Evaluation of three DNA extraction techniques in molecular diagnosis of toxoplasmosis

Introduction

- Conventional laboratory diagnosis of toxoplasmosis is based on the presence of IgM and IgG anti-*Toxoplasma gondii* antibodies; however molecular techniques have emerged as alternative tools due to their increased sensitivity [(1)]

- To perform a sensitive, specific, and reliable PCR-based diagnostic test, the availability of pure DNA lacking PCR inhibitors as well as a rapid and easy to perform DNA extraction protocol are essential [(2)]

- Different DNA extraction techniques had been employed by different researchers for molecular diagnosis of parasitic infections[3]

- QIAamp DNA mini, MagNa pure kits, phenol chloroform, boiling , lysis and centrifugation methods had been using to extract *Toxoplasma* DNA[2,4]


objectives

Aim of the study

To:

1) compare the performance of three DNA extraction techniques: (1) dried blood spot DNA isolation kit,2) genomic DNA isolation kit and 3)FTA elute cards).

2) assess the sensitivity of my Taq blood PCR kit for the molecular diagnosis of toxoplasmosis.

Subjects and methods

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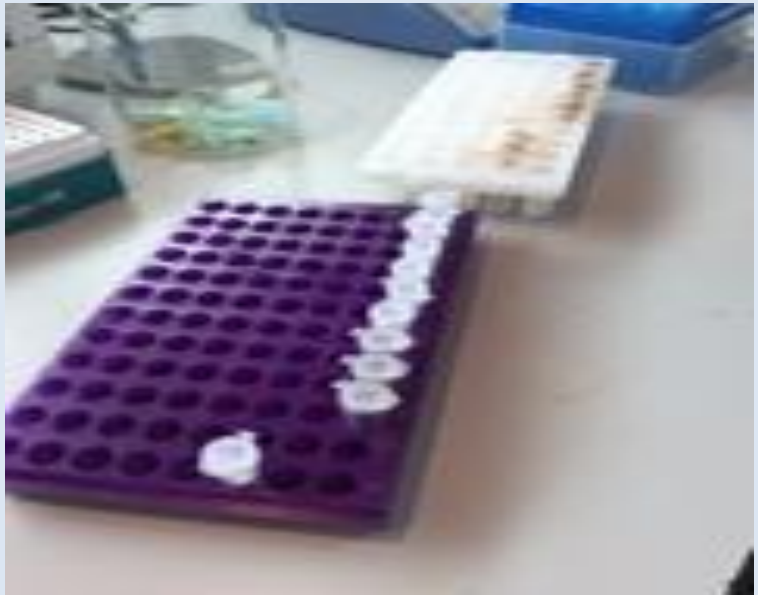
- This study was carried out in the city of Dubai-UAE at the municipality clinic with ninety male participants included in the study.
- Mean age (SD±) 34.544 ± 6.342.

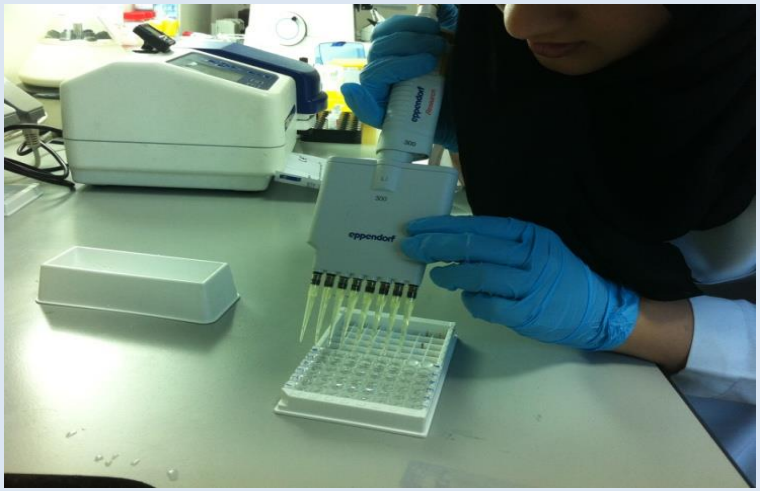
Subjects and methods (Cont.)

Blood samples were collected from participants and sera were screened by ELISA



ELISA

ELISA test kits (Diagnostic automation company-USA – Product # 1102z for detection of IgM Anti-*Toxoplasma* antibodies were used to screen all the test samples.

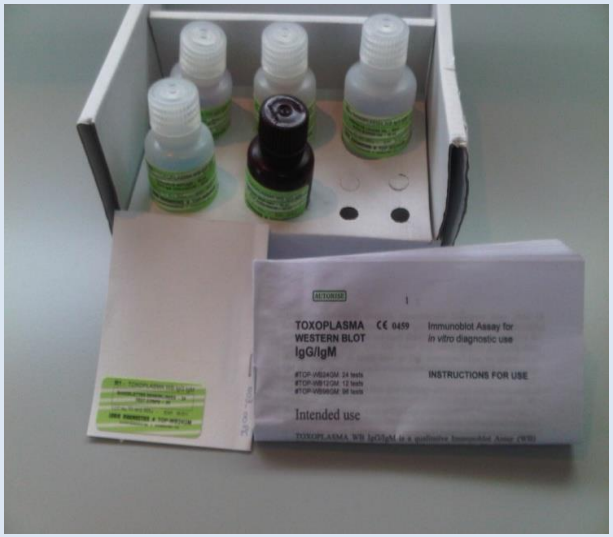


Western Blot (WB)

WB was used to confirm the ELISA screening results

(LDBIO diagnostics –France)

(Product # Top-WB24GM)



PCR

- PCR was done for all positive samples using extracted DNA by three methods.

- Another PCR run was done on positive samples using my Taq blood PCR kit.

DNA extraction using dried blood spot DNA isolation kit

- The kit (Norgen-Bioteck-Canada-Product # 36000) is designed for the rapid preparation of genomic DNA from dried blood spot.

- The blood samples were spotted on filter paper and steps of the kit protocol was followed.

DNA extraction using genomic DNA isolation kits

- The Kit (Norgen –Bioteck-Canada -Product # 24700) , is designed for the rapid preparation of genomic DNA from various samples.
- Purification is based on spin column chromatography using Norgen's resin as the separation matrix.
- Norgen's resin binds DNA under high salt concentrations and releases the bound DNA under low salt.

DNA extraction using Whatman FTA Elute

- Whatman FTA Elute (GE health care Life science , Product #, WB120412) is easy to use.
- It only requires a single drop of whole blood that can be directly spotted.
- Samples were collected, and stored at room temperature.
- Sample processing time to isolate DNA is 15 to 30 min.

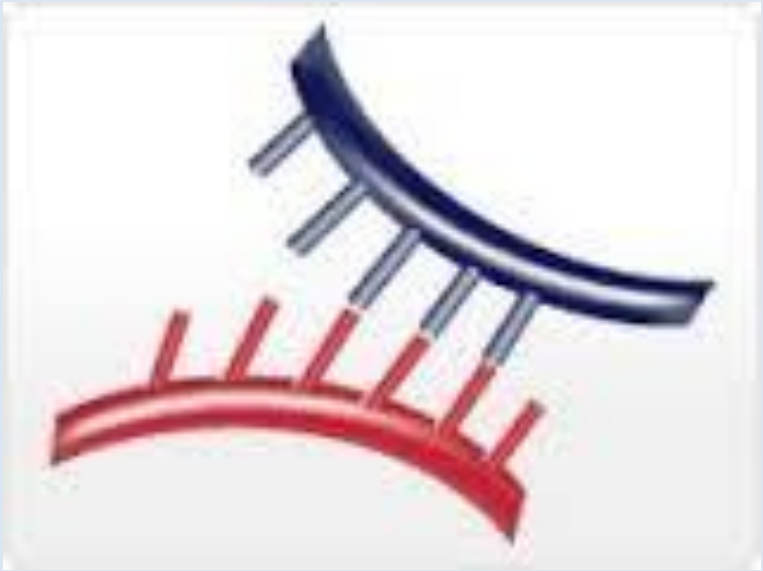
MyTaq™ Blood-PCR kit

- MyTaq™ Blood-PCR Kit (Bioline scientific-Sweden- Product # Bio- 25053) offeres a fast and direct PCR from whole blood samples.

- Extraction-free , it eliminates complex DNA extraction protocols and replaces the need for complicated extraction or purification steps.

PCR Protocol

Toxoplasma gondii primer and positive control were obtained from Norgen BiotecK-Canada



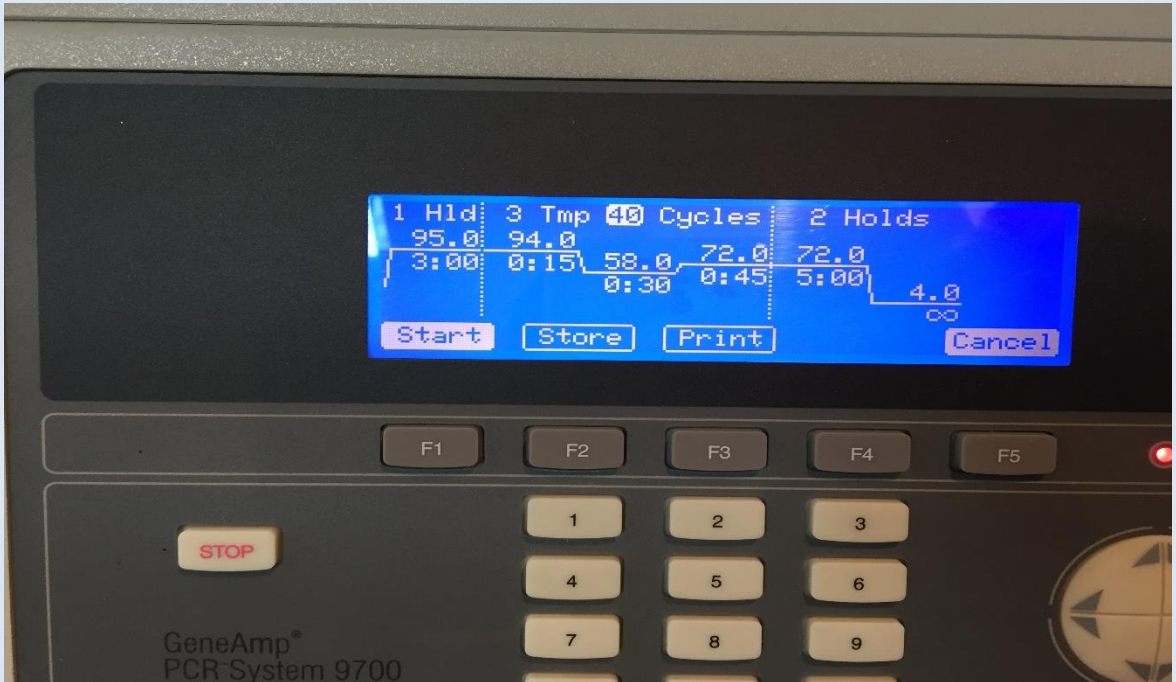
PCR Set-up
Three DNA extraction technique

PCR Components	Volume per PCR Reaction
Master mix	**10ul**
Primer	**2ul**
Nuclease free water	**5.5ul**
DNA Sample	**2.5**
PCR reaction volume	**20ul PCR reaction**

PCR Set-up
My taq blood PCR kit

PCR Components	Volume per PCR Reaction
Whole blood	**1ul**
My taq PCR mix, 2x	**12.5 ul**
Primers	**0.5 ul**
Water(dH2O)	**Up to 25 ul**
PCR reaction volume	**25ul PCR reaction**

PCR Cycling conditions



PCR Cycling conditions

PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1	95°C	**3 minutes**
Cycle 2 (40X)	Step 1	94°C	**15 seconds**
	Step 2	58°C	**30 seconds**
	Step 3	72°C	**45 seconds**
Cycle 3	Step 1	72 °C	**5 minutes**
Cycle 4	Step 1	4 °C	

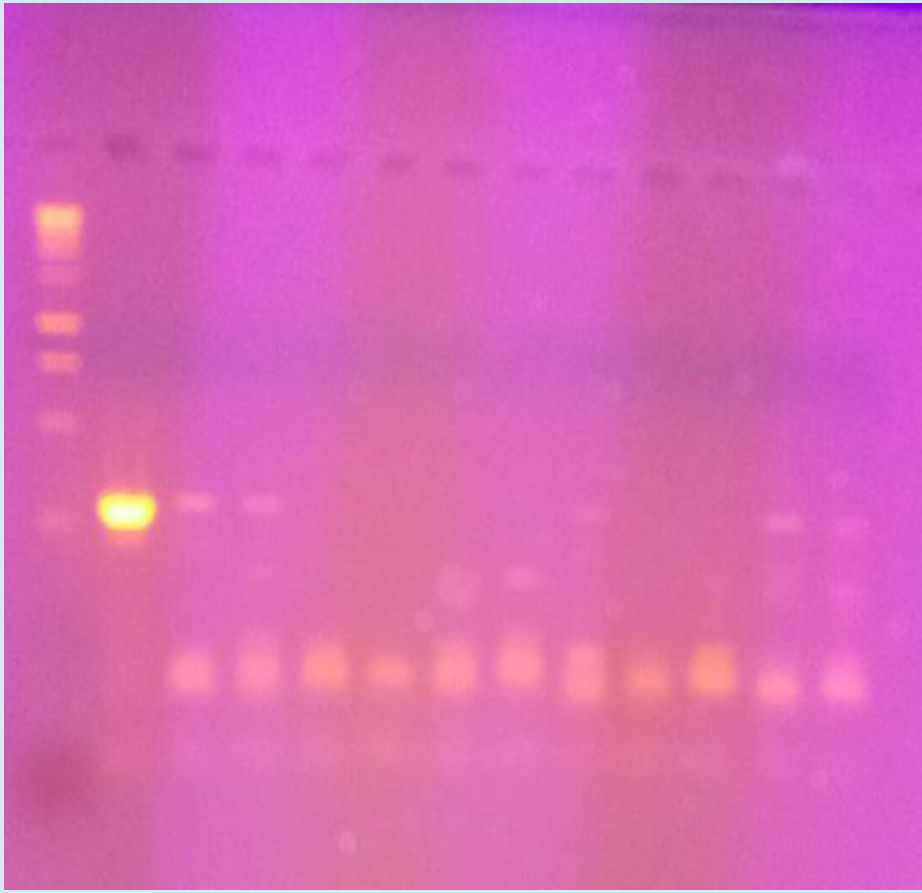
PCR Cycling conditions in my taq blood PCR kit

PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1	95°C	3 minutes
Cycle 2 (40X)	Step 1	95°C	15 seconds
	Step 2	58°C	15 seconds
	Step 3	72°C	45 seconds
Cycle 3	Step 1	72 °C	2 minutes
Cycle 4	Step 1	4 °C	

Toxoplasma PCR Assay Results Interpretation

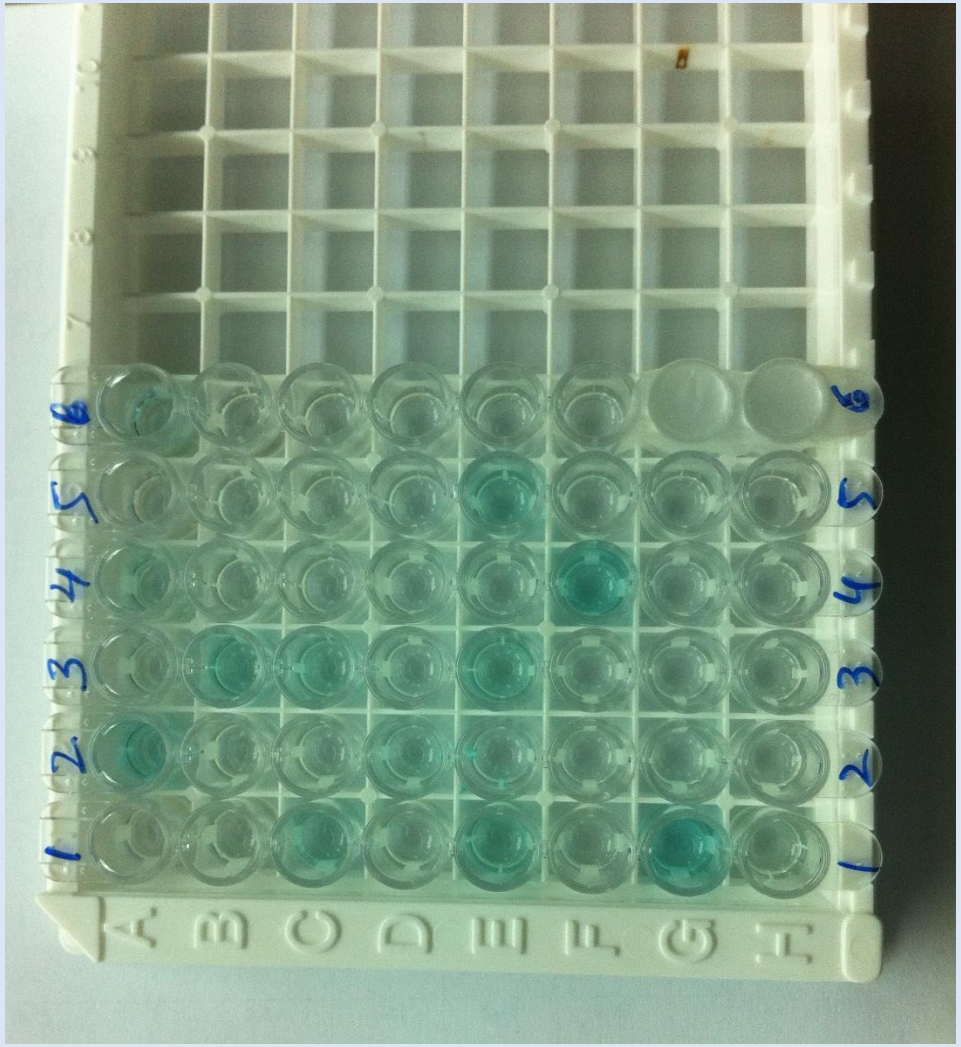
- For the analysis of PCR product, the entire PCR reaction volume was loaded on a 1X TAE 1.5% gel along with 10ul DNA ladder.

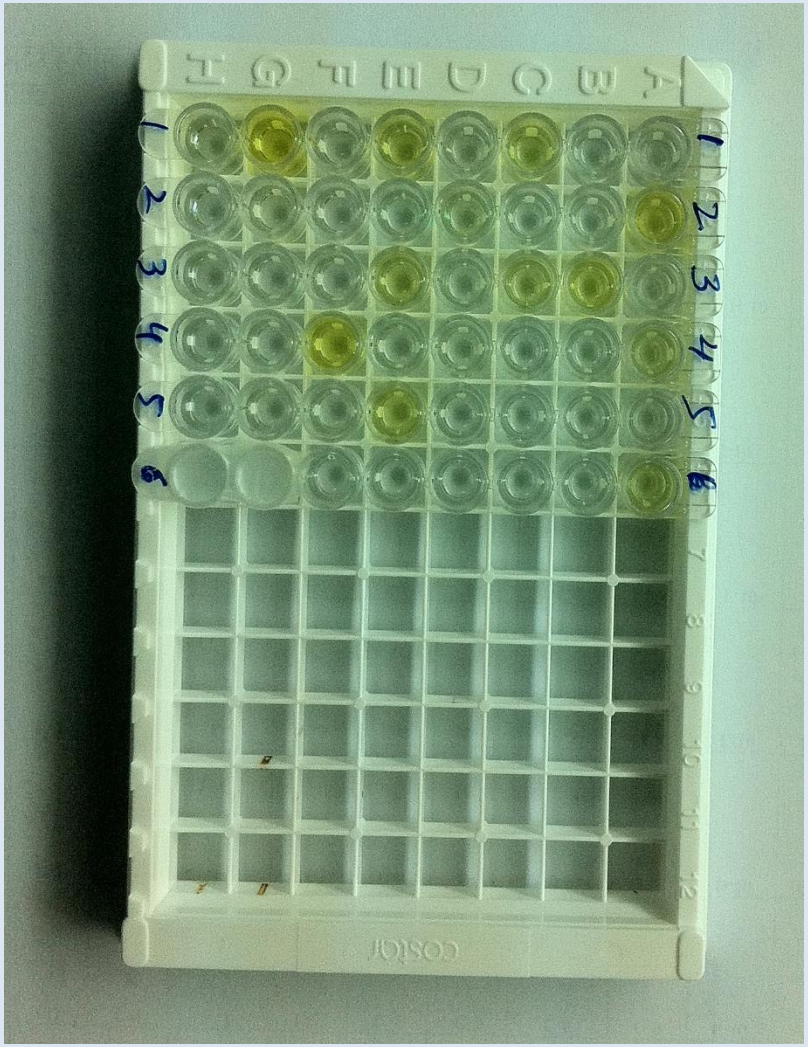
- PCR gel running was at 150 V for 1 hour.



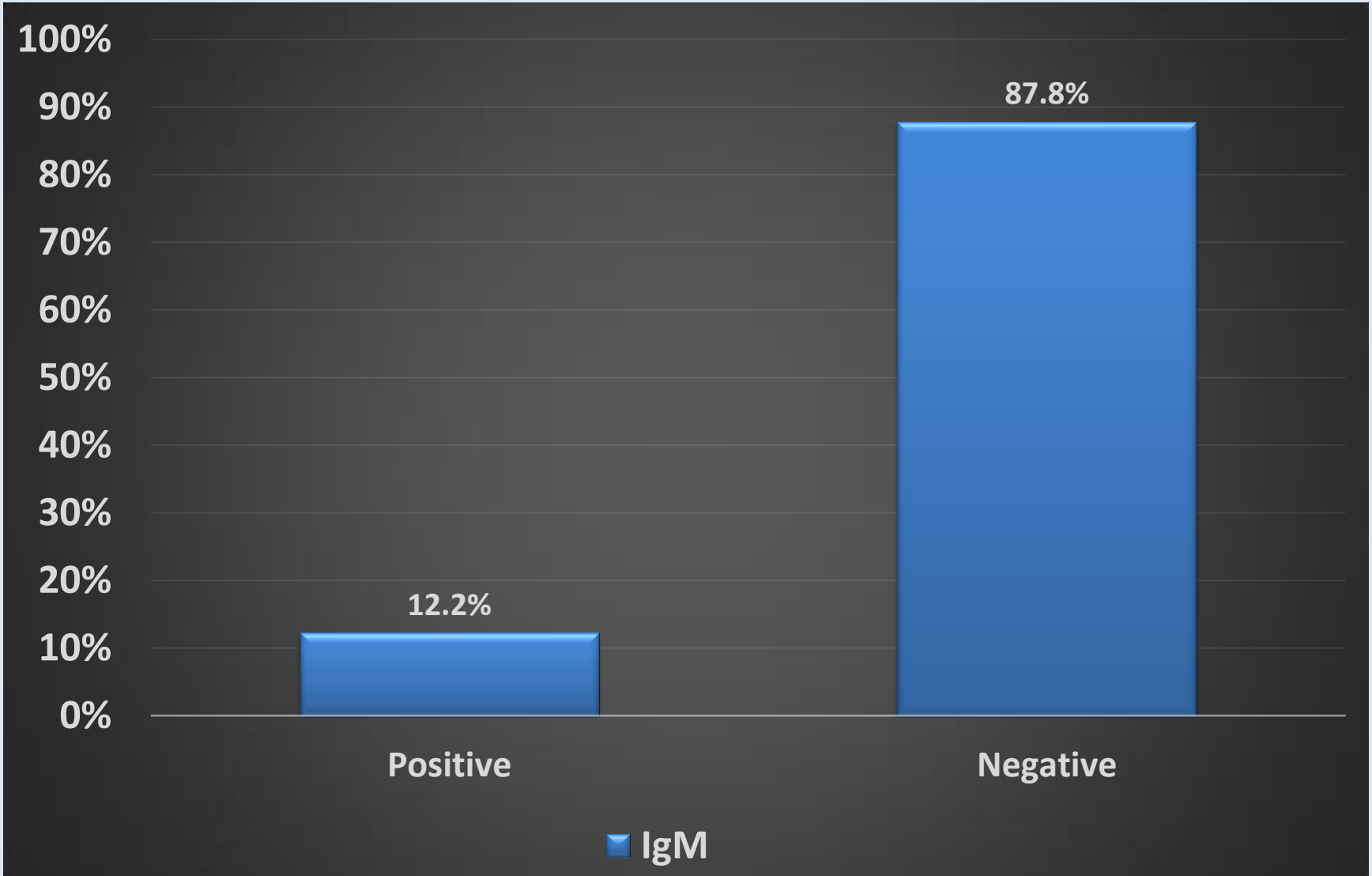

RESULTS

ELISA Results

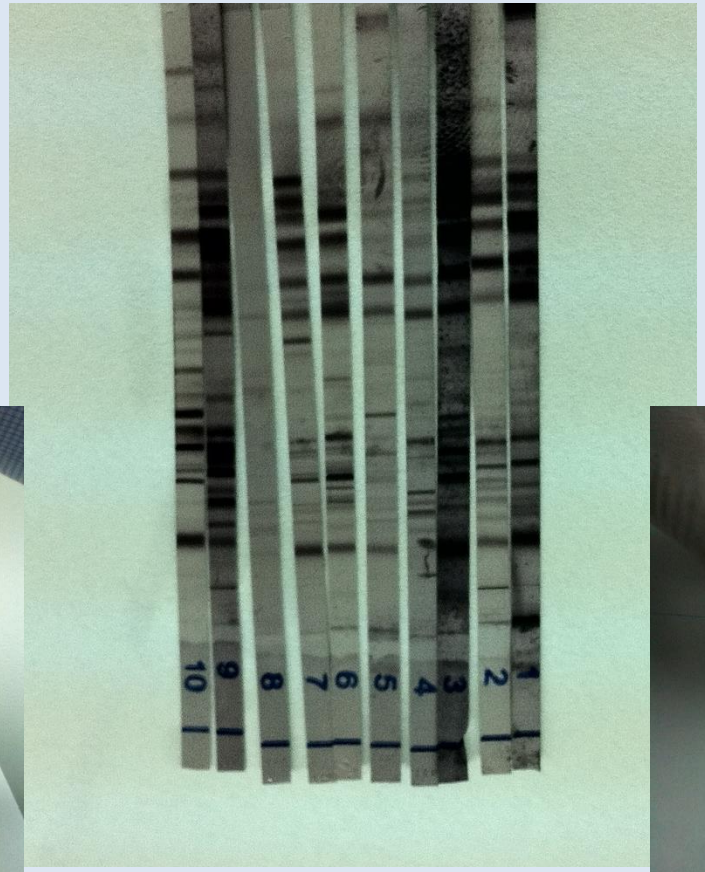


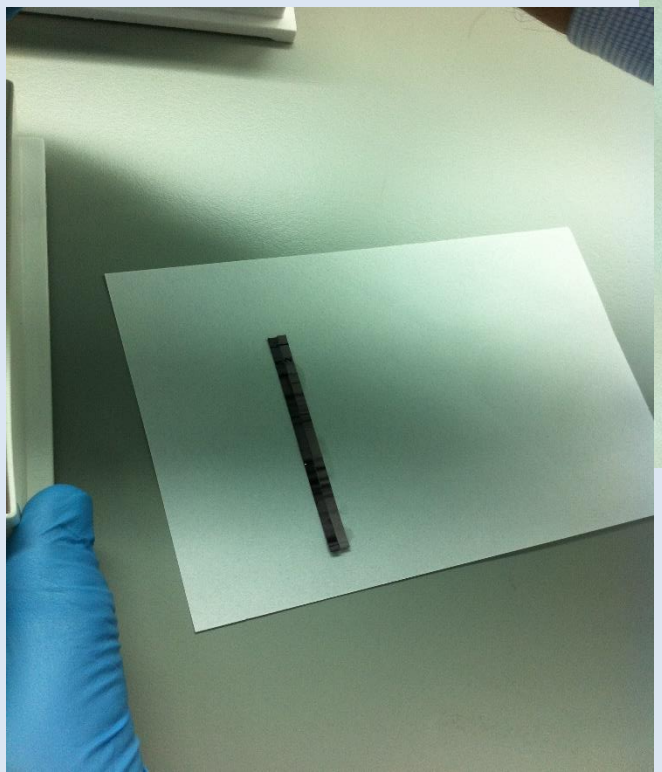


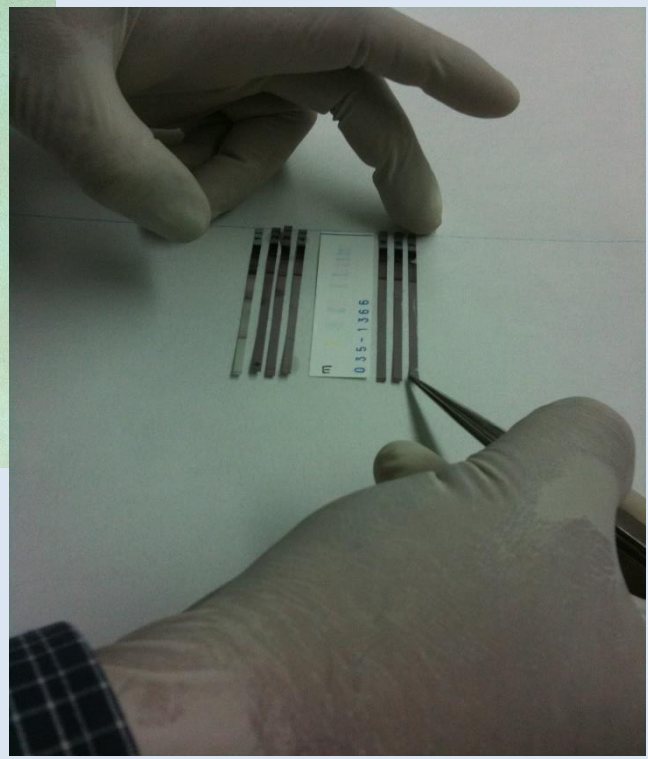
Frequency of infection among participants By ELISA



Western blot (WB) results

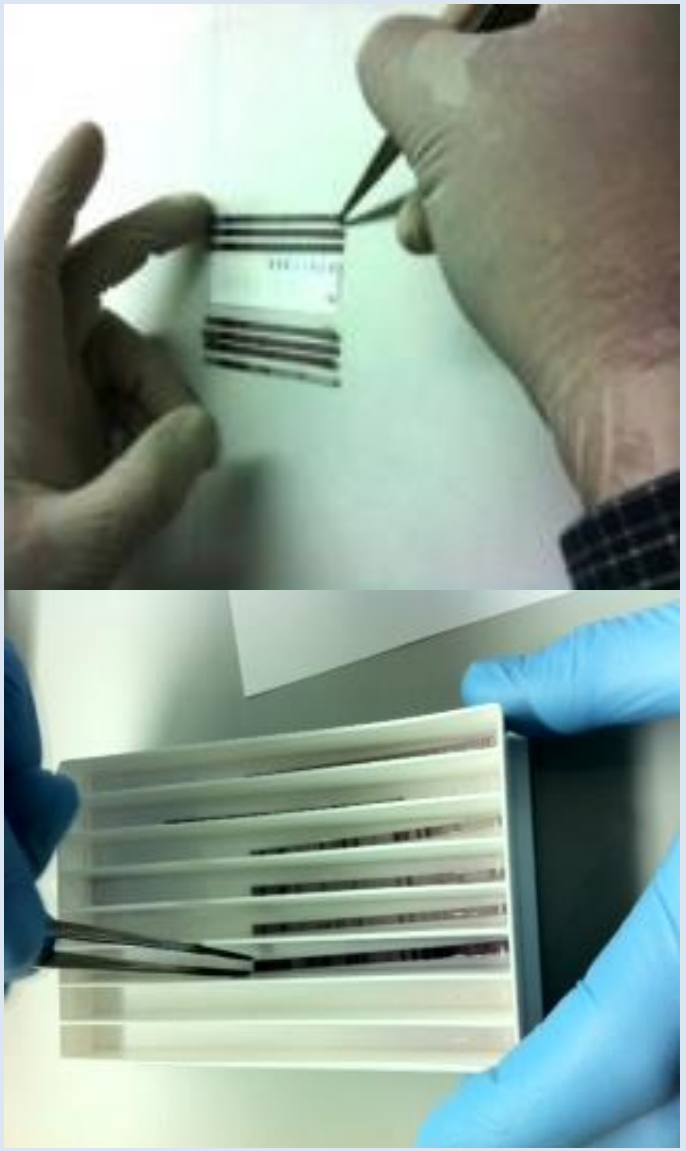






WB results

- The reading was done by comparing the results with the strip obtained by using the positive control (R5).
- With MW identification tools, presence of 30-45kDa specific bands was confirmatory.



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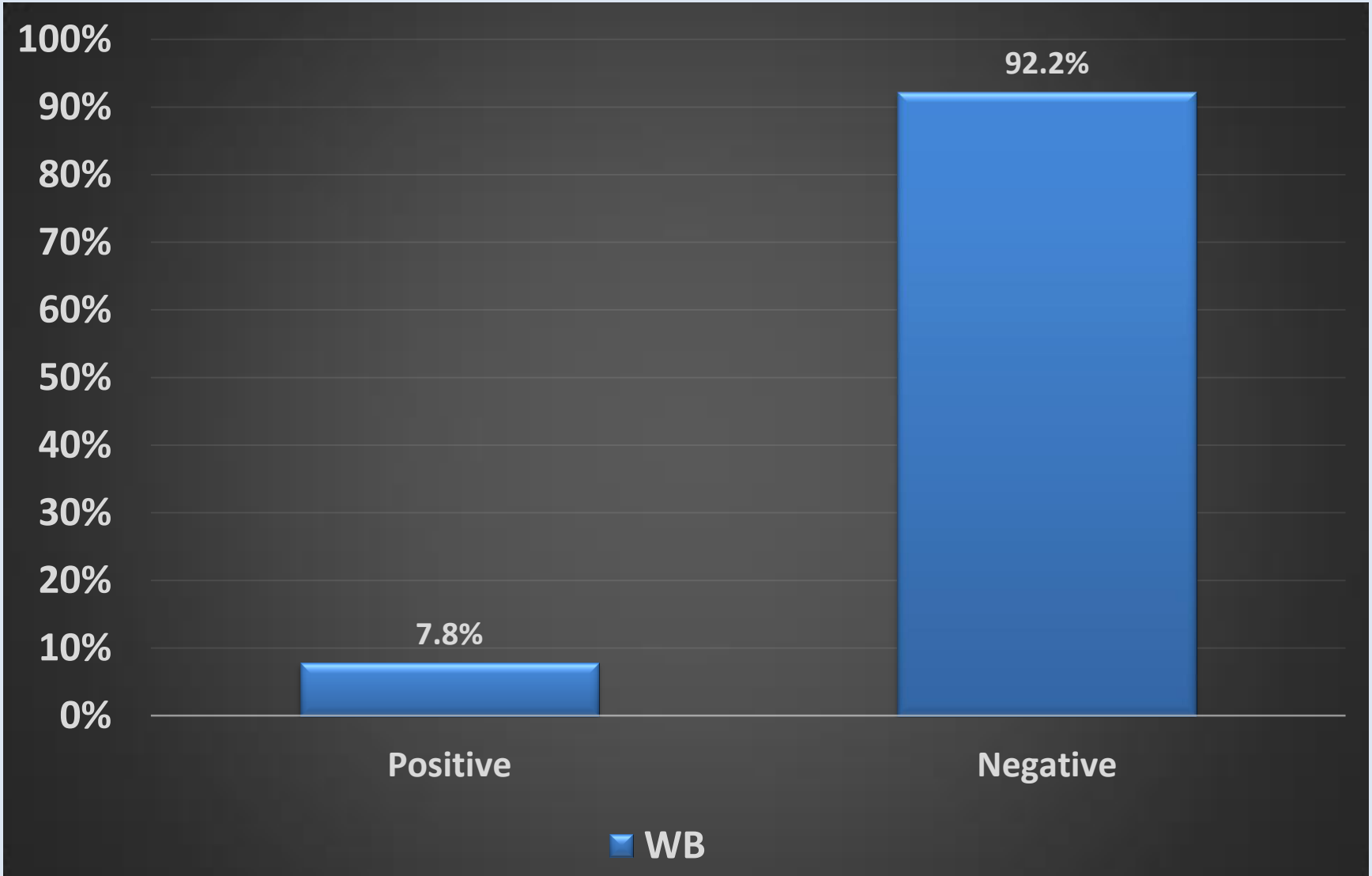
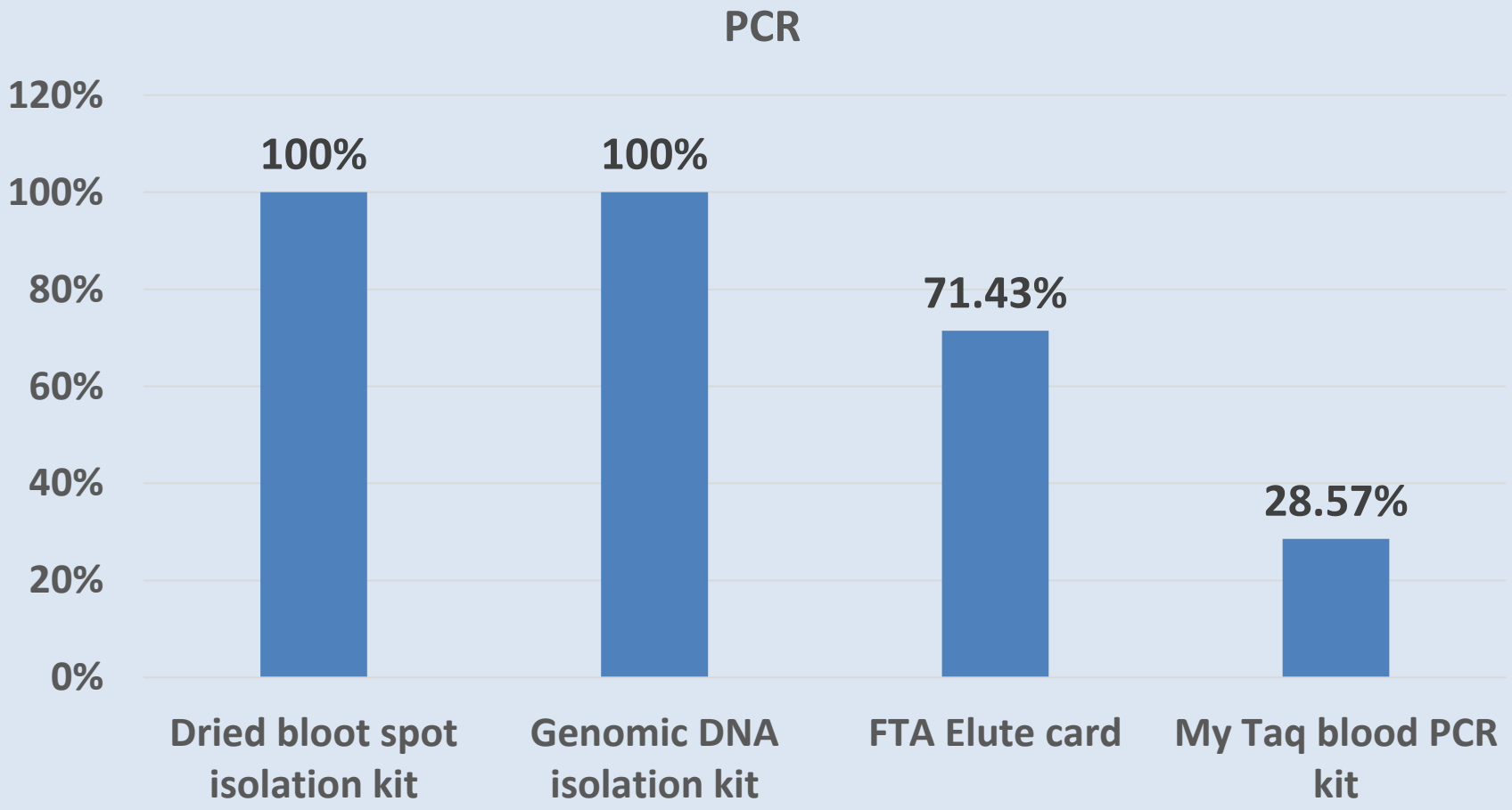
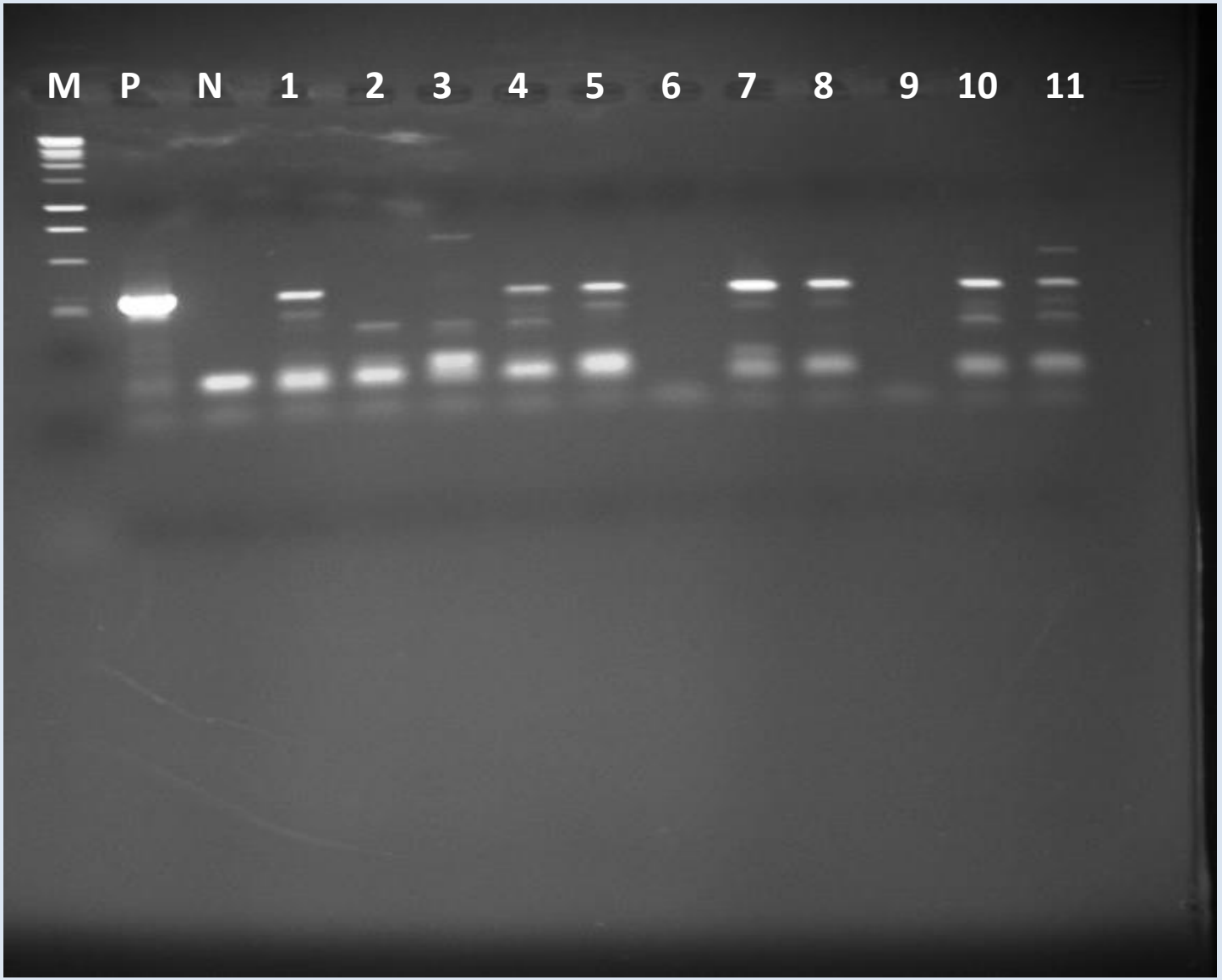


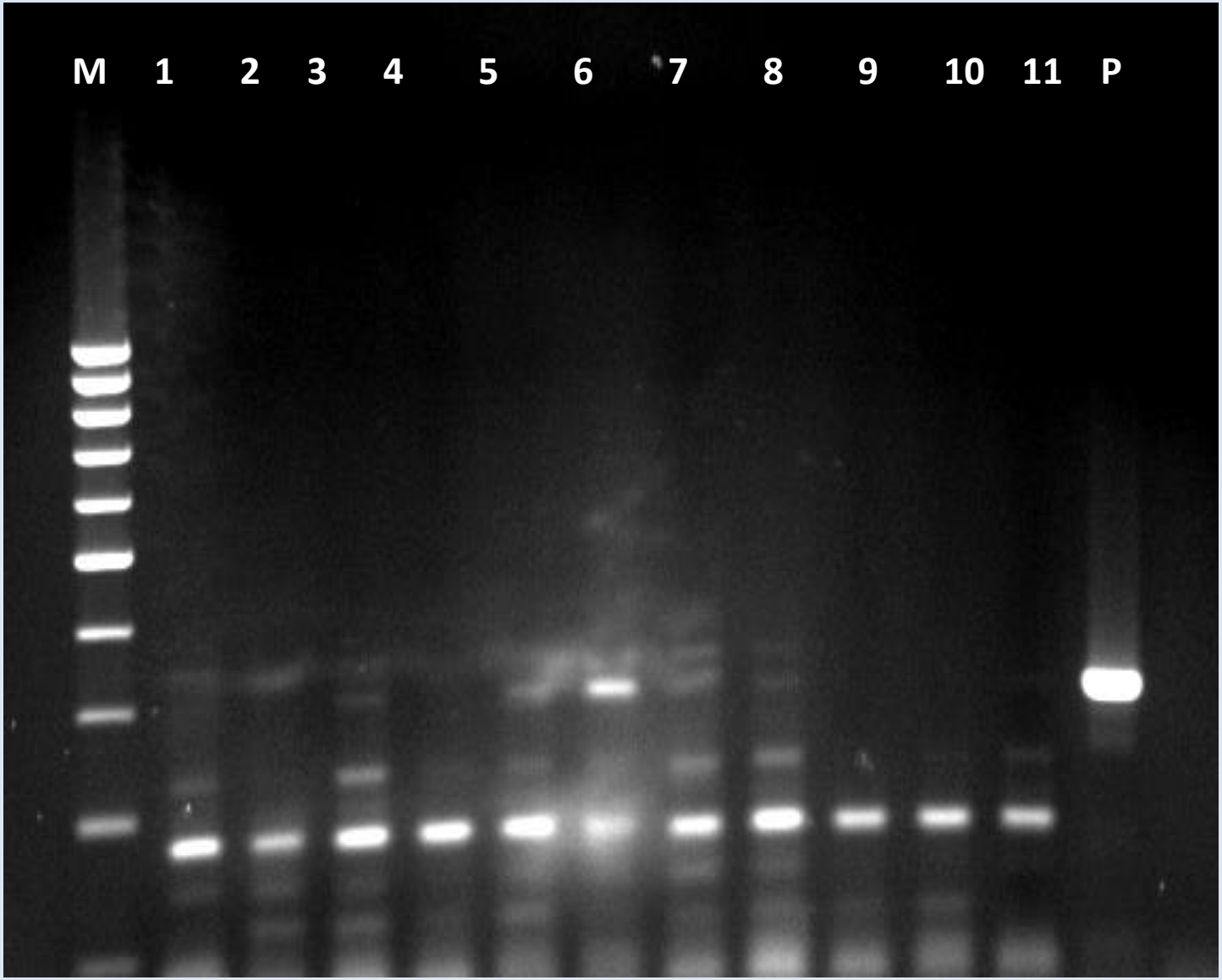
Sensitivity of DNA Extraction Techniques



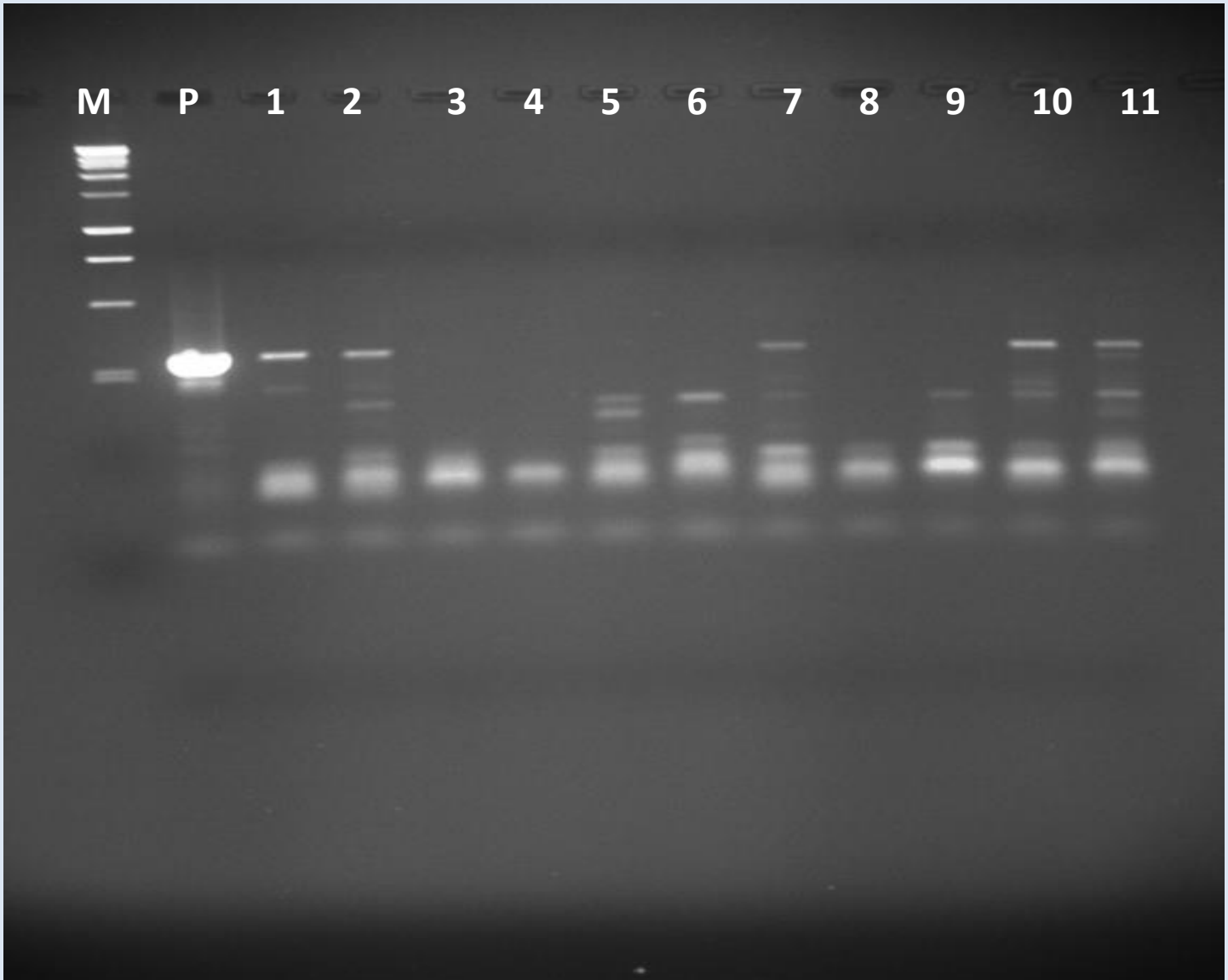
Gel photo using DNA from Dried blood spot



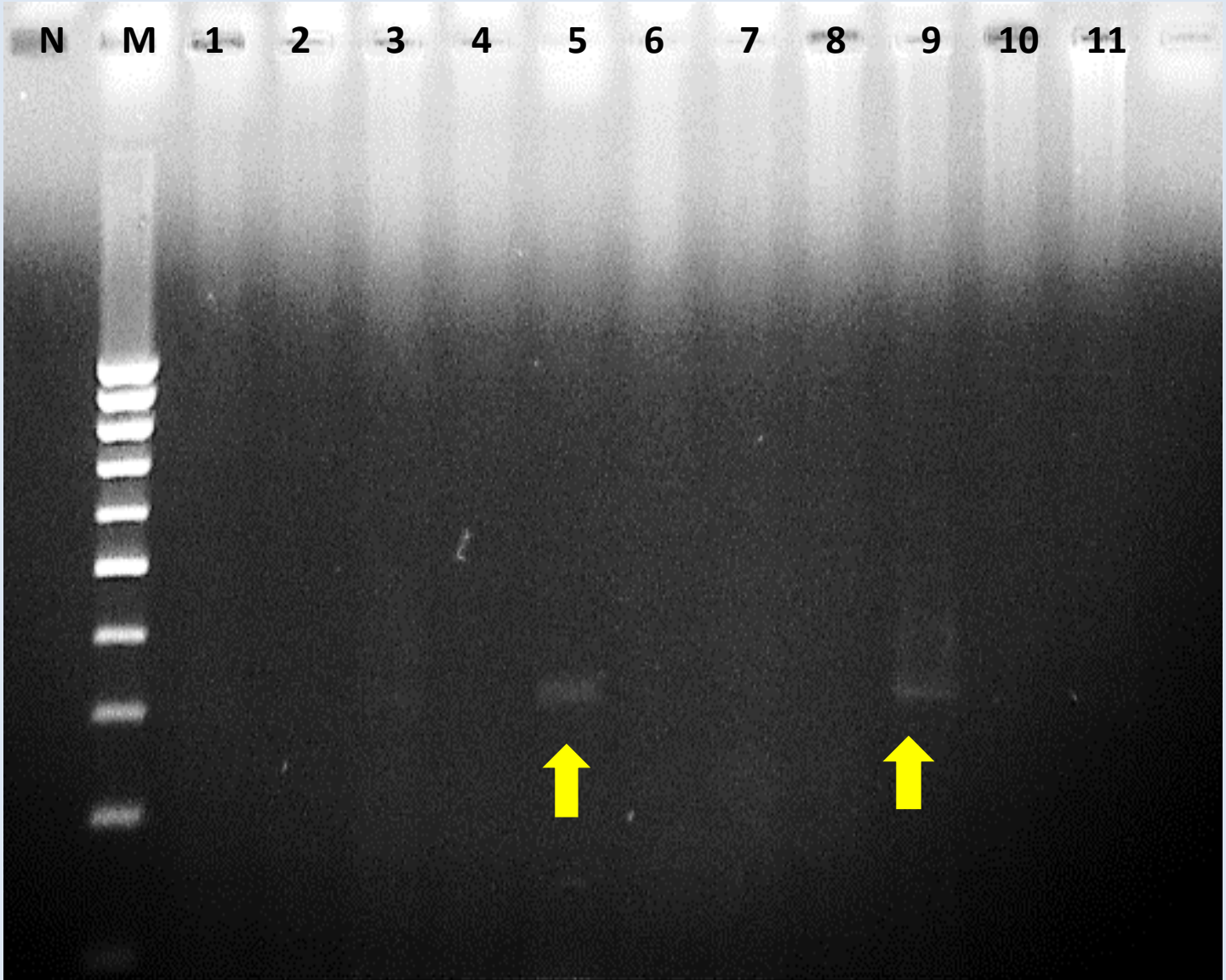
Gel photo using DNA from genomic DNA kit



Gel photo using DNA from Elute cards



Gel photo using MyTaq™ Blood-PCR kit



Conclusion

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- In the view of our results we conclude that dried blood spot extraction kit is a sensitive ,simple and efficient procedure to recover the *T. gondii* DNA present in whole blood.

- Also the easier steps which eliminated the complex and lengthy steps of other DNA extraction protocols which reduces the time required for completion.

- Concerning Taq blood PCR kit, in spite of offering a fast and direct PCR from whole blood sample, the results was not satisfactory according to its low sensitivity.

References

1) **Teixeira LE, Kanunfre KA, Shimokawa PT, Targa LS, Rodrigues JC, Domingues W, Yamamoto L, Okay TS. (2013) :** The performance of four molecular methods for the laboratory diagnosis of congenital toxoplasmosis in amniotic fluid samples. Rev Soc Bras Med Trop. 584-8.

2) **Alfonso Y1, Fraga J, Cox R, Bandera F, Pomier O, Fonseca C, Ginorio D, Torres G, Capo V. (2008) :** Comparison of four DNA extraction methods from cerebrospinal fluid for the detection of *Toxoplasma gondii* by polymerase chain reaction in AIDS patients**.** Med Sci Monit. 14(3):MT1-6.

3) **Sultan, DM , Khalil, MM, Saleh, AA AND MUTHANNA , AM. (2009):** Imported Malaria cases in UAE: Evaluation of a new DNA extraction technique using nested Polymerase chain reaction. Korean J.Parasitol. (47): 3 ,227-233.

4) **Edvinsson B1, Jalal S, Nord CE, Pedersen BS, Evengård B. (2004**): DNA extraction and PCR assays for detection of *Toxoplasma gondii*. APMIS.: (112) 6:342-8.


thank you!