Guidelines for the Blood Transfusion Services

16.4: Reagents

http://aws-lon-jpac.targetservers.uk/red-book/chapter-16-hla-typing-and-hla-serology/16-4-reagents

16.4: Reagents

16.4.1: DNA typing reagents

Methods available for HLA typing of DNA samples rely on identification of polymorphic HLA gene sequence motifs. In all widely used methods, the polymerase chain reaction (PCR) is utilised, either through the use of sequence-specific primers as in PCR-SSP, or to produce a locus-specific DNA template (e.g. HLA-A) which can subsequently be typed using a panel of sequence-specific oligonucleotide probes (PCR-SSOP). The locus-specific template may also be directly sequenced.

DNA can be prepared from various tissues by a variety of methods. The laboratory should prepare DNA by a standard method that has been reported in the scientific literature and validated in the laboratory for the HLA typing method to be used.

16.4.1.1: Instructions for use

In addition to section 11.1.4.12 of these guidelines, the instructions for use must adhere to the relevant EFI Standards and should include the following:

- a statement explaining the test and intended application of the kit
- the principle of the procedure
- reagents and equipment required to perform the test
- detailed instructions for all components of the test
- the gene targeted as a PCR amplification control (PCR-SSP)
- the specificity and nucleotide sequence of all primers and probes used in the HLA typing kit
- a table or diagram indicating the location of the probes and/or primers utilised in the test
- a list of ambiguous combinations of alleles defined for each test kit – this may also be given as part of interpretative software
- the HLA alleles which are claimed to be detected by the HLA typing kit, further divided into the following groups:
    - those HLA alleles which have been detected in appropriately controlled validation tests
    - those HLA alleles which have not been directly detected in validation tests but where the reactivity of the allele is expected to be detected
    - those HLA alleles which have not been directly detected in validation tests and whose reactivity cannot be assumed to be detected by the kit
    - those HLA alleles that are known to produce weak or unreliable signals in the output systems
- the date and the source of the sequence information used in the kit design and a statement that new alleles described following the date of design may not be detected by the kit
- the control tests to be performed to check for contamination (negative control) of the test system
- the control DNA to be included to check for quality of sample DNA used
- the control test to be performed to generate a true positive signal
- acceptable limits of signal intensity should be specified for positive and negative results
- all computer software assisted interpretation of results should be validated on control DNA

- the chemical components of the kits should be listed and reference made to any toxic substances included in the kit with recommendations for their safe disposal. Reference to material safety data sheets should be given.

16.4.1.2: Requirements

Manufacturers should inform all primary users of a DNA-based HLA typing kit when any changes to a kit's ability to perform are detected. All users of DNA-based HLA typing kits should report any kit-related problems directly to the manufacturer and maintain records of such events.

16.4.2: HLA Antibody Testing Reagents

All commercial HLA antibody test kits should be CE and in vitro device (IVD) marked and validated for use. Each batch of commercial test kit or in-house panel should be evaluated against a minimum of three sera of known HLA specificity from different cross-reacting groups.

HLA-specific antibodies may be detected by solid-phase bound, purified HLA molecules, or particle bound, purified HLA molecules. If such techniques are used for screening (i.e. not characterisation of specificity) the following apply:

- There should be discrimination between HLA Class I and Class II-specific antibodies.
- Overall the target cells or molecules should cover either all the known HLA immunogenic epitopes or all HLA specificities (Class I, Class II, or both as appropriate) found in the population at over 0.5%.

16.4.2.1: Instructions for use

The instructions for use must comply with the requirements of EN ISO 18113:2009 and the information required in section 16.6. In addition, the instructions for use should include the following information on each individual preparation or component of a set of HLA screening product:

- the HLA antigens represented in each container
- the expiry life of the HLA screening product following reconstitution or preparation and subsequent storage in conditions recommended by the manufacturer should be stated
- when components of an HLA screening product contains preservatives the name of the chemical preservatives and the components which contain them should be stated.

16.4.3: External Quality Assessment (EQA) Schemes

Laboratories should take part in regular external quality assessment exercises such as the UK NEQAS for Histocompatibility and Immunogenetics schemes. Effective mechanisms should be in place to correct poor performance in EQA schemes.