PREVENTION OF FALSE POSITIVES AND FALSE NEGATIVES:

Specific procedures should be implemented to prevent false-positive and false-negative reactions from the techniques used.

A. False Positive Prevention and Detection

False positives can occur from

- contamination and
- non-specific amplification.

a. Contamination:

Contamination introduced by personnel, positive controls, or positive samples (i.e., cross-sample contamination) can be minimized by following strictly adhering to the protocols defined for personnel, facility design, workflow, equipment, disposables, and cleaning; as well as use of good quality reagents. Physical separation of work areas as described above is the must for contamination prevention.

Contamination in PCR occurs primarily because of:

- Generation and spread of aerosols;
- Contaminating materials present on hands, clothing or hair, introduced into PCR mixes;
- Fomites – Laboratory coats, gloves, vortexes, pipettes etc.;
- Circulating PCR amplicons (aerosols/fomites);
- Positive control specimens; and
- DNA clones used for synthesis of positive control material

There are a number of approaches to control PCR contamination, and the degree of stringency that is required in a laboratory is often determined by the assay being performed. Contamination can be prevented to a great extent by:

- Compulsory wearing of a laboratory coat and disposable latex/ neoprene gloves and changing the laboratory coat and disposing the gloves upon moving from one area to the next in the PCR annex.

- Maintaining strict unidirectional flow of material and personnel in the PCR annex, from the sample processing room to the pre-PCR room to the PCR amplification room to the post-PCR analysis room.
- Keeping all bottles and reaction tubes sealed for as long as possible.
- Using as few manipulations as possible.
- Avoiding vigorous and excessive vortexing, pipetting and spillages in the PCR areas.
- Using separate and dedicated set of equipment such as pipets, tips, vortex, spinner, centrifuges etc. for each area. Should an extreme case arise where a reagent or piece of equipment needs to be moved backwards, it must first be decontaminated with 10% sodium hypochlorite (with minimum contact time to be 10 minutes), followed by a wipe down with sterile water.
- Centrifuging vials containing specimens, nucleic acids, PCR mix ingredients and PCR amplicons, before opening them.
    a) Aliquoting reagents into single-use volumes
    b) Autoclaving /incinerating contaminated utensils (glassware, reaction tubes, pipette tips, buffers etc.)
    c) Cleaning the lab benches periodically with 1N HCl (causes depurination and hydrolysis of contaminant nucleic acid molecules),or expose the workstations overnight to UV light.
    d) Working within an easily-cleaned Biological Safety Cabinet or PCR workstation, preferably one with a built-in UV source.
    e) Treatment of surfaces with psoralene compound may be considered in cases of recalcitrant contamination.
    f) Substitution of dTTP in PCR mix by dUPT
    g) Using "anti-contamination primers" (which amplify sequences from within the contaminating vector itself)

Handling False Positives

One should take all possible precautions in preventing contamination. Once developed it is difficult to handle.

In the worst-case scenario of PCR contamination, when source cannot be pointed, it is advisable to:

- *Shut down the offending PCR laboratory*
- *Discard all the working solutions*
- *Thoroughly decontaminate all work surfaces, laboratory coats, pipettes etc. using UV irradiation.*
- *Order new supplies of PCR reagents.*

ii. Nonspecific Amplification:

False positives can also result from the non-specific amplification of cross-reacting nucleic acids. The redesign of primer sets and/or hybridization probes should be used to reduce this type of false-positive reaction. Various modifications that can be used to reduce nonspecific amplification are:

- Hot start PCR
- Touchdown PCR

A. False Negative Prevention

False negatives can result from inhibitors from the environment, poor experimental design of the PCR, poor quality of reagents, the variability of the sample processing, degraded or too little nucleic acid, not sticking to SOP, poor quality equipment or contaminants like DNase and RNase that destroy the nucleic acid.

Ways to reduce false negatives:

- avoid known PCR inhibitors (avoid using powdered gloves, wooden sticks with cotton/ calcium alginate swabs to collect samples)
- reduce inhibition, by including the incorporation of 200 to 400 ng/µL of BSA (bovine serum albumin) to the PCR (may not always work)
- the rigorous use of each positive control
- Good laboratory practices to prevent contamination with RNase.
- Use of good quality reagent, equipment and following SOPs stringently.