Applied Environmental Microbiology

Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

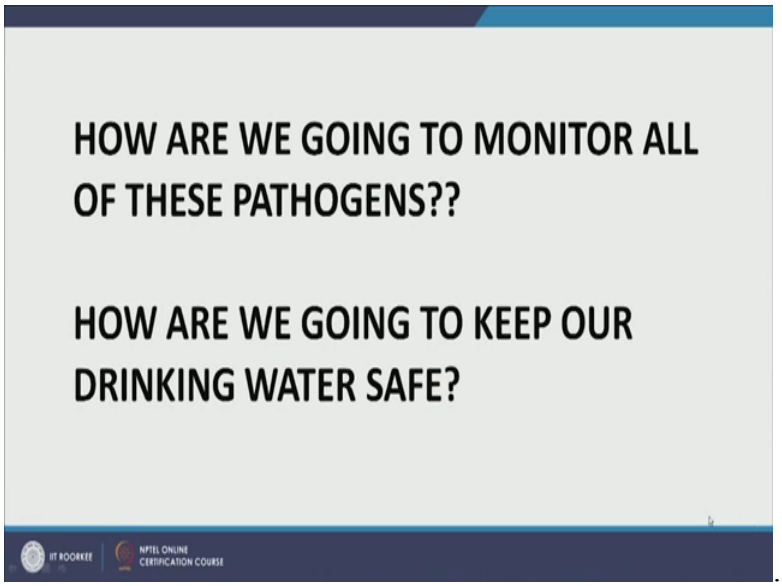
Lecture – 43
Drinking Water Microbiology III

Dear students, in the previous lectures we talked about the pathogens that are present in drinking water and what are the general mechanisms or techniques through which we can detect them. Now, if we want to ensure the safety of our drinking water, it is very important for us to know where pathogens are present in drinking water and in which places they are not present. Unless we have that information we cannot ensure safety, because safety involves two aspects.

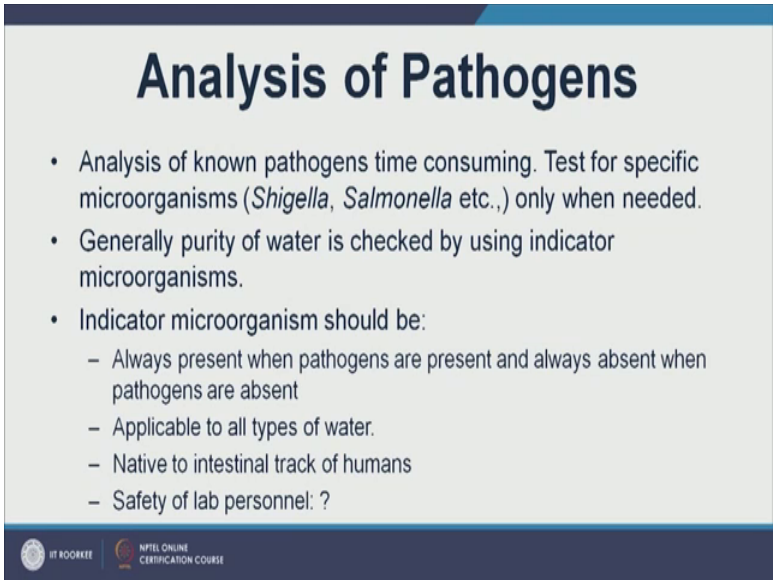
One is proper disinfection and the other is public awareness. Now, there are multiple tools and techniques available for detecting pathogens in drinking water; however, our regulations in our country, are very strict about which techniques should be followed and when and how and each of these techniques, whether it is the one that is mandated by regulations or the months that are preferred by scientist, ones that are preferred by lab personnel.

All of them have certain advantages and certain limitations. So, today let us go ahead and explore the limitations of the techniques, that we have talked about earlier and the ones that we are going to talk about now. So, the primary question that we are trying to answer right away is, how are we going to monitor all of our pathogens and how are we going to keep our drinking water safe.

(Refer Slide Time: 01:37)



(Refer Slide Time: 01:40)



So, step is to analyze pathogens and most of the tests that we use for analyzing the pathogens, are very time consuming for example, a simple bacteria might require at least overnight culturing to grow. Total coli forms, fecal coli forms often require incubating for 24 hours and in that 24 hour, considerable damage to public health is possible.

So, when public health is involved and we want to ensure that the water that our public is getting exposed to and is consuming is safe. This time consuming tests are going to cause great harm and thus, there is need for other kind of technologies and usually our

regulations, not just in our country India, but across the world involve only indicator microorganisms. Now, let us spend some time trying to understand what indicator microorganisms are. So, pathogens are dangerous for us, if I try to grow them in the lab or if I try to test, whether they are present in a sample or not, in case they are present in the sample the only way I can test is, by growing them in suitable media and noticing, if they show a signal of presence or not. This is cultural based detection technique.

Now, when I am growing them or when I am considering them in the lab, I am increasing their concentration many folds, much - much more than the infectious tones and thus I as a lab personal, am at risk of catching the infection. Not only that, but I am also at risk of spreading the infection in people around me and thus, in the general public. That is what our agencies do across the globe is, we use indicator microorganisms. These indicator microorganisms usually colonize our gut and thus, represent our indicator for contamination of fecal matter in drinking water and they are typically not dangerous, not pathogenic for human beings.

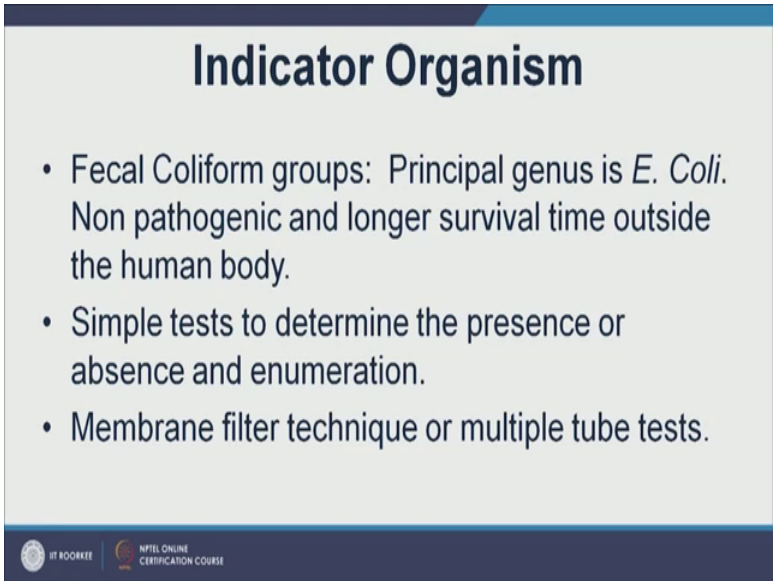
So, if we detect indicator microorganisms, we can be sure to some degree that perhaps, there are pathogens present. Now, what are the general characteristics of indicator microorganisms? How do we decide that F1 forge is a good indicator virus for drinking water, but some other forge or virus is not an indicator? First of all the indicator microorganisms should always be present when, pathogens are present and always absent when, pathogens are absent. So, basically it should be like to use, a given general example for it should be best friends with pathogens, it should be always there when pathogens are there, it should always be absent pathogens are absent.

So, it should have same source as pathogen and same fate as pathogens. It should be applicable to all kinds of water, whether it is a line water, whether it is clear drinking water or whether it is wastewater and because we know that, most of the pathogens are interested in terms of water bone diseases, are usually found in our gut, we know that fecal matter without doubt, is the leading source of contamination in drinking water sources.

So, these pathogens should be native to our intestinal tract because, the pathogens that will eventually go in drinking water sources will come from our gut. The other thing is,

we have to ensure the safety of lab personnel. Otherwise, nobody would want to work in these labs and we will never know if pathogens are present in our drinking water or not.

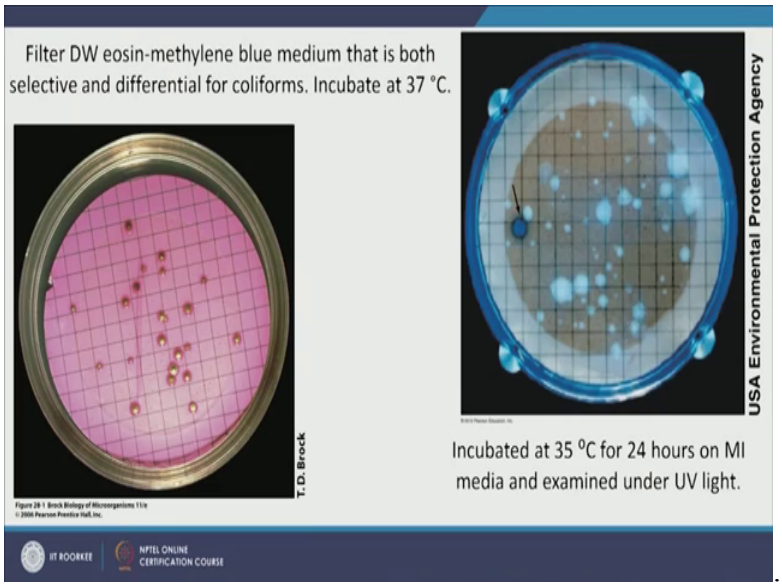
(Refer Slide Time: 05:22)



So, we use indicator organisms and we use fecal coli forms to get an idea of fecal contamination. So, as name suggests fecal, so if the fecal matter is present then we know, the water is not suitable for drinking. If you remember from the last lecture, we talked about fecal coli form detection in any water sample should be zero for it, to be fit for drinking and the principle genus that is, detected and grown in for fecal coli form, when we do fecal coli form test is E coli which is a (Refer Time: 05:50) coli, it is non – pathogenic, the strain that we grow visually and it survives longer outside human body.

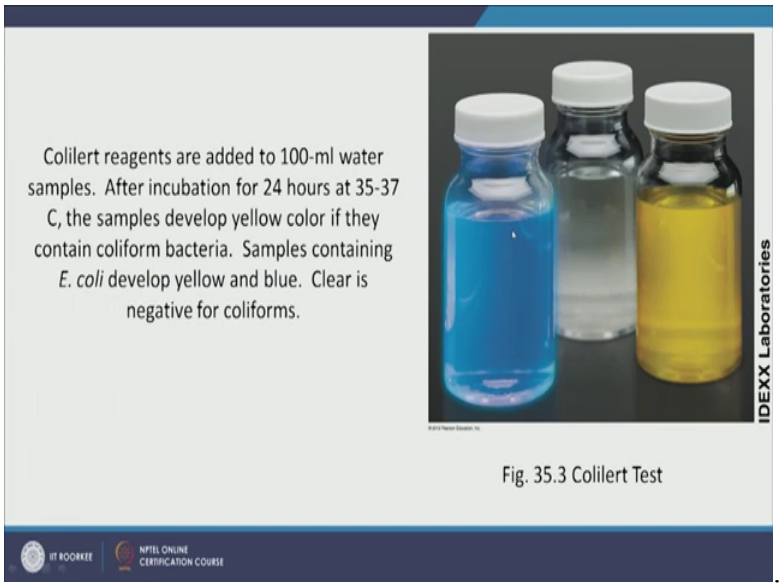
The other advantage of using this particular genius of fecal indicator is that, it tends to live longer than most pathogens and thus, we are on the safer side. It has a very simple test for growing, just requires incubation for one day and we can use either membrane filter technique, where then we grow them on plates, or we can use multiple tube tests, where we have multiple tubes and we notice a change in pH, by noticing change in our indicator dye and know where the fecal coli forms are present or not.

(Refer Slide Time: 06:34)



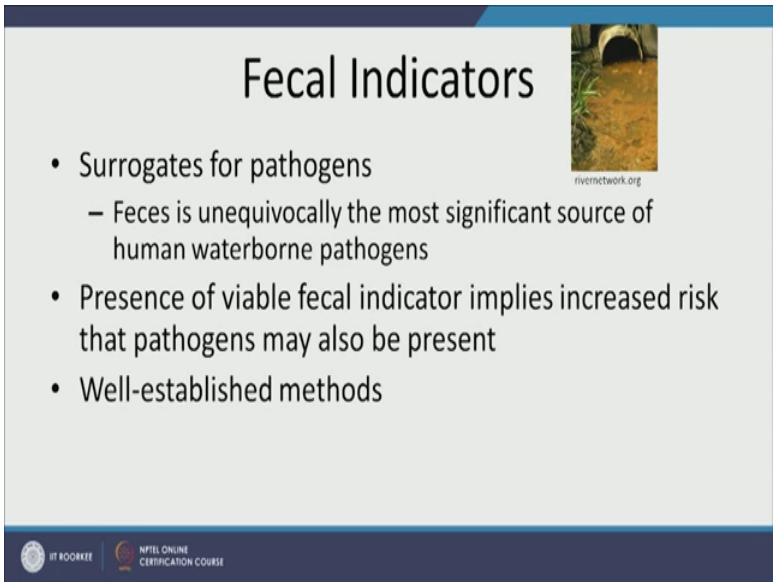
So, I showed you this example in one of the previous lectures. So, here we have total coli forms giving positive sign and here, the dark colony if you can see carefully, is the fecal coli form equal to high.

(Refer Slide Time: 06:44)



And then, another technique that is quite prevalent across the globe and is also used in India is collier kit. So, this is a kit where you just put the reagent, add your water incubates for 24 hours at nearly body temperature and then, depending on change of color, you know whether fecal coli forms are present or not.

(Refer Slide Time: 07:02)



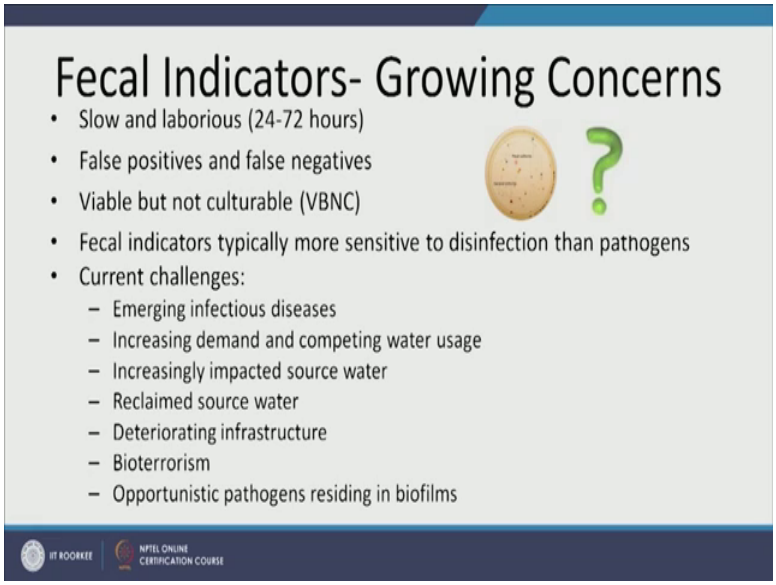
Now, let us take a look at fecal indicators - what are they? They are surrogate for pathogens and we know that feces are, unequivocally the single most important source of pathogens in water. Now, if we notice that feces are present in the water, we are at higher risk of getting exposed to pathogens. So, if viable fecal indicator is present, it means we have higher risk of falling sick. Now, here is a word viable, it is a very important word and I would like to give you some hint on what this word implies.

Now, viable is any microbe that can grow. Not all microbes are viable, as in they are not alive and healthy enough to replicate and increase in their numbers, but viable pathogens are. So, basically anything that we can grow on a plate is viable, anything that can go anywhere is viable, not dead. Now, they are well established methods for detecting E coli and for detecting coli forms and total fecal coli form and thus, it is very useful and easy to use technique for indirectly detecting presence of pathogens and has a long regulatory history.

So, in fact, it has such long regulatory history that despite of all its limitations. It is nearly impossible, not impossible it is very difficult to suggest and apply other techniques for detection of fecal matter pathogens in drinking water and other surface waters and you know, the good news is most of the time it works. So, you go ahead, drink water from the tap and you do not fall sick because, your regulatory agency is doing routine testing, using these same indicator organisms and they are following the

rules, that have been laid and it works; most of us do not fall sick every now and then, but there are growing concerns for fecal indicators.

(Refer Slide Time: 09:03)



First of all is, that it is very slow in the barriers. It can take anywhere from 24 to 72 hours. In fact, there are certain microbes like micro bacterium, which are found in our bio films that grow in drinking water distribution system. They can take up to eighteen weeks to grow. So, these culture based techniques are very slow and laborious, they require skilled personnel, they have many false positives and false negatives.

This is a very important thing because, not all E coli or fecal coli form that is present in water may be fit enough to grow on our plate or grow in our tube and thus, we will get a false negative. False positive is also an option, even another microbe can utilize the similar food that we are giving it, the substrate that we are giving it, either on the plate or on the tube and then, give us a signal and then, there is a big issue of viable, but not culturable. What about E coli or what about other indicator organisms and pathogens that is viable?

So, they are alive and they are kicking it, in which means, they are growing rapidly or just growing, but we cannot culture them and we have noticed this, for quite some time now in microbiology, that some microbes that are e coli will be culturable, but some E coli will not be culturable easily thus, and not just the E coli, but there are many other microorganisms that may be viable, but cannot be cultured and grown in lab. Now, here

is the thing, an issue, most of these fecal organisms, they are more sensitive to disinfection than our pathogens. So, if we have a disinfection event then, they are likely to outlive pathogens.

So, let us say, I do my drinking water treatment plant and I put the raw water in there and as the water gets treated and processed and I am very happy that, that is working well and then, at the disinfection step if you remember, the last step prior to sending it to storage and then, to drinking water distribution system is, of disinfection and let us say I bleach it disinfected and I notice that my total coli form and fecal coli form level is zero and perfectly under limit and I am very happy and I send it to my DWDS (drinking water distribution system).

But it is also possible that while the fecal coli forms died, while the total coli forms reduce in number, the pathogens survived because some pathogens are more persistent in face of disinfectant than, fecal coli forms. The current challenges are emerging infectious diseases, not all diseases are.

But some diseases are new and not all of them can have fecal coli forms and total coli forms as reliable surrogates, then we have increasing demand and competing water usage which means, that we have more and more demand on our raw water supplies. Let us say, on our ground water, on our surface water and less time to do this, very slow and laborious test and also because there is more demand, the duration between the one wastewater treatment plant throwing away their effluent and another drinking water treatment plant taking in their influent is less.

So, there is less time that microbes have to undergo natural attenuation and this is a big issue. Next is, we have increasingly impacted source water. So now, our surface water, our source water is usually dirtier than have ever been before and a very good example would be Agra and Mathura.

So, I do not know how many of you are familiar with Agra and Mathura, in terms of where they are located and this is, a very good opportunity for you to go and Google India's map or look it up and notice where they are and if you already know, very good So, Agra is a historic town, it is also a tourist town and Mathura has lot of religious and historic relevance to it. Now, both of them have a river a common, river that flows to them and upstream of Agra and Mathura is our national capital region.

Now, as you would have guessed, national capital region is heavily populated, heavily polluted and many of the untreated waste are dumped in this river and as such this river in the Yamuna is nearly dead, which means that its quality - water quality is so bad, that we have sort of given up on treating it successfully.

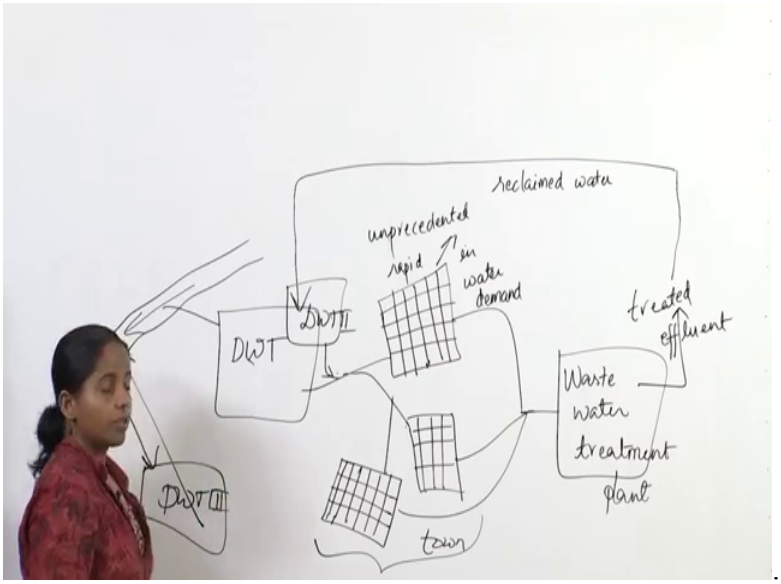
Now, this water serves as a drinking water source for Agra and Mathura. Now, I want to mention here, that not all water treatment plants at Agra and Mathura use Yamuna water as their raw drinking water source, but few do and one of them is very interesting in sense, that it is the only water treatment plant in India, where we have a biological process for treating water.

So, they do not have the usual coagulation or flocculation, sedimentation or filtration followed by disinfection, storage and distribution. They have a treatment train that resembles more treatment, more the treatment for wastewater than for drinking water and in biological treatment, in this particular treatment they use microbes and thick bio films to consume all the organics and all the wastes and remove the excess nutrients in the water.

So, the thing is that, this water is so dirty that even if we do our best in removing the turbidity, in removing the coli forms and fecal coli forms, there is a very good chance that we still have some contaminants, that either we are not regulating for now or some emerging diseases, that we are not aware of or there are viable, but not culturable fecal organisms or the possibility that, the disinfectant killed our indicators, but our pathogen survived.

So, when our source water gets dirtier, it is more challenging to clean water and it is also more challenging to ensure and test whether the water is clean or not. Now, another thing is reclaimed source water, now in developed countries, we use the word reclaimed source water to suggest in a way recycling of water. So, this would seem like this.

(Refer Slide Time: 15:29)



Let us say there is a river. So, let us say, there is a river here and there is a town. So, this is a town or city and this town uses the surface water of this river as its raw drinking water source, now in between, we have a drinking water treatment plant that disinfects and cleans the water, stores it and then supplies it through its drinking water distribution system. So, these three grids that I have made are representatives of three different nodes of drinking water distribution system.

Now, the people in this town utilize this water and generate what is called as waste water. So, they use it for drinking, they use it for washing or cleaning or whatever else they need to use it for, agriculture maybe and when this water has been utilized, it is collected again through a very complicated system and it is diverted towards its waste and this water is now, directed towards its wastewater treatment plant.

Now here, they use a very beautiful biological process to clean the water up to certain standards. It is not, it is nearly very, it is going to be very not feasible and very difficult to clean this wastewater to a standard, that matches our drinking water, but now what is happening is, we have rapid population increase. So, as because of this increase in population, which is very rapid and very difficult on our water sources, what we have is an unprecedented in water demand.

So, now, the water demand is so high that this river can no longer meet the demand for this town and let us say, this town does not want to think about other options such as

importing the water from nearby villages, nearby towns or exploiting its groundwater for some reasons and it might consider, I mean those are options that it should consider and it might, but another option that it has is, it can take this treated effluent of wastewater.

It can take this treated effluent of wastewater and instead of throwing it in the river, because the usual practice is that we take it and we throw it downstream. So, let us say river is flowing in this direction, upstream bit of drinking water, downstream with ruddy their wastewater away, what it can do is, it can recycle it and have a specialized drinking water treatment plant that will treat this water up to drinking water quality standards and then supply it to the grid.

Now, there is a problem here. This reclaimed water, this is called reclaimed water, this has higher number of microbes, most likely very high number of pathogens compared to actual treated drinking water and other contaminants, most of their emerging contaminants and other trade the conventional contaminants at very high level. So, it requires a different kind of drinking water treatment or more thorough drinking water treatment, before it is applied for public health.

Now, because this reclaimed water usually has a very rich chemistry and rich biology, it is possible that when we do our coli form test and our fecal coli form test, we would not get this, we would not get an accurate representation of what is really happening microbiologically and I want to take a moment here and mention that, in a developing country like India or in a developing country like India where we are still beginning to advance our understanding of drinking water treatment and wastewater treatment and what is relevant in India.

And not necessarily, what is working well outside and in a highly populated and growing economy like India, this is a reality, the reclaimed water, whether we intentionally bring our treated effluent into a drinking water treatment plant, or we take our treated effluent and put it here downstream and then here we have, because the city is so populated, here we have another drinking water treatment plant and it directly takes it in, drinking next drinking water treatment plant.

So, whether we have river as an intermediate or not, but we are using our surface water. So, exhaustively that pretty soon the effluent of a wastewater treatment plant becomes the raw source water for another drinking water drinking water treatment plant and so,

whether we are doing it intentionally, reclaiming the water for the same town or another town, nearby town is using this treated effluent as its source for raw drinking water.

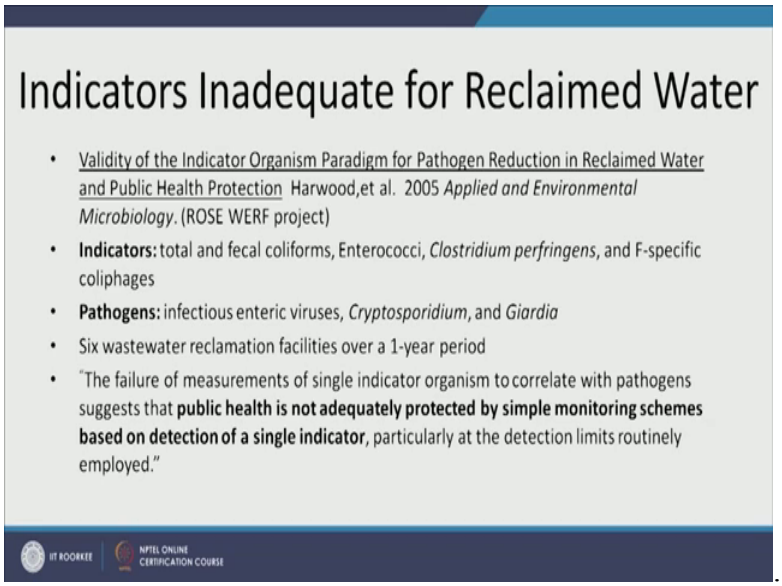
In either case, we are utilizing reclaimed water and thus, drinking water quality and these techniques that are required, to meet those quality in India particularly and other countries, like in the other town cities like India, is very challenging and there is a lot of research that needs to be done here. And then, we have a problem of deteriorating infrastructure, something that we can definitely expect from old cities, old towns because with age, we have corrosion and we have regular breakdowns and also at times, because of poor operation and poor maintenance, we tend to have deteriorating infrastructure.

Other factors that might deteriorate our infrastructure are, for example, intermittent supply may create a water hammer kind of situation and destroy our pipes and then, we have another issue of bioterrorism, which I must admit, is not a big issue in our country, because I mean, it has not been a big issue yet, might be in future, hopefully not. Alright! And then, we have opportunistic pathogens residing in bio films.

So, apart I mean, apart from all the wonderful treatment, that we can do of drinking water and all the tests we can do for fecal coli forms or not and let us say, when I measure my fecal coli forms and total coli forms, at the, after disinfection and before distribution to water network, water distribution network and I say that the water is really good, fecal coli forms is zero, total coli forms are negligible and then, in the distribution system wonders can happen.

For example, pathogens can come in and then, they can make bio films and in bio films, they can grow some of them or they can survive for long time and when they are fit and healthy, they can lead the bio film and. In fact, people. So, in all of these cases, our fecal indicators do not serve us very well, thus, we have growing concerns. So, we know that these indicators are inadequate for definitely for reclaimed water.

(Refer Slide Time: 23:20)



And I want to say, in developed countries, they call it reclaimed water, in developing countries we just call it water, because as I mentioned just few minutes earlier, whether we do it intentionally or not, it is a reality of our situation. So in 2005, there was a wonderful paper in 'AEM', which is a nice journal and they discussed and tested the validity of the usual indicator organisms, when it came to reclaimed water and protecting public health and they measured further, they measured the total fecal coli forms, enterococci, clostridium perfringens and F specific qualifiers, which are bacteriophages indicator for viral pathogens.

So, these are their indicators that they tested and they look for pathogens, that are infectious on enteric viruses, cryptosporidium and Guardia and they looked at 6 wastewater reclamation facilities over one period, 1 year and then, their conclusion was that, I will read it aloud because it is really well written. "The failure of measurements of single indicator organisms to correlate with pathogens suggests that, public health is not adequately protected by simple monitoring schemes based on detection of a single indicator, particularly at the detection limits routinely employed.

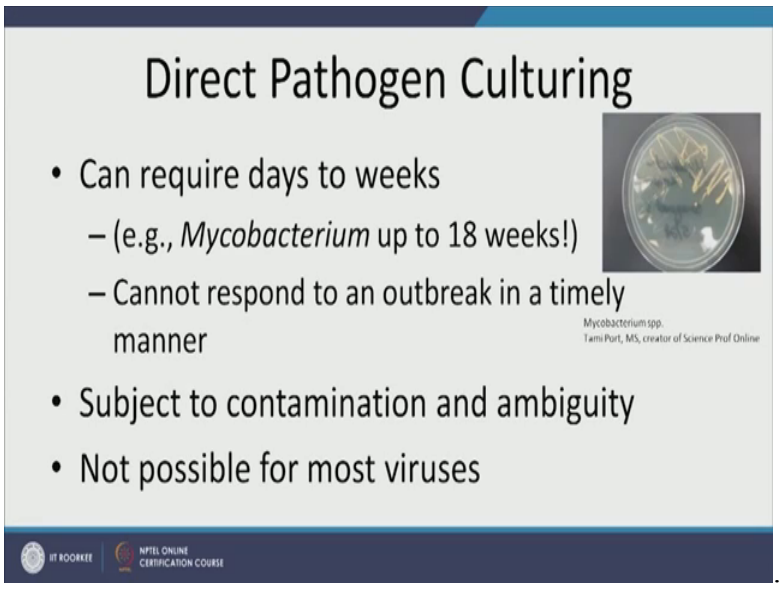
So, there are three things that are happening in this statement. First is that, the failure of measurements of single indicator organisms to correlate with pathogens." So, in this one year study of six wastewater reclamation facilities, they noticed that the fecal indicators

and the total coli form indicators and what other indicators we had, they did not correlate well with actual presence of pathogens.

So, they failed. So, these indicators are not valid, they are inadequate, whenever it comes to reclaiming water. The other thing they noticed that and because of this, the public health is compromised, which is our priority to ensure public health and third thing they are mentioning is about, detection limit which is very important and if you go back to your previous lectures, you will notice how many of the pathogens require very low amount of pathogen to actually infect you, but the cultural culturing techniques and other usual techniques, that are that are applied for these indicator organisms, have a much higher limit of detection.

So, they might be hundred HIV, HEV, hepatitis A, hepatitis E virus particles present, but the quantity of forge F1, that we will measure would be zero, that we would be able to detect and we will require much higher amounts of hepatitis A and E viruses, to get a positive signal in our indicator viruses. So, this is a major challenge in pathogen detection. Let us look at others. So, one is low concentration and low infective doses. I mentioned this, particularly in case of HIV, hepatitis A virus cryptosporidium and Guardia, that anywhere from 10 to 100 amounts of microbes, or particles in case of hepatitis A, are sufficient for a healthy individual to fall sick.

(Refer Slide Time: 26:34)



And then, another issue is direct pathogen culturing. Some pathogens can take up, to for micro bacterium can take a really long time to get cultured and by that time, many people will fall sick.

So, we will not be able to respond in a timely manner to any outbreak. So, think of it in this video is an HIV, hepatitis A, or hepatitis E outbreak happening somewhere and we will take some days to respond, or whether there is an outbreak or not in that days, many thousand people might fall sick. The other thing is, these direct pathogen culturing is subject to contamination and also ambiguity, we are not sure, what we are culturing and this is a tricky situation. It is not possible for more viruses.

For example, in the winter of 2015 and 2016, there was a major jaundice outbreak in Shimla and when they typed, what is causing this jaundice, they found out that it was hepatitis E virus, that was causing the jaundice and detecting the virus, quantifying the virus is, this virus particularly, such a specialized field.

First of all, it requires BSL level three facilities, because HIV hepatitis E virus is highly contagious and dangerous. So, they had to send it all the way to Pune, which is the only place in India, that is certified to do this kind of analysis for hepatitis E virus and when they send it there, it took days for the water samples to go there, in the journey. Some pathogens might have died and then, they got the results back and in between all this, many thousands of people had fallen sick and one of the reasons, why an institute, such as most IIT's and other institutes close to Shimla, could not do these analyses is because they are dangerous.

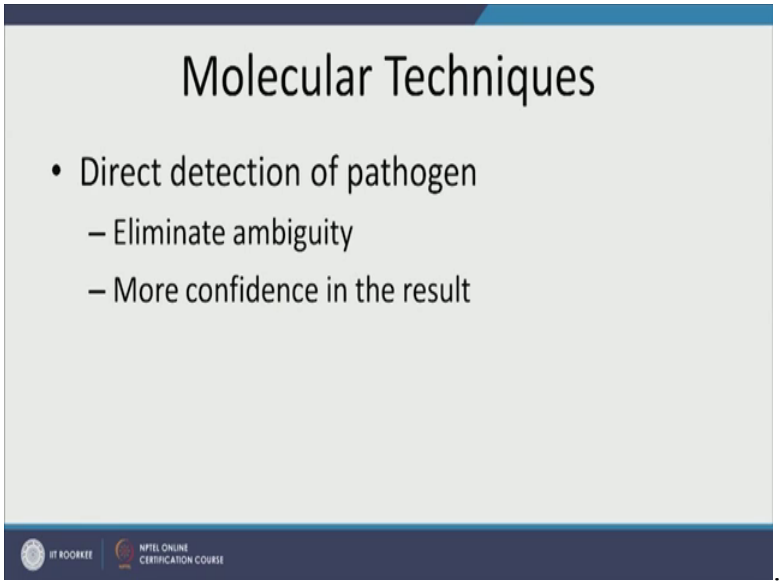
But we could also not do the analysis of the indicator viruses, because these viruses are extremely hard to grow. Even people who work on norovirus or hepatitis virus, different kinds of hepatitis virus, acknowledge the difficulty in just merely growing the viruses.

Now, think of it this way. Virus does not directly eat food. If you know about virus life cycle, it needs to enter the host and then, it uses the resources of the host to replicate, make copies and then, it kills the host. It goes and infects the next host cell. So, as such the most viruses are grown by first growing the host cell line. So, in case of hepatitis A and E viruses, we will have to first grow the human cell line, then in then put the water in it and wait for places where the cell lines die.

So, in case of bacteria we will have colonies growing and here, we will have clock absence, PFE use. So, what is the option? Culturing has a lot of limitations and not just culturing, but using fecal indicators and I have lot of limitations, especially for our current scenario. So, our next option is, using molecular techniques.

Now molecular techniques come with their own set of challenges. For example, but also many advantages, so let us look at our advantages first, and then we will go ahead and look at the challenges So, the advantages that are here is that, they allow us to directly detect the pathogens..

(Refer Slide Time: 29:43)



They eliminate any ambiguity, because we know what we are detecting and I will tell you how they work and then, that will clarify things for you and you have more confidence in the result and it takes less time to do these analyses.

So, we can detect presence of mycobacterium in a day; do not need to wait for eighteen weeks. They have very low detection limit, which is really nice for us. So, they have very high sensitivity, remember as detection limit goes low, we can detect at very low concentration, as our sensitivity increases and they have very high specificity and we can actually quantify their specificity. Specificity is the accuracy. So, if it says hundred out of hundred times, that it says yes pathogen is present, how many times the pathogen really presents, that is specificity.

So, the very talk of molecular techniques, we are often referring to PCR which is 'polymerase chain reaction'. Now, this is a wonderful reaction and I will give you homework to watch two videos later, but in short polymerase chain reaction, is a chemical reaction, where you can make millions and millions of copies of a single genetic element.

So, you choose the genetic element that is of your interest, typically it is the one that is a marker for your pathogen. So, for example, for E coli might have a marker, for shegella, salmonella we have a gene, this gene, if this gene is present we know shegella is present, if this gene is present, we know salmonella is present. So, you can take these samples and then amplify that particular gene, millions of millions of time by in few hours.

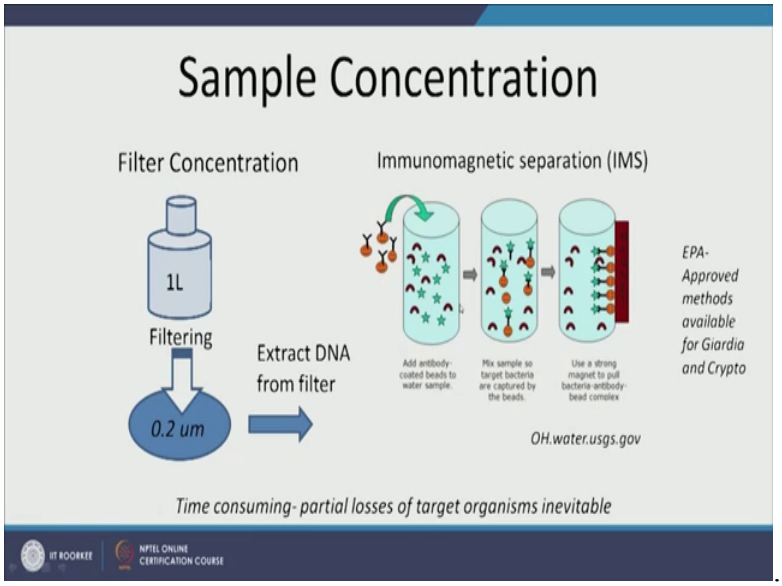
So, if it amplifies and if you see a product, because there it was only ten microbes, ten pathogens, with let us say one gene each. So, we had ten genes in the pool in our water, but now, you have amplified it to millions and millions. So, it is easier to see these millions. You can actually run it on a gel, gel electrophoresis; we will talk about this technique. So, relax. So, you can run it on a gel and see whether the PCR product is present or not, but there are shortcomings for these molecular biology techniques as well.

It is not quantitative you know. It is present, you can do a presence of, but you do know how much and then, you have to concentrate the sample, especially in case of drinking water, you know it is such a sparse sample. It is not like waste water, it is not very rich, and it is an oligotrophic environment. Oligo means less food environment and then, next step is DNA extraction. Lot of pathogens can be lost in the step, when we are actually extracting the DNA, then, we have the issue of PCR inhibition, which is, let us say, we took our sample, we concentrated it, we filtered it and we got our microbes on the filter paper.

If we extracted DNA from it and now, we are trying to amplify the genes of interest and maybe, there are some molecules present like EDTA, which are inhibiting the PCR and I get a false negative and it, pathogen is actually present and then, here is another thing, let us say, there are ten microbes of which five are dead, ten pathogens of which five are dead, the DNA extraction step will take all the DNA that is present and these five of my pathogens also have intact DNA, that will also be taken up in the DNA extraction step.

So, in the PCR, when they are amplifying the gene of interest, all of them will give a signal. So, here is a room for over estimation of the actual pathogens and then, the other thing is, this is very expensive. I can tell you, I do this and it is really consumables are very high in the terms of cost, and it requires specialized expertise, special rooms. The way it works is, first you filter your sample.

(Refer Slide Time: 33:29)



So, this is filtering and then you extract the DNA, and then, you can do different kinds of separations to separate your DNA of interest and then, amplify it or you can skip this step altogether. So, this is all for this lecture, my dear students. In the next lecture, we will look at some new and modern molecular techniques that we have, apart from PCR.

We will go into quantitative polymerase chain reaction and we will go into automated systems, that actually detect, we do not have to do anything, just put in the water, it will extract the DNA, it will do the analysis and it will give you a result, which makes things really easy, especially in areas where we do not have very trained personals and that is all for today then.

Thank you very much.