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Isolation of DNA from spots of old microscopic glass slides with mini column isolation kit for molecular analysis

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ABSTRACT

Aim: Many institutes have a number of old fixed slides for various pathogens. These can serve as the source of DNA reference material as well as conducting the prevalence studies of a pathogen. Moreover, such blood smeared slides can be shipped at room temperature in letter through postal service for molecular diagnostics. But there is no standard and reliable method in the literature on how to isolate DNA from them. Therefore, we decided to develop and standardize the method for DNA isolation from such spots and smears of glass slides. **Methods:** Two different pathogens like *Anaplasma phytocytophilum* and Echinococcus slides were used as examples. The isolations were done with DNA isolation kit (mini column). Real time and conventional PCR assays were used to confirm the isolations of these pathogens. **Results:** The successful isolations of these pathogens were done with mini column kit. The confirmations of these isolations were achieved with pathogen specific real time and conventional PCR assays. Isolated DNA was stored at -200C for more than 3 years. **Conclusion:** We have demonstrated a robust and reproducible DNA isolation method from spots of fixed glass slides for two pathogens as examples. Such slides can be shipped at room temperature with the postal service. This method opens the new opportunities for conducting the molecular analysis of different pathogens like Mycobacterium, blood parasite like *Plasmodium*, *Anaplasma*, *Babesia* as well as for forensic molecular analysis in different laboratories from smears of microscopic slides worldwide.

Introduction

Isolation of nucleic acid is an essential step for conducting the different nucleic acid analysis methods like PCR and hybridization for various viral, bacterial, biomarkers and genetic targets [1-4]. Not only this, but the nucleic acid can also be used as therapeutic as well as vaccine options in many clinical settings, therefore there is an urgent need to isolate the sufficient amount of nucleic acid in highly pure form [5- 8] The nucleic acid can be isolated from different sources e.g., blood, tissue, buccal swabs, nasal samples, plasma, serum, urine and cell cultures. There are different methods available to isolate such nucleic acid, which are magnetic beads, solutions-based and mini column methods for example [9, 10].

Many institutes have old, fixed slides for microbiological purposes. These slides have valuable material of a particular pathogen, which can be used for molecular analysis e.g., as reference control while doing conventional as well as real time

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PCR along with gene sequencing. The problem is that the user has fixed slides with spots and there is no standard protocol available for isolation of DNA acid from such spots as well as smears from these slides.

In 1992 attempts have been made to isolate the DNA from slide smears. [11] Museums have a lot of archival slides, where isolated DNA is needed, and attempts are being made to perform such isolations [12]. A research group from Cuba used different isolations methods for detection Mycobacterium leprae and compared the results and recommended that such slides can be sent to reference laboratory for molecular testing [13]. Similarly, there are a lot of attempts made to isolate the DNA from smears of slides for molecular detection of Mycobacterium tuberculosis [14].

Forensic analysis is another large field, where molecular analysis needs to be conducted on old slides, therefore a group of research conducted STR (also called microsatellites) profiling from histological slides [15].

Similarly, there are blood pathogens, where the blood smears are made to do microscopic analysis, but there is a big scope to reconfirm the results with molecular methods. Such types of attempts are going for detection of *Plasmodium* as well as Mycobacterium in some institutes worldwide [13, 14]. Such ideas can strengthen the diagnostic capabilities of many laboratories, as accurate diagnostic can lead to better monitoring and controlling the pathogens through preventive as well as therapeutic options.

In literature, there is no accurate step by step method written on how to isolate DNA from blood parasite or digestive tract parasites as user has to conduct a large number of experiments to establish the needed method. Therefore, our aim was to develop a first step by step method to isolate DNA with Genekam DNA isolation (mini column) kit to perform molecular detection.

Materials and methods

A. Two slides, one for *Anaplasma phytocytophilum* and another for Echinococcus were used for isolation of DNA. Material used were Genekam Minicolumn kit (Genekam Biotechnology AG), pipettor, centrifuge and heating block to isolate the DNA.

Steps

1. An Edding pen was used to mark the glass slide spot from which DNA is to be isolated but from the other side.

2. The heating block was turned on, where the temperature to 56 °C was adjusted.

3. The slide was put on a heating block while its marked area touching the heating block.

4. A microtube with 300 µl of lysis buffer (Tube A) with proteinase K was prepared and labelled it as tube 1. It was kept at 56 °C.

5. 20 µl of lysis buffer (Tube A) from Genekam DNA isolation kit was added on the spot and kept it for 5 minutes at heating block at 56 °C. The time may vary between 5-10 minutes, but care was taken that lysis buffer should not dry up.

6. The pipettor with pipette tips with filter (10-200 µl) was used to scrape off the spot with it. Usually, spots are dissolved in lysis buffer partial or whole. The fluid from the spot out was transferred into tube 1.

Steps 5 and 6 were repeated to get maximum yield.

7. Tube 1 was kept for 20-30 minutes in the heating block at 56 °C.

8. 400 µl of tube G (second lysis buffer) was added to tube 1 and kept it for 5 minutes at 70°C in the heating block.

Second microtube called EL was labelled and 150 µl of Tube E (elution buffer) was added to it. It was kept at 70°C till its use.

9. Tube 1 from heating block was taken out. 400 µl of molecular ethanol was added into this tube.

10. The mini column with receiver tube was labelled as MC1 and 600 µl of fluid from tube 1 was added into it. It was centrifuged at 10000 RPM for 1 minute. The fluid was discarded.

-Repeat step 9 was repeated, till there was no fluid left after the above step in MC1 now in tube 1.

11. 500 µl of tube B (washing buffer 1) was added and it was centrifuged at 10000 RPM for 1 minute. The fluid from MC1 was discarded.

12. 500 µl of tube C (washing buffer 2) was added and it was centrifuged at 10000 RPM for 1 minute. The fluid from MC1 was discarded.

13. 200 µl of tube C (washing buffer 2) was added and it was centrifuged at 10000 RPM for 1 minute. The fluid from MC1 was discarded.

Now the mini column should be dry.

14. MC1 was centrifuged at 13000 RPM for 3 minutes to remove the rest of fluid in it, if any. The fluid was discarded.

15. A new collection tube called COL 1 was labelled. The mini column MC1 was put in this tube now. To this mini column, add 100 µl from microtube called EL was added.

16. This was kept at room temperature for 2 minutes. It was centrifuged at 13000 rpm for one minute to collect isolated DNA. The microtube COL1 has now isolated DNA, which was used for different analysis.

17. The isolated DNA was stored at -20°C for further use.

Two (2) different slides for different pathogens were used to isolate the DNA.

B. The Control of successful DNA isolation was done through the pathogen specific PCR testing with the help of ready to use PCR kits for different pathogens as examples: Echinococcus was detected with Genekam PCR Kit number K065, where 2 µl of isolated DNA was added to 18 µl solution to have 20 µl total solution, which was subjected to thermocycling in thermocycler (Biometra, Germany). The full protocol can be obtained from the manufacturer. The results were seen in ethidium bromide stained 2% gel agarose (Carl Roth, Germany). The pathogen specific bands were observed at 200 bp in positive samples, but no band in the negative control was found.

Isolated DNA from *Anaplasma phytocytophilum* slides were tested with real time PCR kit number FR054 (Genekam Biotechnology AG) on ABI7500 (Thermofischer, Germany). Total volume of 20 µl per test was used, where 2 µl was pathogenic specific DNA from the above shown isolation. Thermocycling conditions were as follows: 15 seconds at 90 °C and 60 seconds at 60 °C 0C for 40 cycles and manual can be obtained from the manufacturer for exact instructions. The results were obtained as Ct values in form of smooth curves while doing the baseline subtraction in order to remove the noise and to have the baseline at the same level in all positive samples and there were no curves in negative controls.

The different concentrations of DNA were used to find how concentrated isolated was, with above mentioned PCR methods. These serial dilutions were 10-fold dilutions till 1:100.

Results

There are around 11 experiments (6 for Echinococcus and 5 for *Anaplasma phytocytophilum*) conducted to do the DNA isolation from different slides. The slides for Echinococcus used here were for conducting the fluorescent assays, therefore there were small holes (12 spots) with fixed antigen. The slide for *Anaplasma* has only 2 big spots for conducting the fluorescent microscopy. The isolations of DNA were done over the period over 3 years successfully. The results are shown in **table (1),** where it is shown that there was successful isolation in all experiments.

Serial dilutions show that there was the presence of pathogen in all dilutions.

Real time PCR tests for *Anaplasma phytocytophilum* were positive for all DNA isolations, which shows that DNA was successful in each isolation **(Picture 1).** Similarly, Echinococcus PCR test were positive, which indicate the isolations were isolation.

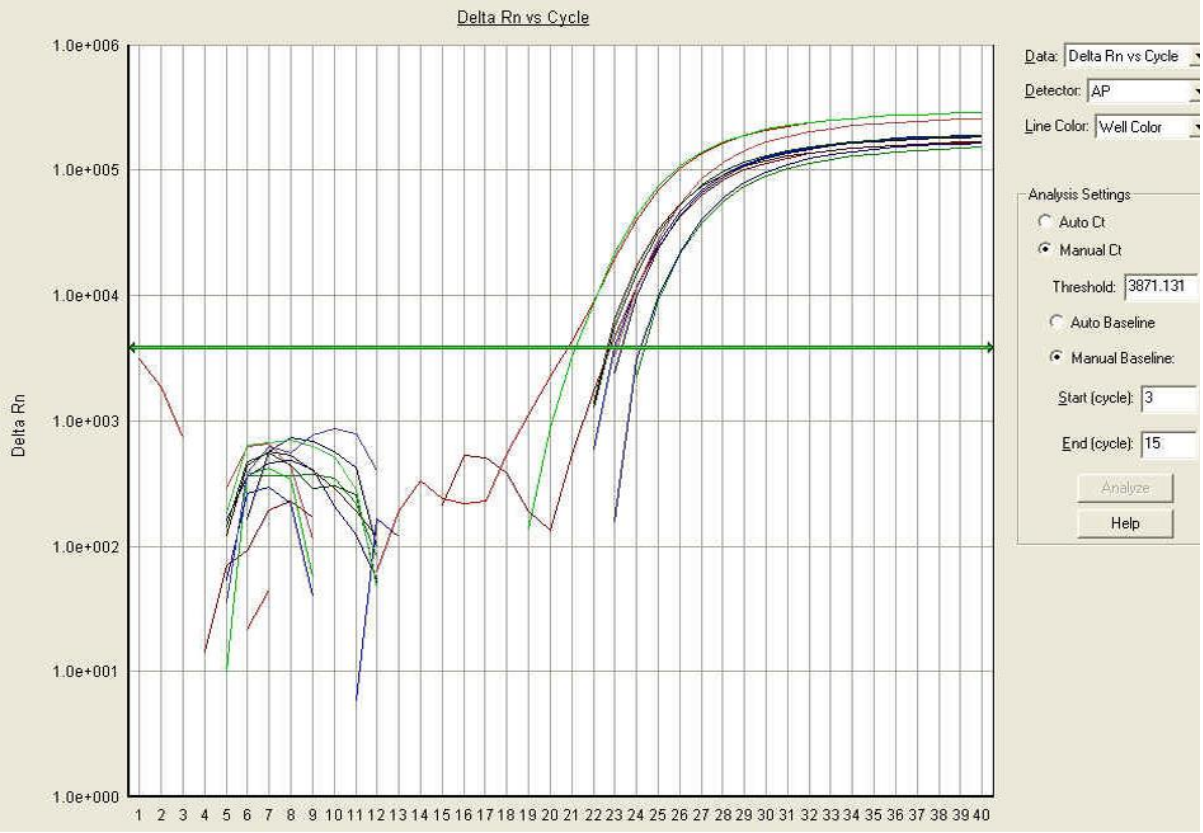
The isolated DNA samples were stored for at -20 0C for more than 3 years and they were functioning still. These isolated DNA were used to validate the studies for development of different ready to use PCR kits. (Data not shown here as it will be published in different publications)

The isolation steps about scientific explanation are shown as picture too, so that user can understand the steps of the method **(Picture 2).**

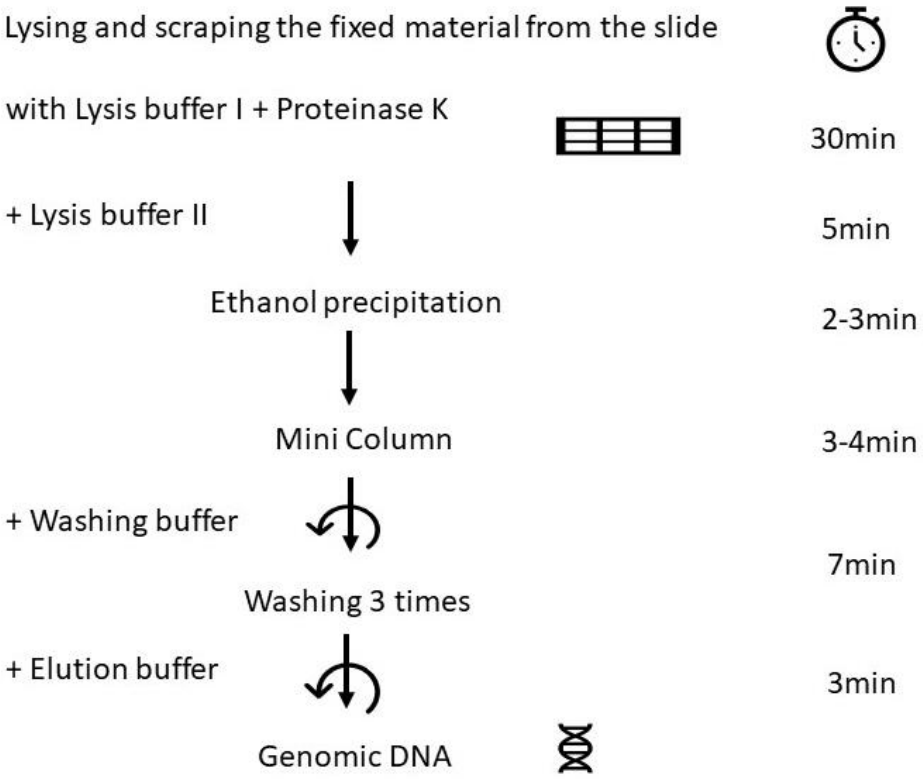
Table 1. Showing the results of successful DNA isolation from the spots of microscopic slides.

Experiment Number	Pathogene name	DNA isolation	Real time PCR	Conventional PCR
1	*Anaplasma phytocytophilum*	successful	+	
2	*Anaplasma phytocytophilum*	successful	+	
3	*Anaplasma phytocytophilum*	successful	+	
4	*Anaplasma phytocytophilum*	successful	+	
5	*Anaplasma phytocytophilum*	successful	+	
6	Echnicoccus	successful		+
7	Echnicoccus	successful		+
8	Echnicoccus	successful		+
9	Echnicoccus	successful		+
10	Echnicoccus	successful		+
11	Echnicoccus	successful		+
12	Echnicoccus	successful		+

Picture1. Showing the results of different concentrations of isolations from *Anaplasma phytocytophilum* in real time PCR.



Picture 2. Shows the scheme of isolation method of almost 50-55 minutes.



Discussion

In this research work, we have shown a reproducible and robust method to do successful isolation of DNA from spots of glass slides in order to conduct the conventional as well as real time PCR. In this work, the method has been written step by step so that any reader of this publication can copy this method successfully from a smear or spot from glass slide.

For blood parasites, this method may be used to reconfirm the microscopic results with a molecular test. The blood smear on glass slides is a standard method for detection of parasites for last many years. These slides can be sent long distances with normal postal service. Other groups are already working to develop such solutions for molecular detection of bacterial pathogens like Mycobacterium and blood parasite like *Plasmodium*. This is opening the doors for a new method to transport the slides from different areas at room temperature. The slides can be fixed to inactivate the pathogens and fixed slides can be stored for many years as in this method, very old, fixed slides are used over a long period of time. [13,14, 16]

Many pathological departments and microbiological departments have old, fixed glass slides; hence DNA can be isolated from them with our method to conduct the molecular studies to see the presence of a pathogen as well as genetic studies. Till we rarely found that there was any problem with any of pathogen DNA isolation from slides, however we faced some issues with some mounted histological fixed slides with human tissues, but for RNA isolation. (data not shown)

This method provides sufficient amount of isolated DNA e.g., 100 µl per isolation, which is sufficient to conduct a number of PCR assays as in our case, it was 50 reactions. But isolated DNA can be diluted, if the isolated DNA is concentrated. It is possible that dilutions up to 1:100 was possible. Not only this, it is shown in this work that one can isolate DNA from spot of a glass slide multiple times.

In our laboratory, we have used this method for DNA isolation from microscopic glass slides, which are many years old, very successfully. Sometimes, many laboratories have slides with fixed material for conducting the fluorescent microscopy or slides for conducting student practical. We have put this isolation protocol step by

step on a website, where users can download the protocol also [17].

Conclusion: We have shown here a step-by-step method how to isolate DNA from old, fixed slides, which were developed for fluorescent microscope. *Anaplasma phytocytophilum* and Echinococcus specific slides were used to obtain their DNA successfully with mini column isolation method and isolated DNA samples were confirmed with pathogen specific real time as well as conventional PCR tests. This method is opening new doors to isolate the pathogen specific DNA from different slides, which are available in many pathology and microbiology departments in many universities in the whole world. Similarly fixed smeared slides for different pathogens can be shipped easily to have molecular confirmation at room temperature.

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Conflict of interest

There are no such conflict.

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