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>> Michael: Good morning, everybody. Viral Replication Cycle How HIV Replicates and How Drugs Work to Control It will start in five minutes. Good day, everyone, with this to Viral Replication Cycle How HIV Replicates and Drugs Work to Control It. I will be the moderator for today's session. The viral replication cycle webinar will be posted on the CATIE website and part of the building Block series. Before we begin I would like to go over a few tips. If you can't hear the audio then hopefully you can see the slide and dial into the audio portion using number listed at the top of the slide. Secondly, you are called in and using a speaker phone to listen to the audio and have any difficulties, try muting your microphone and finally, if you have any technical difficulties that you can always call the Webex customer support line listed in the bottom right-hand slide. For the purposes of accessibility this webinar will

be processed in real-time in process called Communication Access Real-time Translation. This is shown on the bottom right-hand side of your screen in a panel called media viewer. To resize the media viewer move your curser to the top of the title bar until it changes shape. Then drag up or down. So before we start the presentation I just like to remind everyone that this webinar will be recorded and made available on CATIE website soon after the webinar has concluded. Okay. Thank you everyone for attending. It is my pleasure to introduce you to the presenter for this presentation on viral replication cycle and I would like to introduce you to Thomas Egdorf, Regional Health Education Coordinator at CATIE for the Atlantic region. Thomas Egdorf has been at CATIE for two and a half years now and has been at HIV sector for almost 27 years. Here you go, Thomas.

>> Tom: Thank you, Michael. Why is it important to understand the HIV viral replication cycle? We want to build an understanding how HIV works in the body, increase knowledge of the drug classes and understand how the medications work to prevent HIV replication. Our learning objectives are to name and describe the five steps of the viral replication cycle, name the three enzymes involved in viral replication and name the five drug classes and locate where they work in the replication cycle. But first let's look at viruses. Viruses

are tiny organisms that may lead to mild to severe illnesses in human, animals and plant. They may include flu or a cold to something more life threatening like HIV. Are viruses alive? Viruses by themselves are not alive. They cannot grow or multiply on their own and need to enter a human or animal cell and take over the cell to help them multiply. These viruses may also infect bacteria cells. The virus particle or the virion attack the cell and carry out their own life processes of multiplication and growth. An infected cell will produce viral particles instead of usual its products. How big are viruses? Pictured is a graphic illustration of the size of viruses. I hope it is clear on your screen. The virus particles are 100 times smaller than a single bacterial cell. Bacterial cell alone is more than ten times smaller than a human cell and human cell is ten times smaller than the diameter of a single human hair. We can see pictured here the blue sphere is a rain droplet and viruses are the very last little circle on the screen. The structure of a virus, virion or virus particle has three main parts. The nucleic acid. This is the core of the virus with the DNA or RNA or deoxyribonucleic acid or ribonucleic respectively. The DNA or RNA holds all of the information for the virus and that makes it unique and helps it multiply. The protein coat or capsid, this is the covering over the nucleic acid that protects it. Labeled in the image as nucleocapsid top right of this image. The lipid membrane or envelope. This covers the capsid. Many viruses do not have this envelope and are called naked viruses. On this diagram it is called a viral envelope. What is the difference between virus and retrovirus. Simply a retrovirus or a group of viruses so retroviruses have special characteristics which are not seen in viruses. Virus contains genetic material as DNA and RNA but retrovirus contains only RNA. What is the difference between virus and retrovirus? If the virus has DNA it inserts DNA into the host cell and it is integrated directly into the host genome at the lytic phase whereas retrovirus has RNA as genetic material and needs to convert RNA into DNA and into the host genome. So viruses have a transcription process whereas retroviruses have a reverse transcription process. This process will be explained further throughout the webinar. The second generation of the retrovirus may be different from the first generation because of the inaccuracy of the reverse transcription process. Whereas mostly the second generation of a regular virus is similar to the first generation genetically since the virus has a normal transcription process, which is a more accurate process than the reverse transcription. Because of the vast genetic change in second generation of retroviruses, treatments for diseases caused by them are difficult. More than the treatments for virus caused diseases. For an example HIV does not have such specific treatment whereas virus caused diseases have treatments such as rabies or influenza. Now, that we have looked at viruses, it is time to build an understanding of the immune system. The immune system is the body's defense against disease. It protects the body from disease causing germs such as bacteria, viruses, fungi and parasite as well as cancerous cells. Normally the immune system can tell the difference between what belongs in your body and what doesn't. It remembers previous encounters with disease causing germs. For example, the virus that causes the measles you had as a child and knows how to defend against these threats. It also learns how to respond to invaders it hasn't seen before by developing specific defenses against them. Where is our immune system located? On the outside of the body the skin is your immune system's first line of defense. It provides a physical barrier that keeps bacteria and viruses from getting inside the body. HIV, for instance, cannot be transmitted through healthy unbroken skin. However, HIV and other organisms can be transmitted through the body's mucous membranes. These are the wet linings of body cavities such as vagina, rectum and urethra, the pee hole in the penis or vulva. These tissues don't have to be damaged to be infected. HIV can infect cells in the lining of the vagina, rectum and penis, even if the tissues are healthy. Inside the body: when disease causing germs get into the body, the internal part of the immune system comes into play. Its job is to recognize intruders and enjoy them or suppress so they won't cause harm. The immune system involves white blood cells, lymph nodes and body tissues such as nostrils and inside of the intestines. The immune system also includes bone marrow, thymus, spleen and appendix and all of these play different roles in fighting infection. Lymphatic system seen here is especially important with HIV -- in people with HIV. Lymphatic system is made up of vessels that branch out into all parts of the body similar to the veins, arteries and capillaries that carry blood. Instead of blood, lymphatic vessels carry a clear watery fluid called lymph which carries fluid away from the cells. Lymphatic vessels pass through lymph nodes and tissues. Inside these nodes and tissues these cells of the immune system trap, filter and destroy foreign material including bacteria, viruses and other microbes. There are 500 to 1,000 lymph node and tissues scattered throughout the body. Large groups of lymph nodes are found in the neck, armpits and groin but the largest concentration is near the abdomen. Sometimes when you have an infection you can feel what people call swollen glands in some of these areas. These swellings are lymph nodes responding to unwanted germs. Each lymph node is densely packed with millions of immune cells that identify and destroy the microbes that cause disease. These infection fighting cells are known as white blood cells or leukocytes and they are the key players in the response of your immune system. Let's take a closer look. There are many different kinds of white blood cells. Don't worry too much if you can't keep them all apart. CD4 cells are very important for HIV positive people. The other types of white blood cells will rarely come up during doctor's visits. If you’re interested, this list should give you a quick sense of what’s what. Leukocytes are the biggest group including all types of white blood cells. Leuko means white and cytes means cells. T cells are identified by molecules called receptors on the surfaces. Different kinds of receptors carry out different functions. One particular kind of T cell, CD4 cell, is especially important for people with HIV. CD4 cells are named after a protein called CD4, which they carry on their surface like a fingerprint. CD4 cells lead the attack against infections. They release chemical messenger called cytokine which stimulate other immune cells to make antibodies or destroy infected cells. CD4 cells are sometimes compared to the quarterbacks of a football team or the conductors of an orchestra, because they direct the response of your immune -- of your body's immune system. CD8 cells are T cells that have a protein called CD8 on the surface. When CD8 cells recognize a specific infection such as HIV, they can develop into what are often referred to as killer T cells or CDL's cytotoxic lymphocytes and these killer T cells seek out and kill other cells that are already infected or cancerous. B cells make and release antibodies. Antibodies are a type of protein that can lock on to bacteria or viruses. When antibody locks on to a germ, it acts as a signal for other immune cells to destroy the invader. Each B cell is programed to make one specific antibody. For example, one B cell will make the antibody that blocks measles viruses, while another makes antibodies to the bacteria that cause pneumonia. Although the immune system produces antibodies against HIV, these antibodies don't protect the body from HIV infection. Macrophage are immune cells that perform many functions such as warning the immune system of invading microbes and helping to attack and destroy HIV infected cells and cancerous cells. Dendritic cells help alert the rest of the immune system to invading germs and help magnify the immune system response to germs. Natural killer cells are lymphocytes that help prevent the spread of infection by killing infected cells. They kill cancerous cells too. Natural killer cells also help direct the production of other immune cells. They may contribute substantially to the body's effort to control HIV. Now, let us focus on CD4 cells as they play an integral role in HIV replication. As you may know, HIV's primary target for infection are the CD4 cells. They play a central role in the immune system and help recognize infection. They also coordinate all the other parts of the immune system to provide an organized response. They're often described as quarterbacks or general's defense team or Army. If you're not big on sports or the military, you can just think of CD4 cells as central to fighting off germs. Only activated CD4 cells can be infected by HIV. Viral reservoir. HIV is chronic lifelong infection due to its ability to stay hidden within infected blood cells. These cellular reservoirs contain the genetic code of HIV. They remain invisible to the body's Immune defenses and are not sensitive to anti-HIV drugs. Latent reservoirs of HIV are located throughout the body and throughout the brain, lymphoid tissue, bone marrow and genital tract. These reservoirs persist even in the presence of anti-retroviral therapy, today's standard of care. Many people with HIV and their health care providers focus on two test results. The CD4 Count and the viral load. The CD4 cell count or simply CD4 count is a measure of the strength of the immune system. Normal CD4 cell count is generally between 500 and 1500 cells per cubic millimeter about the size of pin head of blood. As with other tests some CD4 counts may naturally fall above or below the values. The CD4 cell count also fluctuates depending on how active the immune system is when the test is taken. Even tests done just 12 hours apart from each other can give different values. If a single count seems out of line with previous values it may be worth repeating it. It is also a good idea not to measure your CD4 cell count if you are feeling unwell or were recently vaccinated. Your immune response to the infection or vaccination may make your CD4 cell count temporarily high or low. The viral load is a measure of the amount of HIV in your blood. The viral load test measures the number of copies of HIV in milliliters. About a tea responsible of blood. In untreated individuals it varies widely. Some people have viral loads of only a few hundred while others may have viral loads greater than 100,000. Perhaps more than any other test the viral load test may show significant changes over time in untreated people. With this test it is especially important not to focus on any one member but to look at the trend over time. Generally speaking the higher the viral load the faster HIV will disable the immune system. For this reason high viral load may prompt a doctor to suggest beginning anti-HIV drugs sooner than if you have a low viral load. If you are in the acute phase of HIV infection, the viral load will tend to be very high. For more information on the relationship between CD4

counts and viral load in untreated HIV, please view the webinar titled HIV basics. Part of this building Block's webinar series. So far we have talked about viruses and the immune system. Now we will play a short video developed by CATIE to explore the HIV replication process and how medications work to stop HIV replication. This will be available for viewing on the CATIE website in the coming weeks as a stand-alone video and as a part of this webinar.
[ Movie being played. ]

>> Speaker: Welcome to video presentation of the HIV viral replication cycle brought to you by CATIE, Canada's source for HIV and hepatitis C information. Section one. What is HIV? Here we see a reputation of HIV. HIV is a virus that affects humans. Acronym HIV stand for human immunodeficiency virus. Here is simplified picture of the virus. HIV has receptors on the surface represented by receptors fuse into host cell receptors and then HIV merges into the host cell. In the middle of the picture there are two squiggly lines. These represent the viral genetic material called RNA. HIV is a virus that weakens your immune system which is the internal system in the disease and infection. HIV can avoid being cleared by your system and actually infect and kill certain immune cells. If your immune system becomes weak enough you can become sick

from other infections and possibly die. Section two. What is CD4 cell? Pictured here is CD4 cell. It is a type of white blood cell found in the immune system. Different kinds of white blood cells work as a team to recognize and destroy intruding germs and play a central role in the immune system. They're often described as the quarterbacks or the generals of the bodies defense team or Army. If you're not big on sports or the military, you can just think of CD4 cells as central to fighting off germs. CD4 cells help recognize infection. They also coordinate other cells of the immune system to provide an organized response. On the outside of CD4 cells are receptors. CD4 cells have a CD4 receptor, hence the name CD4 cell, and co-receptors called CCR5 and CXCR4. Like all cells CD4s have a nucleus at the center. The nucleus houses human genetic material called DNA which is the genetic code that tells the cell what to do and to do it. Section three overview. Demonstrated here we see HIV attaching to the CD4 cell. Once HIV has attached on to the CD4 cell it fuses to the immune cell and releases its RNA and three enzymes into the cell. From there a process called reverse transcription takes place changing the viral RNA into DNA. At this point HIV is able to move to the nucleus of the cell and integrate into DNA. And now ceases to coordinate other immune cells and instead becomes a virus making factory. When HIV replicates it makes long strands of viral material that needs to be cleaved and cut into smaller pieces that then make up new viruses. These new and immature viruses butt out of the cell and eventually killing the cell. When the new viable viruses mature they go on to infect other CD4 cells. Section four. Detailed replication cycle. So now that we have seen the HIV replication cycle, we are going to go through it a second time and add more terminology and details. The first phase HIV infecting a cell is called entry. This is where HIV attaches using two receptors. The CD4 receptor and either the CCR5 or CXCR4 co-receptors. HIV needs both the CD4 receptor and co-receptor in order to enter the cell. Once HIV has connected with the CD4 cell it pulls itself closer to the cell. Fuses with the cell and through a complicated process releases its RNA into the cell. Once the RNA enzymes are in the cell it moves to the second process called reverse transcription. This process uses enzyme called reverse transcriptase to see the single strand RNA seen here into double strand DNA and newly formed virus DNA now enters the nucleus of the cell and integrates itself into the CD4 cells DNA using the assign this phase and this phase is called integration. At this point the cell ceases to be functional and must be cleaved or cut in the enzyme protease into shorter strands that become individual HIV virion. This phase is called assembly. Shortened strands move to the surface of the cell and butt out and become new HIV. After the budding out and will be maturation. HIV will use the cell to make numerous copies of itself. Some will die off quickly while others will mature to a form that can infect other CD4 cells. HIV does not have very good proofreading ability and tends to make many mistakes while replicating its viral material. Section five medication. So far we have talked about HIV, CD4 cells and replication process. It is now time to talk about the anti-HIV medications or drugs and where in the replication process they work or inhibit replication. HIV medication prevents HIV from reproducing. It does not kill HIV directly. Going through the HIV replication cycle in order again we start with the medication class called entry inhibitors. There are currently two classes of drugs that interfere with HIV attaching to and fusing with the CD4 cell called fusion inhibitor and co-receptor inhibitors. The co-receptor inhibitors Block CCR5. In the reverse transcription phase where viral RNA turns into DNA there are two classes of drugs called nukes and non-nukes or to be very technical nucleoside transverse -- nucleoside reverse transcriptase inhibitors and non-nucleoside transverse inhibitors and process of changing RNA into DNA and without this change HIV cannot incorporate itself into the human DNA. The next class of drugs we are going to explore are called integrase inhibitors. And they work by blocking the viral DNA from incorporating into the cellular DNA. The next phase is replication cycle assembly is where protease inhibitors do their work and block the cleaving or cutting process from happening making it impossible for the long viral proteins or chains to become viruses. The final drug class works to prevent HIV from maturing and thus infecting other cells. This class is called maturation inhibitors. There are currently no medications licensed in this class but research continues. And that is the HIV replication cycle. Please remember decisions about particular medical treatments should always be -- consultation with qualified medical practitioner knowledgeable about HIV related illnesses and treatment. For more information with topic or other related HIV and hepatitis C topics please visit us at www.catie.ca or give us a call at 1-800-263-1638. Thank you for taking your time to watch this video.

>> Tom: Again, we want to apologize for the technical difficulties playing the video. By the end of today the video will be posted on our website and I have assurances from our technical staff that it will run properly and as well, it will be corrected in the posting of this webinar so it will play correctly in this webinar. Now to summarize a little bit now that we have reviewed

the HIV viral replication video, it is time for a short recap. Starting with the first step entry and the drug classes entry inhibitors and fusion inhibitors and note that there is no enzyme is at play at this point and we have reverse transcription set and two drug classes that work here are nukes and non-nukes or for those that are technically inclined nucleoside reverse transcriptase inhibitors or non-nucleoside transcriptase inhibitors. And HIV entering into DNA is integration using the enzyme integrase to complete the process. Integration inhibitors block the integration process. Once the cell is taken over by HIV it moves into the production step using the enzyme protease and the medication used is protease inhibitors and maturation which does not have maturation on market at present and again does not use enzyme at this stage. And here we have the chart fully filled in with the steps, enzymes and corresponding medical classes. Pictured here is HIV budding process where HIV emerges from a CD4 cell. As you can see there are many copies of HIV budding at any one time. Control versus cure. As we have seen earlier in this webinar retro viruses through the process of reverse transcription make mistakes in replication and this makes a vaccine very difficult to develop. We also spoke to HIV reservoirs in resting CD4 cells and that anti-retro viral treatment does not reach the reservoirs. And through the video we have seen where HIV medications work to interfere with the replication process of HIV. The medications do not kill HIV but make it difficult to reproduce and spread. This is why all the drug classes have the term inhibitor in the title. They inhibit the replication, not kill the virus. And to recap, I hope through participating in this webinar you have increased your understanding of the five steps of the replication cycle, three enzymes involved in viral replication and have a better understanding of the five drug classes and how they work.

>> Speaker: Thank you very much, Thomas, to this presentation and once again we would like to excuse ourselves for the quality of the animation that was part of this presentation. We are assured that it will be repaired and webinar will be up later today. For those of you that have any questions feel free to type your questions and comments on the left side of your screen and also you can dial pound six to un-mute your line. We don't have any questions at this time. Thank you very much for the patience and participating in this webinar and once again the corrected version of the animation will be online later today. Thank you and have a good day.

the proceedings.