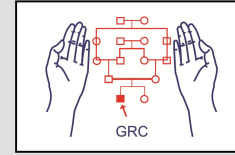


PCR for Infectious Diseases

Genetics Resource Centre (GRC)



Polymerase Chain Reaction (PCR) is an extremely sensitive technique in which the nucleic acid (RNA or DNA) of the infectious agent is first isolated (extracted) from the clinical sample and then it is amplified. It has become an essential requirement for diagnosis and monitoring the response to treatment in many infectious diseases like Hepatitis-B and C. It can also be used for rapid and early diagnosis of infections like Dengue and Malaria. The PCR for infectious diseases may be done as “qualitative” or “quantitative” test. The qualitative PCR test tells about the presence or the absence of the disease and may be useful in diseases like Dengue or Malaria. The quantitative PCR test gives accurate information about the load of the infectious agent. This is very useful in the serial monitoring of response to treatment in chronic infections like Hepatitis-B & C. The quantitative PCR (Q-PCR) is usually done by real time method.

PCR can also be used for genotyping of the viruses. It is most commonly done for HCV because of the differences in response to treatment in infection by various genotypes.

GRC offers high quality and cost-effective testing for qualitative and quantitative PCR of Hepatitis-B, & C, Dengue and Malaria. We also do high resolution HCV genotyping. The PCR of HBV and HCV is done by real time method and high quality CE marked diagnostic kits. The PCR of Dengue, malaria and HCV Genotyping are done by methods developed at GRC.

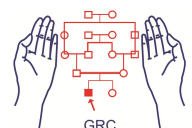
What is the difference between qualitative and quantitative PCR?

“Qualitative PCR” means whether the test is positive or negative and in the “Quantitative PCR” the exact amount of the infectious agent is reported. In the era of real time PCR practically all of the test results are reported as “Quantitative”. The term “Qualitative PCR” stemmed from the crude analytical method of gel electrophoresis that has now become obsolete. Unfortunately many labs are still using the gel electrophoresis and are continuing to report “Qualitative PCR” results. The gel electrophoresis method in addition to being less sensitive is also associated with significantly higher numbers of false positives due to carryover contamination between samples.

At GRC we use the state of the art real time PCR and all of our results are reported as “Quantitative”. Unfortunately, on insistence by some of our clients we have to resort to reporting the “Quantitative PCR” result as “Qualitative”.

What are the reasons for inconsistencies in PCR results between different labs and often between the results of the same lab?

1. The level of infectious agent tends to fluctuate in the blood. For obvious reasons this may cause significant variation in the results of PCR especially between the results of the same lab. This is beyond the control of the lab and even the best quality lab practice cannot control it.
2. Sample mix-up or clerical mistakes are not uncommon. This is almost completely preventable by good lab practice.
3. Prolonged delay between sample collection and processing: The RNA viruses like HCV are delicate and rapidly degrade on exposure to higher temperature after sample collection. Best results are ensured when the sample is collected and processed in the same lab. It has become a common practice for the small labs to collect samples for HCV/HBV PCR and get these tested from bigger labs. This often leads to degradation of the viral RNA and false low/negative results. The problem becomes more pronounced during the summer season with high environmental temperature.
4. PCR failure due to technical reasons may also cause variation in the PCR results. This happens more frequently when cheap and low quality reagent kits are used. The problem can be overcome



to a large extent by using high quality “CE marked” diagnostic kits. Low quality kits can easily detect high to moderate positive samples but these can miss low positive samples. There is also some degree of variation in the duplicate or triplicate measurement of the same sample in the same lab because the real time PCR has an inherent coefficient of variation in the range of 5-10%.

5. A less frequently discussed cause of variation in the results of PCR is the units in which the results are reported. Some labs use copies/ml while others use IU/ml. Reporting in IU is preferred. Similarly some labs report results/ml of blood while others report/ul of blood. While comparing the results it is important to see if the two results have been reported in the same units.

What is the best way to minimize the inconsistencies in the PCR results?

The best would be to get the serial PCRs of each patient done from the same lab. It is always preferable to know through the request form about the previous PCR results. The lab can also maintain a record of all serial PCRs of a particular patient.

When is the right time to get the genotyping done?

The genotyping analysis is usually done by gel electrophoresis that is less sensitive than the real time PCR. Therefore genotyping should ideally be done when the viremia is high. Quite often genotyping is requested when the patient fails to respond adequately to the treatment. At this point in time the viremia may also be low and it may be difficult to pick the genotype.

What is un-type-able genotype?

The viral genomes keep on changing with time that result in different genotypes. There are several HCV genotypes that are known. These genotypes are identified by the subtle differences in the nucleic acid sequence of the sub-types. In a normal genotyping method the labs checks for the known genotypes only. Occasionally a patient sample having positive HCV viremia may show a genotype that is either extremely rare or is not reported previously. Such genotypes are collectively reported as “un-type-able”. The un-type-able genotype must not be confused with a “negative” or “not detected” genotype result that is reported when the viremia is below the detection limit of the analytical method.

What precautions are required in collection and dispatch of samples for PCR of infectious diseases?

PCR of viruses is usually done on serum or plasma of the patient. The best quality of PCR is ensured if the sample is processed without unnecessary delay after collection. However, many a times the samples have to be collected at remote places. For PCR of all types of viruses we require 0.5-1.0 ml of serum/plasma. The samples should be dispatched to us within 24 hours of collection. Samples older than that, especially in the summer season, may give compromised results. The results are usually available in a week. For PCR of malaria we require 1-2 ml of whole blood in EDTA. Since the results of PCR for Dengue and malaria are often required urgently we can provide these results within 24-48 hours.

