KHYBER MEDICAL UNIVERSITY	**Public Health Reference Laboratory (PHRL)**	Public Health Reference Laboratory
	Requisition Form	

Full Name .....

Department/Institution .....

CNIC .....

Address .....

.....

Contact No .....

Full Name .....

Age ..... Gender .....

CNIC..... City .....

Contact No .....

Address .....

.....

Specimen Information/Type			
Collection date	Collection time	Collected by	☐ Swab ☐ Blood ☐ Stool

Sequencing based Genotyping
☐ Targeted Sequencing (Sanger)

What is diarrhea?

Diarrhea is the condition of having loose, watery, and possibly more-frequent bowel movements. It often lasts for a few days and can result in dehydration due to fluid loss.

What are the likely causative agents?

A number of diseases and conditions can cause diarrhea, including:

- **Viruses.** Viruses that can cause diarrhea include Norwalk virus (also known as norovirus), enteric adenoviruses, astrovirus, cytomegalovirus and viral hepatitis. Rotavirus is a common cause of acute childhood diarrhea. The virus that causes coronavirus disease 2019 (COVID-19) has also been associated with gastrointestinal symptoms, including nausea, vomiting and diarrhea.
- **Bacteria.** Common bacteria that cause diarrhea include *Campylobacter*, *Escherichia coli* (*E. coli*), *Salmonella*, and *Shigella*.

Bacterial Vs. Viral Diarrhea

Diarrhea caused by viral infection differs from that caused by bacteria in terms of symptoms, treatment, and causes**.** It is important to determine which kind you have in order to receive proper treatment. Viral infection generally produces diarrhea without blood or mucus, and watery diarrhea is the prominent symptom. Conversely, mucus and blood are more often seen in bacterial diarrhea.

What specimen should be used to detect viral or bacterial diarrhea?

A stool sample may be submitted to confirm the underlying causative agent.

What is acute viral gastroenteritis?

Acute gastroenteritis is the inflammation of the stomach and intestines. Morbidity and mortality of diarrheal diseases in children under 5 years old in many countries are attributable to viral infection.

What is Norovirus?

Human norovirus, family Caliciviridae, is a major virus that causes acute gastroenteritis in all age groups. Norovirus GI and GII are the most predominant genogroups associated with human diseases.

What are the symptoms?

Norovirus causes inflammation of the stomach or intestines. This is called acute gastroenteritis. Some typical norovirus symptoms are Diarrhea, vomiting, nausea and stomach pain. Other symptoms include Fever, headache, and body aches.

How to diagnose a Norovirus infection?

Diagnostic methods for norovirus focus on molecular detection of viral RNA or viral antigen.

What are Astroviruses?

Astroviruses are associated with a spectrum of disease ranging from gastroenteritis to neurological disease and encephalitis, dependent on virus genotype and host factors including species and individual immunity.

What are the symptoms of Astrovirus infection?

While mild diarrhea is the main symptom of astrovirus infections, a person who gets sick may also have other common symptoms, including nausea, vomiting, stomach ache, loss of appetite, body aches and fever

How to diagnose an Astrovirus infection?

Astrovirus-specific RT-PCR has been the screening method of choice to detect human astroviruses in stool samples.

What are Rotaviruses?

Rotavirus is a genus of double-stranded RNA viruses in the family Reoviridae. Rotaviruses commonly cause severe, watery diarrhea and vomiting in infants and young children.

What are the symptoms of Rotavirus Infection?

The most common symptoms of rotavirus infection are severe watery diarrhea, vomiting, fever, and/or abdominal pain.

How to Diagnose a Rotavirus Infection?

RT-PCR is widely used to detect the rotaviral genome.

What is Bacterial gastroenteritis?

Bacterial gastroenteritis occurs when bacteria cause an infection of the digestive system. High fever and bloody diarrhea are more common with bacterial gastroenteritis. Common bacteria that cause diarrhea include *Campylobacter*, *Escherichia coli* (*E. coli*), *Salmonella*, and *Shigella*.

What is *Escherichia coli*?

Escherichia coli (abbreviated as *E. coli*) are bacteria found in the environment, foods, and intestines of people and animals. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.

What are the symptoms of *E. coli* infection?

Symptoms of Shiga toxin-producing *E. coli* (STEC) infection vary for each person, but often include severe stomach cramps, diarrhea (often bloody), and vomiting.

How to diagnose a *E. coli* infection?

E. coli infections are usually diagnosed through laboratory testing of stool specimens (feces). Shiga toxin-positive specimens must be sent to a state public health laboratory.

What is *Shigella*?

Shigella bacteria cause an infection called shigellosis. Most people with *Shigella* infection have diarrhea (sometimes bloody), fever, and stomach cramps.

What are the symptoms of *Shigella* infection?

People with *Shigella* infection (shigellosis) usually experience Diarrhea that can be bloody, fever, stomach pain and feeling the need to pass stool even when the bowels are empty.

How to Diagnose a *Shigella* infection?

Confirming shigella infection involves taking a stool sample to be tested in a lab for the presence of shigella bacteria or their toxins. The test could be a culture that isolates the bacteria or a rapid diagnostic test that detects genetic material of the bacteria.

What is *Salmonella*?

Salmonella bacteria cause diarrhea, fever, and stomach cramps.

What are the symptoms of *Salmonella* infection?

Most people with a *Salmonella* infection experience bloody diarrhea, fever, stomach cramps, nausea, vomiting, or a headache.

How to Diagnose a *Salmonella* infection?

Salmonella infection can be detected by testing a sample of your stool, body tissue, or fluids. The test could be a culture that isolates the bacteria or a culture-independent diagnostic test (CIDT) that detects genetic material of the bacteria.

What is Vibrio cholerae?

Cholera is an acute, diarrheal illness caused by infection of the intestine with the toxigenic bacterium *Vibrio cholerae*. Cholera is most likely to occur and spread in places with inadequate water treatment, poor sanitation, and inadequate hygiene.

What are the symptoms of Vibrio cholera infection?

The main symptoms of cholera are vomiting, diarrhea, and leg cramps.

How can we diagnose Vibrio cholerae infection?

Cholera diagnosis can be made from stool samples, through rapid diagnostic tests, blood antigen tests and through monoplex and multiplex PCR.

What is Influenza?

Influenza is an infectious respiratory illness caused by infection with an influenza A virus.

What are the symptoms of Influenza?

Common symptoms include headache, fever, cough, sore throat, aching muscles and joints and fatigue. It's important to note that not everyone with flu will have a fever.

How to diagnosis Influenza virus?

Diagnostic tests available for influenza include viral culture, serology, rapid antigen testing, reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence assays.

What is the difference between cold and Flu?

Flu is caused by influenza viruses only, whereas the common cold can be caused by a number of different viruses, including rhinoviruses, parainfluenza, and seasonal coronaviruses.

Available Tests?

The ACGT currently conducts genomic testing for respiratory infections including Influenza and COVID-19 and, gastroenteritis.

What is Influenza?

Influenza is an infectious respiratory illness caused by influenza A viruses. Common symptoms include headache, fever, cough, sore throat, aching muscles and joints and fatigue. It's important to note that not everyone with flu infection will have a fever.

How to diagnose Influenza (flu) virus?

Diagnostic tests available for influenza at ACGT include reverse transcription polymerase chain reaction (RT-PCR) and targeted gene sequencing to track emerging genotypes.

Other respiratory pathogens

Apart from Influenza, ACGT conducts molecular testing for pneumonia-causing viral infections such as respiratory syncytial virus and human rhinovirus infections.

Specimen Requirements

Nasopharyngeal or nasal swabs are the preferred respiratory samples for influenza testing. Place respiratory specimens into viral transport medium for transport to ACGT, PHRL, KMU. Store at refrigeration temperature until transport. Freeze specimen if it will not reach ACGT, PHRL within a week time. Label each specimen in a waterproof manner.

What is Gastroenteritis?

Gastroenteritis is an intestinal infection that includes signs and symptoms such as diarrhea, stomach cramps, nausea or vomiting, and sometimes fever. Viral gastroenteritis **differs from that of bacterial.** Viral infection generally produces watery diarrhea. Conversely, mucus and blood are more often seen in bacterial diarrhea.

Some of the common viruses that cause gastroenteritis include, Rotavirus, Norovirus, and Astrovirus while common bacteria that cause diarrhea include *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella* and *Vibrio Cholerae*.

How to diagnose gastroenteritis?

Staff at ACGT executes molecular diagnostics (PCR) to determine the microbiologic etiology of enteric infection.

Specimen Requirements

A fresh stool sample preferably during the period of active diarrhea should be collected in clean, dry containers. In case of rectal swabs, refrigerate them in transport media at 4℃. If possible, test within 48 hours after collection otherwise, freeze samples at -70℃. Frozen samples

should be bagged and sealed in an insulated box embedded in dry ice or with frozen refrigerant packs. Label each specimen in a waterproof manner.

General Guidelines

All samples should be labelled carefully with identification marks (serial no). Blood samples should be stored in refrigerator. Don’t freeze blood samples. Requisition form should be filled for all samples individually and attached to it.

What is Genomics?

Genomics aims to study the entirety of an organism's genes. Beginning with the Sanger family of methods in 1977, there have been a major innovation in the field led by the induction of high-throughput deep or so-called next generation sequencing (NGS) technologies.

Why a Genome Sequencing facility at PHRL?

The rise of highly contagious infectious diseases pose a significant threat to global health, including the potential of major pandemics. To effectively deal with this evolving threat, PHRL-KP, a leading COVID-19 testing lab felt the need of an in-house sequencing facility to reveal genomic diversity of emerging and remerging pathogens at much lower cost and with much greater precision.

The inception of **Advanced Center for Genomic Technologies** (ACGT) as part of the public health reference lab (PHRL), Khyber Medical University was supported by both national and international bodies including the WHO, NIH, and KP Health Department. The facility currently houses a portable single-molecule long-read sequencer from Oxford Nanopore for whole genome and a SeqStudio™ genetic analyzer from Thermo Fisher Scientific for targeted gene sequencing. Induction of a MiSeq system from Illumina is in the pipeline.

What have we achieved?

ACGT has successfully optimized targeted and whole-genome sequencing of SARS-CoV-2. We have sequenced and submitted a total of 120 partial and 30 whole-genome sequences of circulating SARS-CoV-2 strains to GISAID database. This is the highest no of submissions from Pakistan after Aga Khan University in months of February to March 2022.

What we do?

At present the lab focuses on genomics-based surveillance of SARS-CoV-2 using both 3rd generation Nanopore and Sanger Sequencing technologies. The goal is to track spread of SARS-CoV-2, find new variants and guide public health choices. With the currently underway transition, PHRL will be extending its services and will be able to test BSL-II plus level pathogens such as Congo fever, Dengue fever, Typhoid, and Cholera.

Workflow

Like all viruses, SARS-CoV-2 evolves over time through random mutations. Using these little changes, we can draw up phylogenetic trees, much like family trees. We can also make connections between different cases of COVID-19 and gauge whether there might be undetected spread of the virus.

Our Vision

The Genomics Center aims to be the national leader in genomic medicine research offering high-end genomic solutions to researchers working in the fields of microbiology, infectious diseases, cancer biology and human molecular biology. A key component of this transition will be recruiting and retaining personnel with the specialized skills and experience who will train subspecialists in their respective areas to competently address provincial and national healthcare needs.

Our Team:

Dr. Yasar Mahmood Yousafzai (Assoc. Prof & Director PHRL)

Dr. Ishaq N Khan (Assist. Prof. in-charge)

Mr. Muhammad Zakria (Scientific Officer & Team lead)

Dr. Muhammad Iqbal Qureshi (Virologist, Bioinformatician)

Dr. Muhammad Waseem Shah (Molecular Biologist)

Mr. Tariq Rahim (Molecular Biologist)

Mr. Shahzad Ahmad (Lab Technologist)

Mr. Hanif Ullah Khan (Lab Technologist)