Guidelines for the Blood Transfusion Services

14.2: Avoidance of contamination

http://transfusionguidelines.org/red-book/chapter-14-guidelines-for-the-use-of-dna-pcr-techniques-in-blood-establishments/14-2-avoidance-of-contamination

14.2: Avoidance of contamination

DNA should be purified by a standard method that has been reported to the scientific literature and validated in the laboratory. DNA should be suitably stored to protect the integrity of the material.

During the preparation of genomic DNA, great care should be taken to avoid contamination from any other source of DNA. Pre-polymerase chain reaction (PCR) and post-PCR procedures should be undertaken in separate areas and using separate laboratory coats in each area. The laboratory should have documented procedures which have been constructed to eliminate potential causes of contamination, including training of the operator. If contamination does occur, all procedures should be reviewed and appropriate corrective action taken. Proposed change to procedures should be validated prior to their introduction.

In order to avoid contamination, the use of separate working stations or clearly defined work areas is beneficial for each stage of the PCR process. For example:

- One to prepare reagents. This is particularly important to avoid contamination of primers.
- One dedicated to pre-PCR manipulation (e.g. DNA isolation). A Class II laminar flow cabinet should avoid contamination of the sample with DNA from the operator.
- One dedicated to setting up PCRs.
- One for manipulation of PCR-amplified DNA. PCR-amplified products should be kept away from areas used for pre-amplification manipulation and reagent preparation.

Each working station should be adequately and independently equipped. However, the use of such working stations should not absolve the laboratory from ensuring procedures are constructed to eliminate contamination.

Examples of measures which will help to minimise contamination include:

- the use of new sterilised, disposable plastic tubes or glassware for handling DNA
- the use of freshly prepared and sterilised materials and reagents when making up solutions for DNA samples, particularly dH_2O and buffers
- aliquoting reagents in small amounts to minimise the number of repeat samplings
- the changing of gloves and coats when moving between the areas dedicated for pre- and post-PCR manipulations
- the use of positive displacement dedicated pipettes or plugged tips to carry out PCR preparations
- routine wipe-tests of pre-amplification work areas should be performed. If an amplified product is detected, the area must be cleaned to eliminate the contamination, re-tested and measures taken to prevent future contamination
- reagents used for amplification must not be exposed to post-amplification work areas.