Guidelines for the Blood Transfusion Services

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

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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 Blood and Tissue Establishments, but tissue and stem cell donations are now a significant 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 Blood and Tissue Establishments.

9.1: General requirements

All screening must be performed within Blood Safety and Quality Regulations[1] (BSQR) 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] and any other appropriate regulations
- 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 UKCA or CE marked and must have been assessed in respect of sensitivity and, if appropriate or necessary, specificity, and deemed suitable by the UK Blood and Tissue Establishments kit evaluation groups (NHSBT KEG or SNBTS 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 must be assessed prior to being accepted and put into use (Lot Release Testing - LRT). Each additional delivery of an existing lot should be assessed before use (Delivery Acceptance Testing - DAT). Each manufacturer's batch/lot of microbiology test kits must be shown to conform with nationally established minimum criteria for specificity and sensitivity prior to being accepted for use for screening.

Additionally, all testing laboratories must ensure that the expected standard of performance of the assays used is being achieved through the use of the appropriate Quality Control (QC) samples and the statistical monitoring of assay control and QC sample results. Appropriate reactivity with manufacturers' and QC samples must be demonstrated with every series of tests. 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).

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.

9.2: Microbiology screening

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 specific infectious marker to be defined as ‘mandatory’. These include a UK or European Union regulatory 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/regulatory requirement.

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 additional screening is 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 screening 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 screened 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.

Table 9.1 Screening required for blood donations

Table 9.2 Screening required for tissue and stem cell donations[1]

9.2.2: Deceased neonatal and infant tissue donors

- Full microbiology screening of a maternal sample is always required.

- For still births and for neonates up to 28 days after birth, no microbiology screening of the neonate is required.

Infectious agent	Minimum requirement	Comments[1]
HIV 1+2	anti-HIV 1+2+O or HIV 1+2+O Ag/Ab (M) HIV RNA[2]	RNA screening in pools of a maximum of 24 donations[3]
HCV	anti-HCV (M) HCV RNA (M)	RNA screening in pools of a maximum of 24 donations[3]
HBV	HBsAg (M) HBV DNA[2] anti-HBc [+ anti-HBs] (A)[4]	DNA screening in pools of a maximum of 24 donations[3] Donations that are anti-HBc reactive and have anti-HBs >100 mIU/mL, tested in the past 24 months by a UK Blood Service, are considered suitable for release if HBsAg and ID HBV DNA negative
Syphilis	anti-treponemal (M)	
HTLV I/II	anti-HTLV I/II (M)[5]	Serology screening individually or in pools of a maximum of 24 donations[3]
HEV	HEV RNA (M)	RNA screening in pools of a maximum of 24 donations[3]
HCMV	anti-HCMV (A)	Ideally both IgG and IgM, but IgG alone is considered sufficient
Plasmodium sp.	anti-*P. falciparum/vivax* (A)	
Trypanosoma cruzi	anti-*T. cruzi* (A)	
West Nile Virus (WNV)	WNV RNA (A)	RNA screening in pools of a maximum of 16 donations[6]
HAV	HAV RNA (A)	RNA screening in pools of a maximum of 96 donations
Human B19	B19 DNA (A)	DNA screening in pools of a maximum of 96 donations
(M) – mandatory (release criteria) for the purpose of these guidelines		
(A) – additional (release criteria) due to specifically identifiable risk		
[1] All microbiology screening performed on individual donations unless specified otherwise		
[2] Although neither are mandatory for blood donations in most of the UK, HIV RNA and HBV DNA are included in nucleic acid screening as the commercial systems available are now triplex assays. HIV RNA is, however, mandated within Scotland.		
[3] The minimum sensitivity of the molecular screening is dependent upon pool size. The maximum validated pool size for use for mandatory blood screening within the UK Blood Transfusion Services is 24 donations.		
[4] All blood donors are to be screened for anti-HBc at their first donation or their first donation after the introduction of anti-HBc screening. Anti-HBc screening to be repeated if a donor lapses (over 2 years) or has a new HBV risk.		
[5] anti-HTLV screening is only required for blood donations from previously untested donors and for blood donations destined for use to prepare non-leucodepleted products		
[6] The maximum validated pool size for WNV RNA screening is 16 donations		

Infectious agent	Minimum requirement	Comments[1,2]
HIV 1+2	anti-HIV 1+2+O or HIV 1+2+O Ag/Ab (M) HIV RNA (O)	Stem cell donors: as for blood donors

HCV	anti-HCV (M) HCV Ag and/or HCV Ag/Ab (O) HCV RNA (O)	Stem cell donors: as for blood donors
HBV	HBsAg (M) anti-HBc (M) [+ anti-HBs[2]] (O) HBV DNA[3] (O)	Stem cell donors: as for blood donors (for HBsAg and HBV DNA, anti-HBc mandatory for stem cell donors) **Either:** donations that are anti-HBc reactive and have anti-HBs >=100 mIU/mL are considered suitable for release **Or:** donations that are anti-HBc reactive and are HBsAg and ID HBV DNA negative do not require an anti-HBs level of >=100 mIU/mL to be considered suitable for release[3]
Syphilis	anti-treponemal (M)	
HTLV I/II	anti-HTLV I/II (M)[4,5]	Serology screening individually or in pools of a maximum of 24 donations[4]
HEV	HEV RNA (A)	RNA screening in pools of a maximum of 24 donations[4]
HCMV	anti-HCMV (A)	Ideally both IgG and IgM, but IgG alone is considered sufficient
Plasmodium sp.	anti-*P. falciparum/vivax* (A) *Plasmodium spp* DNA (A)[7]	
Trypanosoma cruzi	anti-*T. cruzi* (A)	
West Nile Virus (WNV)	WNV RNA (A)	Maximum of 16 donations[6]
(M) – mandatory (release criteria) for the purpose of these guidelines		
(A) – additional (release criteria) due to specifically identifiable risk		
(O) – optional, genomic screening for HIV, HCV and HBV nucleic acids is not mandated but can be performed on the original donation sample as an alternative to 180 days' quarantine and follow-up serological testing		
[1] All microbiology screening performed on individual donations unless specified otherwise		
[2] 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 screening requirements.		
[3] anti-HBc reactive tissue and stem cell donations do not need to have an anti-HBs level >=100 mIU/ml to be considered suitable for release if both HBsAg and ID HBV NAT are negative on screening		
[4] All screening of deceased tissue donations should be performed on individual samples. Anti-HTLV I/II screening of surgical tissues/stem cells may be performed using pools of a maximum of 24 samples. HEV RNA screening of surgical tissues may be performed using pools of a maximum of 24 samples		
[5] Not mandatory for avascular tissue donations but may be considered good practice		
[6] The maximum validated pool size for WNV RNA screening is 16 donations.		
[7] Certain tissues and cord bloods from donors with malaria risk and which are found to be malaria antibody positive may be released for use if additional testing for malaria DNA is performed and malaria DNA is not detected (see Tissue Donor Selection Guidelines[3])		

- 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 donation which is 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 in both tests in duplicate repeat testing using the same assay.

- Blood donations which are reactive in one or both of the repeat tests are unsuitable for use and must be labelled as biological hazard/not for transfusion.

- Donations which are 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 screened using a second (alternative) screening assay of at least equal sensitivity to the primary screening assay, and can be considered suitable for clinical use if giving a negative result in the alternative screening assay.

See flowchart for screening of blood donations, provided as Figure 9.1.

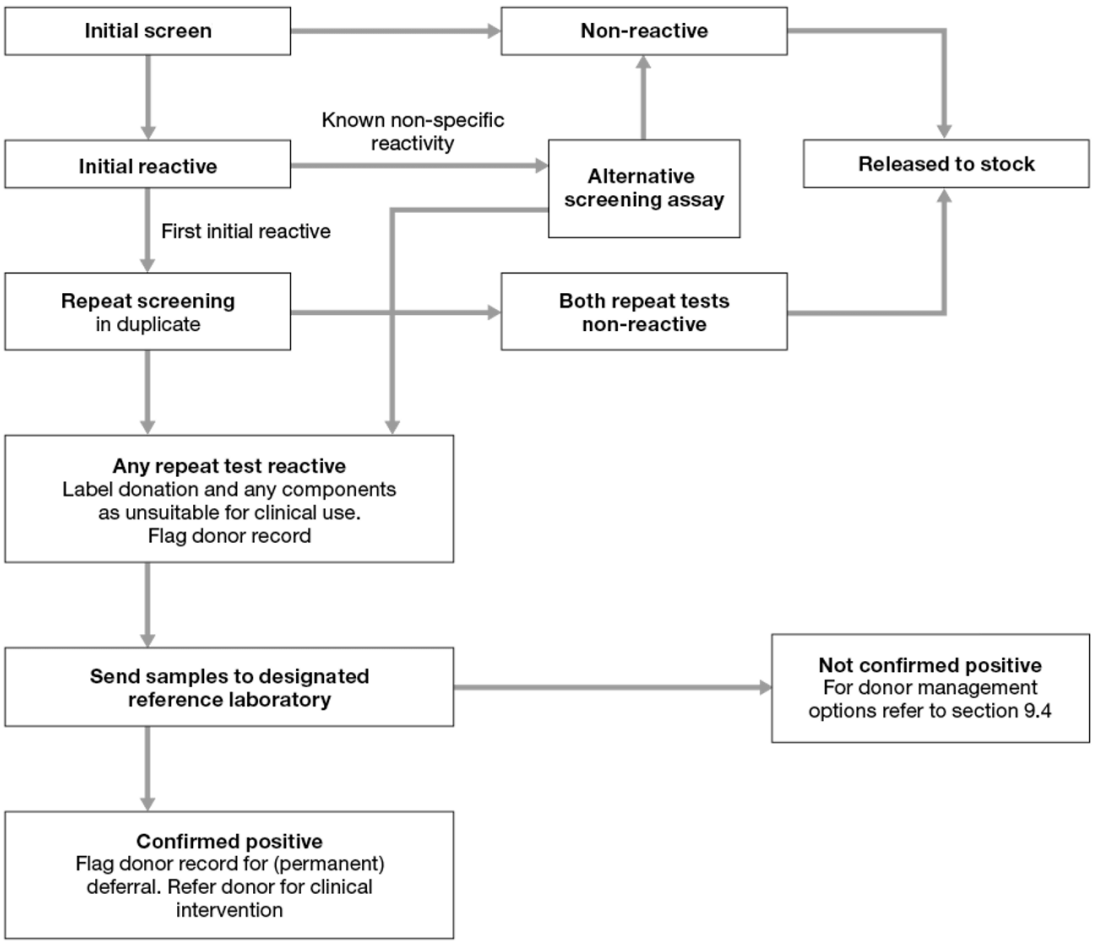

Figure 9.1 Serology screening: blood donations

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 in both of the repeat tests can be considered suitable for clinical use.
- Donations that are reactive in one or both of the repeat tests may in some clinical circumstances, and depending on the confirmatory results, be considered suitable for use (SaBTO Microbiological Safety Guidelines, 2020[3]).

9.2.4: Molecular screening algorithm

- All initially reactive pools (see Figures 9.3 and 9.4) must be resolved to an individual (or more) reactive donation(s). 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 Microbiological Safety Guidelines, 2020[3]).

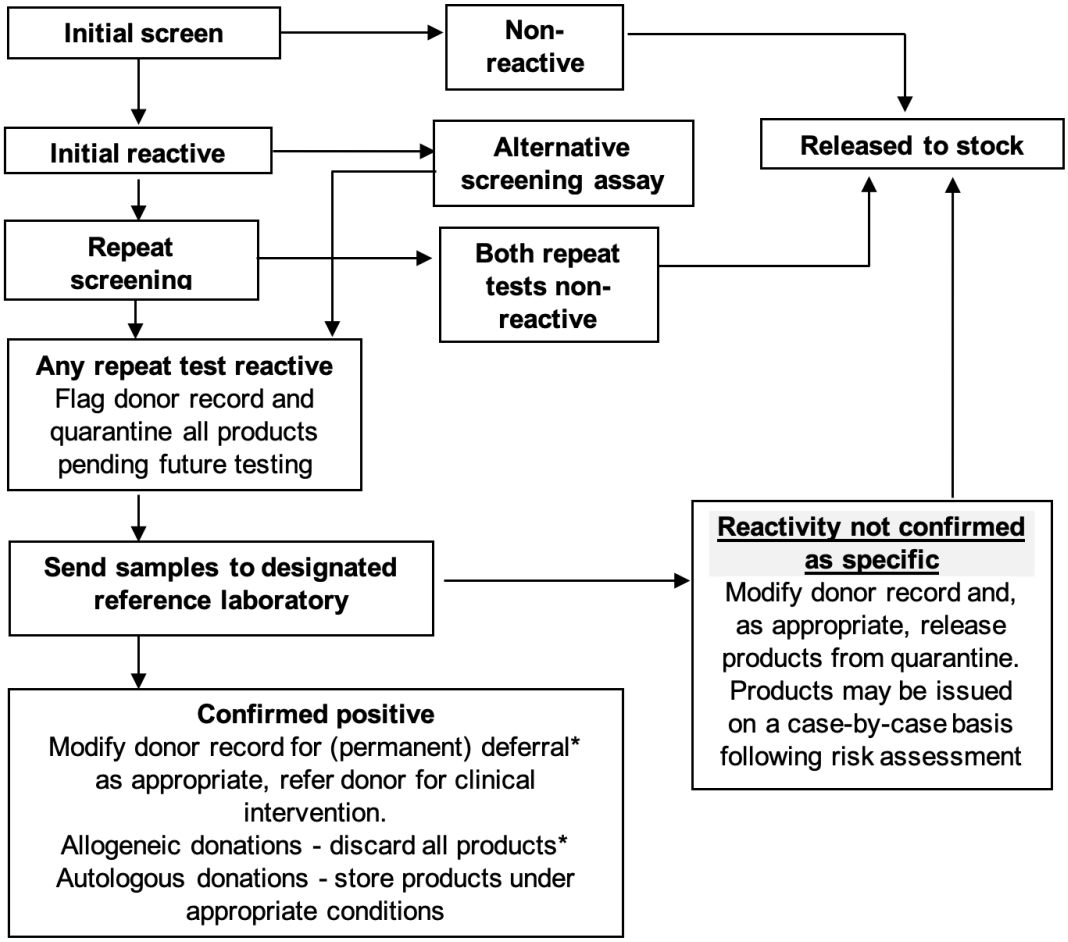

Figure 9.2 Serology screening: tissue and stem cell donors/donations

* Tissue/stem cell donors/donations confirmed to be anti-HBc reactive, and which are HBsAg and ID HBV DNA negative may be considered suitable for release

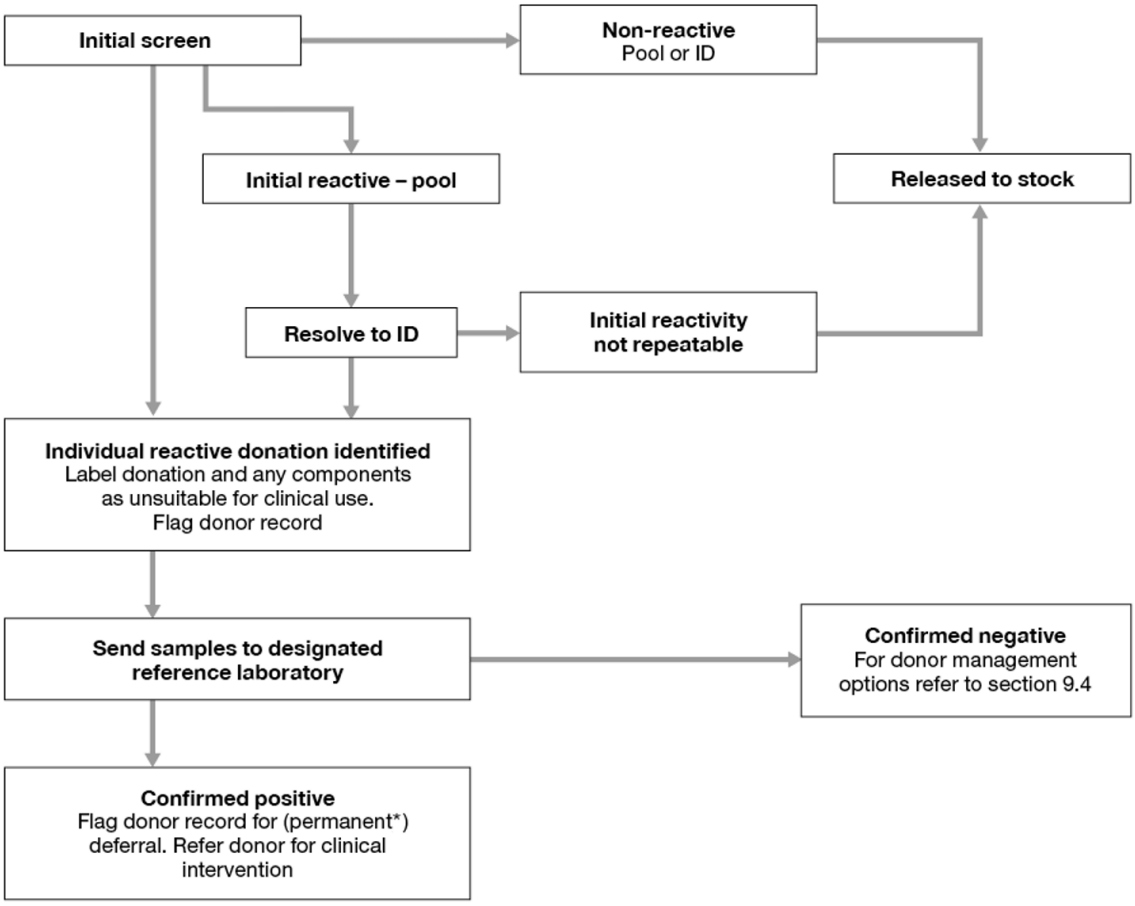

Figure 9.3 Molecular screening: blood donations

* Donors confirmed to be HEV or WNV RNA positive need only be deferred for 6 months from initial detection.

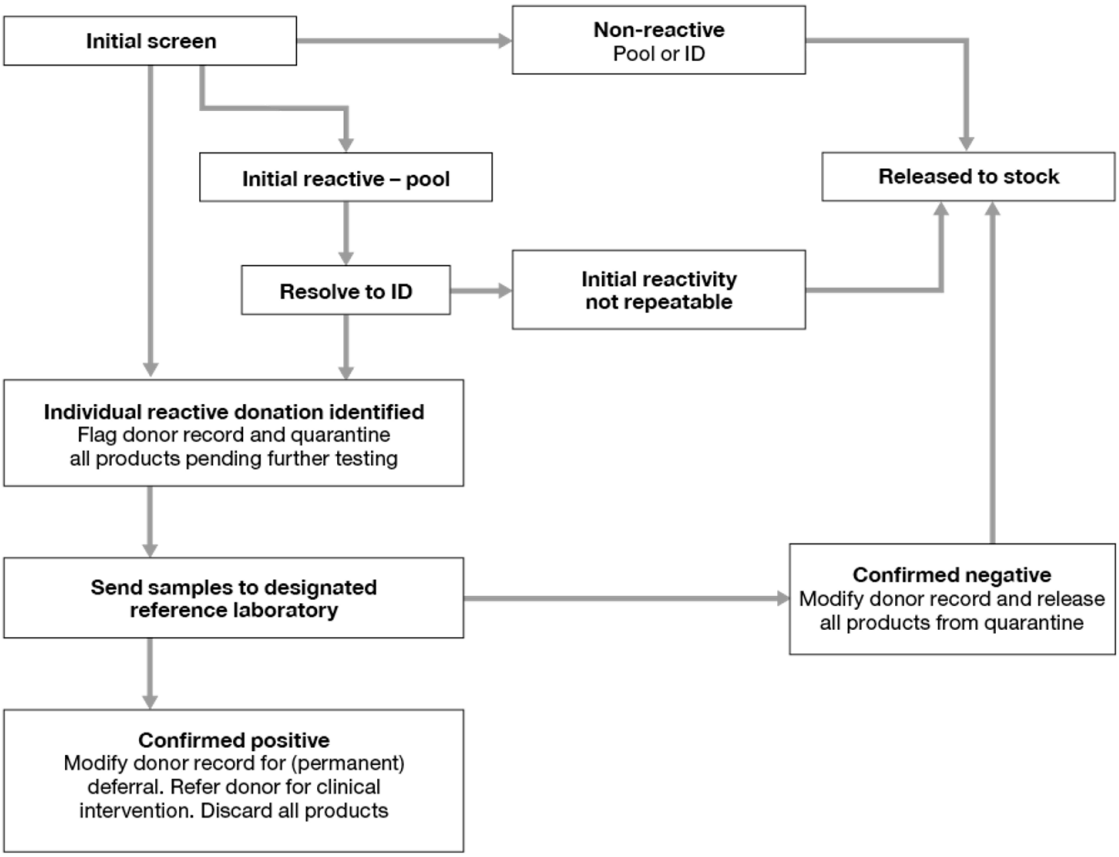

Figure 9.4 Molecular screening for tissue and cell donors

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) samples from the donor/donation must undergo confirmatory testing at a designated reference laboratory.

- For blood, donations that are confirmed positive for anti-HBc from donors with anti-HBs >100 mIU /mL, tested in the past 24 months by a UK Blood Service, are considered suitable for release if HBsAg and ID HBV DNA negative.

- For tissues and cells **either** donations that are anti-HBc reactive and anti-HBs >=100 mIU/mL are considered suitable for release, **or** donations which are anti-HBc reactive and are HBsAg and ID HBV DNA negative are considered suitable for release without the need for anti-HBs level of >=100 mIU/mL.

- Anti-*T. gondii* IgG and IgM screening is recommended for HSC, donor lymphocyte infusions and other therapeutic cells (e.g. selected and cultured products including T-cells, natural killer cells, mesenchymal stem cells, cytotoxic T-lymphocytes, T-regulatory cells, tumour derived cells) and embryonic stem cell lines intended for clinical use derived from human embryos initially created for fertility treatment with the use of IgM positive donors avoided. Confirmation of anti-*T.gondii* IgM only reactivity recommended due to known specificity issues with IgM assays.

- If HEV, HAV 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, HAV or WNV RNA positive donation: see section 9.4.

- If human B19 DNA is confirmed in a donor, the donor record must be flagged as ‘temporary exclusion’ for 4 weeks. The donor can be reinstated automatically at least 4 weeks after the date of the index DNA 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, when appropriate, perform specific neutralisation tests for HBsAg to ensure that donors with low-level HBsAg reactivity, in the absence of other HBV markers, are not incorrectly reported as non-specifically reactive.

9.2.6: Definitions

9.3: Specific screening targets

9.3.1: HBsAg

- The UK specification for the minimum level of sensitivity for the performance of HBsAg screening is 0.2 IU/mL. This level of sensitivity can be demonstrated during assay evaluation/validation /verification through the use of a quality control calibrated to the World Health Organisation (WHO) International Standard. If the WHO International Standard is withdrawn or otherwise unavailable, an alternative quality control reagent can be used that must be validated for use by the UK Blood Service using that reagent.

- In addition to the assay manufacturer’s controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. This control must have a unitage of 0.2 IU /mL or less. If the unitage is not defined by the manufacturer it can be validated by at least one of the UK Blood Services. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

Term	Definition
Non-reactive (NR)	A sample whose reactivity when first tested falls below the assay cut-off as defined by the manufacturer's instructions. May also be referred to as a 'Negative' test result
Initial reactive (IR)	Any sample whose reactivity when first tested falls above the cut-off as defined by the manufacturer's instructions
Repeat reactive (RR)	Any sample reactive on two or more occasions either in the same screening assay (duplicate) or in two or more screening assays that are used in combination sequentially, to determine the suitability of a donation for release for clinical use
Alternative assay screening	When a second assay for the same screening target and of similar sensitivity is used sequentially to screen a sample which is either IR or RR in a first screening assay
Confirmatory testing	Full investigation, in a designated reference laboratory of a repeat reactive sample to determine whether the reactivity is specific to the infectious agent being screened for and indicative of current or past infection in the donor
Positive	A sample whose reactivity in confirmatory testing meets pre-defined criteria. This may indicate current or past infection
Inconclusive	A sample whose reactivity in confirmatory testing is not sufficient and/or specific enough to determine whether it reflects infection or possible non-specific reactivity
Negative	A sample whose screen reactivity, on investigation, is either not demonstrable or is deemed not to reflect infection
Individual nucleic acid test (ID-NAT)	Molecular screening of a donation as an individual sample cf. pooled testing

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

- Screening for both HIV p24 antigen and antibody to HIV 1+2+O in a combination assay is recommended as the serological screening approach for HIV within the UK Blood Services.
- 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 during assay evaluation/validation/verification with a quality control calibrated to the WHO International Reference reagent. There is no specific requirement to demonstrate individual anit-HIV 2 or HIV p24 Ag reactivity. If the WHO International Reference reagent is withdrawn or otherwise unavailable, an alternative quality control reagent can be used that must be validated for use by the UK Blood Service using that reagent.
- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.
- No series of tests should be considered acceptable unless the result of the assay manufacturer's controls and the additional quality control sample have satisfied the criteria laid down.

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 during assay evaluation/validation/verification with a quality control calibrated to the WHO International Standard. In the absence of standardisation against a WHO International Standard, the reagent must be validated for use by the UK Blood Service using the reagent.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

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 during assay evaluation/validation/verification with a quality control reagent calibrated to the WHO International Standard. In the absence of standardisation against a WHO International Standard, the quality control must be validated for use by the UK Blood Service using the reagent.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

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 a positive result should be obtained during assay evaluation /validation/verification with a quality control reagent calibrated to the WHO International Standard. In the absence of standardisation against a WHO International Standard for enzyme immunoassays, the quality control must be validated for use by the UK Blood Service using the reagent.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

9.3.6: Malarial antibody

Donations collected from donors with an identified malarial risk may be released if the donation has been collected following the exclusion period set out in the JPAC Donor Selection Guidelines[3] and malarial antibody is not detected on screening. These guidelines also identify specific situations when donations may be released if malarial antibody is detected and additional testing for malarial DNA is then performed and malarial DNA not detected, and situations when donations may be collected at a timepoint within the standard exclusion period.

- The UK requirement for the minimum level of sensitivity for the performance of malarial antibody (anti-*P. falciparum/vivax* as a minimum) screening is that a positive result should be obtained during assay evaluation/validation/verification with a quality control calibrated to the WHO International Reference reagent. If the WHO International Reference reagent is withdrawn or otherwise unavailable, an alternative quality control reagent can be used that must be validated for use by the UK Blood Service using that reagent.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

9.3.7: *T. cruzi* antibody

The deferral criteria for donors from *T. cruzi* endemic areas are given in the JPAC Donor Selection Guidelines[3]. 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 a positive result should be obtained during assay evaluation/validation/verification with a quality control calibrated to the WHO International Standard. If the WHO International Standard is withdrawn or otherwise unavailable, an alternative quality control reagent can be used that must be validated for use by the UK Blood Service using that reagent.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

9.3.8: anti-HBc

The exclusion period for blood donors who have had body piercing, which includes derma-rolling, ear and body piercing, permanent and semi-permanent make-up, tattooing, platelet rich plasma facial, ritual self-flagellation and acupuncture, are given in the JPAC Donor Selection Guidelines[3].

All blood donors are to be screened for anti-HBc at their first donation or their donation after the introduction of anti-HBc screening. Anti-HBc screening to be repeated if a donor lapses (over 2 years) or has a new

HBV risk. Tissue and stem cell donations have anti-HBc screening as a mandatory requirement at each donation.

- The UK requirement for the minimum level of sensitivity for the performance of anti-HBc screening is that a positive result should be obtained during assay evaluation/validation/verification with a quality control calibrated to the WHO International Standard. If the WHO International Standard is withdrawn or otherwise unavailable, an alternative quality control reagent can be used that must be validated for use by the UK Blood Service using that reagent.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

- Blood donations which are confirmed positive for anti-HBc should be tested for anti-HBs; Tissue and stem cell donations found to be reactive for anti-HBc alone may not require additional anti-HBs testing (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 a positive result should be obtained during assay evaluation/validation/verification with a quality control reagent calibrated to the WHO International Standard. In the absence of standardisation against a WHO International Standard, the quality control must be validated for use by the UK Blood Service using the material.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro,* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

9.3.10: anti-HBs

- The UK requirement for the minimum level of sensitivity for the performance of anti-HBs testing is that a positive result should be obtained during assay evaluation/validation/verification with a quality control reagent calibrated to the WHO International Standard. In the absence of standardisation against a WHO International Standard, the quality control must be validated for use by the UK Blood Service using the material.

- In addition to the assay manufacturer's controls a quality control suitable for the purposes of monitoring the performance of *in-vitro* assays must be included at least once in each series of tests to demonstrate acceptable sensitivity of the test method. Where available, the quality control should be CE or UKCA marked. The quality control must be manufactured by a different manufacturer to that of the assay.

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

9.3.11: Hepatitis C virus RNA

- The UK requirement for the minimum level of sensitivity for the performance of HCV RNA screening is 5000 IU/mL in an individual donation.
- 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 controls and any additional quality control sample have satisfied the criteria laid down.
- If an additional quality control is run on each series of tests, it should be a CE or UKCA marked quality control where available and suitable for the purposes of monitoring the performance of *in-vitro* assays and manufactured by a different manufacturer to that of the assay.

9.3.12: Hepatitis B virus DNA

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of HBV DNA screening.
- 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.
- If an additional quality control is run on each series of tests, it should be a CE or UKCA marked quality control where available and suitable for the purposes of monitoring the performance of *in-vitro* assays and manufactured by a different manufacturer to that of the assay.

9.3.13: Human immunodeficiency virus RNA

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of HIV RNA screening.
- The assay must include a specific internal control for each sample tested.
- The assay must utilise two separate targets within the HIV genome to minimise any risk of failure of detection due to sequence changes in the primer or probe binding regions.
- No series of tests should be considered acceptable unless the result of the assay manufacturer's controls and any additional quality control sample have satisfied the criteria laid down.
- If an additional quality control is run on each series of tests, it should be a CE or UKCA marked quality control where available and suitable for the purposes of monitoring the performance of *in-vitro* assays and manufactured by a different manufacturer to that of the assay.

9.3.14: Hepatitis E virus RNA

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of HEV RNA screening.
- 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 controls and any additional quality control sample have satisfied the criteria laid down.
- If an additional quality control is run on each series of tests, it should be a CE or UKCA marked quality control where available and suitable for the purposes of monitoring the performance of *in-vitro* assays and manufactured by a different manufacturer to that of the assay.

9.3.15: West Nile virus RNA

The exclusion criteria for donors from a WNV risk area is given in the JPAC Donor Selection Guidelines[3]. 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 controls and any additional quality control sample have satisfied the criteria laid down.
- If an additional quality control is run on each series of tests, it should be a CE or UKCA marked quality control where available and suitable for the purposes of monitoring the performance of *in-vitro* assays and manufactured by a different manufacturer to that of the assay.

9.3.16: Other infectious agents

The JPAC Donor Selection Guidelines[3] 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 controls and the additional quality control sample have satisfied the criteria laid down prior to release of the result.

9.3.17: Additional screening of plasma intended for fractionation

All plasma pools intended for the manufacture of medicines are subjected to microbiological screening as described in the current European Pharmacopoeia Monograph on Human Plasma for Fractionation. Dependent on which product the plasma pool is being used to produce, to limit the viral burden in-process

screening of the first homogenous plasma pool for both hepatitis A Virus (HAV) RNA and human parvovirus B19 (B19V) DNA is performed. A maximum level for B19 DNA has been defined in the European Pharmacopoeia, but not for HAV RNA.

There is no mandatory requirement to screen donations for HAV and Human B19V, although UK Blood services may elect to screen donations in minipools to reduce the risk of discard of larger plasma pools.

9.3.17.1: Human parvovirus B19 DNA

- There is currently no specific UK requirement for the minimum level of sensitivity for the performance of human B19V DNA screening. If screening is performed in minipools, UK Blood Services must ensure that human B19V DNA can be detected at a level that will ensure less than 10^4 IU/mL of B19V DNA in the homogenous plasma pool.

- The assay must include a specific internal control for each test performed.

- No series of tests should be considered acceptable unless the manufacturer's QC requirements in the IFU have been met, and the results of any additional quality control samples used have satisfied the criteria laid down.

- If an additional quality control is run on each series of tests, it should be a CE or UKCA marked quality control where available and suitable for the purposes of monitoring the performance of *in-vitro* assays and manufactured by a different manufacturer to that of the assay.

9.3.17.2: Hepatitis A virus RNA

There is currently no specific UK requirement for the minimum level of sensitivity for the performance of HAV NAT. If screening is performed in minipools, UK Blood Services must ensure that HAV RNA can be detected at a level that will ensure a negative HAV NAT in the homogenous plasma pool.

- The assay must include a specific internal control for each test performed.

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

- If an additional quality control is run on each series of tests, it should be a CE or UKCA marked quality control where available and suitable for the purposes of monitoring the performance of *in-vitro* assays and manufactured by a different manufacturer to that of the assay.

Note – International Standards or Reference Reagents do not need to be CE or UKCA marked.

9.4: Reinstatement of blood donors

Where a blood donation sample is found to be repeatedly reactive on screening (except for anti-HCMV), the donation and any components must not be released for clinical use. For anti-HBc/anti-HBs exceptions see 9.2.5. 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).

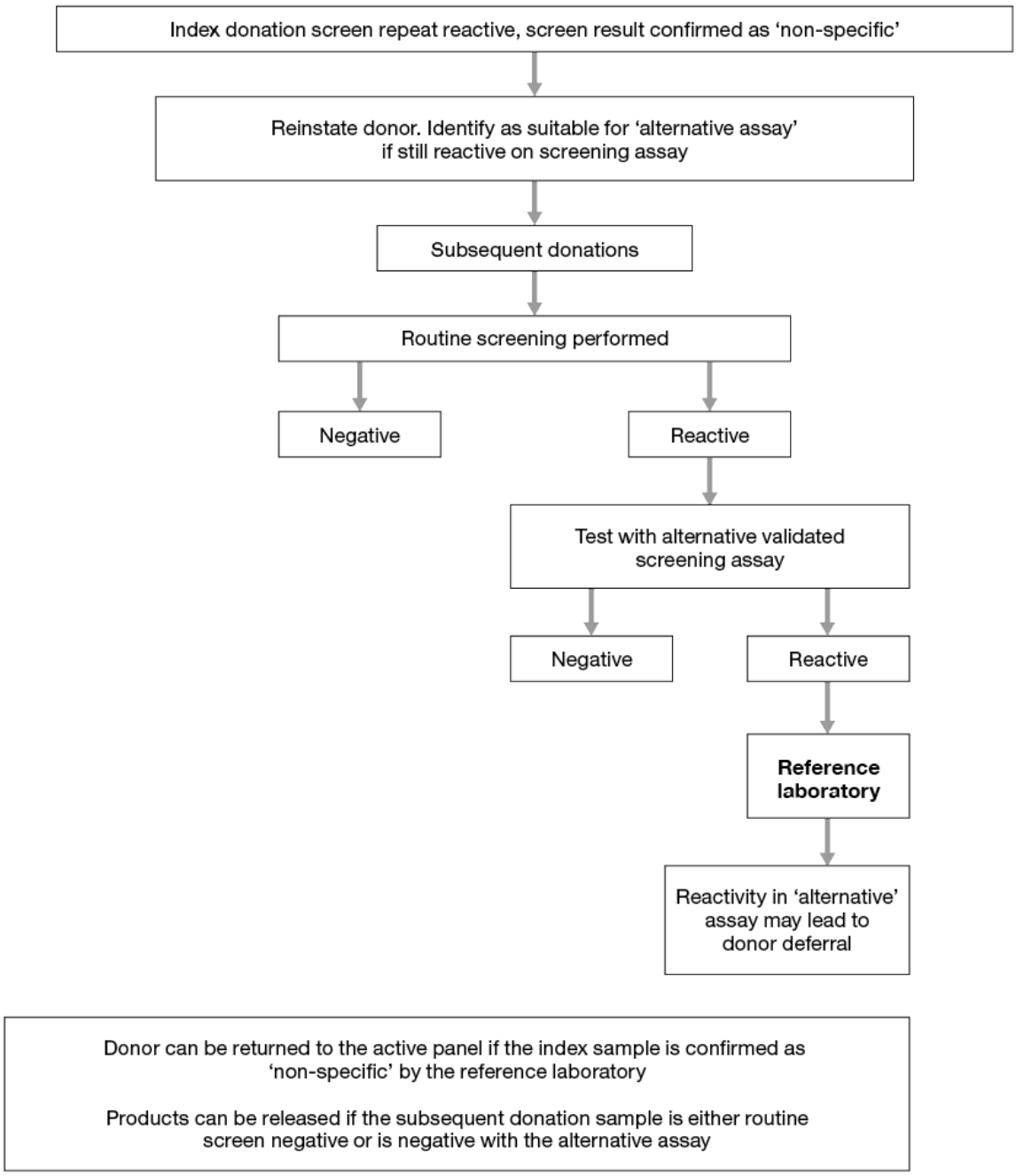

Figure 9.5 Action chart – blood donor reinstatement following confirmation of screen reactivity as non-specific

9.4.1: Donors whose samples are confirmed positive

- Donors whose blood 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.

- For blood, donations that are confirmed positive for anti-HBc from a donor with anti-HBs >100 mIU /mL, tested in the past 24 months in a UK Blood Service, are considered suitable for release if HBsAg and ID HBV DNA negative.

- For tissues and cells **either** donations that are anti-HBc confirmed positive and anti-HBs >=100 mIU /mL are considered suitable for release, **or** donations which are anti-HBc reactive and are HBsAg and ID HBV DNA negative do not require an anti-HBs level of >=100 mIU/mL to be considered suitable for release.

- Donors with confirmed HEV, HAV or WNV infection should be deferred for 6 months from the date of first detection of HEV/HAV/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 on individual testing and HEV IgG positive at >=1 IU/ml using the PEI International standard for HEV IgG (HEV IgM may or may not still be present), the donor may be reinstated immediately.

- Donors with confirmed human B19 DNA should be deferred for 4 weeks from the date of first detection of B19 DNA. These donors may be reinstated without further testing 4 weeks from the date of the index DNA positive donation.

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).

- Blood donations in which reactivity in an anti-HBc screening assay is subsequently confirmed as non-specific do not require any additional screening (unless the donor lapses, has a defined exposure incident or reports a hepatitis-like illness). The confirmed negative result can impart anti-HBc negative 'status' to the donor’s record.

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.
- For anti-HBc confirmed non-specific reactivity, 'anti-HBc negative' status can be applied. No additional screening is required in subsequent donations unless the donor lapses, has a defined exposure incident or reports a hepatitis-like illness.

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

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[4]. Adherence to the principles, protocols and practices relating to the correct use of the specified skin-cleansing system shall be regularly audited by periodic observation and corrected if found to be lacking.

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 continuous monitoring with alarm notification and (iii) reliable detection of bacteria at a level indicating potential 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 incubated culture bottles to allow immediate withdrawal of contaminated and associated units.

The use of an automated microbial detection system using the following protocol has been shown to give a substantial risk reduction regarding transfusion transmission of bacteria in platelet components[5]. This requires a minimum hold period of at least 36 hours before sampling and a minimum of 8 mL inoculated into both anaerobic and aerobic culture bottles. Continuous incubation and monitoring need to be performed until the end of shelf life, which can be extended from 5 to 7 days.

9.5.3.1: Single-test protocol

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

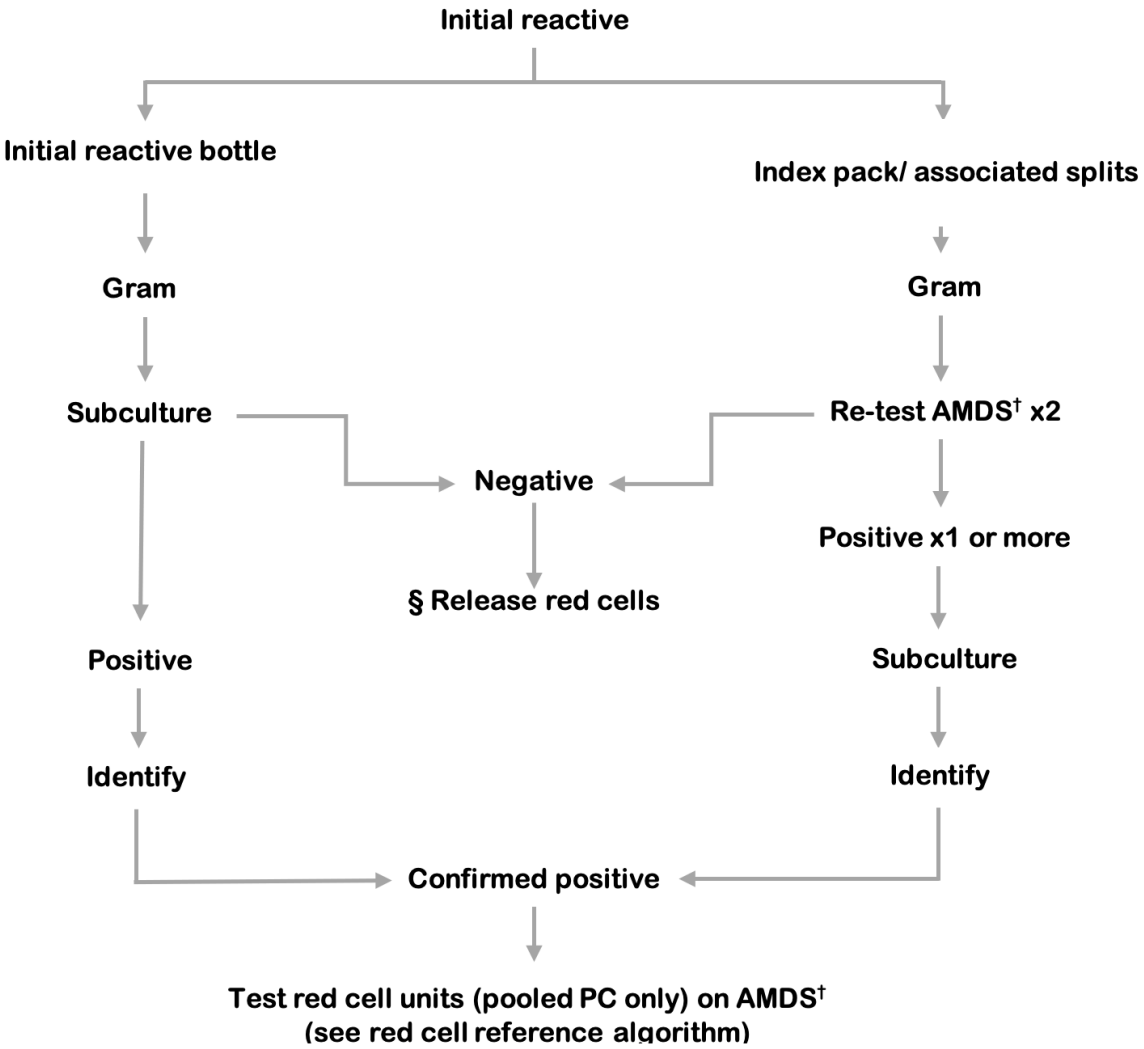

Figure 9.6 Platelet components testing algorithm. If the index pooled platelet component is not available to re-test, the associated red cell unit must be tested.

§ 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.

The following definitions of screening test results are recommended.

Initial reactive: Index culture bottle(s), from initial screening, with positive signal from an automated microbial detection system.

Repeat reactive: Repeat culture bottle(s), from repeat sampling of the index unit, with positive signal from an automated microbial detection system.

Associated reactive: Associated culture bottle(s), from sampling of associated components, with positive signal from an automated microbial detection system.

Confirmed positive: Matching speciation from positive subculture of the initial reactive AND the repeat reactive OR the associated reactive.

Indeterminate positive: A combination of results that includes positive subculture but does not satisfy the definition for ‘confirmed positive’.

- Positive subculture from the initial reactive, BUT no positive signal from sampling of the index or associated components OR no index or associated components returned for sampling.
- Positive subculture from ONLY ONE of the initial reactive OR the repeat reactive OR the associated reactive.
- Non-matching speciation from positive subcultures of the initial reactive AND the repeat reactive OR the associated reactive.

(In most cases there is positive subculture from the initial reactive bottle but the index unit is not available for culture because it has been transfused).

Indeterminate negative: Negative subculture from the initial reactive, BUT no index unit available for testing.

(Where assessed, negativity would also require no organisms on Gram stain and negative growth curves from the automated microbial detection system).

False positive: Negative subculture from the initial reactive AND no signal from repeat sampling of the index unit.

False negative: Negative screening test to the end of the incubation period, BUT positive subculture from sampling of the platelet unit during investigation of a visually abnormal unit OR a post-transfusion reaction.

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

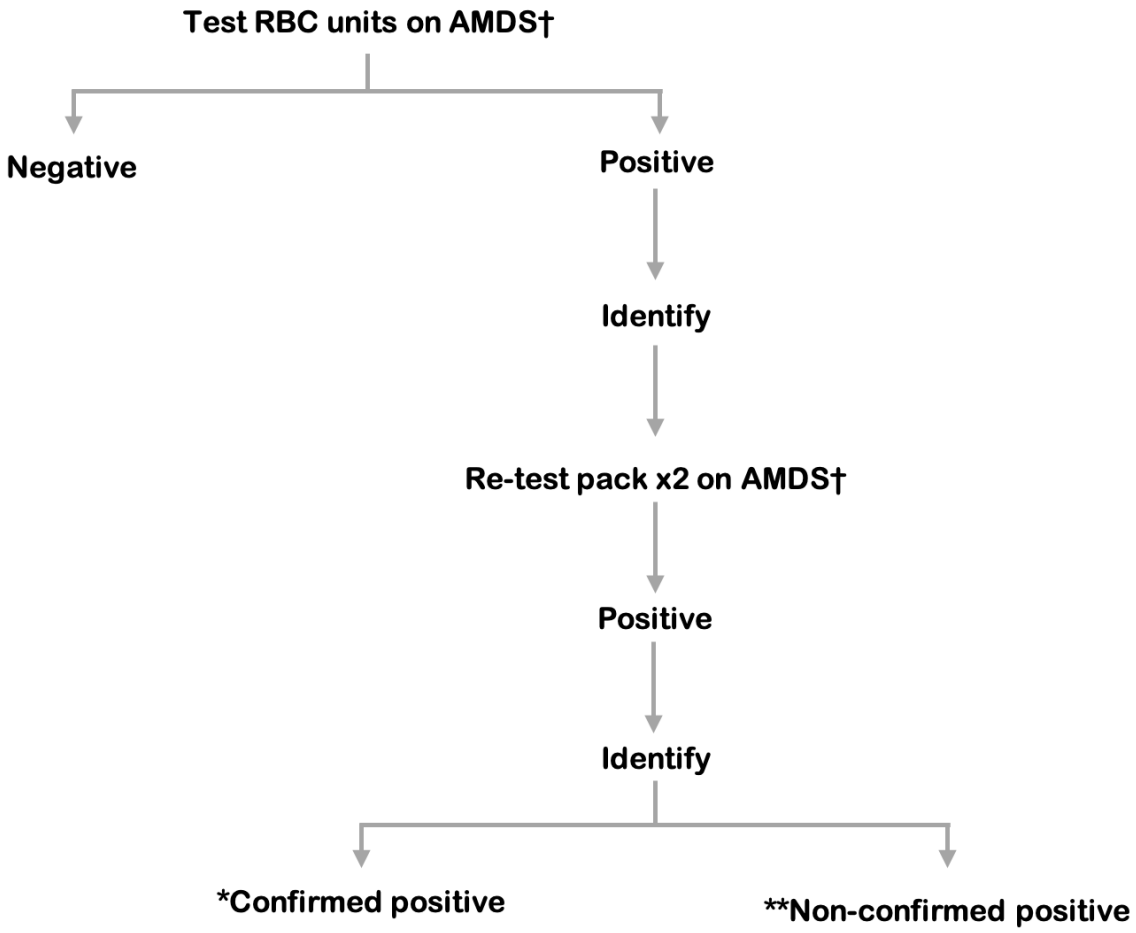

Figure 9.7 Red blood cell reference algorithm

* 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.

Release of red cells requires a negative result from both the index culture bottles and testing of the platelet component.

Testing algorithms shown in Figures 9.6 and 9.7 are guidelines and may be modified to be Blood Service specific.

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 must 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.) must be obtained aseptically and placed in appropriate culture media at the time of retrieval or processing. Samples must 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 must not be used for transplantation unless it is effectively sterilised by a process such as gamma irradiation. It is considered good practice to test cardiovascular tissues 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 must 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 must 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, Banking and Release for Administration[6]. 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 unrelated donations collected for public banking found positive for microbial growth must 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 must be performed periodically to maintain quality.

9.6.3: Stem cells

Stem cell products (peripheral blood stem cells, bone marrow and whole blood) are subject to the FACT-JACIE International Standards for Haematopoietic Cellular Therapy Product Collection, Processing, and Administration[7].

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 must be identified to species level and antimicrobial susceptibilities determined. A trend analysis of data must be reviewed by relevant experts to identify potential sources of contamination.

9.6.4: Serum eye drops

Eye drops made from serum are used to treat ocular surface disorders. The serum is diluted with saline or used neat and dispensed under closed aseptic conditions and bacteriologically tested (European Pharmacopoeia 2.6.27). Samples must be tested for sterility in accordance with regulations.

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 (EM) of processing facilities

EM programmes must be in place for both uncontrolled and controlled (GMP graded areas) processing facilities and must meet the requirements of appropriate regulatory bodies. Uncontrolled facilities include blood-processing laboratories and controlled facilities include cleanrooms used for the aseptic processing of tissues, stem cells and associated products.

The main aim of microbiological EM is to provide a means of monitoring trends over time thereby ensuring that processing facilities continue to operate within acceptable bioburden limits and comply with GMP recommended limits for microbial contamination and airborne particulate concentration for controlled (GMP graded) areas. The EM programme must form part of the quality risk management system (QRM) ensuring that products are processed to the highest possible standards and that microbial, particulate and pyrogen contamination associated with microbes is prevented in the final product.

The EM programme must be part of the contamination control strategy document. The locations, frequency, volume and duration of monitoring must be determined based on a risk assessment method (EU GMP i.e. Hazard Analysis Critical Control Points (HACCP)) and from the results obtained during room qualification.

9.7.1: Key elements of an EM programme

The monitoring programmes must define and document:

- The sites to be monitored and the rationale behind the selection of these sites.
- The formal risk assessment study for each process and GMP processing area listing the Critical Control Points, which must be monitored
- A location map of monitoring sites on local data sheets.
- Airflow visualisation studies (i.e. smoke tests) to define EM sites (N/A uncontrolled rooms).
- 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 as defined by EU GMP Annex 1[8]. Routine monitoring for clean rooms, clean air devices and personnel must be performed 'in operation' during processing (N/A uncontrolled rooms).
- Which personnel are authorised to perform EM.
- The incubation regime for samples.
- The setting of limits (alert and action limits). Alert limits for controlled rooms must be established based on results of Performance Qualification (PQ) tests or trend data and must be subject to periodic review.
- The requirement for data and trend analysis.
- A procedure for the investigation of out-of-specification (OOS) results including the identification of colony growth and the possible causes of the contamination.
- A procedure for corrective and preventive action in the event of OOS results. A root-cause analysis (RCA) followed by a corrective and preventive action (CAPA) protocol.

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.

- Passive air sampling using settle plates and in addition, in GMP graded areas, active air sampling and particle counting.
- Glove prints for assessing potential transfer of bacterial contamination to sterile product during aseptic processing in GMP grade A and B areas.

Viable and Non-viable EM techniques must comply with EU GMP Annex 1. Guidance and QRM principles[8].

In controlled facilities, monitoring for fungal in addition to bacterial contamination must, as a minimum, be achieved by settle plates with media and incubation regimens specific for each type of contamination.

9.7.3: Culture media

Culture media used for EM 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, that will either individually or in combination, 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 being met.

9.7.4: Alert and action limits

9.7.4.1 Controlled Rooms (Cleanrooms)

In cleanroom facilities, alert and action limits must be set for the results of particulate and microbiological monitoring. Action limits are specified in Annex 1 of the EU Guidelines to GMP (Manufacture of Sterile Medicinal Products)[8].

Action limits are initially set in alignment with EU GMP Annex 1 guidance values. However, if trend data for Grade B, C or D GMP areas indicates a consistently lower value, the action limits may be lowered to improve control. Alert limits must also be set 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.

Alert limits must be established based on results of Performance Qualification (PQ) tests or trend data and must be subject to periodic review.

9.7.4.2 Uncontrolled facilities

Action limits must be established using historical data. The monitoring programmes must define how the action limits in uncontrolled rooms are to be determined.

9.7.5: Data and trend analysis

Trends may include:

- Increasing numbers of action or alert limit breaches
- Consecutive breaches of limits
- Regular but isolated breaches of limits that may have a common cause
- Changes in flora type and numbers

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 on a six-monthly basis. This formal review must involve senior cleanroom/processing staff and representatives from the quality and microbiology departments.

9.7.6: Cleanroom gowning

EM 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 assessment and confirmation of compliance with aseptic gowning procedures. This must be reassessed periodically, at least annually, and must involve both visual and microbiological assessment (using surface monitoring methods for locations such as hands (glove prints), arms, neck and chest)
- The monitoring of personnel after critical operations
- The monitoring of staff upon leaving an aseptic area as a means of assessing operator bioburden limits
- Exit suit monitoring must 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 must include a process simulation test using a nutrient medium. The process simulation test must imitate as closely as possible the routine process including all critical subsequent manufacturing steps. It must also take into account various interventions known to occur during the routine process as well as worst-case situations. Process simulation tests must 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). Acceptance criteria must be defined and documented, and any contamination investigated.

9.7.8: Cleaning and disinfection

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

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

The cleaning and disinfection of controlled rooms is particularly important and must be performed in accordance with a written programme. Where disinfectants are used, more than one type must be employed on a rotational basis. Disinfectants must be validated for their effectiveness and compatibility with the cleaning agents used. A sporicidal disinfectant must be included as one of the rotational disinfectants if practical. Detergents and disinfectants must be monitored for microbial contamination and when used in grade A and B areas, must be sterile prior to use and where possible single 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 must 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 sterile wash out of the pack, must be sampled in the laboratory taking care to minimise the introduction of contaminants. A Gram stain may be informative, but the sample must 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 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 must 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

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