A COMPARISON OF DIFFERENT METHODS FOR RNA AND DNA EXTRACTION FROM FORMALIN – FIXED PARAFFIN-EMBEDDED TISSUES FROM DIFFERENT CANCER SAMPLES

Nuha **J**oseph **K**andalla *, **M**ajeed **A**rsheed **S**abbah **, **R**amina **M**ekhael **Kh**oshaba * and **D**iaa **A**bdulsalam *
* Biotechnology Dept. , College of Science, Baghdad University, Iraq.
** Biotechnology Research Center, Alnahrain University, Iraq.
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Abstract

RNA and DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue is problematic due to chemical modifications and continued degradation over time. we compared quantity of RNA extracted by two different protocols from 14 recently archived from patients suffered from different cancers distributed among formalin-fixed paraffin-embedded (FFPE) breast cancer tissues ,thyroid cancer tissues and Cervical uterus carcinoma tissues by using Guanidine isothiocyanate (GTC)with phenol-chloroform (protocol-1) and Silica Gel Column(SGC) dependent on spin column purification-based (protocol- 2), to assess, which technique is the most efficient and reproducible in terms of total yield and purity. The results showed RNA isolated with SGC technique was characterized by higher mean concentration in a range (80-180) µg/ml ,but it give positive results to 12 sample with degradation in comparison with RNA isolated by the (GTC) technique (protocol-1), comparison with total RNA extraction from human blood (two distinguished bands) .

In this study comparative methods have been performed to analyze the efficiency for extraction and purification of Genomic DNA from six selective FFPE Tissues samples, revealing that the extraction of DNA by using extraction modified method give good result with yield higher mean concentration of DNA in a range (160-260) µg/ml.

The lysis of FFPE Tissues was enhanced by increased the concentration of proteinase K to30 mg\ml for 2 hour at 65C, which considered the best time for lysis tissues and heated comparable with results that obtained from lysis tissues by using 20mg\ml for (24 -48 hours) at 55C .

Key Words : DNA extraction, RNA extraction, Formalin – Fixed Paraffin-Embedded Tissues (FFPE).

Introduction

The identification and validation of molecular markers in formalin-fixed, paraffin-embedded (FFPE) tissue is currently an area of intense and exciting research activity. This is due in part to the fact that expression profiling, genotyping, and mutation analysis have been shown to aid in diagnosis and to provide guidance, particularly in the treatment of cancer. Despite this concentrated effort, to date, only a limited number of individual markers or panels of markers using extracted nucleic acids from histopathological tissue specimens have been introduced into routine clinical practice (Hennig, *et al.*,2010)..

Formalin-fixed, paraffin-embedded (FFPE) tissue is one of the most widely practiced methods for clinical sample preservation and archiving. It is estimated that, worldwide, over a billion tissue samples, most of them FFPE, are being stored in numerous hospitals, tissue banks, and research laboratories. FFPE samples pose a major challenge for molecular pathologists, because nucleic acids are heavily modified and trapped by extensive protein-nucleic acid and protein-protein cross linking. Historically, Recently, however, it has been discovered that with appropriate protease digestion, it is possible to release microgram amounts of DNA and RNA from FFPE samples. The purified nucleic acids, although highly fragmented, are suitable for a variety of downstream genomic and gene expression analyses, such as polymerase chain reaction (PCR), quantitative reverse transcription PCR (qRT-PCR), microarray, array comparative genomic hybridization (CGH), microRNA, and methylation profiling. Several commercial kits are currently available for FFPE extraction (Weining *et al*.,2009)..

The recovery of nucleic acids (DNA and RNA) from fixed, paraffin-embedded specimens is challenging. Although formaldehyde (HCHO), a principal ingredient of most commonly used fixatives, does not physically degrade nucleic acids, it leads to the generation of DNA-protein and RNA-protein. crosslinkages. Furthermore, the nucleic acids will fragment in situations where the fixative solution is unbuffered, as the pH can be extremely low (Thomas *et al*.,2007). Furthermore, many methods for extracting nucleic acid from FFPE tissue, particularly those that are highly manual, are not standardized protocols , In addition, these manual protocols

are time consuming and did not require to use of hazardous and flammable materials like xylene and ethanol for deparaffinization. The GTC technique for isolation of RNA, is very popular because it requires much less time than other classical methods. Moreover, GTC salt denatures the cellular proteins and inactivates RNases ensuring that isolated RNA is not degraded and separates rRNA from ribosomes,. Such factors have required individualized adaptation to create standardized protocols. As a result, these isolation protocols have been incompatible with high-throughput formats (Benavides.*et al*.,2006). Several attempts have been made to isolate RNA from formalin-fixed paraffin-embedded tissue using modifications of currently available techniques for RNA extraction . However, the interpretation of results is often difficult and therefore, alternative methods for long-term storage of tissues prior to gene expression profiling are greatly required (**Muyal *et al*.,2009).** So the present study was designed to test the efficiency of RNA and DNA extraction from archival formalin fixed, paraffin-embedded tissues based on quantity of the nucleic acid extracted: The goals included development of a simpler and more effective protocol for RNA and DNA extraction .

MATERIALS AND METHODS

Formalin-fixed paraffin- embedd (FFPE)tissue blocks

This study included 14 FFPE blocks of different cancer tissues from different ages (those with better fixation& processing) collected from the department of Histopathology –Teaching Laboratories that belongs to the Medical City Teaching Hospital during the period of study from November 2011 to February 2012., by archival paraffin-embedded tissue blocks along with the histopathological reports for 14 patients with tumors patients` ages and tumors stage were . These samples were distributed as follows: Breast cancer (6), Thyroid cancer (6) , Cervical uterus carcinoma (2), in different ages (25-65).

Sectioning the paraffin –embedded blocks.

For each paraffin block, one 10 micrometer thick section was cut using rotary microtome (Leica, Germany) (more than 5 section for each sample) and collected in each sterile eppendorf tube, ensuring that an equivalent amount of tissue was placed in all the eppendorf tubes(Thomas *et al*, 2007).

Deparafinization method

Deparafinization was carried out by adding 1ml of xylene to each tube containing the tissue sections, and this was vigorously vortexed and Incubated at 55 C 20 minutes. Centrifugation was then performed at full speed for 5 minutes, and the resulting supernatant was discarded. The deparaffinization step was repeated once again, followed by the addition of 500ML of absolute ethanol, and this was mixed by vortexing for 1 minute .Incubated at 55 for 15 min The solution was then centrifuged at full speed for 5 minutes, and the resulting supernatant was discarded ,This step was repeated once again,.

Extracted RNA From tissue after Deparafinization (protochol-1)

After the steps of Deparafinization of the sections, to extracted RNA from tissue, the pellet was resuspended in 500 µl of Lysis buffer (SDS 2%, Na2-EDTA 1mM, Tris – HCl ,20mM , Guanidine isothiocyanate (GTC)1M, Mercabto ethanol , 25mM , DEBC (ddH2O), and homogenized using vortex for minutes , then Add 300 µl of a freshly prepared solution of Proteinase K (6 mg /ml) and incubated at 55ºC for (2- 24) hours. After lysis cells and extracted RNA from tissue we continuous other steps for purified RNA by addition 500 µl phenol : choloform: isopropanol alcohol at 25:24:1, followed by vigorous vortexing and centrifugation at 12,000 x g at room temperature for 10 minutes. The solution at the aqueous phase was transferred to a new 1.5 ml microfuge tube and an equal volume of chloroform was added, followed by mixing by vortexing and centrifugation at 12,000 x g for 5 minutes. RNA precipitation was performed by the addition of 0.1 volume of 3 mol/L sodium acetate (pH 4.0), an equal volume of isopropanol and 1 µl of 10 mg/ml carrier glycogen, followed by incubation overnight at – 20 C. The mixture was centrifuged at 12,000 x g at 4 C for 5 minutes. The supernatant was discarded, followed by washing of the RNA pellet with 500 µl of 70% ethanol and air-dried aseptically. The air-dried RNA pellet was resuspended with 30 µl of RNase-free water (Specht *et al*., 2001) .

Extracted RNA From tissue after deparafinaization (protochol-2)

After the steps of Deparafinization of the sections, RNA extracted from tissue , The pellet was resuspended in 500 µl of Lysis buffer (SDS 2%, Na2-EDTA 1mM, Tris – HCl ,20mM , Guanidine isothiocyanate (GTC)1M, Mercabto ethanol , 25mM , DEBC (ddH2O), and

homogenized using vortex for minutes , then Add 300 µl of a freshly prepared solution of Proteinase K (6 mg /ml) and incubated at 55ºC for 2 hours only. After lysis cell and extracted RNA from tissue we continuous other steps for purified RNA according to the Total RNA Mini Kit instead of manual method(protochol-1) for purification as described in a manual of mini kit by using silica column (Rupp and Locker 2001).

Extracted RNA From Blood

In this study ,Total RNA Mini Kit (Blood Cultured Cell protocol , promega \USA) was used to extract RNA from blood (normal person as a standard).

Extracted DNA from tissue after deparafinaization

In this study the method described by Thomas *et al* (2007) was used with some modification to isolate genomic DNA from 3 FFPE samples of breast cancer type and 3 FFPE samples of thyroid cancer type .After the steps of Deparaffinization of the sections ,Adding 300µl of Proteinase K buffer in final concentration 20mg/ml to the extracted sample with lysis buffer 1 .Incubation at 55°C for 24 hours, to assure the sample lysate is clear, during incubation the sample should be inverted every 30min (We repeated this step when the sample lysate is not clear by adding 300µl of Proteinase K buffer in final concentration 20mg/ml to the sample extraction and Incubated 24 hours at 55°C until the sample lysate become clear) . Adding 500µl of chloroform: isoamyl alcohol in percentage(24:1) to extract and mixing by shaking vigorously for 30 min until the mixture emulsified, and then centrifuged at 12000 rpm for 20 minutes.The aqueous phase was transferred to another tube (avoiding touching the interphase layer), two volumes of cold absolute ethanol was added to the aqueous phase and immediately mix by shaking vigorously for 10 seconds. and then were kept at –20 ºC overnight. The samples were centrifuged at 12000 rpm for 20 minutes, the supernatant was discarded and the precipitate was left to dry completely. Adding 500µl of 70% ethanol. and discarding the flow-through. The precipitate was dissolved in 50 µl of TE buffer .The samples were then kept at –20 ºC.

Extraction treatments: the effect of cell lysis buffers

To investigate the effect of cell lysis buffers on nucleic acid quality, samples were compared across the following buffers: cell lysis 1(SDS 10%, Na2-EDTA 0.5M, Tris – base 1M) (Shi *et al* , 2002), cell lysis2(SDS 0.5%, Na2-EDTA 0.1mM, Tris – base 10mM)(Bohmann *et al*., 2009) and cell lysis 3(SDS 2.0%, Na2-EDTA 2.5mM, Tris – base 25mM ,Sodium citrare 25mM, CaCl2 5mM (Thomas *et al*., 2007).

Extraction treatments: the effect of incubation time Lysis buffer and temperature .

To investigate the effect of incubation time in nucleic acids extraction, pairs of samples were tested across the following incubation times: 2, 24 hours. After we determined the best cell lysis buffer, the samples were tested across the incubation times 2 hours at 65 ºC.

DNA Extraction from Human blood

This method described by Sambrook and Rusell (2001) to isolate genomic DNA from human blood (used as control) ,Approximately 3-5 ml of blood was taken from donor by sterile syringe and places in EDTA tubes..

Agarose Gel Electrophoresis

Agarose gel was prepared according to the method described by Sambrook and Rusell (2001). Agarose gels were stained with ethidium bromide (0.5µg/ml) for 30 minutes. DNA bands were visualized under U.V transilluminator at 365 nm wavelength. A gel documentation system was used to document the observed bands.

Estimation of the nucleic acid concentration by the Spectrophtometer

The purity and concentration of the extracted DNA and RNA were determined by UV-Spectrophotometer (Eppendorf, Hamburg, Germany), according to the Protocol as described by Sambrook and Rusell (2001).

Results and Discussion

Isolation of Nucleic acid

The Comparison of Two Techniques for Total RNA Isolation from FFET Samples

In this study comparative methods have been performed to analyze the efficiency for extraction and purification of total RNA from 14 samples of FFET from different cancer types, revealing that the extraction RNA according to protocol 1 suffer from low efficiency, mainly due to incubated the samples over night and purified by phenol-chloroform this lead to degraded [figure(1),lane 11, lane12 , lane13 (present in small amount), in compare with

extracted RNA according to protocol 2 by using Guanidine Isothiocyanate (GTC) and Silica Gel Column(SGC) techniques (spin column purification-based protocols) , although the protocol 2 give positive results to 12 sample with degradation as showed in (figure -2) comparison with total RNA extraction from human blood (figure (1) lane2, lane3). While we did not get any result from samples of cervical uterus carcinoma by using two protocol (data not shown).

As we know about Formalin fixation, the most widely used fixative in histopathology, has many advantages such as the ease of tissue handling, the possibility of long-term storage, an optimal histological quality and its availability in large quantities at low price. But RNA is a particularly labile bio-molecule and is much more susceptible to degradation by endogenous- and exogenous-nucleases and to non-specific degradation by divalent cations, heat, elevations in pH, and storage of tissue or cells over extended periods prior to RNA extractions, which result in falsely altered gene expression patterns. However, this technique poses many problems due to the fact that formalin fixation cross-links nucleic acids and proteins. Further, mono-methylol is added to the amino groups for all four RNA bases ($N\text{-}CH_2OH$) and subsequently methylene bridges are formed between neighbouring bases that resulted to continue degradation over-time (Masuda *et al* .,1999). or the extremely low pH (<1). Of the fixative.(Gillio *et al*.,2007). The two most reliable and widely used techniques for high throughput RNA isolation are: 1- Guanidine Isothio Cyanate-phenol:chloroform (GTC)-based RNA isolation technology and , 2- Silica-gel column (SGC)-based RNA isolation technology. In contrast, the principle of SGC technology is a combination of the selective binding properties of a silica-based membrane with the speed of microspin technology, which allows saving time, money, and efficient use of small and precious biological samples.

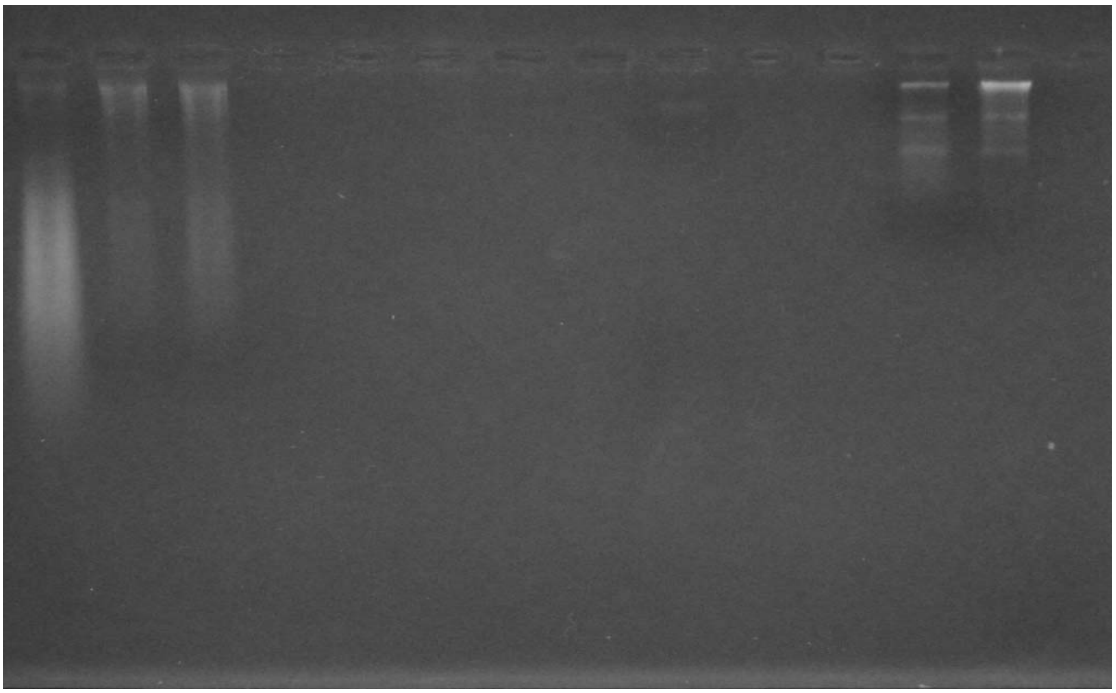

Figure (1) : Agarose gel electrophoresis of RNA Extraction from FFPE Tissues from different cancer types By using protocol 1, with agarose concentration (1.5%),voltage 5volt/cm,during 2 hr

Lane 2 and 3 : RNA extract from human blood (control).
Lane 1,4,5,6,7,8, : RNA extract from Thyroid cancer type.
Lane 9,10,11,12,13,14 : RNA extract from breast cancer type

Here in this study , compare RNA extraction protocols on a set of 14 FFPE different cancer samples, testing spin column purification and phenol-chloroform technologies, That we designate Unfortunately formalin fixation induces RNA-tissue protein cross-links, which can prevent obtaining a good results or degraded RNA In addition, nucleic acid fragmentation may occur in formalin fixed tissue due to aging of the specimen or the extremely low pH (<1). of the fixative.

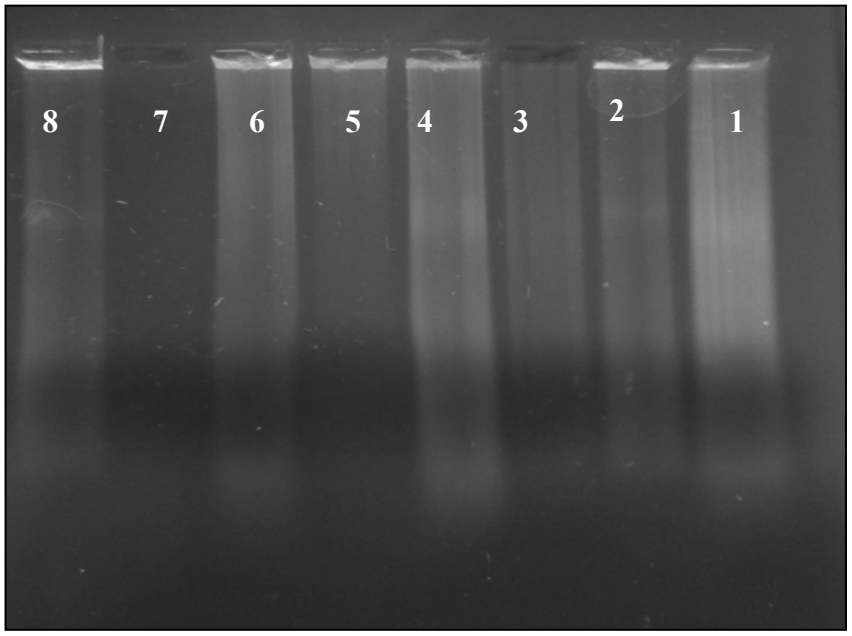

Figure (2) : Agarose gel electrophoresis of RNA Extraction from FFPE Tissues from different cancer types By using protocol 2, with agarose concentration (1.5%),voltage 5volt/cm,during 2 hr

Lane 1 ,2,3,4 : RNA extract from Breast cancer type.
Lane,5,6,7,8, : RNA extract from Thyroid cancer type.

The Modified Extraction methods To extract DNA from FFPE Tissues samples

This study shows how the modified extracted method can actually give good yield of DNA. Three different lysis digestion buffers of DNA extraction were tested with six FFET samples , and the results showed the best lysis buffer extract was type (3) (figure -5) in DNA concentration range (200-260) **µg/ml** , compare with other results as showed in (figure - 3) and (figure - 4). In figure (6) three sharp band appeared in three samples of FFPE Tissues, this results revealed to the modified DNA extraction method (the incubation time 2 hours , the temperature of lysate cell 65 C , the concentration of proteinase K 30mg\ml and add GTC(1M) to the best digestion buffer).In other words, thermal energy does not lead to an increase of extracted nucleic acids; it merely makes whatever available DNA and RNA more amenable to amplification, presumably by making it less cross-linked (Banerjee *et al* .,1995).

So the data (only three samples) shows no indication that even 24 hour or 48 hour digestions at 55C adversely effect the DNA yields with increased incubation temperatures, this may become an important factor However, comparisons between digestions at 65C and higher temperatures provide evidence , the higher temperatures significant DNA and RNA degradation occurs (85C versus 65C, up to 8000 times less PCR amplifiable, average/standard deviation Therefore, there is good evidence to argue that increased digestion temperature can be useful with regard to obtaining greater levels of PCR amplifiable DNA; however, these digestion temperatures should be limited to 65C(Shi *et al* .,2002).

In this study , lysis of FFPE Tissues was enhanced by increased the concentration of proteinase K to30 mg\ml for 2hr at 65C , which considered the best time for lysis tissues and heated comparable with results that obtained from lysis tissues for 24 -48 hours. The data presented here indicate that proteinase K digestion is required for obtaining DNA of sufficient quality by all 4 extraction methods. DNA depended on the extraction method. On types of lysis buffers extraction and heat-

treatment in combination with proteinase K digestion resulted in the good quantity of DNA concentration were found in four extracts of each samples after proteinase. K digestion and heat-treatment. This may in part explain why it is not unusual to find conflicting findings in previously published studies. Such as, in a comparison of the effect of time of incubation during tissue digestion, Isola *et al.* (1994) argue that prolonged time is better, while Banerjee *et al.* (1995) argue that no more than 3 hours are required. While others study pointed to importance of adding Proteinase K in high concentration and incubation at high temperature (60 to 70°C) in DNA extraction method (Thomas *et al*., 2007) ,this also lead to removes part of the methylol additions induced by formalin fixation . The predominant alternative to Tris-based digestion buffers are guanidinium thiocyanate/proteinase k containing buffers, favored by those who perform DNA extractions using commercially available kits (e.g. Qiagen's QIAamp DNA micro kit) that are based on the silica-binding principle described by Boom et al. (1990). In this study we succeeded in finding a modified method for DNA isolation especially that from FFPE Tissues samples by decreased the incubation time to 2 hours and increased the temperature of lysate cell to 65 C with increase the concentration of proteinase K to 30mg\ml with addition the GTC(1M) to the best digestion buffer .

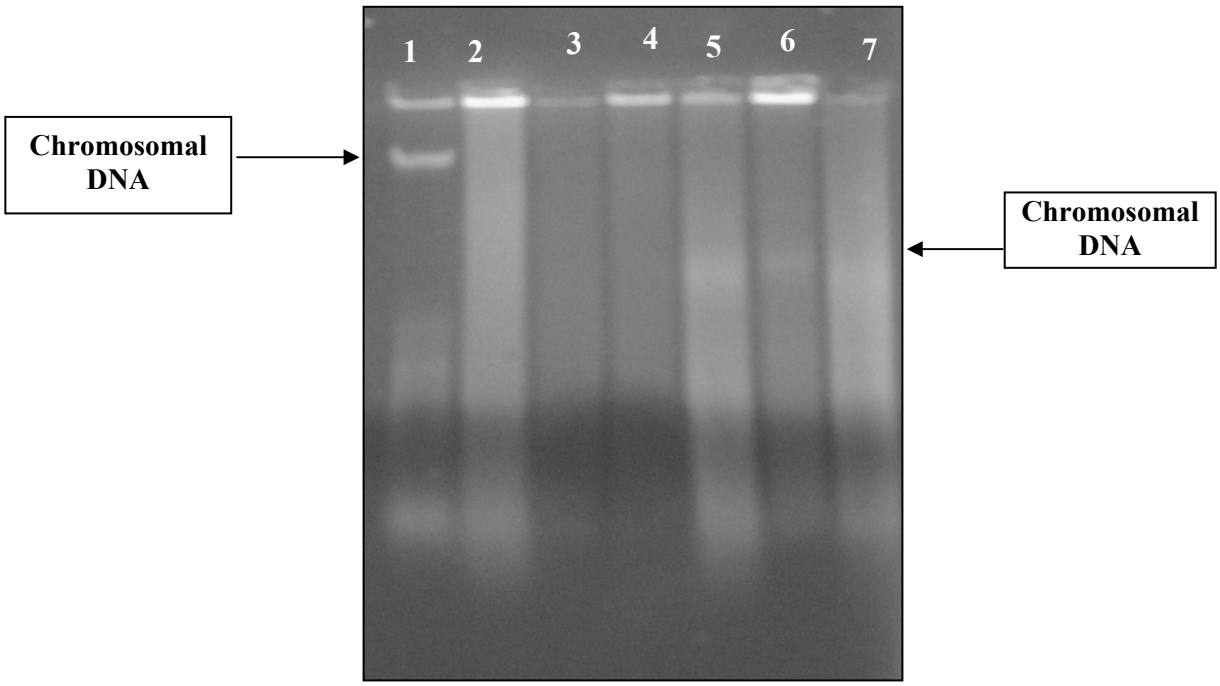

Figure (3): Effect of cell lysis buffer type(1) on detection the quantity of DNA extraction from different samples of cancer by agarose gel electrophoresis (with agarose concentration (0.8%),voltage 5volt/cm,during 1.5hr)
Lane 1 : DNA extract from human blood (control).
Lane 2,3,4, : DNA extract from breast cancer type.
Lane 5,6,7, : DNA extract from thyroid cancer type

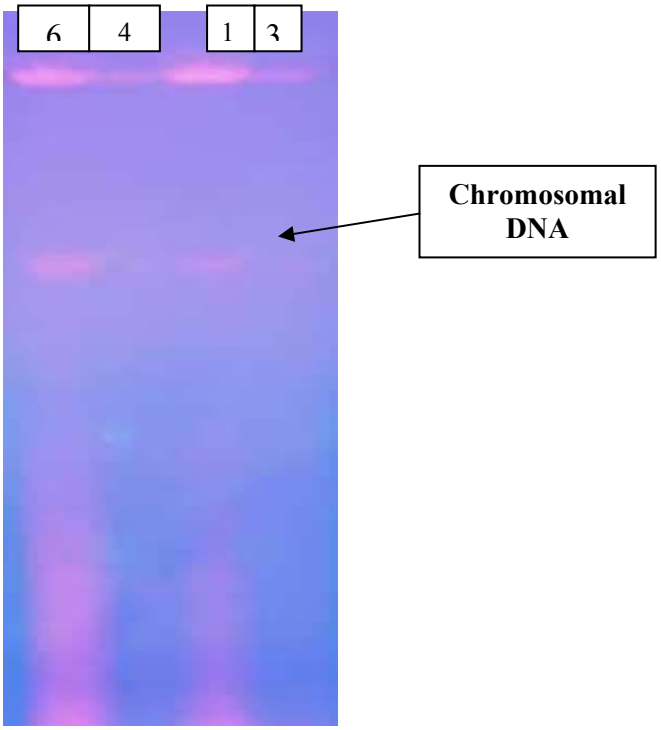

Figure (4): Effect of cell lysis buffer type(2) on detection the quantity of DNA extraction from different samples of cancer by agarose gel electrophoresis (with agarose concentration (0.8%),voltage 5volt/cm,during 1.5hr .

Lane 1 , 3 : DNA extract from breast cancer type.(B)
Lane 4, 6 : DNA extract from thyroid cancer type.(B)

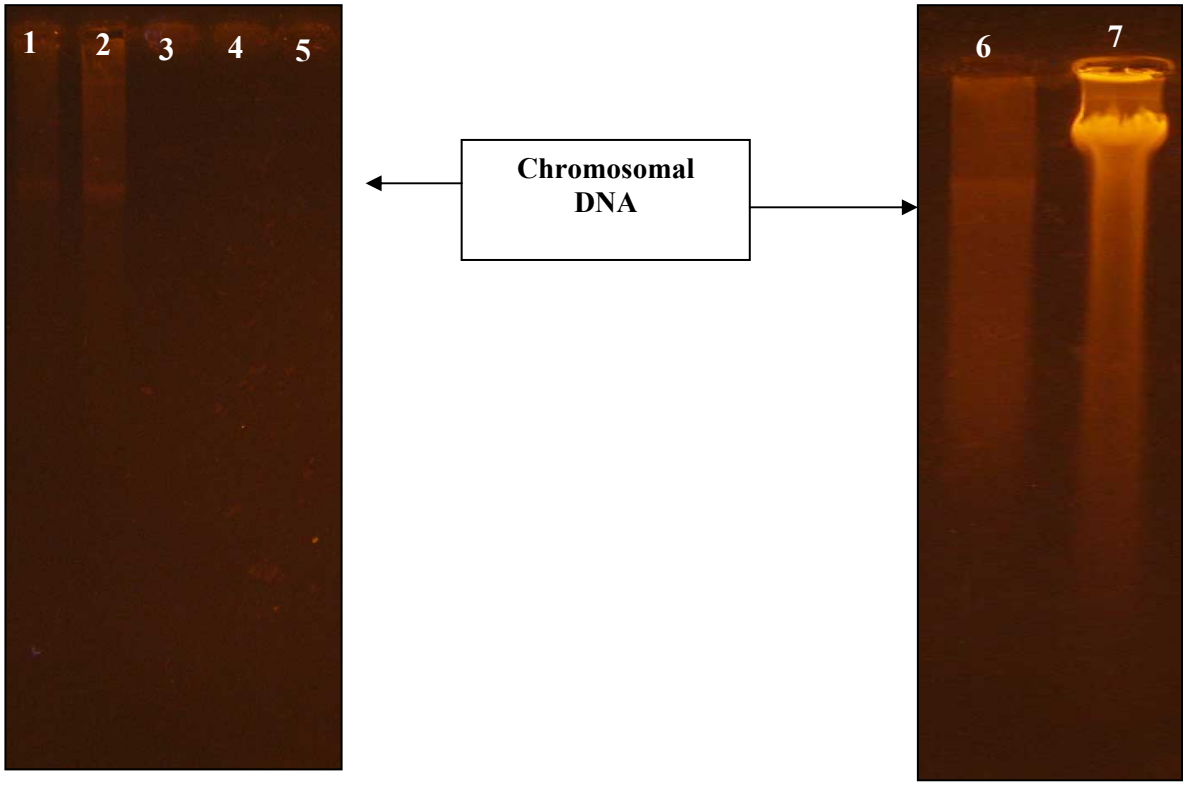

Figure (5): Effect of cell lysis buffer type (3) on detection the quantity of DNA extraction from different samples of cancer by agarose gel electrophoresis (with agarose concentration (0.8%),voltage 5volt/cm,during 1.5hr).
Lane 1 ,2,3, : DNA extract from breast cancer type.
Lane 4,5,6, : DNA extract from thyroid cancer type.
Lane,7 :DNA extract from Human blood sample

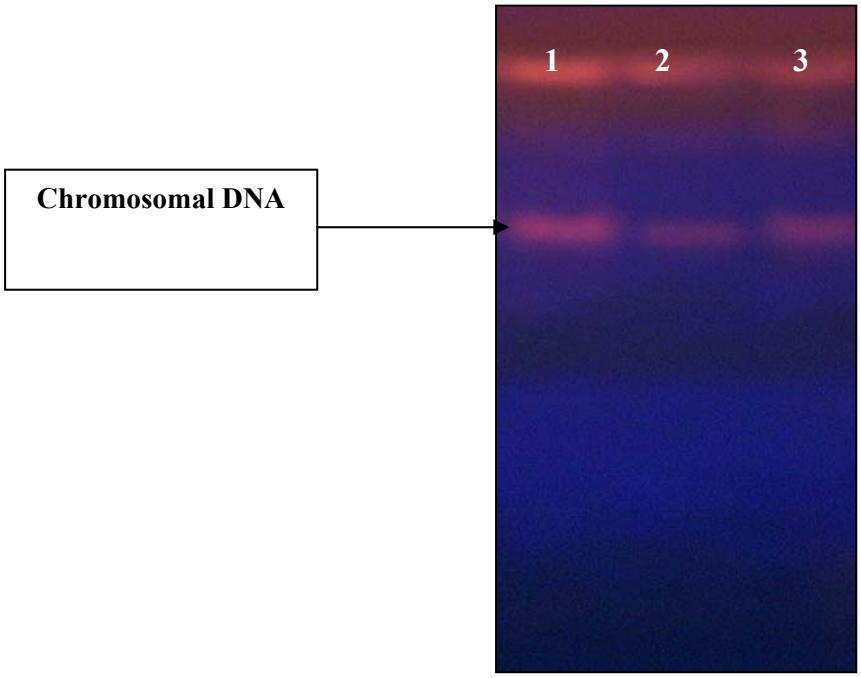

Figure (6): Agarose gel electrophoresis of DNA extracted by using modified methods from different samples of cancer by agarose gel electrophoresis (with agarose concentration (1%),voltage 5volt/cm,during 1.5hr)
Lane 1, 2 : DNA extract from breast cancer type.
Lane, 3 : DNA extract from thyroid cancer type.

Assessment of total RNA yield and purity

The amount of total RNA extracted for each samples was measured by a UV and Vis-Spectrophotometer with the exception of the samples that contained no RNA. According to the results of methods extracted total RNA as mentioned .

The results of protocol (1) showed only three breast cancer samples give result, while tweleve samples from breast cancer and thyroid cancer showed positive result according to protocol 2 .The estimated mean total RNA extracted for protocol (2) was 130 µg (2.6 µg\ µl)(2600 ng/µL) for breast cancer samples with values ranging from 80 to 180 µg (1.6 to 3.6 µg\ µl)(1600 to 3200 ng/µL) , for thyroid cancer samples the estimate mean total RNA extract was125 µg (2.5 µg\ µl)(2500 ng/µL) with values ranging from 70 to 180 µg (1.4 to 3.6 µg\ µl)(1400 to 3600 ng/µL), . The meanA260/A280 ratio for protocol 2 was 1.8 ± 0.2 , The desired ratio A260/A280 is in the range of 1.7 to 2.1 and is dependent on the extraction conditions (Table-1).

The purity of the extracted RNA was comparable throughout the samples and was close to a ratio (A_{260}/A_{280}) of 1.85, the GTC and the SGC technique,. A ratio close to 1.8 indicates that there were only limited protein contamination.

Table (1): Comparative data of total RNA Extracted from different cancer type by modified protocol2,

Types of cancer	A260/A280 ratio.	Yield : µg/ml
Breast cancer (6)	**2, 1.25,1.4,2,1.7,1.7**	**180,80,100,160,110,120,**
Thyroid cancer(6)	**1.7,1.3,2,1.7,1.8,2.3,**	**120,70,150,120,123,180**
Cervical uterus carcinoma	**-**	**-**

* The total yield, 1.8 < A260/A280 ≥ 2 indicates pure RNA

Assssement of DNA yield and purity

According to the results of methods extracted DNA as mentioned above . The results of Modified methods by using different lysis buffers, showed only three breast cancer samples give positive result, while others samples from thyroid cancer did not show any result . To evaluate the purity of the extracted DNA, absorbance ratio at 260/280 nm (DNA/protein) was determined. (Table 2). The estimated mean total DNA extracted by using digestion lysis buffers type(1) was 170 µg (3.4 µg\ µl)(3400 ng/µL) for breast cancer samples with values ranging 160-180 µg (3.2-3.6 µg\ µl)(3200 -3600 ng/µL) , for digestion lysis buffers type(2) the estimate mean total DNA extract was200 µg (4 µg\ µl)(4000 ng/µL) with values ranging from 160 to 240 µg (3.2 to 4.8 µg\ µl)(3200 to 4800 ng/µL), for digestion lysis buffers type(3) the estimate mean total DNA extract was230 µg (4.6 µg\ µl)(4600 ng/µL) with values ranging from 200 to 260 µg (4 to 5.2 µg\ µl)(4000 to 5200 ng/µL), while the value for modified method the estimate mean total DNA extract was250 µg (5 µg\ µl)(5000 ng/µL) with values ranging from 220 to 280 µg (4.4 to 5.6 µg\ µl)(4400 to 5600 ng/µL),.. The meanA260/A280 ratio for Extract DNA with different lysis buffers was 1.6 and for modified method 1.8 – 2 , The desired ratio A260/A280 is in the range of 1.6 to 2 and is dependent on the extraction conditions .

Table (2): Comparative data of total DNA Extracted from different samples of cancer by modified protocol,

Types of cancer	A260/A280 ratio.	Yield : µg/ml
Breast cancer (1)	2* , 1.7**, 2***	180, 160, 210
Breast cancer (2)	1.7 * , 0 ** , 1.8***	160, 0 , 200
Breast cancer (3)	1.6* , 0** , 0 ***	160 , 0 , 0
Thyroid cancer (4)	-	
Thyroid cancer (5)	-	
Thyroid cancer (6)	0 *, 1,8** , 1,9***	0 , 240 , 260
	Modified method	
Breast cancer (1)	1.88	260
Breast cancer (2)	1,8	220
Thyroid cancer (6)	2	280

*** The total yield, 1.8 < A260/A280 ≥ 2 indicates pure RNA**

* result of digestion lysis buffer (1) ** result of digestion lysis buffer (2) *** result of digestion lysis buffer (3).

References

Banerjee SK, Makdisi WF, Weston AP, Mitchell SM, Campbell DR (1995). Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. Biotechniques 18: 768–773.

Benavides J, Garcia-Pariente C, Gelmetti D, Fuertes M,and Ferreras MC,.(2006). Effects of fixative type and fixation time on the detection of Maedi Visna virus by PCR and immunohistochemistry in paraffin-embedded ovine lung samples. *J Virol Methods.* .137:317–324.

Bohmann, K.; Guido ,H.; Uwe ,R. ;Christopher, P. ;Berit ,M.M., Peter, F.; Stephan ,S., and Karl-L. S.(2009). RNA Extraction from Archival Formalin-Fixed Paraffin-Embedded Tissue: A Comparison of Manual, Semiautomated, and Fully Automated Purification Methods . Clinical Chemistry .55(9):1719–1727.

Boom, R. ; Sol, C.J,; Salimans, M.M,; Jansen, C.L,; Wertheim-van ,;Dillen ,P.M, van der.; Noordaa, J. (1990) .Rapid and simple method for purification of nucleic acids. J Clin Microbiol. *28(3):495-503.*

Gillio-Tos A. ; De Marco L. ; Fiano V. ; Garcia-Bragado F. ;Dikshit R. 'Boffetta P;. Merletti and F. Efficient .(2007). DNA extraction from 25-year-old paraffin-embedded tissues: study of 365 samples. Pathology. 39:345–348.

Hennig, G.; Gehrmannn, M.;. Stropp, U. ; Brauch, H.; Fritz, P.; Eichelbaum, M. Schwab, M.(2010). Automated extraction of DNA and RNA from a single formalin-fixed paraffin-embedded tissue section for analysis of both single-nucleotide polymorphisms and mRNA expression..Clinical Chemistry,56(12): 1845-1853.

Isola J,; De Vries S,' Chu L,'Ghazvini S and Waldman F (1994) Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archivalparaffin-embedded tumor samples. Am J Path .145: 1301–1308 .

Masuda ,N, ;Ohnishi, T, ;Kawamoto, S, ;Monden ,M,; Okubo,K.(1999).Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such

samples. Nucleic Acids Res.;27:4436–4443. .

Muyal, J. P.; Vandana, M.; Brajesh ,P .; Kaistha, C; S.and Heinz, F.(2009). Systematic comparison of RNA extraction techniques from frozen and fresh lung tissues: checkpoint towards gene expression studies. Diagnostic Patholog. 4(9) : 1746-1596-4-9.

Sambrook J, and Rusell DW. (2001). Molecular cloning. A laboratory manual. Third ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press, N.Y

Shi,S-R,; Richard J. ;Cote, L. ;Wu, C.; Liu, R.; Datar, Y. S. ,and Dongxin L. (2002). DNA Extraction from Archival Formalin-fixed,(Paraffin-embedded Tissue Sections Based on the Antigen Retrieval Principle: Heating Under the Influence of pH . The Journal of Histochemistry & Cytochemistry. 50(8): 1005–1011.

Specht ,K,; Richter ,T, ;Müller, U,; Walch ,A, ;Werner, M, ;Höfler,(2001).Quantitative gene expression analysis in microdissected archival formalin-fixed and paraffin embedded tumor tissue. Am J Pathol. .158:419–429. 7- Rupp,G.M, and Locker, J. (1988).Purification and analysis of RNA from paraffin-embedded tissues. Biotechniques.;6:56–60.

Thomas, M.P.; Gilbert,P.; Tamara ,H, Michael ,B, Juan , ; J. Sanchez, S. B.; Lucas, L.; D. Jewell,' E. Van Marck, and Michael ,W.(2007). The Isolation of Nucleic Acids from Fixed, Paraffin- Embedded Tissues–Which Methods Are Useful When? Research Article.2(6):537-550.

Weining, T. ; Freedom , B.; David, M, M. ;Wilson,1. ; Benjamin ,G. ; Barwick,1.; Brian R.; Leyland-J and Mark M. B. (2009). DNA Extraction from Formalin-Fixed, Paraffin-Embedded Tissue. *Cold Spring Harb Protoc*; doi:10.110.

بەکارهێنانی ڕێگەی جیاواز بۆ جیاکردنەوەی ناوکە ترشی DNA وRNA لە شانانەی چاندێندراو لە ناو مۆمدا لە سامپڵە شێرپەنجە جیاوازەکاندا

پوختە

چەند کێشەیەك هەیە لە ڕێگاکانی جیاکردنەوەی DNA وRNA لە شانە چاندێندراوەکانی ناو مۆم ئەمەش بەهۆی گۆرانکاری کیمیای و ئەو پارچانەوەی کە دروست بوون بە تێپەڕبوونی کات ، ئەم توێژینەوەیە پێکهاتبوو لە ڕێگا چەندێتیەکان لە جیاکردنەوەی RNAلە ١٤ سامپڵی شێرپەنجەیی چاندێندراوەلە مۆمدا بۆ ماوەی (٢-٥ مانگ) لە چەند نەخۆشێکی شێرپەنجەیی جیاواز وەرگیرابوون وەکو شێرپەنجەیی ممەك و گلاندنی دەرەقی و شێرپەنجی ملی منداڵدان ، بە بەکارهێنانی دوو ڕێگا جێ بەجێ کرا یەکەمیان بە بەکارهێنانی Guanidine isothiocyanate (GTC)لەگەڵphenol-chloroform وە ڕێگەی دووەم بە بەکارهێنانی(Silica Gel Column(SGC بۆ دیاریکردنی باشترین ڕێگا بۆ پوختە کردن و بەتوانیی پوختێتی وە چڕی RNA. لە دەرەنجامدا ڕێگەی دووەم بە تواناتر بوو SGC لە پوختەکردنی RNAلە هەر ١٢ سامپڵە کەی شێرپەنجەی مەمك و گلاندی دەرەقی و بە چڕی (٨٠-١٨٠)مایکرۆگرام / ملبە بەراوردکردنی لەگەڵ ڕێگەی یەکەمدا کە(GTC)کە تەنها لە٣ سامپڵی شێرپەنجەیی مەمکی بەکارهێنا بۆ جیاکردنەوەی RNAبە بەراورد کردنی لە RNA جیاکراوەکان لە خوێنی ئادەمیزاد (دەرکەوتنی دوو گورزە).

هەروەها ئەم توێژینەوەیە چەند شتێکی تریش ئەگرێتەوە وەك دۆزینەوەی ڕێگەی باش و بە توانا بۆ تەکنیکی جیاکردنەوە DNA بۆ ٦ سامپڵی هەڵبژێردراو لە شانە چێندراوەکانی ناو مۆم، ئەنجام بە توانای ڕێگایی پوختە کردنەکە گۆراوەکەی دەرخست بە بەکارهێنانی ٣ گیراوەی جیاواز بۆ تێکشکاندنی خانەکان کاتێك کە چڕیەکی DNA ی کرد بە (١٦٠-٢٦٠) مایکروگرام/ مل ، وەباشترین چڕی بۆ ئەنزیمی شیکەرەوەی شانە ٣٠ مل گرام / مل لەکاتی ماوەی هەڵگرتن ١٢٠ خولەك و پلەی گەرمی ٦٥ سەدی بە بەراورد کردنی لەگەڵ ئەو ئەنجامانەی کە دەست نەکەوت لە شیکردنەوەی شانەکانبە چڕی ٢٠ مللگرام / مل بۆ ماوەی (٢٤-٤٨) کاتژمێر لە پلەی گەرمی ٥٥ سەدیوە چڕیDNA (٢٢٠-٢٨٠)مایکروگرام /مل.

استخدام طرق مختلفة لاستخلاص الـ RNA و DNAمن الانسجة المطمورة في الشمع من عينات سرطانية مختلفة

الخلاصة

تعاني طرق استخلاص RNA و DNA من الانسجة المطمورة في الشمع من مشاكل عديدة ويعود هذا الى التحويرات الكيميائية والقطع الناجمة بمرور الزمن , تضمنت الدراسة مقارنة بين الطرق الكمية في استخلاص الرنا من ١٤ عينة سرطان مطمورة في الشمع جمعت من مرضى يعانون من امرا ض سرطانية مختلفة توزعت مابين عينات سرطان الثدي وسرطان الغدة الدرقية وعنق الرحم، باستخدام طريقتين تمثلت الاولى باستخدام (GTC) Guanidine isothiocyanate مع phenol–chloroform والطريقة الثانية باستخدام (SGC)Silica Gel Column لتحديد الطريقة الكفوءة في الاستخلاص ودرجة النقا وة وتركيز الرنا . واظهرت النتائج كفاءة الطريقة الثانية SGC في استخلاص الرنا من ١٢ عينة لسرطان الثدي وسرطان الغدة الدرقية بتركيز (٨٠–١٨٠) مايكروغرام \مل مقارنة بالطريقة الاولى باستخدام (GTC) اذ اسخلص الرنا من ٣ عينات من سرطان الثدي فقط مقارنة مع الرنا المستخلص من عينة دم بشرية (ظهور حزمتين).

شملت الدراسة ايضاالتحري عن طرق كفوءة في استخلاص الـ DNA وتنقيته من ستة عينات منتخبة من انسجة مطمورة في الشمع، واظهرت النتائج كفاءة طريقة استخلاص محورة باستخدام ثلاث محاليل مختلفة لتكسير الخلايا عندما اعطت تركيز للدنا تراوح (١٦٠–٢٦٠) مايكروغرام \ مل ، وافضل تركيز للانزيم الحال للنسيج ٣٠ مللغرام \ مل عند فترة حضانة ١٢٠ دقيقة بدرجة حرارة ٦٥ مئوي مقارنة مع النتائج التي حصل عليها من تحلل الانسجة بتركيز ٢٠ مللغرام \ مل للمدة(٢٤–٤٨) ساعة بدرجة حرارة ٥٥م .