Chula Med J Vol. 65 No. 3 July - September 2021;339-348 DOI : 10.14456/clmj.2021.45

Review article

Compelling evidence of viral shedding of SARS-CoV-2 in stool and laboratory safety suggestion for stool examination amid COVID-19 era

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COVID-19 pandemic unfolds in December of 2019 as a number of unknown pneumonia cases arose in Wuhan, China. Patients who contracted the disease usually have respiratory symptoms such as cough and dyspnea. With no established treatment and vaccines, the rise in the number of infections has been unrelenting. SARS-CoV-2 transmits via droplets and aerosols. However, evidence has shown that they are infectious through stools as well. There are increasing numbers of reports of virus particles in feces as well as systematic reviews and meta-analysis to identify the extent of viral fecal excretion. The findings posed a very serious threat upon laboratory technicians who perform routine stool examination.

In this review, our topics cover the natural history of SARS-CoV-2 infection and gastrointestinal tract, viral shedding in stool, virus survival in the environment, and disinfection for SARS-CoV-2 in stool samples, and laboratory safety suggestion for stool examination in post-COVID-19 outbreak.

Keywords: COVID-19, disinfection, fecal viral shedding, SARS-CoV-2, stool exam, stool specimen, viral shedding.

SARS-CoV-2 (COVID-19) emerged in Wuhan, Hubei, China. The current unprecedented pandemic began with a cluster of pneumonia cases with unknown etiology at the end of December of 2019. The novel coronavirus genome is similar to those found in bats. Therefore, it is assumed that the virus is a zoonotic causing epidemic resembling the emergence of the SARS-CoV epidemic in 2002 and MERS-CoV in 2012. The confirmed transmission modes of the COVID-19 virus announced by World Health Organization (WHO) include contact, droplet, airborne, and fomite transmission. Other possible modes can be fecal-oral, bloodborne, and mother-to-child transmission since SARS-CoV-2 RNA has been detected in urine, feces, plasma and/or serum of some patients, and in a few breast milk samples of infected mothers.[1] The coronavirus has infected more than 66 million people worldwide causing roughly 1,500,000 deaths. The United States is worst afflicted with over 278,000 lives taken by the virus (Accessed Dec. 8th, 2020).[2] Patients infected with the coronavirus usually present with fever, fatigue, cough, and diarrhea. The impact is larger for patients aged over 60 years old or had particular underlying diseases. The effective treatment for the coronavirus (SARS-CoV-2) has not yet been established. To date, a few vaccines have shown positive results in trials. A vaccine from Pfizer/BioNTech was already granted an emergency approval in the United Kingdom. Nonetheless, the amount of vaccine that will be produced is going to be halved due to shortage of supplies.[3]

New measures are introduced to every aspect of daily life globally to help plateau the curve. Such policies encompass social distancing, work-from-home policy, and wearing face masks in public. Apart from respiratory specimens, studies have demonstrated viral secretion via stool even in asymptomatic or recovered patients.[4 - 11] Viral shedding has also been found in blood and, in one study, urine.[5, 12] The SARS-CoV-2 spreads through the respiratory tract through angiotensin-converting enzyme 2 (ACE2) receptors.[13] Zhang Y, *et al*. have demonstrated

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Received: September 14, 2020
Revised: November 30, 2020
Accepted: December 15, 2020

abundant angiotensin-converting enzyme 2 (ACE2) receptors in the glandular cells of the stomach, duodenum, and rectum.[11] Consequently, viral penetration into the gastrointestinal tract occurs as well. The study helps elucidate the mechanism of diarrhea as presenting symptoms together with reasons behind positive viral genetic data in stool examination. Another research has been reported that live virus has been found 14 days later in stool after nasopharyngeal swab-confirmed diagnosis of SARS-CoV-2 infection.[11]

According to these data, fecal-oral route transmission cannot be ruled out

Generally, stool specimens have been sent to several laboratories to test for gut health and pathogens. Stool examination for some protozoan and helminth infections is responsible by Department of Parasitology. A number of patients with SARS-CoV-2 infection presenting at the clinic for other purposes without any respiratory symptoms is an intriguing issue needed to be explored further regarding the necessities of universal precaution in every routine stool examination as a new normal in the COVID-19 era. Therefore, this review is intended to gather information regarding the magnitude of stool viral shedding and suggest a new normal for stool examination until the introduction of a vaccine against COVID-19.

Natural history of SARS-CoV-2 infection and gastrointestinal tract

SARS-CoV-2 is well-known for its strength to attack the human respiratory system. In general, clinical course ranges from asymptomatic to severe symptoms, such as acute respiratory failure.[14, 15] Initially, patients usually report to have fever, cough, and dyspnea.[14, 15] For those with certain co-morbidities and aged more than 60, risks for intensive care unit admission rose sharply.

As SARS-CoV-2 is regarded as a fatal and highly contagious newly emerged disease, with no standardized effective treatment established, attention is much paid to it from all leading medical centers around the world. During the course of the disease, a considerable number of researches recorded gastrointestinal involvement as patients develop diarrhea upon hospitalization.[16] Some reports claimed diarrhea as one of the presenting symptoms.[14, 15, 17] A wide range of gastrointestinal manifestation percentages was recorded ranging from thirteen to around forty percent. A meta-analysis of 60 studies by Cheung KS, *et al*. found that seventeen percent of COVID-19 patients have gastrointestinal symptoms.[18] From the study, loss of appetite ranked the first accounting for 26.8% with diarrhea and nausea/vomiting accounting for 12.5% and 10.2% respectively. Nevertheless, the meta-analysis observes significant heterogeneity. The gathered data reiterates the variability of symptoms. The ratio of alimentary involvement should still be further followed as it might be underestimated due to the lack of gut involvement information in the early days. However, once compare the proportion of digestive manifestations among human coronaviruses (COVID-19, SARS-CoV, and MERS), COVID-19 has a significantly lower ratio of GI involvement.[19] The first case of COVID-19 in the United States was a patient who came back from Wuhan. Initially, he presented with cough and fever. He later developed diarrhea during hospitalization. His diarrhea began on the 6th day of admission and lasted for two days. Stool *real time-polymerase chain reaction* (RT-PCR) for the coronavirus revealed positive at day 7.[20] Multiple trials utilized RT-PCR to detect SARS-CoV-2 in different types of clinical specimens, stool RT-PCRs for the virus were found to be highly positive.[8, 9, 11, 13, 17] Xiao F, *et al*. reported of the seventy-two patients admitted due to COVID-19, 100.0% revealed positive for SARS-CoV-2 in feces subsequently after hospital admission.[13] However, stool results do not correlate with the patients' characteristics as only twenty-six patients (36.1%) had diarrhea. The study also showed that 50.0% of the patients might be able to pass on the virus via stool despite their not having any signs of alimentary involvement. A paper conducted released an appalling figure of the average for the period of stool viral detection of 27.9 days in comparison to 16.7 days for respiratory specimens.[21] This gave rise to the questions of SARS-CoV-2 impacts on the alimentary system and its possibilities of transmission via fecal-oral route.

Wrapp D, *et al*.[22] have conducted a study which found that SARS-CoV-2 morphology is almost homologous to SARS-CoV. The 2003 SARS-CoV exploits ACE2 receptor for cell entry.[23] The novel virus is no different.[24] It uses ACE2 receptor and serine protease TMPRSS2 to approach human-being cells. Interestingly, a study showed that the novel virus has a much higher affinity to the receptor ranging

from 10 to 20 folds compared to its ancestor.[22] The inhibitor of the receptor and enzyme embraces hopes to tackle the virus. ACE2 is abundantly distributed in human pulmonary alveolar type 1 cells as well as gastrointestinal epithelia.[13] Viral nucleocapsids were detected via biopsy in gastric, duodenal, and rectum glandular epithelial cells confirming gastrointestinal infectability.[13] Despite clues behind its pathophysiology, the mechanism behind diarrhea remains unclear. According to the study by Hashimoto T, *et al*. [25], it is assumed that disturbances of the gut microbiome, innate immunity, and altered amino acid regulation may play an important role.

Viral shedding in stool

The knowledge of gut susceptibility to COVID-19 helps explain the positive results of viral RT-PCR in stool. Accordingly, it is unsurprising to detect the virus in the feces of patients presenting with diarrhea. Several studies have demonstrated evidence of SARS-CoV-2 in either anal swab or stool exam.[5, 8, 9, 11, 13 - 15, 17, 18] The aforementioned meta-analysis by Cheung KS, *et al*. illustrated the magnitude of gastrointestinal viral excretion reporting positivity of 48.1% (data from COVID-19 138 patients confirmed by at least one nasopharyngeal swab) during the course of the disease.[18] It is under the discussion whether the majority of the virus detected is its RNA fragments or live virus.

According to Zhang W, *et al*., stool viral RT-PCR from six out of sixteen patients reversely reported positive after 5 days from the initial collection.[9] Few reports showed that viral detection in anal swab is higher amid later stages of the disease as well.[7, 9] Interestingly, some studies found that the phase of viral shedding from the gastrointestinal tract can extend as long as two weeks or up to four weeks especially in pediatric cases.[5, 7, 17, 26] Moreover, the virus could still be detected in stool after the patient met the criteria for discharge (two negative nasopharyngeal swabs at least 24 hours apart).[8] The meta-analysis also noted that roughly 70.0% of the patients who had their stool RT-PCR performed for follow-ups alarmingly reported positive after two negative nasal swabs, the longest evidence of viral shedding documented was 33 days from the beginning of the illness.[18] It is thus now questionable whether nasopharyngeal swab alone is qualified for diagnosis and criteria for discharge.

Additionally, Han C, *et al*. found that COVID-19 patients with any digestive symptoms had a longer interval of viral clearance when compared to patients with respiratory symptoms alone (viral clearance definition: two consecutive negative nasopharyngeal swabs twenty-four hours apart).[27] Unsurprisingly, patients with gastrointestinal symptoms had a higher proportion of positive fecal viral RT-PCR exam compared with ones without. Despite high positivity of stool viral shedding in symptomatic patients, patients without digestive symptoms can exhibit positive fecal RT-PCR as well.[8, 15] Additionally, cases that underwent corticosteroid treatment had a prolonged viral excretion via respiratory and gastrointestinal tracts. They also exhibited a prolonged period of stool viral excretion compared to the steroid-free group.[28]

Due to the high percentage of respiratory involvement in SARS-CoV-2, the regulations imposed make the greatest effort to halt any droplet, and according to mounting evidence, aerosol transmission.[14, 29] However, regulations against fecal oral transmission are still not widely recognized and practiced. From the review, evidence of prolonged viral emission from gastrointestinal rather than respiratory specimens is striking. Moreover, patients with COVID-19 can be symptomless throughout the entire course of the disease. A cohort study on Diamond Cruise ship, Japan, revealed that ninety out of seven hundred and twelve patients (12.6%) tested positive for SARS-CoV-2 remained asymptomatic from the beginning until disease resolution (two serial negative RT-PCRs).[30] Pre-symptomatic cases developed symptoms after the first positive viral test on average of four days. Also, the patient remained symptomless despite the positivity of the RT-PCR test for several days. The high positive rate of stool viral RT-PCR, extended period of viral shedding from the digestive tract in claimed-as-recovered COVID-19 cases regardless of their wellbeing, and viral shedding in asymptomatic cases illustrate the unpredictability of the novel virus status of the stool sample. Accordingly, medical personnel are at risk to perform routine stool exams the usual way. Incidental outbreak emerging from the laboratory should be prevented at all costs. Therefore, lab workers had better constantly remain vigilant.Gastroenterologists have also acknowledged that endoscopy is a high-risk procedure for the disease transmission and thus has released a review regarding safety guidance during COVID-19.[31]

As of fecal specimen collection, it is observed that stool exam is more popular than rectal swab.[32, 33] We assumed that stool exam is seen to be more convenient in patients with gastrointestinal

symptoms such as diarrhea which is not uncommon in COVID-19 cases. Detection of virus in stool has not been the compulsory investigation to fulfil the criteria for diagnosis and usually serves as an additional exam when patient develops alimentary symptoms. Out of 1008 severe patients, the positive rate of the stool exam and anal swab were relatively close to each other holding at 12.3% and 11.2% respectively.[33] Another small study conducted in 132 patients revealed similar results, the positive rate of SARS-CoV-2 for feces was 9.8% (24/244 times) and was 10.0% (12/120 times) for anal swabs.[34] There is still no well-designed study to compare the sensitivity between two modes of specimen collection for the novel corona virus. However, a study of the gut microbiota using 16S rRNA gene sequence comparing the two techniques in the same individual declared only minimal differences.[35] The research concluded that the two procedures are interchangeable in the context of gut microbiota detection. Therefore, there is a high chance that the two techniques are replaceable for viral detection as well. Moreover, anal swab was sometimes the only specimen which alarmed positive in few discharged patients among other specimens. Suggestions have been made to include the viral detection from anal swab to the discharge criteria. Stool exam is appropriate for patients with frequent bowel movements in our perspective.

The targets for coronaviruses detection for RT-PCR are ORF1ab/RdRp, E, N, and S genes.[36] There are currently 32 approved molecular diagnostic tests available in the United States of America.[37] The assays of different companies offered different selection of target genes. The interested gene varies from single to three target genes such as detecting single E gene or all RdRp, N, and E genes per kit. A study claimed all seven commercial kits effective despite targeting different gene/genes. [36] The efficacy of these tests was measured using the standard naso-oropharyngeal swabs. At present, only one study is available for stool specimens comparing two commercial kits. The results between the tool using ORF1a region and both the E and N2 genes were acceptable supporting future stool sample protocols.[38]

Virus survival in environment

Virus is an obligate intracellular organism. Once barely exposed in the environment, the virus degrades over time. However, the rate at which it diminishes depends on various factors, some viruses can live up to nine days in a certain environment. Water facilitates viral spreading. This principle supports fecal-oral transmission in a number of viruses such as the rotavirus, and for this time, the SARS-CoV-2 virus. A review studied the survival of viruses in the water among different conditions; such as temperature, sun exposure, oxygen levels, presence of local microorganisms, and organic matters.[39] Temperature strongly affects its existence. Lower temperature prolongs viral survival whereas higher temperature destroys them. Most trials divide the temperature into three ranges; 1. Low (4/10^{o}C) 2. Ambient (20 - 25^{o}C) 3. Body (37^{o}C). A study demonstrated that 5 log viral reduction was achieved in less than a week in the laboratory at 37^{o}C compared to one year in 4^{o}C.[40] In high temperature, the virus is rendered inactive via protein denaturation, nucleic acid destruction together with capsid dissociation.[41] Likewise, solar light destroys the virus by targeting its nucleic acid and forming pyrimidine bonds. It was shown that 1 log unit of virus was reduced in four hours and fifteen minutes in light simulating winter and summer conditions, which has a higher concentration of sunlight, respectively.[40] Another study conducted suggested that chlorinated and dechlorinated tap water exhibited no distinction among SARS-CoV-1 virus survival which ranged between two to three days.[29] Additionally, the presence of indigenous microorganisms disturbs viral prevalence. Sterile water proved to contain a higher amount of virus in comparison with raw water under controlled conditions. However, hints of organic matter extend its lifespan. Moreover, the virus survives longer once dwelled in fungus spores or seed.

SARS-CoV-2 is very sensitive to high temperatures. It lasted fourteen days and one day in Dulbecco's Modified Eagle Medium (DMEM) at 4^{o}C and 37^{o}C respectively. The time was reduced to ten minutes and one minute at 56^{o}C and 70^{o}C in the order stated.[42]

The RNA of SARS-CoV-1 could be detected in air specimen from hospitals in China confirming airborne-transmission.[43] Growing evidences have suggested the same for SARS-CoV-2. Few experiments aerosolized SARS-CoV-2 into the air and collected data regarding its persistence and infectability. Results revealed that the virus was detected active after sixteen hours.[29]

A study tested SARS-CoV stability in various conditions and compare it with other three human-pathogenic viruses.[44] The results showed that in dry conditions, SARS-CoV infectibility lasted as long as nine days whereas other two viruses lost their potential at seventy-two hours. It was described that at the same temperature, in aerosols, other coronavirus persisted longer in high relative humidity conditions. Conversely, on fomites, coronaviruses favored lower moisture.[29]

A review conducted confirmed that human coronaviruses can maintain its infectability on an inanimate surface at room temperature up to nine days (the duration reversely correlates with the temperature).[45] On non-porous surface, they lasted longer. Studies have shown that SARS-CoV-2 losses its infectability roughly four to five days on a plastic surface. The duration varies upon room temperature and humidity. Few researches displayed SARS-CoV-2 survival on stainless steel, the duration ranges three to four days. It was marked that, apart from the environmental factors, types of metal also have an impact on its status. Coronaviruses have a shorter lifespan on copper, copper-nickel, and brass compared to stainless steel and zinc surfaces. The survivability also relies on different types of coronaviruses. SARS-CoV-2 remained infectious for two days on a glass surface. On the other hand, the novel virus lives shorter on a more porous compound. It can live up to merely eight hours on a cardboard and one day on cloth and banknotes. Fortunately, the virus can survive no longer than thirty minutes on paper.[29]

For SARS-CoV-1, several studies proved that their infectability in feces lasted up to four days.[46,47] The survival also depends on the pH and other elements such as the temperature. It could survive two days in domestic sewage. A study conducted in the Netherlands found a correlation between the prevalence of SARS-CoV-2 and the concentration level of virus particles in sewage.[48] It is noted that virus detection in wastewater can serve as a potential tool for the epidemiology of the disease especially in the beginning of an epidemic. The infectability via used water is believed to be minimal.

Disinfection for SARS-CoV-2 in stool samples

To protect medical personnel, it is of great importance to know of how to safely handle the potentially COVID-19 infected specimens while causing minimal impact on stool analysis results. The methods are broadly divided into two categories; chemical and physical disinfection.

Chemical disinfection

Alkalinity and acidity's impact on viruses rely on multiple factors. The duration of exposure, temperature, and virus types have a role in virus survival. There is no clear cut for SARS-CoV-2 yet but the virus could survive in the pH between 3 - 10 at room temperature for one hour.[49] Hydrogen peroxide vapor was effective against the novel coronavirus in the vapor decontamination of N95 masks.[50]

For fixation procedures, it was found that no residual infectability was detected among the listed methods; ice-cold acetone for 90 seconds, acetone: methanol 40:60 for 10 minutes, 70% methanol for 10 minutes or 100% ethanol for 5 minutes. [44] 100% methanol or 4% paraformaldehyde fixation can both kill the virus and maintain the cellular structure.[51,52] Regarding commercial hand disinfectants, the virus was completely inactivated within 30 seconds of contact with 100% isopropanol, 70% isopropanol, 78% ethanol, and a combination of 45.0% 2-propanol and 30% 1-propanol (Table 1).[44]

Hospital benchmark disinfectant sodium hypochlorite at the concentration level of 0.1% (diluted 5% sodium hypochlorite at 1:50 ratio) claimed effective in coronavirus setting. The WHO also recommended 70% ethanol for cleaning of smaller surface areas.[53] The study of chlorhexidine's impact on SAR-CoV-2 was disturbed by multiple factors, but it is assumed to possess some degree of virus inactivation qualities.[42] Formaldehydes or formalin kills the coronavirus completely at the concentration as low as 0.7%. [54,55] Benzalkonium chloride was assessed to be effective against the SARS-CoV-2 as well as quaternary ammonium.[56] Chloroxylenol (0.05%) is potent against the novel coronavirus.[56]

Chemicals proved effective for the treatment of specimen when incubated at room temperature are 0.5% sodium dodecyl sulfate, 0.5% Triton X-100, 0.5% NP-40, and 0.3% tri(n-butyl)phosphate (TNBP) with 1.0% Triton X-100. Some lysis buffers in nucleic acid extraction kits also offer viral deactivation property.[51]

Table 1. Common Chemical and Physical disinfectants with known efficacy against SARS-CoV-2.

Chemical		Physical		
	Chemical Substance			Dose
1	Ice-Cold Acetone	1	Heat 60°C	N/A
2	Acetone:Methanol 40:60	2	UV-C 260 nm	10 J/cm^2
3	70% Methanol			
4	100% Ethanol			
5	100% Isopropanol			
6	70% Isopropanol			
7	78% Ethanol			
8	Combination of 45% 2-propanol and 30% 1-propanol			
9	0.1% Sodium Hypochlorite			
10	Chlorhexidine			
11	0.7% Formaldehydes (Formalin)			
12	Benzalkonium Chloride			
13	Quaternary Ammonium			
14	Chloroxylenol			

Physical disinfection

Heat sterilization

The virus was undetectable when exposed under 56°C heat for 30 minutes. However, this is untrue in samples with FCS (Fetal Calf Serum). The most effective way to eradicate the virus in any condition is to incubate it at 60°C for the same amount of time. [44] For the aspect of SARS-CoV-2 deactivation before nucleic testing, heat sterilization proved unwelcomed due to possible false negative results. Thermal energy disrupts nucleic acid integrity.[57]

Ultraviolet disinfection

Ultraviolet (UV) disinfection has been known as an alternative for microorganism eliminations. It is preferred in some circumstances over other conventional germicidal methods due to its simplicity, energy-saving, and indifferent potential to disinfect air, surfaces, and liquids. In general, ultraviolet radiation can be divided into 4 ranges depending on wavelength. The effective range that is well-absorbed by the viral RNA is UV-C (200 - 280 nm). UV-C inactivates the virus by pyrimidine formation upon their bases similar to that of solar energy. Of note, different organisms respond to a different spectrum of UV-C. The suitable wavelength for the coronavirus is at 260 nm in a laboratory setting. The upper limit of normal dose required for viral inactivation is at 10 mJ/cm^2.[58] Treatment of SARS-Co-V 2 under 222-nm irradiation proved success in both killing the corona virus and preserving its copies.[59] To date, no standardized protocol is yet established in terms of dose due to the unmatching end-point viral viability criteria among researches and lack of real-world experiments.

Parasitology department is responsible for parasite identification in stool exam. Apart from safety, accurate yields for the detection of pathogen is also expected. As for standard practice in parasitology department, all stool samples are treated with 10% formalin for its optimal capacity to preserve the morphology of the parasites.[60] Colliding with its potency to deactivate SARS-CoV-2, 10% formalin is considered a suitable option for the field of parasitology during this pandemic. The disadvantage is the movement of the parasites will be obscured.

Laboratory safety suggestion for routine stool examination amid COVID-era

1. All lab workers are required to wear Personal Protective Equipment (PPE).
2. All stool specimens retrieved from Emerging Infectious Diseases Clinical Center should be investigated at Parasitology Laboratory. The examination is performed strictly under Biological Safety Cabinet (BSC) class II. As for viral culture, the procedure must be carried out under BSC III.[61, 62] Specimens of confirmed or suspected COVID-19 patients must be delivered as UN3373, "Biological Substance Category B". As for samples for viral cultures, the specimen must be transported as UN2814, Category A.[63]

3. For simple smear, 10% formalin is recommended for mounting the slide instead of routine normal saline. The process must be executed in BSC class II as previously mentioned. Due to formalin's potential to kill all microorganisms, observation of protozoa movements will not be feasible. However, its benefit weighs out its disadvantages. After mounting formalin in BSLII at 25°C, the specimen is safe to be examined under a microscope[64] (Figure 1).
4. Upon finishing, it is mandatory to use disinfectants to clean areas or equipment that might contain residual contagious microorganisms. Then, the cabinet is once again sterilized with installed UV lamp for at least 30 minutes.

Conclusion

SARS-CoV-2 is a fast-spreading emerging disease. It has created global health crisis that affects the world population in every aspect like never before. Stringent measures have been imposed to control the disease, but rates of infections and mortality remain high in many countries. Studies have depicted the possibilities of viral shedding and spreading via stool. The disturbing fact that stools of the patients with negative nasopharyngeal swabs are still infectious (46.0%) is a threat for lab workers who routinely perform stool exams. Moreover, it is claimed that the virus retains its infectability up to four days in feces. Therefore, it is concluded that lab technicians should omit the usual practice and remain cautious by following the suggested guidelines to protect themselves from contracting the virus during this challenging time.

Author contributions

The authors confirm contribution to the paper as follows: Study conception: Padet Siriyasatien, Narisa Brownell; Data collection: Nirin Seatamanoch, Switt Kongdachalert; Analysis and interpretation of results: Nirin Seatamanoch, Switt Kongdachalert, Vivornpun Sanprasert, Narissara Jariyapan, Narisa Brownell, Padet Siriyasatien. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgements

This review article would not be possible without the access to scientific data provided by Chulalongkorn University.

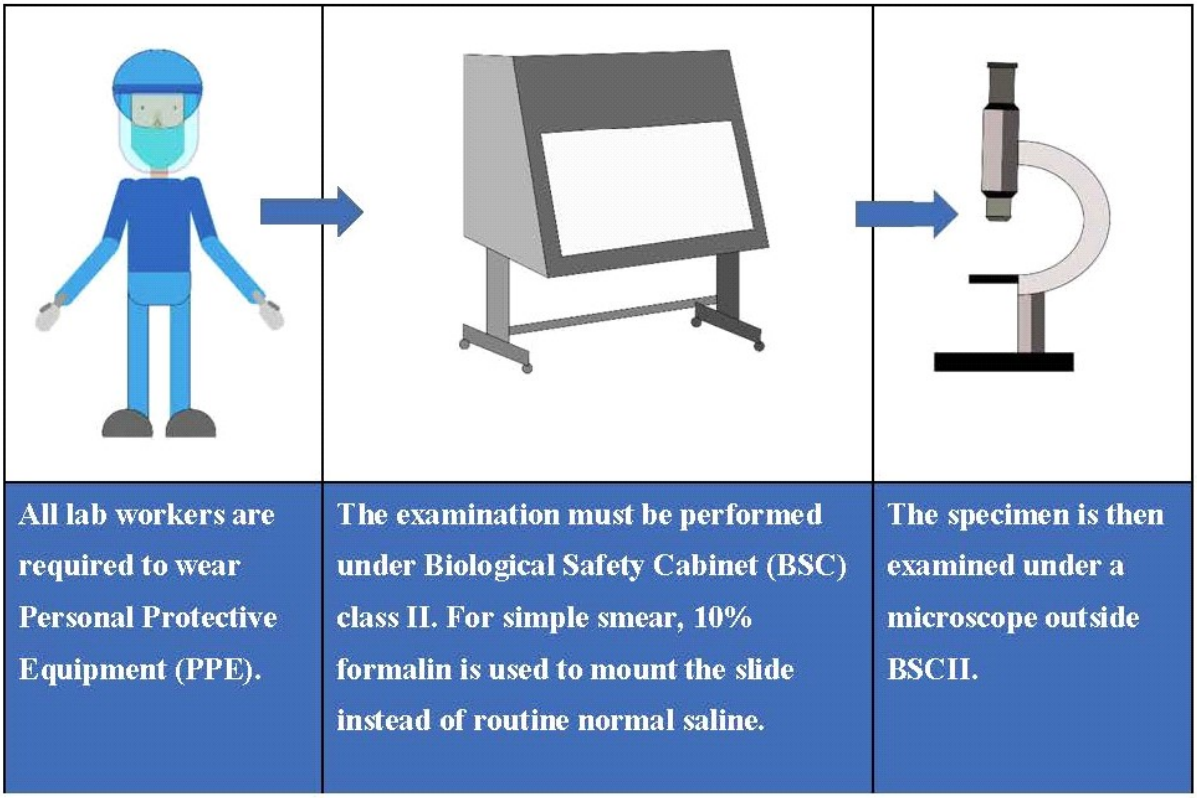

Figure 1. Laboratory safety suggestion for Routine Stool Examination.

Conflict of interest

None of the authors disclose any conflict of interest.

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