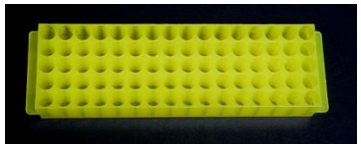
FLOW SHEET #	01	SCENARIO #	01	DATE	01/03/2016	PERSONNEL	Sergeeva Zhanna, Chaban Nikolay
TITLE	**PCR script performance description** *All manipulations are carried out in a specially equipped laboratory (in a laminar flow cabinet equipped with an UV lamp), in medical overalls, and be sure to wear gloves.*						
SOURCES/ REFS	https://www.youtube.com/watch?v=7jf_zNvK6Yw&t=150s (*YouTube* podcast: PCR mixture preparation) https://github.com/EMICVL/ONU_Team_25.01.17/blob/master/PCR_script.pdf (*GitHub: written script*) https://github.com/EMICVL/ONU_Team_25.01.17/blob/master/PCR_flowchart.pdf (*GitHub*: Flow Chart) https://www.youtube.com/watch?v=cvxHjyRe78M (Simulation: Video simulation)						

PODCAST TIME (min:s)	ACTION	OBJECTS		PHOTOS OF OBJECTS	POSSIBLE ERRORS	NOTES/ SCREENTEXT /GRAPHICS
SCENE #	**1**	**1. DNA isolation**				
0:00	1.1. Number and position properly in the rack clean polypropylene Eppendorf tubes 1.5 ml. Fix in the protocol.	Eppendorf tubes  The rack 			Be sure to number the tubes!	

		Marker				
0:15	1.2. According to the corresponding optimal method chosen to isolate DNA carry out an extraction. (kit, heat lysis)	DNA isolation kit Pipets and tips Centrifuge		  		

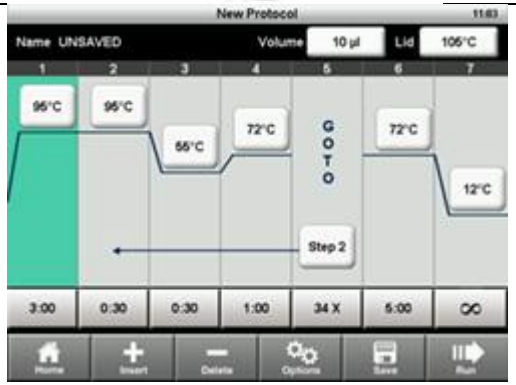
		Termo Heater				
0:45	1.3. A solution of the purified DNA can be stored at -18 ...- 20 ° C for two weeks.	Tubes with DNA Freezer		 		
SCENE #	**2**	**2. PCR performance (amplification)**				
0:50	2.1. Prepare and number thin-walled tubes for amplification (0.5 ml or 0.2 ml) according to the				Be sure to number the tubes!	

	number of samples analyzed for pathogen DNA presence of. Prepare and label the tubes for the positive (marked "C+") and negative (marked "C-", “no template control”) controls.	Thin-walled tubes for amplification Marker		 		
0:55	2.2. Approximately 30 minutes before the preparation of the working amplification PCR mixture thaw reagents on an ice bath at room temperature.	Ice bath				

0:58	2.3. Add to tubes necessary amounts of amplification reagents to the final volume of 20 µl. (determined empirically for max number of amplicons of predetermined length, to avoid non-specific products)	Pipets and tips -DH_2O -10xPCR buffer -2mM deoxynucleoside triphosphate dNTPs - 10 mM of each pair of primers - 50 mM Mg^{2+} -Taq-polymerase, 50 g/ml (2 AU)		 	Be sure to choose the right volume pipets, set the needed volume and constantly change tips!	While the preparation of the reaction mixture, it is necessary to add all the components with individual tips!

3:25	2.4. After the addition of Taq-polymerase, which is added the last, the mixture must be stirred thoroughly by pipetting or vortexing (3-5 s).	Pipets and tips Vortex		 		
3:35	2.5. Add 5 µl of the sample DNA to the appropriate tube. Mix.	Pipets and tips Tubes with DNA		 	Be sure to add DNA with sterile tips!	

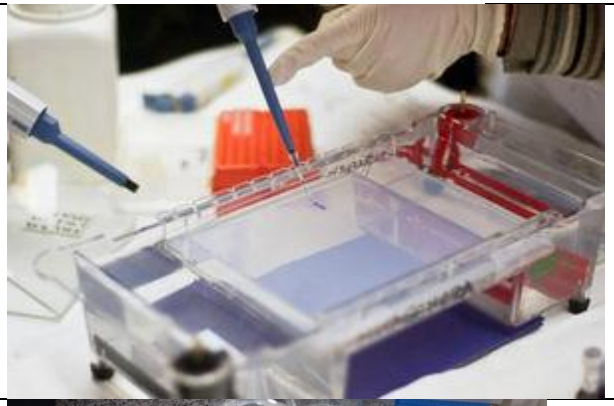
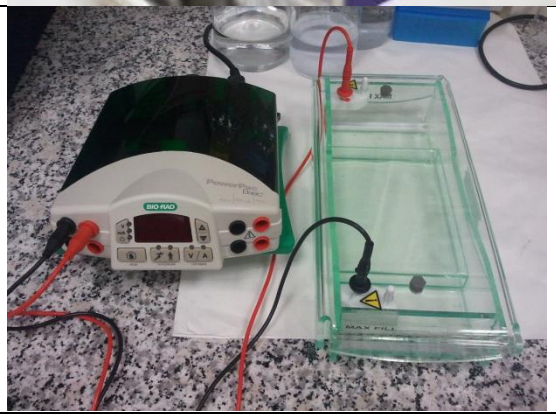
3:45	2.6. Add 5 µl of the corresponding positive control DNA sample to tubes for positive control samples, and all reaction components except DNA to the test tube for the negative control sample.	Positive and negative control samples		 		
3:55	2.7. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture in all tubes with 1 drop (about 25-30 L) of mineral oil.	Pipets and tips Mineral oil			Be sure to add mineral oil with sterile tips!	

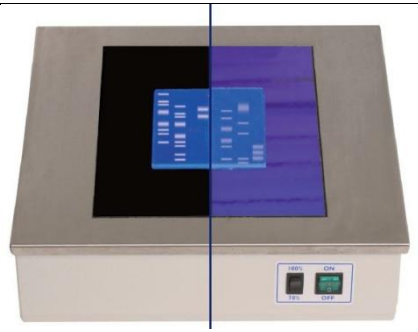
4:05	2.8. Close the tubes and move them to a thermal cycler.	Thermal cycler				
4:12	2.9. Apply the appropriate program on the 35-40 cycles.	Program protocol			Be sure to chose the right program!	Depending on the primer chain annealing can take up to 10 minutes, the last stage about 7-10 minutes 306 bp IS1081 1 min 94 ° C (1st cycle of 4 min) denaturation 1.5 min, 68 ° C annealing 2min 72 ° C (the 35th cycle of 10 min) extension 4 ° C ∞ BW- 6 (5` CGA

						CAC CGA GCA GCT TCT GGC TG 3`) BW -7 (5` GTC GGC ACC ACG CTG GCT AGT G 3`)
SCENE #	**3**	**3. The detection of PCR products** Separation of the amplification products by horizontal electrophoresis.				
4:25	3.1. Fill the electrophoresis camera with 1xTAE buffer, prepared in distilled water by diluting 50xTAE 50 times.	The electrophoresis camera TAE buffer		 		

4:30	3.2. Add 2.0 g of agarose to 2 ml of 50x TAE buffer and 100 ml of distilled water.	Agarose Weighing-machine Flask Distilled water		   		

4:40	3.3. Melt the obtained mixture. Add 10 µl of a 1% ethidium bromide solution to 100 ml of agarose. Mix.	Heater Ethidium bromide solution		 		
4:50	3.4. Cool the agarose to a temperature of 50-60 °C and pour into a gel casting. For agarose gel samples pockets set the comb. After solidification of the agarose carefully remove the comb from the gel and transferee gel to the electrophoresis chamber.	The gel casting The comb				

5:05	3.5. Add dye to the samples.	DNA dye				
5:15	3.6. Add 10-15 µl of samples to the gel in accordance with the protocol. Add positive and negative controls and molecular weight marker. Fix the scheme.	Samples				
5:22	3.7. Connect the electrophoresis chamber to the power supply and set the voltage corresponding to the electric field of 10-15 V/1 cm^2 of gel.	The power supply			Perform electrophoretic separation of the amplification products in the direction from the cathode (-) to the anode (+). Control	Control of the electrophoretic separation is carried out visually by the movement of the dye band. Band dye must pass from the start 1,5-2 sm.

					the phoresis time.	
5:30	3.8. To visualize the results of the electrophoresis transfer gel to the UV glass transilluminator.	The UV glass transilluminator. Nitrile gloves		 		WARNING! Agarose gel should be contact with only in nitrile gloves. Ethidium bromide is a potential mutagen.
5:45	3.9. Analyze the results of the analysis. DNA fragments analyzed appear as red-orange luminescent bands upon irradiation with UV radiation with a wavelength of 310 nm.	Photo camera			Be sure to record the results.	Accounting to the presence or absence of amplicons of the given size. - the absence of orange-red color stripes strictly at the level of the positive control (PC) indicates the absence of the pathogen DNA in the sample; -presence of

						the band corresponding to the electrophoretic mobility of the positive control indicates the presence of the pathogen DNA.
END						
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