td>
<td></td>
</tr>
<tr>
<td>West Nile Virus (WNV)</td>
<td>WNV RNA (A)</td>
<td>RNA screening in pools of a maximum of 16 donations****</td>
</tr>
</tbody>
</table>

(M) – mandatory (release criteria) for the purpose of these guidelines
9.2: Microbiology screening

Update notice: Section 9.2 has been updated following the issue of Change Notification 12 - 2016.

Note: The meanings of certain terms used in this section are defined in section 9.2.6.

9.2.1: Screening of donations/donors

Donation/donor screening can be broadly divided into two main categories:

- Mandatory: Absolute requirement prior to the release of components. There are, however, different reasons for a test to be defined as ‘mandatory’. These include a European Union requirement, a specific instruction from the Department of Health, including its Advisory Committees, and an Act of Parliament.

- Additional (also known as Discretionary): Performed because of specific additional and identifiable donor or recipient risk.

Importantly, the mandatory requirements for blood donation and for tissue and stem cell donations are different, with some tests that are defined as ‘Additional’ for blood donations being ‘Mandatory’ for non-blood donations (Tables 9.1 and 9.2). Although not required for all donations, where

Table 9.2 Screening required for tissue and stem cell donations*

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Minimum requirement</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV 1+2</td>
<td>anti-HIV 1+2 or HIV 1+2 Ag/Ab (M)</td>
<td>Maximum pool size of 24 donations**</td>
</tr>
<tr>
<td></td>
<td>HIV RNA (O)</td>
<td></td>
</tr>
<tr>
<td>Pathogen</td>
<td>Tests</td>
<td>Notes</td>
</tr>
<tr>
<td>----------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>HCV</td>
<td>anti-HCV (M)</td>
<td>Maximum pool size of 24 donations**</td>
</tr>
<tr>
<td></td>
<td>HCV Ag and/or HCV Ag/Ab (O)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCV RNA (O)</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>HBsAg (M)</td>
<td>Donations that are anti-HBc reactive and have anti-HBs &gt;100 mIU/mL are considered suitable for release</td>
</tr>
<tr>
<td></td>
<td>anti-HBc [+ anti-HBs] (M)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBV DNA** (O)</td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td>anti-treponemal Ab (M)</td>
<td></td>
</tr>
<tr>
<td>HTLV I/II</td>
<td>anti-HTLV I/II(M)***</td>
<td>ID or maximum pool size of 24 donations**</td>
</tr>
<tr>
<td>HCMV</td>
<td>anti-HCMV (A)</td>
<td></td>
</tr>
<tr>
<td>Plasmodium sp.</td>
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<tr>
<td>Trypanosoma cruzi</td>
<td>anti-T. cruzi (A)</td>
<td></td>
</tr>
<tr>
<td>West Nile Virus (WNV)</td>
<td>WNV RNA (A)</td>
<td>Maximum pool size of 16 donations****</td>
</tr>
</tbody>
</table>

(M) – mandatory

(O) – optional, genomic screening for HIV, HCV and HBV is not mandated but can be performed on the original donation sample as an alternative to 180 days’ quarantine and follow-up serological testing

(A) – additional due to specifically identifiable risk

* UK screening requirements. Other testing, e.g. Epstein-Barr virus, toxoplasmosis, may be required as additional tests depending upon specific additional risk and/or special requests for individual recipients. For certain product types that are exported there may be additional end user testing requirements.
** All screening of deceased tissue donations is performed on individual samples. HCV and HIV RNA and anti-HTLV I/II screening of surgical tissues/stem cells can be performed using pools of a maximum of 24 samples. HBV DNA screening should be on individual samples.

*** Not mandatory for avascular tissue donations but may be considered good practice.

**** The maximum validated pool size for WNV RNA screening is 16 donations.

additional tests are required, the results are an integral part of the criteria for the release of that donation/component/product. In addition, for certain donation types, there is the option of quarantine and follow-up serological testing before issue or the inclusion of genomic screening at donation.

Donations and any associated components/products must not be released to stock unless they have been tested and found negative for the mandatory, and any additional, microbiological screening required. In certain circumstances, for certain donation/component types, a reactive screen result may not preclude release of the donations/component.

### 9.2.2: Deceased neonatal and infant tissue donors

- Full microbiology screening of a maternal sample is always required.
- For still births and neonates less than 48 hours after birth, no microbiology screening of the neonate is required.
- For neonates between 48 hours and 28 days after birth, a neonatal sample is only required when there are identifiable risks of possible viral transmission. In this scenario only nucleic acid amplification techniques (NAT) testing of the sample is required.
- For infants more than 28 days after birth, full microbiology screening of an infant’s sample is required.

### 9.2.3: Serology screening algorithms

#### 9.2.3.1: Blood donations

- No sample which tests initially reactive for the first time in the routine screening assay can be released for clinical use unless subsequently shown to have a negative result on both tests in duplicate repeat testing using the same assay.
- Blood donations that are reactive on one or both of the repeat tests are unsuitable for use and must be labelled as biological hazard/not for transfusion.
- Samples which test initially reactive in the routine screening assay, but which originate from donors who have been previously investigated in a reference laboratory and have been shown to be demonstrating non-specific reactivity, may be tested on a second (alternative) screening assay of at least equal sensitivity to the primary screening assay, and can be considered suitable for clinical use if the reaction in the alternative screening assay is negative.

See flowchart for screening of blood donations provided in Figure 9.1.
Figure 9.1 Serology screening: blood donations
9.2.3.2: Tissue and stem cell donations

- All initially reactive samples (see Figure 9.2) must be re-tested in duplicate using either the same assay or using an alternative assay that has been specifically evaluated to have at least equal sensitivity and ideally is based on different antigens and/or antibodies, and/or principles.

- Donations that are non-reactive on both of the repeat tests can be considered suitable for clinical use.

- Donations that are reactive on one or both of the repeat tests may in some clinical circumstances, and depending on the confirmatory results, be considered suitable for use (SaBTO Guidelines 2011\textsuperscript{2}).

9.2.4: Molecular screening algorithm

- All initially reactive pool samples (see Figures 9.3 and 9.4) must be resolved to an individual (or more) reactive donation. All other non-reactive donations can be considered suitable for clinical use.

- Individual reactive donations are unsuitable for clinical use and must be labelled as biological hazard /not for transfusion.

- Stem cell donations from known infected individuals that are reactive on screening may in some clinical circumstances be considered suitable for use (SaBTO Guidelines 2011\textsuperscript{2}).
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* Donors confirmed to be HEV or WNV RNA positive need only be deferred for 6 months from pick-up.

* Figure 9.3 Molecular screening: blood donations
9.2.5: Confirmatory testing

When a donation is screen reactive for any of the serological or molecular mandatory or additional microbiology tests described above (except for anti-HCMV and anti-HBc, where anti-HBs is present at a level \(\geq 100\) mIU/mL), samples from the donor/donation must undergo confirmatory testing at a designated reference laboratory.

- If HEV or WNV RNA is confirmed in a donor, the donor record must be flagged as ‘temporary exclusion’ for 6 months. The donor can be reinstated automatically at least 6 months after the date of the index HEV or WNV RNA positive donation: see section 9.4.

- In all other cases, the donor record must be flagged as ‘permanent exclusion risk – not to be used for clinical use’ or equivalent.

- In all cases where a positive result is confirmed, arrangements should be made to inform the donor and to ensure that the donor is given appropriate advice.

Note: Autologous stem cell donations may be collected from individuals who are known to be infected with one or more of the infectious agents for which donations are routinely screened. Such individuals are not generally classified as donors for the purposes of these guidelines.

- If a negative, inconclusive or indeterminate result is reported following confirmatory testing, and the initial reactivity is determined by the reference laboratory to be non-specific, use of further donations or the same donation (tissue and stem cell donors only) may be possible, as covered in section 9.4.
9.2.5.1: Specific requirements for HBsAg confirmation

The designated reference laboratory should, where appropriate, perform specific neutralisation tests for HBsAg to ensure that donors with low-level HBsAg reactivity are not incorrectly described as non-specifically reactive.

9.2.6: Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-reactive (NR)</td>
<td>A sample whose reactivity when first tested falls inside the assay cut-off as defined by the manufacturer’s instructions. May also be referred to as a ‘Negative’ test result</td>
</tr>
<tr>
<td>Initial reactive (IR)</td>
<td>Any sample whose reactivity when first tested falls outside the cut-off as defined by the manufacturer’s instructions</td>
</tr>
<tr>
<td>Repeat reactive (RR)</td>
<td>Any sample reactive on two or more occasions either in the same screening test (duplicate) or in two or more screening tests that are used in combination sequentially, to determine the suitability of a donation for release for clinical use</td>
</tr>
<tr>
<td>Alternative assay testing</td>
<td>When a test of similar modality and sensitivity is used sequentially to screen a sample which is either IR or RR in a first screening assay</td>
</tr>
<tr>
<td>Confirmatory testing</td>
<td>Further testing of a repeat reactive sample using a number of different assays in a reference laboratory to define whether the reactivity is specific to the microbe being screened for and indicative of potential infectivity</td>
</tr>
<tr>
<td>Positive</td>
<td>A sample whose reactivity in confirmatory testing meets pre-defined criteria. This may indicate current or past infection depending on the markers and microbe concerned</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>A sample whose reactivity in confirmatory testing is not sufficient and/or specific enough to determine whether it reflects infection with the microbe being screened for</td>
</tr>
<tr>
<td>Negative</td>
<td>A sample which is either non-reactive in confirmatory testing or whose reactivity in confirmatory testing is deemed not to reflect infection</td>
</tr>
</tbody>
</table>

9.3: Specific assays

Update notice: Section 9.3 - Information has been updated following the the issue of Change Notification 12 - 2016.

9.3.1: HBsAg
• The UK specification for the minimum level of sensitivity for the performance of HBsAg screening is 0.2 IU/mL. A UK HBsAg working standard (07/288 or equivalent) containing 0.2 IU/mL HBsAg is available from the National Institute for Biological Standards and Control (NIBSC). Laboratories using an assay of high analytical or dilutional sensitivity where the working standard reacts too strongly are advised to utilise the NIBSC HBsAg monitoring standard (07/286 or equivalent) set at 0.05 IU/mL in place of the working standard.

• In addition to the assay manufacturer’s controls, the UK working standard must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of HBsAg test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.2: anti-HIV 1+2 or HIV 1+2 Ag/Ab combination

• The HIV 1+2 Ag/Ab combination assay is recommended for use within the UK Blood Services as the serological screening assay of choice.

• The UK requirement for the minimum level of sensitivity for the performance of HIV 1+2 serological screening is that a positive result should be obtained with the UK anti-HIV 1 working standard, available from NIBSC (99/750 or equivalent). Laboratories using an assay of higher analytical or dilutional sensitivity where the working standard reacts too strongly are advised to utilise the NIBSC HIV working standard 1/5 dilution (99/710 or equivalent) in place of the working standard. There is no specific requirement to demonstrate individual anti-HIV 2 or HIV p24 Ag reactivity.

• In addition to the assay manufacturer’s controls, the UK working standard must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of HIV test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening, including demonstrating specific anti-HIV 2 reactivity and, as appropriate specific HIV p24 Ag reactivity.

9.3.3: anti-HCV

• The UK requirement for the minimum level of sensitivity for the performance of anti-HCV screening is that a positive result should be obtained with the UK anti-HCV working standard (06/188 or equivalent), available from NIBSC. Laboratories using an assay of higher analytical or dilutional sensitivity where the working standard reacts too strongly are advised to utilise the NIBSC HCV working standard 1/8 dilution (06/190 or equivalent) in place of the working standard.

• In addition to the assay manufacturer’s controls, the UK working standard must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.
• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of anti-HCV test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.4: anti-HTLV I/II

• The UK requirement for the minimum level of sensitivity for the performance of anti-HTLV I/II screening is that a positive result should be obtained with the UK anti-HTLV working standard, available from NIBSC (03/104 or equivalent).

• In addition to the assay manufacturer’s controls, the UK working standard must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of anti-HTLV I/II test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.5: Syphilis antibody

• The UK requirement for the minimum level of sensitivity for the performance of syphilis (specific treponemal antibody) screening is that, in the absence of a specifically defined UK working standard produced by NIBSC, a positive result should be obtained with the appropriate Health Protection Agency (HPA) syphilis quality control preparation.

• In addition to the assay manufacturer’s controls, the HPA syphilis quality control preparation must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of anti-treponemal test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.6: Malarial antibody

The exclusion period for donors from malarial areas is given in the Joint UKBTS/HPA Professional Advisory Committee (JPAC) Donor Selection Guidelines. These guidelines specify situations where donations may only be released if a test for malarial antibodies is negative.

• The UK requirement for the minimum level of sensitivity for the performance of malarial antibody (anti-P. falciparum/vivax) screening is that, in the absence of a specifically defined UK working standard produced by NIBSC, a positive result should be obtained with the HPA malaria antibody quality control preparation.
In addition to the assay manufacturer’s controls, the HPA malaria antibody quality control preparation must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

Each manufacturer’s batch/lot of malarial antibody test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.7: anti-T. cruzi

The deferral criteria for donors from T. cruzi endemic areas are given in the JPAC Donor Selection Guidelines. Donors at risk of T. cruzi must be tested for anti-T. cruzi and negative results obtained prior to the release of any donation for clinical use.

The UK requirement for the minimum level of sensitivity for the performance of anti-T. cruzi screening is that, in the absence of a specifically defined UK working standard produced by NIBSC, a positive result should be obtained with a formally validated in-house anti-T. cruzi quality control preparation.

In addition to the assay manufacturer’s controls, the anti-T. cruzi quality control preparation must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

Each manufacturer’s batch/lot of anti-T. cruzi test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.8: anti-HBc

The exclusion period for blood donors who have had body piercing, acupuncture etc. are given in the JPAC Donor Selection Guidelines. Certain of these categories may require donations to be tested for anti-HBc and negative results obtained prior to release of any blood component for clinical use. Tissue and stem cells donations have anti-HBc screening as a mandatory requirement.

The UK requirement for the minimum level of sensitivity for the performance of anti-HBc screening is that, in the absence of a specifically defined UK working standard produced by NIBSC, a positive result should be obtained with the HPA anti-HBc quality control preparation.

In addition to the assay manufacturer’s controls, the HPA anti-HBc quality control preparation must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.
• Each manufacturer’s batch/lot of anti-HBc test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

• Donations found to be reactive for anti-HBc should be tested for anti-HBs (see section 9.3.10).

9.3.9: anti-HCMV

• The UK requirement for the minimum level of sensitivity for the performance of anti-HCMV screening is that, in the absence of a specifically defined UK working standard produced by NIBSC, a positive result should be obtained with the HPA anti-HCMV quality control preparation.

• In addition to the assay manufacturer’s controls, the HPA anti-HCMV quality control preparation must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of anti-HCMV test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.10: anti-HBs

Donations found to be reactive for anti-HBc with levels of anti-HBs <100 mIU/mL are deemed unsuitable for release, whereas those with levels >100 mIU/mL can be considered suitable for release.

• The UK requirement for the minimum level of sensitivity for the performance of anti-HBs testing is that, in the absence of a specifically defined UK working standard produced by NIBSC, a positive result should be obtained with the HPA anti-HBs quality control preparation.

• In addition to the assay manufacturer’s controls, the HPA anti-HBs quality control preparation must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down.

9.3.11: Hepatitis C virus RNA (HCV NAT)

• The UK requirement for the minimum level of sensitivity for the performance of HCV NAT is 5000 IU/mL in an individual donation. An HCV international standard is available from the NIBSC.

• The assay must include a specific internal control for each sample tested.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and any additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of HCV RNA test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.
9.3.12: Hepatitis B virus DNA (HBV NAT)

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of HBV NAT. An HBV international standard is available from the NIBSC.

- The assay must include a specific internal control for each sample tested.

- No series of tests should be considered acceptable unless the result of the assay manufacturer's and any additional quality control samples have satisfied the criteria laid down.

- Each manufacturer's batch/lot of HBV DNA test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.13: Human immunodeficiency virus RNA (HIV NAT)

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of HIV NAT. An HIV international standard is available from the NIBSC.

- The assay must include a specific internal control for each sample tested.

- No series of tests should be considered acceptable unless the result of the assay manufacturer's and any additional quality control samples have satisfied the criteria laid down.

- Each manufacturer's batch/lot of HIV RNA test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.14: Hepatitis E virus RNA (HEV NAT)

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of HEV NAT

- The assay must include a specific internal control for each sample tested

- No series of tests should be considered acceptable unless the result of the assay manufacturer’s and any additional quality control samples have satisfied the criteria laid down

- Each manufacturer's batch/lot of HEV RNA test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening

9.3.15: West Nile virus RNA (WNV NAT)

The exclusion criteria for donors from a WNV risk area is given in the JPAC Donor Selection Guidelines. These guidelines specify some situations where donations may only be released if a test for WNV RNA is negative. WNV RNA screening can be performed on donations provided by donors within the exclusion period and the donations released if WNV RNA negative.

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of WNV NAT.
• The assay must include a specific internal control for each sample tested.

• No series of tests should be considered acceptable unless the result of the assay manufacturer’s and any additional quality control samples have satisfied the criteria laid down.

• Each manufacturer’s batch/lot of WNV RNA test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.3.16: Other infectious agents

The JPAC Donor Selection Guidelines3 may identify other infectious agents and specify some situations when screening may be applied in addition to donor deferral. In such situations any screening performed must:

• use assays specifically evaluated and validated for the screening of the donation type

• identify and utilise an independent quality control in each series of tests in addition to the manufacturer’s assay controls

• ensure that the results of the assay manufacturer’s and the additional quality control samples have satisfied the criteria laid down prior to release of the results

• require that each manufacturer’s batch/lot of kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

9.4: Reinstatement of blood donors

Update notice:

Section 9.4.2 and Fig. 9.6 - Technical information has been removed following the issue of Change Notification 23 - 2015.

Further information has been added to Section 9.4 following the issue of Change Notification 12 - 2016.

Where a blood donation sample is found to be repeatedly reactive on screening, the donation and any components must not be released for clinical use. The donor’s record must be flagged in accordance with standard operating procedures to prevent the issue of subsequent donations while awaiting the results of confirmatory testing in the reference laboratory.

The screen repeat reactive sample must be sent to a designated reference laboratory for confirmatory testing.

If the donation sample is determined by the reference laboratory to be demonstrating non-specific reactivity, subsequent donations from the donor may be considered suitable for issue provided that the associated donation samples are negative in the primary or an alternative screening assay (Figure 9.5).
Donors whose samples are confirmed positive cannot normally be reinstated, even after successful treatment, as screening test reactivity will persist in serological assays, for example anti-HCV and TPHA.

Donors with acute HBV infection may be reinstated provided that they meet the criteria for an individual with previous (recovered) hepatitis B virus infection laid out in the current edition of the UK Donor Selection Guidelines.

Donors with confirmed HEV or WNV infection should be deferred for 6 months from the date of first detection of HEV/ WNV RNA. These donors may be reinstated without further testing 6 months from...
the date of the index RNA positive donation

- If a previously confirmed HEV infected donor is tested prior to the end of the 6 month deferral period and found to be HEV RNA negative and HEV IgG positive, the donor may be reinstated immediately

### 9.4.2: Donors whose samples are repeatedly reactive, but concluded after reference testing to represent non-specific reactivity

Where a blood donation sample is found to be repeatedly reactive on screening, the donation and any components must not be released for clinical use.

- The donor’s record must be flagged in accordance with standard operating procedures to prevent the issue of subsequent donations while awaiting the results of confirmatory testing in the reference laboratory
- The screen repeat reactive sample must be sent to a designated reference laboratory for confirmatory testing
- If the donation sample is determined by the reference laboratory to be demonstrating non-specific reactivity, subsequent donations from the donor may be considered suitable for issue provided that the associated donation samples are negative in the primary or an alternative screening assay (Figure 9.5).

### 9.4.3: Process to reinstate a confirmed non-specific reacting blood donor

A donor with screen reactivity that is confirmed by the reference laboratory as ‘non-specific’ may be immediately returned to active status with no restrictions on any subsequent donations (see Figure 9.5).

However, in order to reinstate a donor whose sample remains reactive in the original screening assay, but confirmed by the reference laboratory to be demonstrating non-specific reactivity, the Blood Service must have the facilities to run appropriate alternative screening assays and to the same standard as primary screening. The following conditions must be met for this to be acceptable:

- The alternative assay must be of equivalent sensitivity to the original screening assay in which the index donation gave a repeatable non-specific reaction and conform to the UK requirements for microbiology screening tests.
- Donations taken subsequent to the return of the donor to the active panel may be used provided that the donation is non-reactive by the alternative assay.
- The donor’s record must remain flagged with the information identifying previous non-specific reactivity for the marker.

### 9.5: Recommended standards for the reduction of bacterial contamination of blood components

Update notice: Section 9.5.3.1 - Single-test system has been updated following the issue of Change Notification 16 - 2013.
In recent years bacterial contamination of blood has been significantly reduced by the introduction of improved donor arm cleansing using 70% isopropyl alcohol/2% chlorhexidine gluconate applied as a single-step procedure, and diversion of the first 20–30 mL of the blood donation. The risk of bacterial contamination can be further reduced, but not eliminated, by screening of blood components.

**9.5.1: Arm cleansing**

There should be an effective, specified and validated method of arm cleansing, using an approved skin-cleansing system. 70% isopropyl alcohol/2% chlorhexidine gluconate is recommended by the National Evidence-Based Guidelines for Preventing Healthcare-Associated Infections in NHS Hospitals in England. Adherence to the principles, protocols and practices relating to the correct use of the specified skin-cleansing system shall be regularly audited by periodic bacterial sampling and observation, and corrected if found to be lacking.

Periodic bacterial sampling of the skin of donors’ arms may be carried out as an audit of correct use of the specified skin-cleansing system. If such sampling is performed, it will give an indication of how well staff are complying with the use of the system. In practice, it should be expected that bacterial sampling after skin cleansing with 70% isopropyl alcohol/2% chlorhexidine gluconate will reveal bacteria at a rate of no greater than 2 cfu per standard contact plate. Such levels may be difficult to achieve with other cleansing systems. Consistent finding of higher levels may require a review of compliance/re-education of relevant staff and further observational audits.

Periodic bacterial sampling may also take the form of anonymous sampling of staff fingertips after hand hygiene and after dealing with donors to assess levels of hand contamination and effectiveness of hand washing and decontamination in practice. Findings can then be fed back to staff as an educational tool.

**9.5.2: Diversion of donation**

A minimum of 20 mL of the first part of every blood donation should be diverted into a side-arm pouch, in order to minimise the level of bacterial skin contaminants in the collection bag. This diverted volume can be used as a source of blood samples for mandatory and other testing of the donation.

**9.5.3: Screening of platelet components**

There should be a means of detecting bacterial contamination of platelet components, using validated methods. The key requirements of a detection system are (i) effective sample size, (ii) a rapid test result or automated 24-hour readout with alarm notification and (iii) reliable detection of bacteria at a level indicating emerging risk to recipient.

Bacterial culture using an automated microbial detection system represents the most widely used and efficient method for screening of components. Its key feature is the continuous monitoring of incubation to allow immediate withdrawal of contaminated units.

The following protocols are considered to be optimal for single and two-test systems for the screening of platelets using an automated microbial detection system. Both require the use of a minimum 16 mL sample for aerobic and anaerobic culture and allow a 7-day shelf life. A two-test protocol is the ideal method for optimal performance, but this has significant operational issues. A single-test protocol with a minimum hold period of 36 hours before sampling will allow extension of shelf life of the product to 7 days providing incubation and monitoring is continued for the duration. Services may choose to initiate testing earlier than the 36-hour holding period (e.g. 18 hours), but these platelets will not qualify for a 7-day shelf life unless a second test is performed.

**9.5.3.1: Single-test system**
1. Platelet components are held for at least 36 hours after collection

2. Minimum 8-mL samples are inoculated into each aerobic and anaerobic bottle.

3. If samples are negative after a minimum of 6 hours of incubation, release product on a negative-to-date basis with 7-day shelf life and continue incubation and monitoring for the shelf life of the product.

4. A suitable protocol must be in place for confirmation of the presence of contamination.

5. Discard unused platelets on Day 8. (Time-expired units may be referred to the relevant bacteriology laboratory for surveillance testing.)

9.5.3.2: Two-test system

1. Platelet components are held for at least 18 hours after collection.

2. Minimum 8-mL samples are inoculated into each aerobic and anaerobic bottle.

3. If samples are negative after 24 hours of incubation, release product on a negative-to-date basis with 5-day shelf life and continue incubation and monitoring for the shelf life of the product.

4. A suitable protocol must be in place for confirmation of the presence of contamination.

5. Re-sample and test remaining stock at 4 days after collection and if negative at 24 hours release for use with a 7-day shelf life.

6. Discard unused platelets on Day 8. (Time-expired units may be referred to the relevant bacteriology laboratory for surveillance testing.)
* Release of red cells requires a negative result from both the index culture bottles and testing of the platelet component.

† AMDS: Automated microbial detection system, re-test aerobic and anaerobic culture in duplicate.

**Figure 9.7 Platelet components testing algorithm. If the index pooled platelet component is not available to re-test, the associated red cell unit should be tested.**

The following definitions of screening test results are recommended.

**Initial reactive:** Positive bottle or bottles from which bacteria are isolated on initial screen.

**Repeat reactive:** Positive bottle or bottles from repeat testing of the index unit from which bacteria are isolated.

**Confirmed positive:** Bacteria detected from the initial and repeat tests which are of the same species.

**Indeterminate:** Bacteria detected in only the initial or repeat test, but not both, or bacteria detected in the initial or repeat reactives which cannot be matched at species level.

**False positive:** A positive signal is obtained for a culture bottle but no bacteria are detected on subculture.
*Confirmed positive = a match at species level on the initial RBC test and re-test.

** Non-confirmed positive = negative repeat test and no match at species level with the PC confirmed positive result.

† AMDS: Automated microbial detection system, re-test aerobic and anaerobic culture in duplicate.

**Figure 9.8 Red blood cell algorithm**

**False negative**: Initial test negative but component associated with a post-transfusion reaction is subsequently positive on re-testing.

If the initial test is positive any components (plasma, red cells etc.) from the same donation must be quarantined pending the result of the repeat test and a recall procedure should be initiated for any platelet units or other components already issued.

The algorithms shown in Figures 9.7 and 9.8 are recommended for the confirmation of bacterial contamination of platelet components or red blood cells using an automated microbial detection system.

**9.6: Recommended standards for microbiological screening**

**9.6.1: Tissues**
All microbiological culture testing is subject to quality control tests in accordance with national accreditation standards and guidelines. This ensures that the risk of disease transmission is minimised and that tissue allografts are suitable for their intended use.

A written policy documenting the bacteriological acceptance criteria for specified tissues should be drawn up in consultation with a designated microbiologist.

Tissues must be screened for bacterial and fungal contamination by validated methods in accredited laboratories. Samples for bacterial screening (e.g. swab culture, bone chips etc.) should be obtained aseptically and placed in appropriate culture media at the time of retrieval or processing. Samples should be culture tested before and after exposure to decontaminating agents by enrichment liquid cultures to maximise the recovery of aerobic and anaerobic bacteria, and fungi. If pathogenic, highly virulent bacteria are recovered (e.g. Clostridium spp, Streptococcus pyogenes, Staphylococcus aureus, Candida spp.) the tissue should not be used for transplantation unless it is effectively sterilised by a process such as gamma irradiation. Cardiovascular tissues must be tested for the presence of Mycobacterium spp. Tissues contaminated with opportunist species of low virulence must be decontaminated by a validated process. Tissues which cannot be terminally sterilised (e.g. heart valves, amnion, menisci, osteochondrals) must be discarded if post-decontamination tests prove positive. An exception is cryopreserved skin allografts, which can be transplanted if non-pathogenic bacteria are present.

If no suitable sample is available for screening for bacterial and fungal contamination, then the products should be handled in the same way as those which have positive culture results for highly virulent bacteria: either discard or terminal sterilisation with a process such as gamma irradiation.

If a tissue fails culture testing, other tissues from the same donor should be discarded unless processed separately or an assessment of the risk shows otherwise.

9.6.2: Cord blood

Cord blood donations are subject to the NetCord-FACT International Standards for Cord Blood Collection, Processing and Release for Administration. Cord blood collections must be screened for bacterial (aerobic and anaerobic) and fungal contamination using a system permissive for the growth of these microorganisms (European Pharmacopoeia 2.6.27). All donations positive for microbial growth should be discarded. Identification of any organism isolated needs to be undertaken and results reviewed by a microbiologist to identify potential sources of contamination. A trend analysis of contamination rates should be performed periodically to maintain quality.

9.6.3: Stem cells

Stem cell products (peripheral blood stem cells, bone marrow, whole blood and directed cords) are subject to the FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing, and Administration.

All products (fresh and cryopreserved) must be tested for microbial contamination (European Pharmacopoeia 2.6.27) unless the total sample volume is specifically requested by the transplant surgeon to optimise dose for the recipient. Microbial isolates recovered from products should be identified to species level and antimicrobial susceptibilities determined and stored in a cryobank. A trend analysis of data should be reviewed by a microbiologist to identify potential sources of contamination.

9.6.4: Autologous serum eye drops
Eye drops made from diluted autologous serum are used to treat ocular surface disorders. The serum is diluted with saline and bottled under aseptic conditions and bacteriologically tested (European Pharmacopoeia 2.6.27). It is recommended that 5% of batches should be tested for sterility by culture for aerobic and anaerobic bacteria. Identification of positive cultures needs to be performed and advice sought from a medical microbiologist regarding the suitability of a product for use via a quality concession.

9.7: Recommended standards for environmental monitoring of processing facilities

Environmental monitoring programmes must be in place for both uncontrolled and controlled processing facilities and must meet the requirements of appropriate regulatory bodies. They must form part of the quality management system ensuring that products are processed to the highest possible standards. Uncontrolled facilities include blood-processing laboratories and controlled facilities include cleanrooms used for the aseptic processing of tissues and stem cells.

The main aim of microbiological environmental monitoring is to provide a means of monitoring trends over time thereby ensuring that processing facilities continue to operate within acceptable bioburden levels. Individual test results, whether high or low counts, are rarely significant.

9.7.1: Key elements of an environmental monitoring programme

The monitoring programmes must define and document:

- The sites to be monitored and the rationale behind the selection of these sites.
- The types of samples to be taken and the techniques used.
- The monitoring frequency and the conditions under which the monitoring is to be performed, i.e. in the ‘at rest’ or ‘in operation’ states.
- Which personnel are authorised to perform environmental monitoring.
- The incubation regime for samples.
- The setting of limits (alert and action levels).
- The requirement for data and trend analysis.
- A procedure for the investigation of out-of-limit results including the identification of colony growth and the possible causes of the contamination.
- A procedure for corrective action in the event of out-of-limit results.

9.7.2: Monitoring techniques

Monitoring must be performed using standardised techniques and the main areas of sampling should include:
Surface sampling using contact and swab plates with the latter being used in areas inappropriate for contact plates.

Air sampling using settle plates and, in addition, in cleanroom environments, active air sampling and particle counting.

Glove prints for assessing potential transfer of bacterial contamination to sterile product during aseptic processing (cleanrooms).

In controlled facilities, monitoring for fungal in addition to bacterial contamination must, as a minimum, be achieved through the use of settle plates with media specific for each type of contamination.

9.7.3: Culture media

Culture media used for environmental monitoring must be appropriate for the type of environment in which it is to be used, i.e. irradiated and triple wrapped media for use in cleanrooms, and for the range of organisms likely to be isolated. Media used for post-disinfection monitoring must contain agents, either individually or in combination, that will neutralise any residual surface disinfectant. Neutralising agents must be validated against the disinfectant(s) in use within the facility. Media storage must be in compliance with the manufacturer’s recommendations and the monitored facility must be able to provide monitoring data to show that these storage requirements are met.

9.7.4: Alert and action levels

In cleanroom facilities, limits must be set for the results of both particulate and microbiological monitoring. These limits are specified in Annex 1 of the EC Guidelines to GMP (Manufacture of Sterile Medicinal Products).

The action levels for microbiological monitoring in controlled rooms are taken as the limits given in the EU Guide. Alert levels must also be set in order to provide a warning of a possible deviation from normal operating conditions that may not require direct action but may need to be monitored more closely.

In uncontrolled facilities, action levels must be established using historical data. The monitoring programmes must define how the alert levels in controlled rooms and the action levels in uncontrolled rooms are to be determined.

9.7.5: Data and trend analysis

Monitoring results must be entered on a suitable database to allow data and trend analysis. The results must be reviewed by staff of the monitored facility on a regular basis with a formal documented review being held at a minimum of four times a year. This formal review must involve senior cleanroom/processing staff and representatives from the quality and microbiology departments.

9.7.6: Cleanroom gowning

Environmental monitoring programmes for controlled rooms also need to include procedures for:

- the qualification of staff with respect to cleanroom gowning for grade A and B environments
- the monitoring of staff upon leaving an aseptic area as a means of assessing operator bioburden levels.
Gowning qualification and exit suit monitoring should be performed for each cleanroom operator on a regular basis with the frequency, sampling method(s) used and monitoring sites clearly defined in the procedures.

9.7.7: Process simulations

Validation of aseptic processing should include a process simulation test using a nutrient medium. The process simulation test should imitate as closely as possible the routine process including all critical subsequent manufacturing steps. It should also take into account various interventions known to occur during the routine process as well as worst-case situations. Process simulation tests should be performed as initial validation with three consecutive satisfactory tests and repeated at defined intervals and after any significant modification to the heating, ventilation and air conditioning (HVAC) system, equipment or process.

Normally process simulation tests should be repeated twice a year (per shift and process). Alert and action levels should be defined and documented and any contamination investigated.

9.7.8: Cleaning and disinfection

Cleaning/disinfection validation should be performed in order to confirm the effectiveness of a cleaning /disinfection programme. As part of the validation, pre- and post-cleaning/disinfection environmental monitoring should be used to verify the acceptability of the frequency and efficiency of the programme in terms of microbiological contamination. Pre- and post-limits should be established and documented within the cleaning/disinfection programme. The monitoring results should be reviewed and, where limits have been exceeded, the contamination investigated and corrective action implemented.

Typically, three consecutive applications of the cleaning/disinfection procedure should be performed and shown to be successful in order to prove that the method is validated.

The cleaning and disinfection of controlled rooms is particularly important and should be performed in accordance with a written programme. Where disinfectants are used, more than one type should be employed on a rotational basis. Detergents and disinfectants should be monitored for microbial contamination and, when used in grade A and B areas, should be sterile prior to use.

9.8: Investigation of suspected bacterial contamination of blood components

Suspected cases of bacterial contamination of blood components may be notified by reports from the hospital of a significant transfusion reaction or, following a severe reaction, the identification of bacteria either within the pack or in a patient’s blood culture.

A record of the original notification, clinical details and investigations carried out by the hospital must be made by the Blood Centre. The pack remains should be sealed and transported as soon as possible to a specialist bacteriology laboratory along with any bacterial isolates subsequently recovered from the patient’s blood. If the patient has died without blood samples being obtained after the transfusion, it may be necessary for a post-mortem blood sample to be collected.

The contents of the pack, or if empty, a 20 mL saline wash out of the pack, should be sampled in the laboratory taking care to minimise the introduction of contaminants. A Gram stain may be informative but the sample should be cultured for bacteria (aerobic and anaerobic) and fungi using a system permissive for the growth of these microorganisms. If cultures prove negative no further action/investigation is necessary.
Where bacterial contamination is indicated, action must be taken to safeguard the safety of the blood supply by recalling all other components from the same donation(s) and these must be subjected to bacterial investigation. The possible source of a contamination needs to be investigated in consultation with a specialist microbiologist and appropriate swabs and other samples from the donor obtained for culture. If isolates of the same species are obtained from the pack and donor these must be submitted for molecular typing to establish the strain identity and possible route of transmission. Further decisions about the use of subsequent donations from the donor will depend on the circumstances and the type of contamination. An assessment should be carried out on a case-by-case basis to determine the risk of bacterial contamination through the use of further blood donations from the donor, and appropriate action taken.

9.9: References