

Introduction: Viral infectious diseases represent an important portion of global public health concerns with thousands of deaths annually. From serious pandemics and highly contagious infections to common influenza episodes, clinical prognosis often relies on early detection of the infectious agent. Thus, effective identification of viral pathogens is needed to help prevent transmission, set up appropriate therapy, monitor response to treatment and lead to efficient disease management and control.

Virology Division, functioning under the Department of Microbiology at Era's Lucknow Medical College had been doing testing of viral markers and Elisa testing for viruses.. In the wake of Corona outbreak in the year 2020, a fully functional state-of-the-art Virology Laboratory was established and RT-PCR testing became available in July 2020. Our BSL 2 laboratory had features like negative pressure in extraction area and separate entry /exits for all areas with separate donning/doffing areas. The RT-PCR lab was accredited by NABL in October 2020 and has done more than 5000 COVID RT-PCR tests till date. Currently this laboratory is fully dedicated for the testing of COVID-19 as well as HCV quantitative PCR testing. . The laboratory is well equipped and has the technical expertise to diagnose most human pathogenic viruses.

PCR plays an important role in the detection, quantification, and typing of viral pathogens. This is because detection of important clinical viruses using culture methods is time-consuming while ELISA tests are not universally available and suffer from comparatively low sensitivity and specificity. qPCR (with the inclusion of reverse transcription for the diagnostics of RNA viruses) provides the appropriate sensitivity and specificity and can also be used for determination of the viral load by (RT)-Qpcr which is used as an indicator of the response to antiviral therapies. For these reasons (RT)-qPCR has become an indispensable tool in virus diagnostics.

Equipment's Available in Lab at present:

1. **Quant 5 Studio Real Time PCR System:** The Applied Biosystems® QuantStudio® 5 Real-Time PCR System is designed for users who need superior performance, maximum dye versatility, and security options in a real-time PCR system that is affordable and easy to use. The optimized Design and Analysis software is ideal for both first-time and experienced users. When connected to the Thermo Fisher Cloud, the QuantStudio® 5 system provides access to your data wherever and whenever you want. Using proven OptiFlex® technology (featuring 6 decoupled channels and white LED) and featuring six independent Veriflex® temperature zones, the QuantStudio® 5 96-well system enables improved data accuracy and sensitivity for a broad range of genomic applications. The QuantStudio® 5

system also offers the built-in software features of electronic record security and prevention of unauthorized instrument access to assist with 21 CFR Part 11 compliance.

Features of Quant 5 Studio RT PCR:

- Proven Optiflex technology (6 decoupled channel / 21 filter combination) with white LED for trusted result
- User Account management, locked workflow, and pause features for greater control of experiments
- Built in software feature
- Secure Cloud based software provides increased accessibility and storage option.
- Simplified instrument software which is more user friendly.
- 96-well 0.2 ml format or 0.1 ml format

2. **King Fisher Duo Prime for extraction of different biological samples:**

KingFisher Duo Prime system. Isolates DNA, RNA and proteins from a variety of starting materials including cell-free body fluids, blood, bacteria, cell cultures, tissue and plant samples; with volumes ranging up to 5mL.

KingFisher instruments are the most versatile sample preparation instruments in the lab, and are elegantly designed to support multiple applications.

- Various throughputs—process 6–96 samples per run depending on the instrument model
- Interchangeable formats—choose 24- or 96-well plates so you can process a wide range of input volumes
- Protocol customization—easily edit, modify, or create new protocols
- Optimized reagents—compatible with multiple magnetic-bead reagents.

3. Laboratory has all other needed instruments like refrigerated centrifuges, high end Biological safety cabinet by ThermoFisherScientific Type2A2, Deep freezers, and Laminar work stations etc

4. The work flow is unidirectional to avoid any cross ccontamination and the laboratory has separate work areas for nucleic acid extraction, master mix preparation, template addition , amplification , sample receiving and reporting.

Other tests being carried out in Virology Lab:

HCV Detection and quantitation:

HCV is an RNA virus of the Flavivirus group transmitted in 60% of the cases due to drug abuse. Other modes of transmission seen are following accidental needle punctures in health care workers, dialysis patients and rarely from mother to infant. Sexual transmission accounts for 10% of cases. Chronic infection with HCV occurs in about 85% of infected individuals leading to fibrosis of the liver and Cirrhosis in about 20% of these patients. Risk for Hepatocellular carcinoma in a patient with chronic HCV is 1-5% after 20 years.

Uses: This test is used in conjunction with clinical presentation and other laboratory markers to determine infectivity; predict & monitor response to antiviral therapy in chronic Hepatitis C patients.

PCR methods. Real-time PCR instrumentation requires considerably less hands-on time and testing is much simpler to perform than conventional PCR methods. Additionally, accelerated PCR thermocycling and detection of amplified product permits the provision of a test result much sooner for real-time PCR than for conventional PCR. The combination of excellent sensitivity and specificity, low contamination risk, ease of performance and speed, has made real-time PCR technology an appealing alternative to conventional culture-based or immunoassay-based testing methods used in the clinical microbiology for diagnosing many infectious diseases. This review focuses on the application of realtime PCR in the clinical microbiology laboratory.

Future vision: The department will soon be starting PCR based viral diagnostics for Dengue, Chikungunya, Hepatitis B and other serious infections.