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Technical Note

A Chemist's Guide to the COVID-19 Outbreak

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The COVID-19 pandemic has created a plethora of misinformation regarding viruses, transmission, eradication, and prevention. This information brief is an attempt to organize all of the pertinent information from a chemistry point of view.

The COVID-19 pandemic is not humanities first (nor will it be our last) pandemic. Outbreaks of disease which have led to pandemics have been frequent throughout history, including the smallpox outbreaks which decimated the Incan empire in the 1500's to the more recent SARS pandemic and the Ebola epidemics of the past decades.

The first description of a virus was in 1892 when tobacco plants were dying from an unknown non-bacterial agent. Later, in 1898 this agent was found to be the tobacco mosaic virus. It wasn't until the first electron microscopes that the actual virus structures were observed in the 1930's. Since those early discoveries, there have been over 5,000 additional viruses detailed with millions more still uncharacterized in the environment. Viruses are persistent and found in almost all types of earth ecosystems.

What are Viruses?

The term virus comes from the Latin word for poison. A virus is a very small infectious agent which can only replicate inside living cells of another organism (microorganisms, plants and animals). Most viruses can range in size from 20 to 300 nanometers (Figure 1). In essence, viruses are parasites which cannot live and replicate outside the host cells. Viruses lack the cellular organelles and processes to be able to survive and replicate on their own. The host cell's replication processes allow the viruses to hijack the machinery and produce their own genetic material.

Viruses are often compared to bacteria or confused with bacteria when it comes to infections and human health. Bacteria, unlike viruses, are prokaryotic single celled microbes that are larger than viruses and are able to live outside a host organism. There are very few bacteria that can cause human disease or infection but when they do, bactericides, called antibacterials or antibiotics, can treat those infections. These agents do not work against viral infections and diseases.

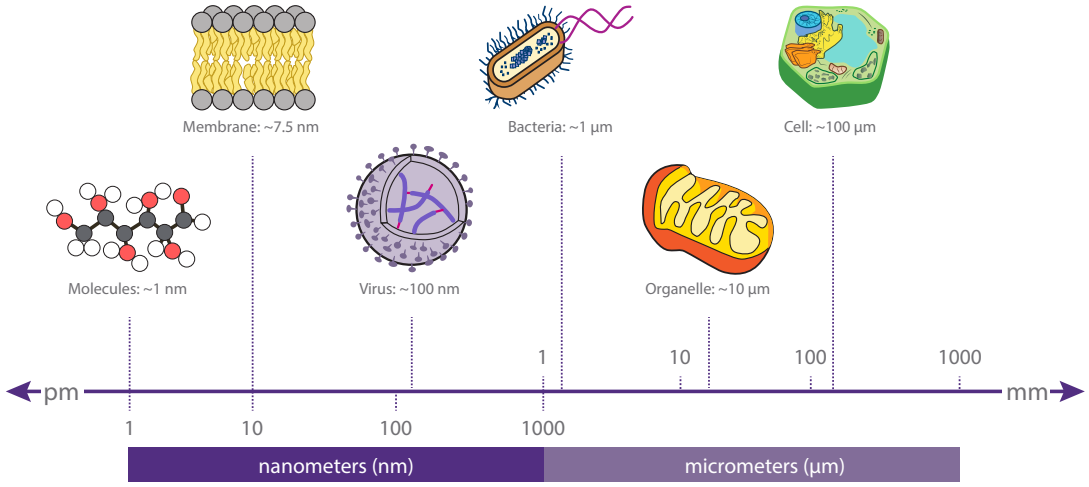

Figure 1. Relative sizes of common microorganisms, molecules and cellular components

Virus Structure

Outside of a host cell, viruses are independent particles called virions. There are three basic parts to viruses: the genome (genetic material - either DNA or RNA); the capsid (a protein capsule surrounding the genetic material); and in some cases, a lipid or protein outer envelope.

Viruses can be found in a variety of shapes from simple to complex. There are four morphological forms of viruses: icosahedral, spherical, helical, and complex (Figure 2). The viruses can also vary in size from under 50 nm to over 900 nm in length (helical or filamentous viruses). (Table 1).

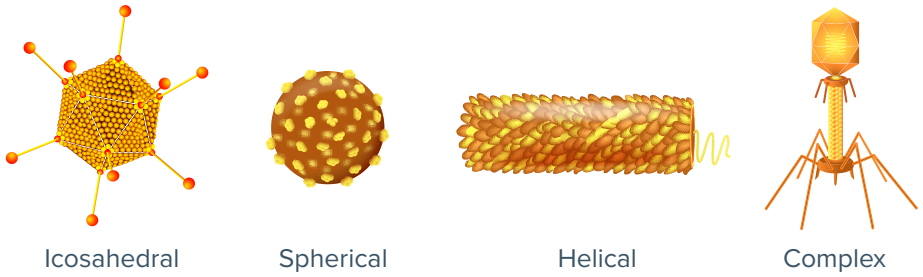

Figure 2. Viral shapes and sizes of common viruses

Table 1. Average size and shape of common viruses

Virus	Average Size (nm)	Shape
Polio	30	Icosahedral
Hepatitis	40	Icosahedral/Spherical
Rotavirus	80	Spherical
Adenovirus (Cold)	90	Icosahedral
Influenza A	100	Spherical
COVID-19	100	Spherical
HIV-1	120	Spherical
SARS	120	Spherical
Herpes Simplex I	125	Icosahedral
Epstein-Barr	140	Icosahedral
Measles	150	Spherical
Rabies	80 x 180	Helical
Tobacco Mosaic Virus	40 x 300	Helical
Variola	360	Spherical
Ebola	80 x 970	Helical

The COVID-19 virus is a new virus and part of the Coronavirus family (*Coronaviridae*). The coronavirus was named for the Latin word for crown when the projections on the surface of the spherical virus looked like points on a crown (Figure 3). The COVID-19 and other coronaviruses have a membrane enveloping the capsid. These viruses are in the middle to large range of the virus size and scale at around 100 nm. The coronavirus is a RNA virus meaning its genetic material is ribonucleic acid (RNA) rather than deoxyribonucleic acid (DNA). RNA viruses have higher mutation rates than DNA viruses because the enzymes which catalyze RNA replication (RNA polymerases) lack the ability to proofread or error correct the genetic material being coded unlike DNA and DNA polymerases. This reason is why these types of viruses mutate so quickly and are difficult to create vaccines to combat.

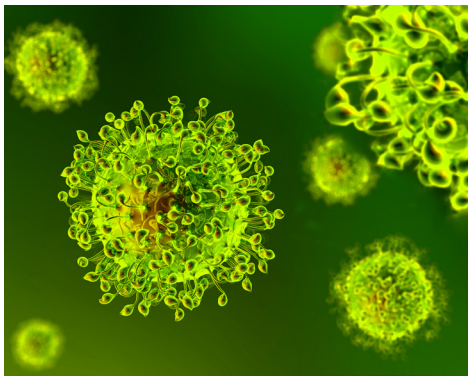

Figure 3. Coronavirus example

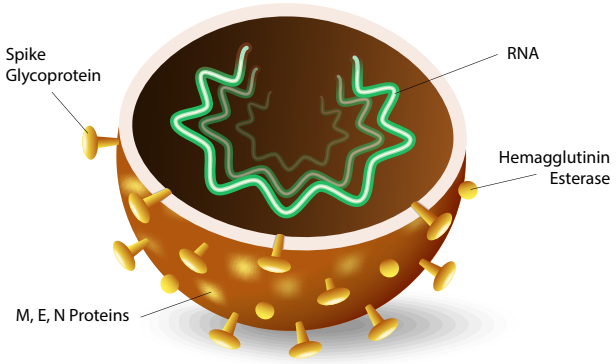

Figure 4. Coronavirus structure

Coronaviruses have a very large genome with a roughly 30KB strand of RNA. The RNA is protected by a nucleocapsid protei, and this is surrounded by a spherical lipid bilayer membrane. There are five different types of proteins embedded in this membrane: M-proteins, E-proteins, N-proteins, hemagglutinin esterase, and spike glycoproteins. These proteins serve several functions that protect its genome and help it recognize and duplicate within the host cells (Figure 4).

The characteristic “crown-like” proteins that give the virus its name are the large spike-like proteins that project from the surface. The spike protein is heavily glycosylated, which tricks our immune system into thinking that the virus is not a foreign antigen, a common strategy amongst this class of virus.

A predecessor to COVID-19, COVID-1’s structure was elucidated by electron microscopy, as a trimer of two large domains, called S1 and S2. S1 is a globular domain that extends outward, and binds to a variety of human cells with an ACE2 receptor like many other viruses such as SARS. This protein receptor called ACE2 (Angiotensin-converting enxyme 2) is located on the surface of human cells. Typically ACE2 has roles in reducing inflammation and reducing blood pressure but for viruses it provides a route to infiltration of the virus into the host cells. The primary function of ACE2 is to counteract the ACE enzyme involved in vasodilation. ACE2 is found on cells in most organs and particularly in alveolar lung tissue cells and cells of the small intestine. The S2 domain has a long, extended shape that pushes the S1 domain as far out as possible, The COVID-19 spike protein has the same basic structure as other similar viruses, but there are a significant number of mutations in the S1 receptor binding domain.

The most significant difference is the introduction of a binding site that can be cleaved by furin, a ubiquitous human enzyme able to break down proteins (proteases). Furins are found in many human tissues including lungs, liver and small intestines that allow the virus to latch onto these systems. The ability of furin to cleave part of the S-protein allows for the spread of the virus within the furin rich tissues. This adaptation did not exist in other viruses such as SARS and MERS. This adaptation allows for rapid spread of COVID-19 in the lung tissue, and the liver in more serious cases. This furin activation enables the virus to spread more efficiently between humans by easily integrating into human cells. It is vitally important to understand the chemistry of viral infections, so we can identify all potential drug targets. Most of the current clinical trials are aimed at various features of the spike protein, as well as its target in the ACE2 receptor host cells.

Viral Mutation

Viruses can change DNA and RNA in host cells in a variety of ways which produce new and unique combinations of genetic material, and in some cases produce novel viruses. These evolutionary adaptations make the mutations of known viral strains into new viral strains very common. Point mutations or substitutions occur when small pieces or bases of genetic material are changed, inserted or deleted from a genome (Figure 5). These types of mutations can occur naturally and can cause small changes in a viral strain to make a new strain. It is the reason we have seasonal variations of the influenza virus.

A second method of mutation viral recombination is when two different viruses or viral strains invade the same host cell and interact during replication and produce new viruses with characteristics of both parent viruses. Recombination mainly occurs between members of the same virus type like coronaviruses or influenza viruses. Recombinant viruses can also be created to produce a vaccine for viral strains. A third type of mutation occurs in RNA viruses with segmented genomes (each gene only codes for one protein) called reassortment. In reassortment, multiple RNA viruses can invade a host cell and shuffle the genomic codes producing a new virus. In all of these mutations, quasispecies can be created where the same virus has different resistance, virulence or symptoms.

Virus, Disease and Health

Most viruses, many other parasites, are species-specific, meaning an equine virus does not spread to a human but there are viruses that are zoonotic viruses and can spread back and forth from animals to humans. In some cases, a virus can mutate and jump to other species, which seems to be the case with the COVID-19 virus.

Viruses are often the cause of common diseases or can be the catalyst for other diseases. Many childhood illnesses are caused by viruses like measles, mumps, pertussis, and chickenpox. There are many widespread persistent viruses which humans deal with continually or sporadically in their life from a single exposure to a virus like herpes, HIV, papilloma virus, or chickenpox (shingles). Then there are the common viruses that produce seasonal colds and flu.

In most cases, viruses are infamous for being instigators of disease, but viruses can also be used to combat illness and diseases, such as cancer, by hijacking the cellular reproduction of cancer cells. Viruses are also instrumental in human health. It is well known that humans have beneficial bacteria in their body. Another resident of the human microbiome are viruses (called the virome). The virome inhabits humans within the first months of life. Each person has a unique virome, but there are some commonalities such as bacteriophages. Bacteriophages are viruses that target bacteria. One of the most common bacteriophage viruses is called CrAssphage and it targets common gut bacteria called Bacteroides. (1)

It is also true that many beneficial evolutionary changes to species can be traced back to viruses subtly changing bits of RNA and DNA. Retroviruses are RNA viruses that change the genome of the host cell. The host cell then incorporates the new genetic material into its own code when it reproduces. Endogenous retroviruses, viruses that originate within an organism, tissue or cell, are prevalent in humans (up to 8% of our genetic material). (1) These viruses were once considered junk material but have been discovered to have a role in human health.

In a recent study, it was hypothesized that since humans diverged from chimpanzees, up to 30% of protein adaptations have been due to viruses. (2)

Viral Infections

Each type of virus can enter a host cell in a different process. Bacteriophages can inject their genomes into bacterial cells without the capsid entering the bacterium. Other types of viruses (SARS and COVID-19) attach to receptors on host cell surfaces (such that has been discussed previously with ACE2 and furin receptors) which can result in membrane fusion that allows the virus to penetrate the host cell.

Once inside the host cell, the capsid is degraded by enzymes or dissociation and releases the viral genome into the host cell. Once inside the cell, the genome hijacks the genetic replication machinery of the host cell and produces more copies of the virus (Figure 5).

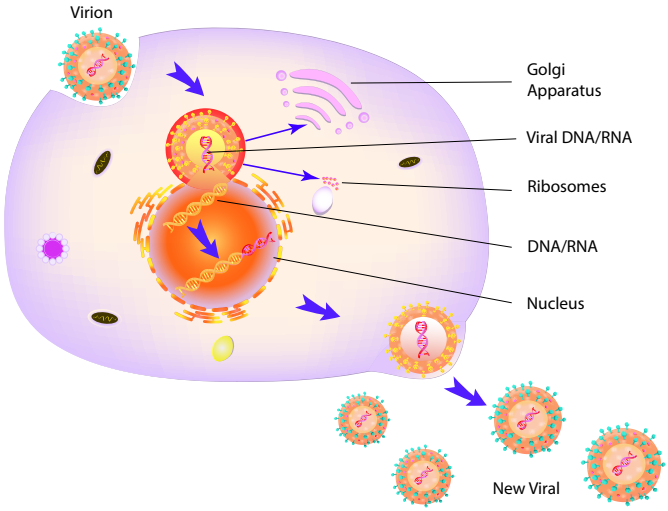

Figure 5. Host cell infection by virus

If more than one virus is present in the host cell, then reassortment or recombination can occur. Once replication has completed and the host cell is at capacity to hold the virus, the virus is released. This release or shedding occurs by either rupturing the host cell (lysis) resulting in cell death; budding creating enveloped viruses or exocytosis in which viral particles are transported through the host cell wall by vesicles and leaves the host cell intact. Individuals who are shedding virus are contagious. Some viruses have symptomatic shedding where the person is infectious upon showing symptoms or shortly before showing symptoms. Other viruses have asymptomatic shedding or a silent infectious period where the individual is contagious for a longer period of time before or if symptoms occur. COVID-19 is one of those viruses with asymptomatic shedding resulting in infections being transmitted without the carrier knowing they are infected. The timeline of an infection starts with the exposure of an uninfected individual to the virus. As the virus infects the host cells and replicates, there is a period of incubation where the virus is latent. At some point during incubation, the first infected host cell begins to shed virus particles and begins the infectious period. This period may or may not include symptoms as was stated previously (Figure 6).

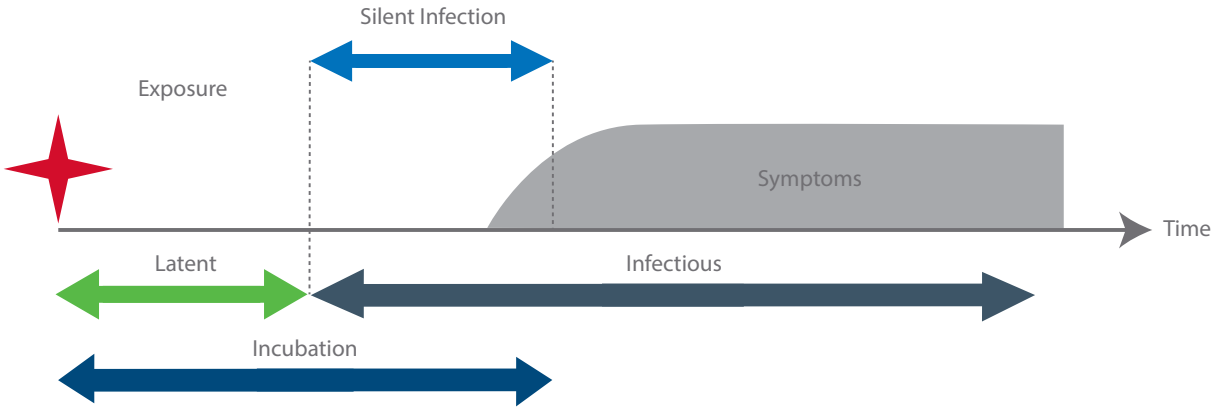

Figure 6. Viral infectious disease periods

The rate at which viral infections increase is dependent upon several risk factors including: population density, geography, sanitation, age, sex, immunity, viral stability, virulence, ease of replication, and mode of transmission. Geography determines if a virus can reach the stage of an outbreak, epidemic or pandemic. An outbreak is a localized number of infections beyond normal for the area. An epidemic is an increased number of infections in a larger geographical area than in an outbreak. Finally, a pandemic is spread of an infection over several countries or continents. Geographically isolated areas have a better ability to contain outbreaks. The relatively easy access and world connectivity of the outbreak center for the COVID-19 outbreak in Wuhan made it difficult to contain and spread into an epidemic and then a pandemic.

Viruses increase rapidly in areas of high density which is why it is recommended to limit groups and practice social distancing during outbreaks, epidemics and pandemics. This strategy reduces contact rates, which are the rates at which an infected individual is in contact with other people. Individuals in high density living arrangements, cities, or professions have a higher contact rate and have a higher incidence of exposure to infected individuals or infecting others. Two other factors in rates of infection are the mode of transmission of a virus and the basic reproduction number (R0).

The mode of transmission of a disease is the way in which a disease causing agent is transferred from an infected individual to another uninfected individual. A virus can be transmitted via direct physical contact or indirect physical contact. Direct physical contact includes sexual contact, kissing or exposure to secretions and usually occurs within friends, households or families. Indirect physical contact can be contamination from physical surfaces. The COVID-19 virus has been shown to spread through direct contact with infected oral and nasal fluids and through indirect contact on surfaces where droplets of the virus can be deposited. The COVID-19 virus can live from several hours to several days on various surfaces. The half-life of a virus is how long up to half of the virus initially deposited will remain viable and the viability is the amount of time in total viable virus particles can be detected (Table 2).

Table 2. COVID-19 persistence on surfaces [3]

Surface	COVID-19 Viability (hours)	Half-Life (hours)
Aerosols	3	1.2
Copper	4	0.8
Cardboard	24	3.5
Stainless Steel	72	5.6
Plastic	72	6.8

Additional modes of transmission include airborne and/or droplet transmission from coughing, sneezing and breathing. Airborne transmission occurs in viruses which are small particles under 5 µm which can persist in the air for long periods of time. Respiratory droplet transmissions stay in the air for shorter periods of time and are larger viruses over 5 µm. Viruses can be transmitted by poor hand washing or sanitary conditions in which fecal matter infects food or water sources. Finally, viruses can be transmitted from other organisms or vectors such as flies, mosquitoes or an intermediate host. In the case of the coronavirus, there is evidence that initial human transmission was due to zoonosis where the original animal virus jumped to humans.

The COVID-19 virus at this time appears to be primarily spread by person to person contact when an infectious individual exposes others to infectious airborne droplets through sneezing, coughing or spitting. A cough and/or a sneeze can travel a fair distance reported from 6 or more feet for the flu virus with the exposure to over 100,000 viral particles at speeds up to 100 mph. The virus is greater than 5 microns so transmission and exposure most likely occur while the infected person is in the vicinity of the exposed individual(s). The virus can also stay viable on surfaces up to several days but this route of exposure is not as frequent as in-person contact. Scientists continue to investigate all the modes of transmission of COVID-19 and the theories of transmission alter daily.

The number of individuals on average that an infectious individual will expose to a contagion is called the basic reproductive number or R_0 (pronounced R naught). Each virus has its own predicted R_0 and factors such as environmental conditions, behavior, etc. can play a role on the estimates. The higher the value, the more infections will result from exposure. COVID-19 currently has a R_0 value of 2-5 (this value is constantly changing as the pandemic progresses). Some common diseases such as measles and whooping cough are highly infectious with R_0 values over 10 (Table 3).

Table 3. Common viruses, modes of transmission and basic reproduction number (R_0)

Virus	Mode of Transportation	R_0
Measles	Airborne	12–18
Pertussis	Airborne Droplet	12–17
Chickenpox	Droplet	8–9
Rhinovirus (Cold)	Airborne or Transfer	5–7
Polio	Fecal-Oral Route	5–7
Smallpox	Airborne Droplet	5–7
COVID-19	Airborne Droplet	3–5
SARS	Airborne Droplet	2–5
AIDS	Body Fluids and Sexual Contact	2–5
1918 Influenza	Airborne	2–3
Seasonal Influenza	Droplet	1–2
Rabies	Saliva	1–2
Ebola	Body Fluids	1–2

Basic reproduction numbers are calculated on a population without any immunity. If a population has some immunity, then less people will become infected and if a population is vaccinated increasing the population immunity, then the virus is not able to spread efficiently (Figure 7). COVID-19 is a new virus and the population at large appears to have little natural immunity from it. There were also no vaccines available to treat the population to increase immunity at the time of the start of the pandemic.

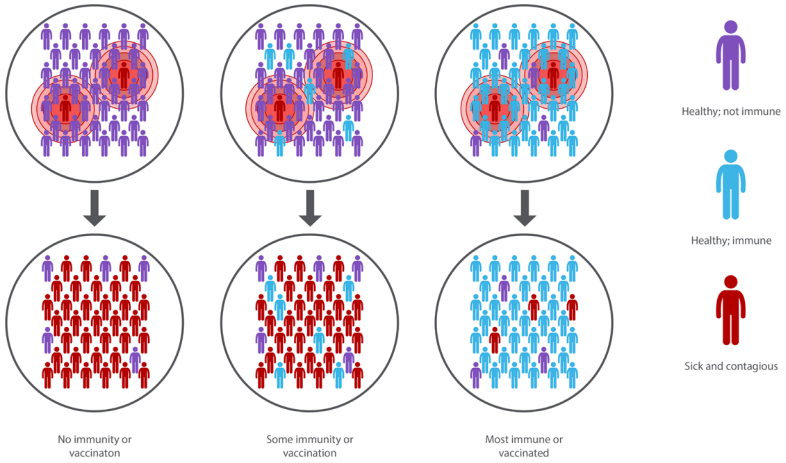

Figure 7. Immunity and infectious disease spread

A vaccine is a biological preparation of potentially infectious disease that can impart immunity to the disease. The term vaccine is derived from variolae vaccine (cow smallpox) which Edward Jenner called cowpox in his 1978 work describing the use of cowpox to combat smallpox. Vaccines are produced in stages. The first stage involves generating an antigen (a molecule capable of creating an immune response and produces an antibody). In the case of viruses, antigens will be part of viral structures such as the capsid, membrane, etc. The antigens are grown on primary cells (chicken eggs) or continuous human cell lines (cultured human cells). It is also possible to grow proteins from viruses in yeast, bacterial or cell cultures. The antigen is then isolated from the growth medium, inactivated, purified and stabilized.

The process of creating a new vaccine can take from months to years. There is the possibility that the vaccine is developed for a strain that has dramatically mutated into another resistant form before the vaccine is ready for distribution.

Vaccine Production

The development of a vaccine is a complex multi-step process. According to the CDC, there are six basic phases of vaccination development. (4)

- Exploratory
- Preclinical
- Clinical development
- Regulatory review and approval
- Manufacturing
- Quality control

During the exploratory phase, intensive research is carried out to identify and study potential antigens that might be useful in disease prevention or treatment. This is achieved by using the pathogen's genetic material, DNA or RNA. The generic material is isolated from a variety of sources including tissue which then must be homogenized using physical or mechanical techniques such as a ball mill grinder. Ball mills or ball-medium mills are very popular laboratory tools which grind through impact of a grinding media such as balls, rods, etc. The grinding media and the material to be ground are moved around the mill body or grinding container. The rotation of the mill and the impact of the media create a mixture of impact and attrition forces. The SPEX SamplePrep Geno/Grinder® series of ball mills are efficient at homogenizing and extracting genetic material for subsequent analysis.

The genetic material that is extracted, undergoes the process of reverse transcription to convert the proteins to a complimentary DNA (cDNA). Polymerase chain reaction (PCR) amplifies certain sequences of the cDNA that have been identified to produce a possible antigen protein. The majority of PCR processes expose reactants to repeated cycles of heating and cooling (thermal cycling) to create temperature dependent reactions (i.e. DNA melting and DNA replication by enzymatic actions). The main reagents of PCR are primers and DNA polymerase. Primers are short single strands of DNA fragments or oligonucleotides that complement the targeted DNA sequence. DNA polymerase synthesizes DNA from deoxyribonucleotides.

In the first step of PCR, the targeted DNA strands are separated at high temperatures (denatured). Then the temperature is lowered and primers bind to the DNA (annealing). In the final step the DNA polymerase assembles the new DNA strand from nucleotides (elongation) (Figure 8).

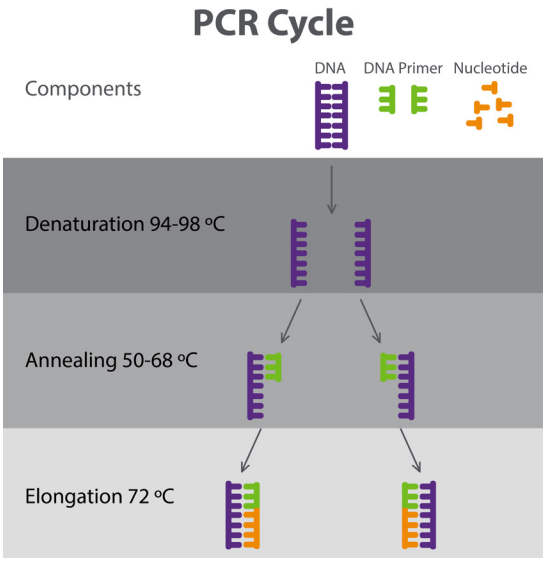

Figure 8. Steps of PCR

The amplified sequences are engineered into a plasmid vector followed by insertion into a living cell for replication and expression of the desired protein/antigen. The plasmids are propagated in cell or tissue cultures to generate multiple copies that are then purified and extracted producing a potential vaccine. To enhance the vaccine quality, stabilizers, adjuvants, and preservatives might be added to the formulation. Figure 9 shows the general steps of the exploratory and preclinical development of a vaccine.

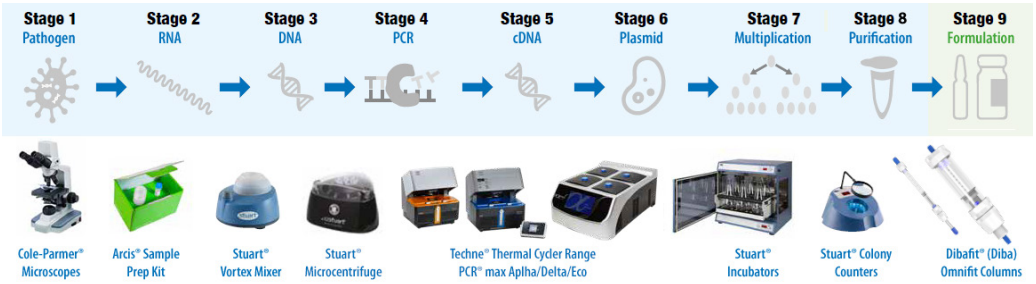

Figure 9. Steps of exploratory and preclinical phases of vaccine development with example equipment used for processes (courtesy of Cole-Parmer) (5)

Next is the preclinical phase, this is when tissue or cell cultures and animal tests are used to determine if a potential vaccine will produce immunity. It is during this phase that most vaccines stall out because they do not produce either immunity or show harmful to the test subjects.

When a potential vaccine moves into clinical development, it requires approval by the FDA for three or more rounds of human trials. Each round of analysis includes the safety of the proposed vaccine, immunogenicity, immunization schedule, dose, size, and overall effectiveness. If a vaccine passes the clinical development phase of human trials, it is then subject to regulatory review and approval by the FDA.

The vaccine developer submits a Biologics License Application to the FDA where approval can take quite some time. Once approved the vaccine goes into manufacturing, usually with a major drug manufacturer, where the personnel and equipment is provided to mass-produce the approved vaccine and provide it to the public. The new vaccine is continually in a cycle of quality control that monitors its performance and safety by the Vaccine Adverse Event Reporting System and the Vaccine Safety Datalink.

After the vaccine is formulated, tested and approved it goes into production, storage and transportation to the public (Figure 10).

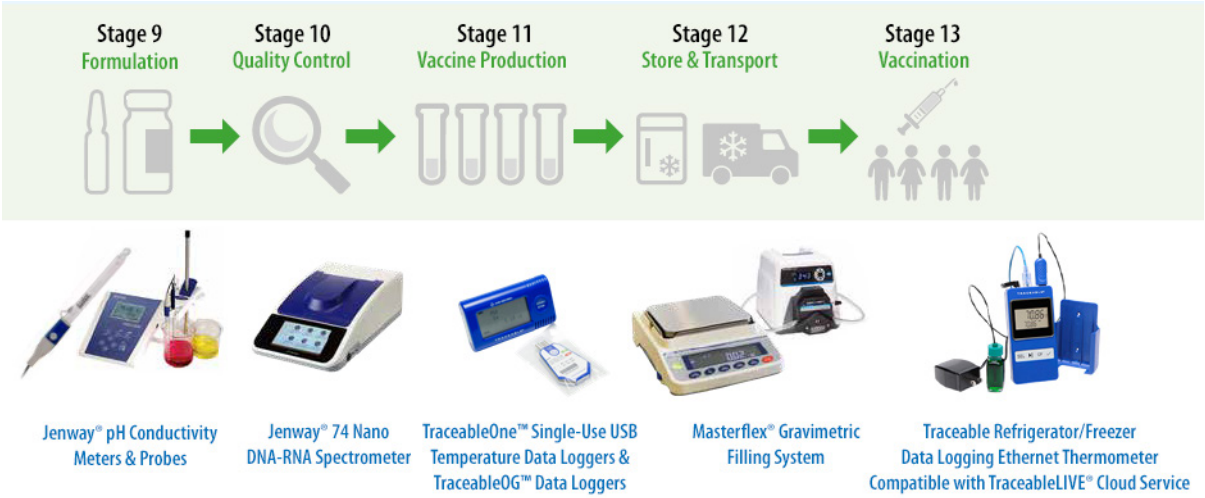

Figure 10. Downstream processing of production of vaccine after testing and approval (courtesy of Cole-Parmer) [5]

Stopping Transmission of Viruses

As a disease progresses from an initial outbreak to an epidemic, it becomes important to employ policies to contain and slow the spread of the disease before it hits pandemic proportions. In other historical outbreaks, it was easier to contain viral outbreaks due to limitations of travel and geography. In our modern world, however, our international community and ability to travel easily and frequently has only aided in the spread of COVID-19.

The essential methods of dealing with an outbreak or pandemic is a multi-pronged attack to limit exposure by isolating those infected, keep risk of exposure to a minimum for the uninfected, practice good health and hygiene practices, develop and employ effective viricides to stop transmission and alleviate symptoms, and develop prophylactic measures such as vaccines to stop future emergence.

The CDC has issued guidelines for limiting exposure for the general population (Figure 11). [6]
(https://www.cdc.gov/coronavirus/2019-ncov/prepare/prevention.html)

KNOW HOW IT SPREADS		PROTECT YOURSELF				PROTECT OTHERS		
Person to person within 6 feet	Droplets	Wash hands > 20 seconds with soap and water	Hand sanitizer > 60% alcohol	Avoid touching face	Avoid close contact	Stay home when sick	Cover coughs and sneezes	Wear face mask when sick

Figure 11. CDC basic steps for protecting oneself and others from disease transmission

One of the main problems encountered during this pandemic is COVID-19 has a silent transmission period meaning that many of the infected expose others before symptoms appear. In some cases, the individuals do not know they have been infected by COVID-19 at all because they exhibited few or no symptoms of the infection. The most common symptoms reported bring fever, dry cough, tiredness, and loss of taste and smell. This fact has made isolation or quarantine of infected populations difficult.

The concept of social distancing and reduced groups is part of the strategy involving reducing the contact rate of infectious patients and not letting COVID-19 infected individuals pass on the disease to other people and end the reproductive rate for that avenue of the disease. These social distancing and quarantine practices slow the growth of the infections but allow health care agencies to keep up with the demand for resources. This practice has come to be known as 'flattening the curve' which originated with a study of the 1918 Spanish influenza epidemic where the spread of the disease was examined in two cities that took different approaches to controlling the disease. In Philadelphia, there was no early isolation or social distancing practices mandated and the number of cases grew sharply over a small period of time overwhelming resources. In Saint Louis, social distancing and quarantine practices were instituted quickly upon outbreak and their growth was slowed and the rate of cases reduced allowing for better handling of resources (Figure 12). [6,7]

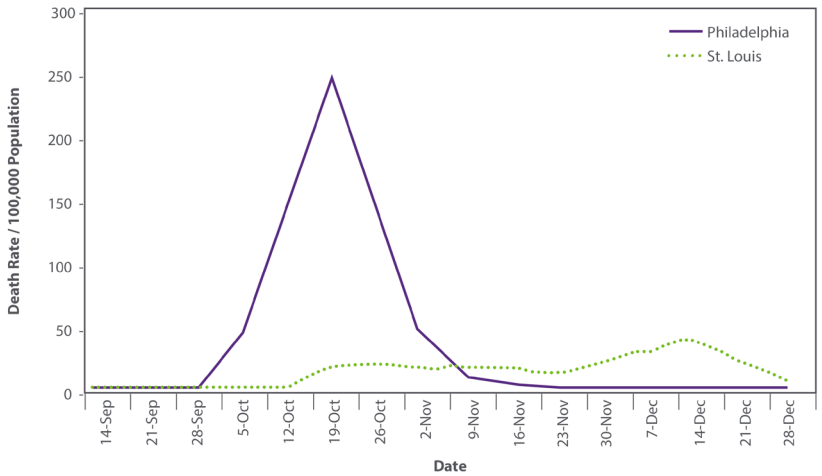

Figure 12. Death rate from Spanish influenza in two cities during 1918 outbreak

The second line of defense is for all individuals to practice behaviors to limit exposure which include cleaning of common areas, hand washing, and isolating oneself when sick. The laboratory approach to the COVID-19 crisis should follow the same advice but expand to encompass the laboratory space, equipment and personnel (Figure 13).

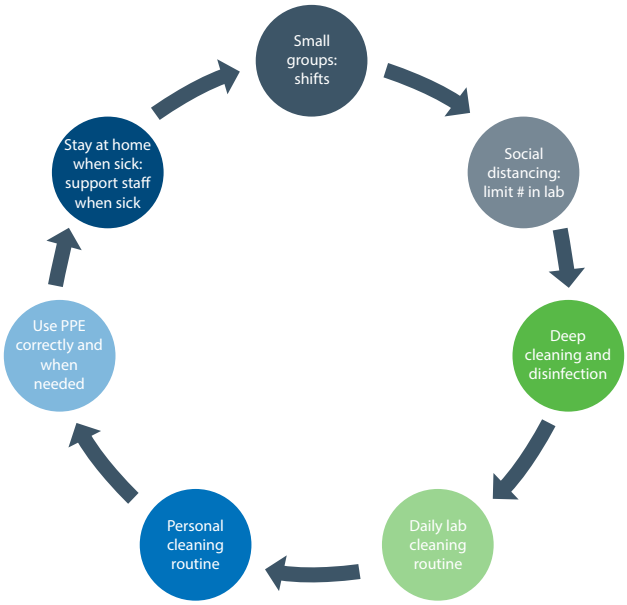

Figure 13. Elements for stopping virus transmission in the laboratory and business

Social Distancing in the Laboratory

In the laboratory, social distancing and not gathering in groups translates into several policies such as staggering work schedules and changing to shifts. Non-essential personnel can be asked to work from home. Some other policies can include stoppage of travel and in-person meetings. Meetings can be arranged by phone or online with our current technology. Contact should also be limited from outside vendors, sales people, visitors, and food delivery.

During times of crisis, it is imperative that managers or HR departments are given accurate contact information, schedules and locations of all employees for health and safety. Essential personnel should be issued all the guidance and equipment to perform their functions legally and safely. Beyond personal protection equipment for the lab personnel, letters of essential work and travel documents should be supplied for the employees to display to authorities if requested.

Cleaning and Disinfecting in the Laboratory

The first step in disinfecting a lab is general cleaning. Dust and dirt attract and collect particles of mold and viruses. The entire lab environment must be cleaned from ceiling to floor to remove dirt and debris. Filters must be changed in hoods and environmental control systems. Trash must be removed and clutter discarded. After general cleaning, the process of disinfection can then occur. There are many chemical agents that can be used to disinfect a laboratory for viruses.

There are many commercial products for all types of settings from home to health care and laboratory. Most of these products have familiar active chemical agents such as alcohols, acids, chlorides, etc. The mode of action for these products is usually one of the three processes. The first mode of interaction is dehydration where the virus or biological agent is dehydrated by the chemical and is rendered inactive. The second mode

of interaction is disruption of the cell or capsule by denaturing proteins or dissolving lipid capsules thereby spilling cell or viron contents out and drying them out before they can replicate. The final mode disrupts genetic, protein or amino acid processes and inhibits replication.

The EPA has published an extensive list of all of the commercial products for use in cleaning against viruses and COVID-19 on their website: https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2 and with the appropriate cleaning times. [8] Table 4 summarizes the most common active ingredients and their suggested dilution times which are dependent upon the product, method of application and concentration. The EU has also provided guidance on agents which have been tested on viruses in the Coronavirus family with their concentrations for use (Table 5). [9,10]

Table 4. Common active ingredients used in commercial disinfectants and their contact time for disinfection

Active Ingredients	Average Suggested Contact Time (Dependent Upon Concentration)
Citric Acid	5–10 minutes
Ethanol	1–5 minutes
Formaldehyde	5–10 minutes
Glutaraldehyde	5–10 minutes
Hydrochloric Acid	10 minutes
Hydrogen Peroxide	1–5 minutes
Iodine	10 minutes
Isopropanol	1–5 minutes
Ortho-phthalaldehyde	10 minutes
Peroxyacetic Acid	1–5 minutes
Phenolics	5–10 minutes
Quaternary Ammonium	5–10 minutes
Sodium Chlorite	5–10 minutes
Sodium Hypochlorite	1–10 minutes

Table 5. Concentration of disinfection agents tested against viruses in the family of Coronavirus [9,10,11]

Agent	Concentration
Benzalkonium Chloride	0.1%
Ethanol	70%
Formaldehyde	0.7%
Glutaraldehyde	2.0%
Isopropanol	50%
Povidone-iodine	10% (1% Iodine)
Sodium Chlorite	0.23%
Sodium Hypochlorite	0.05-0.5%

There are instructions on use and dilution of common laboratory chemicals such as ethanol, sodium hypochlorite, etc. on the disinfection pages of the CDC for healthcare settings [11]:

https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html

The common theme for all of these products, whether they are commercial products to laboratory produced disinfectants, is that the products must be applied and allowed to disinfect for a period of time before being wiped away. This time is called the dwell time or contact time. Surfaces must be wet to be effective. There are very few instantaneously effective products and in most cases the solution needs to be applied for up to ten minutes before wiping or washing the surface clean in order to ensure proper disinfection. There are also appropriate surfaces for each type of cleaner. The manufacturer should list all of the surfaces approved for the agent. Disinfectants will not work universally on all surfaces. Different classifications for surfaces are listed in Table 6. All of these chemical products and chemical agents are effective in disinfection but they can also pose additional hazards for the user and contamination for the laboratory.

Table 6. Porosity of common materials (12)

Porous	Semi-porous	Non-porous
Carpeting	Wood	Some Tiles
Clothing	Drywall	Some Sealed Countertops
Pillows, Bedding & Mattresses	Tile Grout	Glass
Upholstered Furniture	Hardwood Floor	Metal
Fabrics and Leather	Linoleum	
Wall Insulation and Ceiling Tile	Concrete	

An additional method of sanitization is the use of heat and steam. Steam cleaners, ovens and autoclaves can raise the temperature of viruses above 75° C where they will begin to destabilize, denature and dissolve. Temperatures from 75° C to 100° C are best for disinfecting viruses. These methods reduce the risk of chemical contamination from commercial or lab-based cleaners but do have risks for moisture sensitive laboratory areas or chemicals.

No matter which method of cleaning is selected, it is important to create and maintain checklists to remind employees of common touch points in the laboratory and office. These touch points must be cleaned as if they were contaminated and include obvious candidates such as phones, keyboards and light switches but also include less considered areas, such as, water fountain knobs, pens, scissors, laboratory spray bottles, etc. Figure 14 is a general summary of common touch points in an office and laboratory to be included in a checklist.

Laboratory Surfaces Checklist

General Cleaning	Laboratory Related
Computers, Keyboards, Monitors	Instrument Computers
Power Buttons	Instrument Knobs
Doorknobs, Light Switches and Thermostats	Balance Buttons
Phones: Office and Cell	Pipette Handles
Air Filters	Frequently Used Chemical Bottles
Desk	Hood Controls
Pens, Scissors, Staplers, Tape Dispensers	Spigots and Facets
Copiers and Printers	

Figure 14. Common office and laboratory surfaces to be disinfected

It is important to remember in the use of cleaners and cleaning agents that they are still chemicals capable of contaminating daily processes and operations. Alcohols and other solvents are common laboratory materials and may contaminate sensitive areas which may be used to measure volatile organics. These compounds can also kill microbiological experiments if not properly vented from the laboratory workspace. Chlorine compounds and acids can cause oxidation and contaminate experiments, metal components of instruments and equipment. Additional actions may be needed to ensure normal laboratory processes are not contaminated by the updated cleaning procedures.

Before cleaning and disinfecting an area, all porous materials such as paper, paper towels, etc. should be removed from the areas to be cleaned so as not to absorb chemicals. Select cleaning agents appropriate for the area to be cleaned with thought in mind as to the type of work which occurs in these areas and how that work will be affected by these agents. If possible, airflow and hood flow should be increased to drive fumes away from work areas. Chemical odor traps can be used to absorb volatile chemical fumes. Hoods and sensitive areas should be decommissioned during cleaning and allowing several hours for fumes to dissipate.

Multiple spot cleanings should be scheduled during a shift with a plan for more extensive cleaning on a periodic basis. Deep cleaning plans and services should be outlined in a cleaning plan upon an exposure within the laboratory. Personal cleaning and hygiene plans and expectations should be discussed or notices posted to remind everyone to keep a cleaning plan.

One of the most important acts a person can do to reduce their exposure is to wash their hands for a minimum of twenty seconds with soap and water. Most soap compounds are composed of materials whose molecules have a dual nature. One end of the soap molecule is hydrophilic and binds easily to polar solvents such as water. The opposite end is lipophilic and binds to long hydrocarbon chains, proteins and lipids. The action of the soap and water together allows for viral particles to become bound to the soap's lipophilic structure and allows water to wash the particles away (Figure 15).

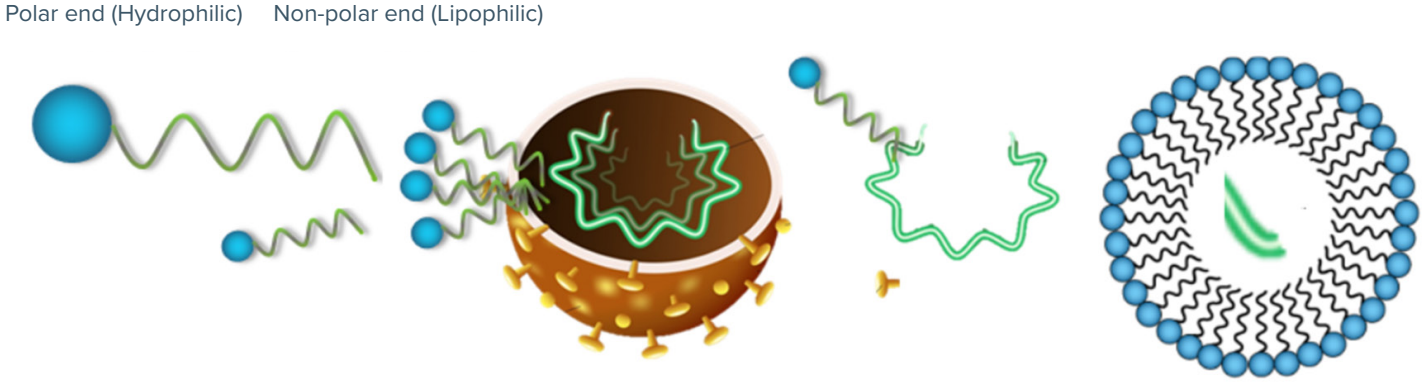

Figure 15. Soap molecules process for breaking down viral particles

The soap does need time to be in contact with the virus particles before being washed away with water. This reason is why it is suggested a minimum of twenty seconds of hand washing is necessary. There are many pneumonics used to count down twenty seconds including singing songs and reciting rhymes. Many schools teach children to sing the 'ABC song' or sing 'Happy Birthday' twice to get their twenty second cleaning done. While these common memes happen to be true, there are a lot of misconceptions about viruses and infections being perpetuated in the news and online. There are a lot of 'common' cures and remedies that simply do not work for a viral infection.

Antibacterials agents from soaps to cleaners to medications do not work for viral infections with the only caveat being if an antibacterial contains greater than 60% ethanol or alcohol, that agent does kill viruses. This concept leads to another misconception where vodka can kill the virus. The concentrations of many consumer alcohols do not have high enough alcohol content, about 60%, to kill viruses.

Another misconception is that antibiotics can treat viruses. As scientists, many are aware that antibiotics are for living organisms such as fungi and bacteria. Viruses are not living organisms, they are genetic particles with some, but not all, aspects of life. Save the antibiotics for bacterial or fungal infections.

Antiviral medications can treat viral infections but they are often specific to a particular viral strain or group of viruses. For COVID-19, investigators are trying to exploit some of its unique binding capacities to create treatments. Some suggested treatments block the interaction with the ACE2 receptors or interrupt other proteases (furin) used by COVID-19 by either blocking the receptors of host cells (which can also block needed biological activity) or bind the portions of the spike protein that usually bind with these receptors. Currently companies are working on both these approaches to treat COVID-19.

Investigators are also trying now to determine if existing antiviral treatments for influenza, hepatitis, HIV, and other viral infections can impart some effect on this viral outbreak. Most antiviral drugs are nucleoside analogues or homologues which can be inserted in the replication process of the virus to disrupt replication. These drugs often have to be taken very shortly after the appearance of symptoms in order to produce a positive effect.

Some medications for other diseases are also being investigated during the COVID-19 outbreak including the use of malaria medications and the use of plasma from recovered COVID-19 patients to impart antibodies against the COVID-19 virus. Hydroxychloroquine has received a great deal of attention because the United States has been purchasing large amounts of this drug and has been suggesting it can be used off-label. Several clinical trials are in place to investigate the efficacy of this drug against COVID-19. Hydroxychloroquine is currently used to treat several immune system disorders, such as Lupus. There is anecdotal evidence of the drug's efficacy, but the current data is limited and somewhat controversial. The drug has been used for many years, but it does carry some potentially serious side effects.

All of these treatments are still under investigation and heavy scrutiny. The best way to fight the virus is not to get the virus. This means following protective guidelines and in some cases, especially in the laboratory or potentially infected areas, using proper personal protective equipment (PPE).

Personal Protective Equipment

Most laboratory personnel are familiar with common laboratory PPE but there are some differences in equipment and use that one often takes for granted as correct. There are different uses for each type of PPE and different ratings for equipment such as masks, respirators, gloves, etc. which are dependent on the function they are intended for in the laboratory. There are some specialized PPE's that are only used in specific settings which tend to benefit the laboratory's clean setting rather than the laboratory technician (Figure 16).

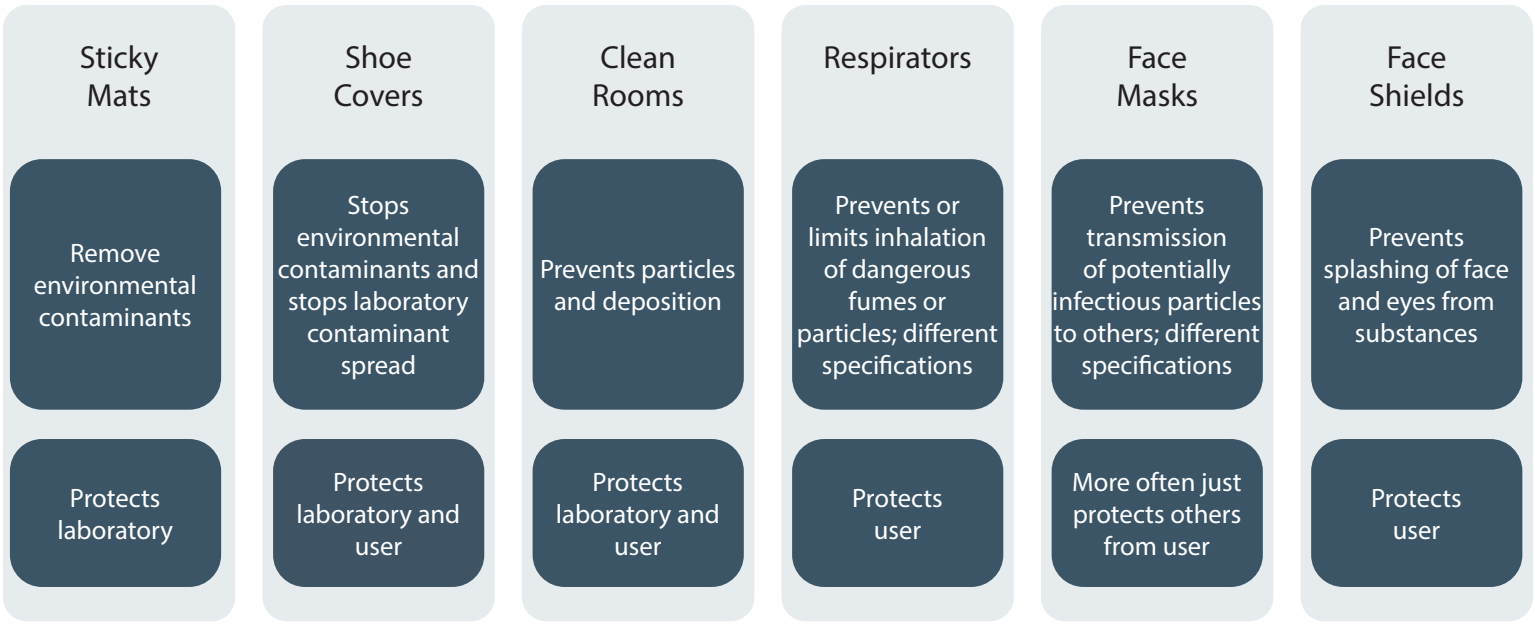

Figure 16. Specialized PPE and their uses

Items such as sticky mats, shoe covers and clean rooms stop the transfer of particles (dirt and otherwise) to and from locations. In chemistry laboratories, these items are used mostly to protect the laboratory from added contamination. In a hospital or healthcare setting, these items can also protect the environment from the transfer of contaminated particles outside a quarantined or contaminated area. Clean rooms or controlled quarantine rooms have sealed air flow and ventilation with HEPA filters to reduce particle transmission. Most clean rooms are rated by the amount and size of particles that are filtered by the system. Table 7 shows the specifications for different classes of clean rooms.

Table 7. ISO 14644 clean room specifications

Maximum Particles/m^3						
Class	**≥ 0.1 µm**	**≥ 0.2 µm**	**≥ 0.3 µm**	**≥ 0.5 µm**	**≥ 1 µm**	**≥ 5 µm**
ISO 1	10	2.37	1.02	0.35	0.083	0.0029
ISO 2	100	23.7	10.2	3.5	0.83	0.029
ISO 3	1,000	237	102	35	8.3	0.29
ISO 4	10,000	2,370	1,020	352	83	2.9
ISO 5	100,000	23,700	10,200	3,520	832	29
ISO 6	1.0×10^6	237,000	102,000	35,200	8,320	293
ISO 7	1.0×10^7	2.37×10^6	1,020,000	352,000	83,200	2,930
ISO 8	1.0×10^8	2.37×10^7	1.02×10^7	3,520,000	832,000	29,300
ISO 9	1.0×10^9	2.37×10^8	1.02×10^8	35,200,000	8,320,000	293,000

Respirators, face masks and face shields cover different parts of the face but generally cover the mouth and nose. Face shields offer the least amount of respiratory protection since they are only physical barriers to splashes and respiratory expulsions directed at the face. Respirators filter particles, chemicals and fumes depending on their specification using filtration chemicals or materials. Respirators are meant to protect the wearer from these agents and must be properly fitted and tested by a professional to ensure good seal and appropriateness for use. Face masks can also potentially be a tool for the filtration of particles depending on their rating. Fitted face masks are very different from the surgical masks being seen in pictures during this outbreak (Figure 17). All of the world organizations warn that a generic face mask is not a substitute for a fitted and regulated face mask or respirator. [13,14] Table 8 shows the OSHA particulate mask ratings. [13,15]

Figure 17. Examples of (a) surgical face mask, (b) particle mask and (c) canister respirators

Table 8. OSHA and NIOSH particle filtration of masks

Rating	Filtration Rate (Airborne Particles)	Oil Resistance
N95	95%	Not Oil Resistant
N99	99%	Not Oil Resistant
N100	99.97%	Not Oil Resistant
R95	95%	Oil Resistant
R99	99%	Oil Resistant
R100	99.97%	Oil Resistant
P95	95%	Oil Proof
P99	99%	Oil Proof
P100	99.97%	Oil Proof

During the COVID-19 pandemic, the use of N95 respirators is often called for and requested. These particle respirators trap up to 95% of particles. For more information, refer to CDC, OSHA, NIOSH and ISO guidelines and instructions on selection and use of respirators and masks.

It is more common that chemists and other laboratory scientists use basic PPE such as goggles, glasses, gloves, and lab coats. All of these PPE items are needed for chemical protection but can also be used for protection against biological agents. As with the respirators, there are different classes of goggles, glasses and gloves which are dependent upon use. Figure 18 shows the uses for common PPE in the laboratory.

Figure 18. Common laboratory PPE and their uses

Goggles and glasses protect the eyes from splashes and can be made from a multitude of materials resistant to a range of agents. Gloves as well can be made from a variety of materials which is important to understand since each type of glove has its own strengths and weaknesses. Many gloves are subject to issues of chemical or biological resistance meaning not all materials are resistant to all agents and therefore offer limited protection (Table 9). Cole-Parmer offers an interactive tool on their website to find glove compatibility (Figure 19): https://www.coleparmer.com/safety-glove-chemical-compatibility (16)

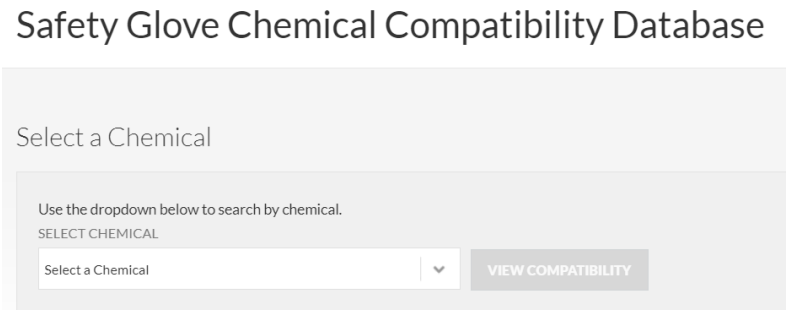

Figure 19. Cole-Parmer glove compatibility database

Table 9. Chemical resistance for glove materials against laboratory materials

Compound	Natural Rubber	Neoprene	Butyl	PVC	Nitrile
HCl 37%	3	3	4	3	3
Ammonium Hydroxide 70%	1	3	4	2	3
NaOH 70%	4	4	4	4	3
Aromatic Hydrocarbons	1	1	1	1	Varies
Methylene Chloride	1	1	1	1	2
Acetone	1	1	4	1	1
Ethanol	1	2	4	1	3
IPA	1	3	4	2	4
Methanol	1	1	4	1	1
Hydrogen Peroxide	4	2	4	3	4

1 = Not recommended; < 1 hour
2 = Fair; breakthrough at 1 hour
3 = Good; 4 hours
4 = Excellent; > 8 hours

The choice of the proper PPE is not the only factor in protection for the wearer. The matter in which PPE is put on and removed after use is important. Many laboratories or healthcare settings have isolation PPE procedures for strict quarantine and contamination control. Smaller commercial laboratories with lower risk for infection often have simple, if any, procedures for proper PPE use. There are some tips to help use PPE efficiently.

Gloves, as was stated previously, should be of a compatible material for the purpose. If a laboratory technician is cleaning for COVID-19 using alcohols such as ethanol, they should not be using latex or other incompatible gloves which might start to become permeable and expose the individual to contaminants, solvents or other agents. Gloves must fit snugly but not so tight that they stretch and become compromised more quickly during use. Gloves should also not gap at the fingers. After gloves are contaminated, they can be removed by pulling one glove off with the still gloved hand and then using the inside portion of the glove to remove the second glove folding them into each other so the glove disposal packet has the contaminated or exposed areas contained inside the packet.

Lab coats should fit properly and button. The cuffs should not hang down into the work area nor should the cuffs be too short to not cover the tops of the gloves when worn. Pockets should not contain items that cannot be exposed to contamination or infection such as personal phones. Lab coats should be changed frequently. The removal of the lab coat is similar to the gloves where the sleeves are removed inside out and the outside is folded inside to contain the contaminated area.

In the event of a known viral exposure, all PPE items and trash should be isolated from the common waste stream and disposed of in a separate location.

Conclusions

During this time of heightened anxiety, it is good to know most common laboratory procedures used to keep scientists safe from chemical exposures also work well for limiting biological exposures. More diligence must be paid in common areas and with common touch points in our offices, laboratories and lives. Check on all of the protective equipment used in the laboratory and make sure it is up to the task that is being set. Isolate all potential contaminants and dispose of them quickly. Most importantly, use your knowledge, training and understanding of science to promote calm and educate your coworkers, employees, families, and others of the real facts of infectious diseases and prevention.

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