

# World Journal of *Clinical Oncology*

*World J Clin Oncol* 2019 September 24; 10(9): 300-317



**EDITORIAL**

- 300 Gangliocytic paraganglioma: An overview and future perspective  
*Okubo Y*
- 303 Novel mechanism of drug resistance to proteasome inhibitors in multiple myeloma  
*Zhou J, Chng WJ*

**ORIGINAL ARTICLE****Observational Study**

- 307 DNA extraction from paraffin embedded colorectal carcinoma samples: A comparison study of manual *vs* automated methods, using four commercially kits  
*Kovacs Z, Jung I, Csernak E, Szentirmay Z, Baniás L, Rigmanyi G, Gurzu S*

**ABOUT COVER**

Editorial Board Member of *World Journal of Clinical Oncology*. Sang Moo Lim, MD, PhD, Chief Doctor, Department of Nuclear Medicine, Korea Cancer Center Hospital, Seoul 139-706, South Korea

**AIMS AND SCOPE**

The primary aim of *World Journal of Clinical Oncology (WJCO, World J Clin Oncol)* is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

*WJCO* mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

**INDEXING/ABSTRACTING**

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Responsible Electronic Editor: *Mei-Yi Liu*  
 Proofing Production Department Director: *Xiang Li*

**NAME OF JOURNAL**

*World Journal of Clinical Oncology*

**ISSN**

ISSN 2218-4333 (online)

**LAUNCH DATE**

November 10, 2010

**FREQUENCY**

Monthly

**EDITORS-IN-CHIEF**

Hiten RH Patel

**EDITORIAL BOARD MEMBERS**

<https://www.wjgnet.com/2218-4333/editorialboard.htm>

**EDITORIAL OFFICE**

Ruo-Yu Ma, Director

**PUBLICATION DATE**

September 24, 2019

**COPYRIGHT**

© 2019 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjgnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjgnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjgnet.com/bpg/gerinfo/240>

**PUBLICATION MISCONDUCT**

<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## Observational Study

**DNA extraction from paraffin embedded colorectal carcinoma samples: A comparison study of manual vs automated methods, using four commercially kits**

Zsolt Kovacs, Ioan Jung, Erzsebet Csernak, Zoltan Szentirmay, Laura Baniás, Genoveva Rigmanyi, Simona Gurzu

**ORCID number:** Zsolt Kovacs (0000-0002-1038-7769); Ioan Jung (0000-0001-6537-2807); Erzsebet Csernak (0000-0002-4311-5364); Zoltan Szentirmay (0000-0003-1624-5398); Laura Baniás (0000-0002-2240-2540); Genoveva Rigmanyi (0000-0001-9093-6598); Simona Gurzu (0000-0003-3968-5118).

**Author contributions:** Kovacs Z drafted the article and contributed to the DNA purifications; Jung I contributed to the diagnosis and immunohistochemical assessment; Csernak E contributed to automated and manual DNA isolation; Szentirmay Z contributed to the research design; Baniás Laura performed DNA collection; Rigmanyi G performed manual DNA extraction; Gurzu S designed research and confer the final agreement for publication; Zsolt Kovacs and Laura Baniás have equally contribution to the paper.

**Supported by** the University of Medicine, Pharmacy, Science and Technology Research Grant, No. 275/11.01.2017.

**Institutional review board**

**statement:** The agreement of the Ethical Committee of University of Medicine and Pharmacy, Targu-Mures, Romania, was obtained.

**Informed consent statement:** This is a retrospective study. No consent was necessary.

**Conflict-of-interest statement:** The

**Zsolt Kovacs, Ioan Jung, Laura Baniás, Simona Gurzu,** Department of Pathology, University of Medicine, Pharmacy, Sciences and Technology, Targu-Mures 530149, Romania

**Genoveva Rigmanyi, Simona Gurzu,** Research Center (CCAMF), University of Medicine, Pharmacy, Sciences and Technology, Targu-Mures 540139, Romania

**Corresponding author:** Simona Gurzu, MD, PhD, Chief Doctor, Research Center (CCAMF), University of Medicine, Pharmacy, Sciences and Technology, 38 Gheorghe Marinescu Street, Targu-Mures 540139, Romania. [simonagurzu@yahoo.com](mailto:simonagurzu@yahoo.com)  
**Telephone:** +40-745-673550  
**Fax:** +40-265-210407

**Abstract****BACKGROUND**

Nucleic acid isolation from formalin-fixed, paraffin-embedded tissue (FFPET) samples is a daily routine in molecular pathology laboratories, but extraction from FFPET is not always easily achieved. Choosing the right extraction technique is key for further examinations.

**AIM**

To compare the performance of four commercially available kits used for DNA extraction in routine practice.

**METHODS**

DNA isolation was performed on 46 randomly selected formalin-fixed, paraffin-embedded (FFPE) colorectal adenocarcinoma (CRC) surgical specimens. Four commercially available extraction kits were used: two for manual DNA extraction (the PureLink Genomic DNA Mini Kit from Invitrogen and the High Pure FFPE DNA Isolation Kit from Roche) and two for automated DNA extraction (the iPrep Genomic DNA Kit from Invitrogen and the MagnaPure LC DNA Isolation Kit from Roche). The DNA concentration and quality (odds ratio) among the four systems were compared. The results were correlated with the clinicopathological aspects of CRC cases: age, gender, localization, macro- and microscopic features, lymph node metastases, and the lymph node ratio.

**RESULTS**

The highest DNA concentration was obtained using the manual kits:  $157.24 \pm$

authors have declared no conflicts of interest.

**Data sharing statement:** No additional data are available.

**STROBE statement:** The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE statement-checklist of items.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Received:** March 12, 2019

**Peer-review started:** March 20, 2019

**First decision:** April 15, 2019

**Revised:** August 19, 2019

**Accepted:** September 4, 2019

**Article in press:** September 5, 2019

**Published online:** September 24, 2019

**P-Reviewer:** Vynios D, Isik A, Kopljar M

**S-Editor:** Tang JZ

**L-Editor:** A

**E-Editor:** Liu MY



62.99 ng/ $\mu$ L for the PureLink Genomic DNA Mini Kit and 86.64 ng/ $\mu$ L  $\pm$  43.84 for the High Pure FFPE DNA Isolation Kit ( $P < 0.0001$ ). Lower concentrations were obtained with automated systems: 20.39  $\pm$  21.19 ng/ $\mu$ L for the MagnaPure LC DNA Isolation Kit and 8.722  $\pm$  6.408 ng/ $\mu$ L for the iPrep Genomic DNA Kit, with differences between the systems used ( $P < 0.0001$ ). The comparison between age, gender, tumor localization, pT or pN stage and the lymph node ratio indicated no statistically significant difference in DNA concentration using any of the nucleic acid isolation kits. DNA concentration was influenced by the macroscopic features and grade of differentiation. A higher DNA concentration was obtained for well-differentiated polypoid colorectal adenocarcinomas (CRCs), compared with undifferentiated ulcero-infiltrative carcinomas, irrespective of the kit used.

## CONCLUSION

For research or diagnosis that needs high DNA concentrations, manual methods of DNA isolation should be used. A higher amount of DNA can be obtained from polypoid-type differentiated CRCs. Automated systems confer comfort and a lower amount of DNA that is, however, sufficient for classic polymerase chain reaction (PCR) and real-time quantitative PCR molecular examinations. All four commercially available kits can be successfully used in daily practice.

**Key words:** DNA isolation; Colorectal cancer; Paraffin-embedded; PureLink Genomic DNA Mini Kit; High Pure FFPE DNA Isolation Kit; iPrep Genomic DNA Kit; MagnaPure LC DNA Isolation Kit

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this paper we proved the advantages and disadvantages of manual vs automated methods of DNA extraction. The original aspect refers to the correlation between DNA concentration and colorectal adenocarcinoma (CRC) features. We conclude that manual methods are more suitable for obtaining high DNA concentrations, especially from differentiated polypoid-type CRCs. In CRC samples, a higher DNA concentration is associated with a lower OD value.

**Citation:** Kovacs Z, Jung I, Csernak E, Szentirmay Z, Baniás L, Rigmanyi G, Gurzu S. DNA extraction from paraffin embedded colorectal carcinoma samples: A comparison study of manual vs automated methods, using four commercially kits. *World J Clin Oncol* 2019; 10(9): 307-317

**URL:** <https://www.wjgnet.com/2218-4333/full/v10/i9/307.htm>

**DOI:** <https://dx.doi.org/10.5306/wjco.v10.i9.307>

## INTRODUCTION

Friedrich Miescher<sup>[1]</sup> performed the first isolation of DNA in 1868/1869. In 1988, Miller *et al*<sup>[2]</sup> described the simple salting-out procedure of DNA extraction from human cells compared to the classic phenol-chloroform method. They found that the salting-out procedure was as good as the classic method using chloroform. In 1991, Lahiri *et al*<sup>[3]</sup> demonstrated that the salting-out procedure is even better for RFLP (restriction fragment length polymorphism). Regarding the type of preserved tissue, although fresh tissues are preferred, Goelz *et al*<sup>[4]</sup> demonstrated in 1985 that DNA can also be isolated from formalin-fixed, paraffin-embedded tissues (FFPETs).

Irrespective of the source of commercially available kits, manual nucleic acid isolation methods are based on the same principle: cells must be disrupted and digested with Proteinase K and proteins and other contaminants need to be washed out in order to achieve pure DNA. Extraction from FFPETs needs an additional deparaffinization step with 100% xylene in order to get rid of the paraffin<sup>[5]</sup>.

Automated magnetic bead methods are time-saving procedures. Starting from sample lysis to DNA elution, everything is done by a machine. However, isolation from formalin-fixed, paraffin-embedded tissue requires the same additional step as manual methods, namely deparaffinization<sup>[6]</sup>.

The aim of this paper was to compare the advantages and disadvantages of four DNA extraction kits used in daily practice for DNA isolation from formalin-fixed, paraffin-embedded (FFPE) colorectal adenocarcinoma (CRC) surgical specimens: two manual and two automated magnetic bead kits. An analysis of the correlation between the results and the clinicopathological features of colorectal adenocarcinomas (CRCs) was also conducted.

## MATERIALS AND METHODS

Forty-six consecutive cases of CRC were randomly selected for DNA isolation, with the approval of the Ethical Committee of Clinical County Emergency Hospital and the University of Medicine, Pharmacy, Science and Technology of Tirgu-Mures, Romania. No preoperative chemo- or radiotherapy was administered in any of the examined cases. The used paraffin blocks from CRC surgical specimens were archived at the Department of Pathology of Clinical County Emergency Hospital of Tirgu-Mures, Romania, during the period 2010-2015.

### *Tissue preparation*

DNA was extracted from FFPE-CRCs. Hematoxylin and eosin stains were first performed to mark the most appropriate area. The selection of the tumor area was based on the presence of tumor cells in over 80% of the marked tissue, without necroses, hemorrhages, inflammatory or highly fibrotic stroma. After macrodissection of the tumor, 3 x 5 µm sections were created and inserted into 1.5 mL Eppendorf SafeLock tubes (Eppendorf, Hamburg, Germany).

### *DNA isolation with manual systems*

For manual DNA isolation, two commercially available kits were used: the PureLink Genomic DNA Mini Kit from Invitrogen Carlsbad, CA 92008, United States and the High Pure FFPE DNA Isolation Kit from Roche GmbH, Mannheim, Germany. For each of the 46 included cases, two manual isolations per case were performed according to the manufacturers' protocols (Table 1).

Invitrogen kits were tested at the Pathology Department of Mures County Hospital, while Roche kits were tested at the Molecular Pathology Laboratory of the National Institute of Oncology, Budapest. The same team performed all isolations.

The manual methods (for both kits) were performed on an anion-exchange resin. It is a macroporous silica-based resin with a high density of diethylaminoethyl (DEAE) groups. The purification is based on the interaction between the negatively charged phosphates of the nucleic acid backbone and the positively charged DEAE groups on the surface of the resin<sup>[7]</sup>.

### *DNA isolation with automated methods*

For automated DNA isolation, two commercially available automated magnetic bead kits were used: the iPrep Genomic DNA kit from Invitrogen and the MagnaPure LC DNA Isolation kit from Roche. Similar to the manual methods, for each of the 46 included cases, two automated isolations per case were performed according to the manufacturers' protocols (Table 1).

Both of the automated purification techniques use magnetic bead isolation principles. Positively charged magnetic beads can form an ionic bond with the negatively charged DNA backbone at low pH values. At high pH values, the magnetic beads lose their charge and DNA binding ability. In deparaffinized tissues, after a standard automatic tissue lysis step which takes 15 min, the genomic DNA is isolated in a 15-minute procedure that involves binding the genomic DNA to the magnetic beads in a low pH buffer, immobilizing the beads with a magnet, washing and finally, elution in a higher pH buffer (Table 1).

### *DNA concentration and quality*

DNA parameters (concentration and quality) were determined using a Nanodrop machine (ThermoScientific, United States). Readings were taken at wavelengths of 260 nm and 280 nm. The optical density (OD) ratio (A260/A280) was automatically calculated.

As a standard parameter for purity, an OD ratio value of 1.8-2.0 was used. A ratio less than 1.8 indicated protein contamination, while a ratio above 2.0 indicated contamination by chloroform, phenol or other organic compounds.

### *Statistical analysis*

Statistical analysis of the data took into account the DNA parameters, which were compared for all four systems used. They were then correlated with tumor

Table 1 DNA isolation protocols

PureLink Genomic DNA mini kit (Invitrogen–manual)	Time	iPrep genomic DNA kit (Invitrogen–automated)	Time	High Pure FFPE DNA isolation kit (Roche manual)	Time	MagnaPure LC DNA Isolation kit (Roche–automated)	Time
3 sections of 5 $\mu$ m	5-10 min	3 sections of 5 $\mu$ m	5-10 min	3 sections of 5 $\mu$ m	5-10 min	3 sections of 5 $\mu$ m	5-10 min
Deparaffinization using Xylo 3 times	3 x 10 min	Deparaffinization using Xylo 3 times	3 x 10 min	Deparaffinization using Xylo 3 times	3 x 10 min	Deparaffinization using Xylo 3 times	3 x 10 min
Lysis and digestion (20 $\mu$ L of ProtK)	From 30 min to overnight	Sample lysis		Lysis and digestion (70 $\mu$ L ProtK)	30-90 min	Sample lysis and binding	
Binding DNA to silica membranes (columns)	15 min	Binding DNA to magnetic beads	30 min	Binding DNA to silica membranes (columns)	15 min	ProtK	30 min
Washing out contaminants	15 min	Magnetic separation of beads		Washing out contaminants	15 min	Binding DNA to beads	
Eluting	3 min	Separation of liquid solutions from beads Washing beads eluting		Eluting	3 min	Washing out contaminants Washing beads eluting	
~ 1.96 Euro/sample		~ 7.8 Euro/sample		~ 3.66 Euro/sample		~ 6.81 Euro/sample	

localization, macroscopic and microscopic features, the depth of infiltration, the lymph node ratio, and the tumor stage, which were determined according to the latest classification rules<sup>[8]</sup>. Chi-squared and Fisher's exact tests were used for statistical analysis using GraphPad Prism 8.0.1 software. A *P* value lower than 0.05 with a 95% confidence interval (CI) was considered statistically significant. GraphPad Prism 8.0.1 software, using Chi Square test and Fisher's exact test, was used for statistically assessment. A *P* value lower than 0.05, at 95%CI, was considered statistically significant.

## RESULTS

### Manual systems

In all 46 cases, after the deparaffinization step with xylene, the time required for manual DNA isolation for both manual kits according to the manufacturers' protocols (Table 1) ranged from 60 min to over 12 h when overnight lysis was necessary. This time was respected for the isolation of a few probes (< 5). In cases of incomplete lysis, re-centrifugation was conducted and Proteinase K was added. These supplementary steps prolonged the isolation time irrespective of the kit used.

The major difference between the two manual isolation kits in terms of the indicated protocol (Table 1) is the quantity of Proteinase K that should be added to the tissue lysis buffer. While Invitrogen suggests using 20  $\mu$ L of Proteinase K, Roche indicates 70  $\mu$ L.

The average DNA concentration isolated with the Invitrogen manual kit was  $157.24 \pm 62.99$  ng/ $\mu$ L (37.6-316 ng/ $\mu$ L), while with the Roche kit a lower median value was obtained ( $P < 0.0001$ ) at  $86.64 \pm 43.84$  ng/ $\mu$ L (4.2-168.9 ng/ $\mu$ L). In three of the 46 cases, a higher DNA concentration was obtained with the Roche kit, compared with the Invitrogen manual kit (Table 2).

DNA purity was adequate at 1.8-2.0, without any protein or organic compound contamination, irrespective of the method used. Only four out of the 46 cases had an OD ratio lower than 1.8. A significant difference in the OD value was found between the two manual methods ( $P = 0.019$ ).

In three of the 46 probes (6.46%), the OD ratio was lower than 1.8 using the Roche manual kit, while using the Invitrogen kit, 16 of the 46 DNA samples (37.78%) had a low OD value. A higher OD value (> 2.00) was found in 29 of the 46 cases using the

**Table 2** DNA concentration (ng/ $\mu$ L) using four commercially kits for DNA isolation

	PureLink Genomic DNA mini kit (Invitrogen–manual)	iPrep genomic DNA kit (Invitrogen–automated)	MagnaPure LC DNA Isolation kit (Roche–automated)	High Pure FFPE DNA isolation kit (Roche–manual)
Mean	157.24	8.72	20.39	86.64
Standard deviation	62.99	6.40	21.19	43.84
Minimum	37.60	0.70	0.30	4.20
Maximum	316	29.80	121	168.90

Roche system and in no cases using the Invitrogen system (Table 3).

### Automated methods

For both automated methods, the protocol indicated by Invitrogen and Roche, using the magnetic beads principle, was similar (Table 1). For one run, the total time was 30 min for 12 probes with the iPrep Genomic DNA kit from Invitrogen and 30 min for 11 probes with the MagnaPure LC DNA Isolation Kit from Roche. For each run, one template control was used to check the probes for contamination.

Compared with the manual kits, the DNA concentration obtained was significantly lower ( $P < 0.0001$ ) irrespective of the automatic system used (Figure 1).

A significantly lower ( $P < 0.0001$ ) DNA concentration ( $8.72 \pm 6.4$  ng/ $\mu$ L, 0.70-29.80) was obtained with the automatic iPrep Genomic DNA Kit from Invitrogen, compared with the automatic MagnaPure LC DNA Isolation Kit from Roche ( $20.39 \pm 21.19$  ng/ $\mu$ L, 0.30-121) (Tables 2 and 3).

Regarding DNA purity, no significant difference in the OD value was found between the two automated methods ( $P = 0.56$ ).

In 19 of the 46 probes (41.30%), the OD ratio was lower than 1.8 using the Roche automated system, while using the Invitrogen automated system, 21 of the 46 DNA samples (45.65%) had low OD values. Higher OD values ( $> 2$ ) were found in 12 of the 46 cases using the Roche system and in four of the 46 cases using the Invitrogen system (Table 3).

Compared to manual isolation methods, the OD values obtained with automated systems were similar for Invitrogen kits ( $P = 0.32$ ), whereas automated DNA extraction was associated with lower OD values ( $P < 0.0001$ ).

### Clinicopathological factors and DNA parameters

The comparison between age, gender, tumor localization, the depth of infiltration (pT), lymph node status (pN stage), and the lymph node ratio found no statistically significant difference in DNA concentration using any of the nucleic acid isolation kits (Tables 4-7).

DNA concentration was influenced by the macroscopic aspects and grade of differentiation. A higher concentration of DNA was obtained for polypoid in comparison to ulcero-infiltrative carcinomas, with both Roche systems (Tables 6 and 7) and using the automated system from Invitrogen (Table 5). The manual kit from Invitrogen allowed good concentrations to be extracted, but in half of the cases (23 of 46 cases) a value below 150 ng/ $\mu$ L was obtained (Table 4). For this reason, the  $P$  value was considered to be at the limit of statistical significance.

Regarding the microscopic aspect of CRC, the concentration of nucleic acids was higher in well-differentiated (G1) carcinomas, compared with G2 + G3 cases (Tables 4-7).

## DISCUSSION

In FFPETs, after deparaffinization, the first step in DNA isolation is cell disruption/lysis<sup>[3]</sup>. After DNA exposure, membrane lipid removal is conducted by adding detergents, proteins and even proteases (an optional step, but almost always included). Precipitation of the DNA is then performed with alcohol (usually ice-cold ethanol or isopropanol). At the end of the procedure, solubilizing the DNA must be conducted in an alkaline buffer or in ultra-pure water.

During DNA isolation, a chelating agent can be added in order to bind divalent cations and stop DNase activities. Cellular or histone proteins bound to DNA can be removed by adding a protease or by precipitating proteins with sodium/ammonium acetate, or extracting them with a phenol-chloroform mixture prior to the DNA

**Table 3** DNA quality<sup>1</sup> using four commercially kits for DNA isolation

	PureLink Genomic DNA mini kit (Invitrogen–manual)	iPrep genomic DNA kit (Invitrogen–automated)	MagnaPure LC DNA Isolation kit (Roche–automated)	High Pure FFPE DNA isolation kit (Roche–manual)
Mean	1.80	1.78	1.84	2.19
Standard deviation	0.04	0.14	0.52	0.37
Minimum	1.67	1.42	0.99	1.60
Maximum	3.10	2.25	4.36	3.10

<sup>1</sup>Quality-OD: A260/A280.

precipitation.

The most commonly used protease in DNA extraction is Proteinase K (protease K or endopeptidase K), which is a broad-spectrum serine protease. It digests and removes proteins as a nucleic acid decontamination step. Proteinase K also inactivates nucleases that might induce DNA or RNA degradation during DNA purification. In this study, it was observed that protein contamination was the same when manual protocols were used, highlighting the fact that it is not affected by the amount of Proteinase K (20  $\mu\text{L}$  vs 70  $\mu\text{L}$ ). On the other hand, the manual probes showed a higher median DNA concentration (157 ng/ $\mu\text{L}$  vs 87 ng/ $\mu\text{L}$ ). Irrespective of the manufacturer, the automated DNA extraction was associated with a higher protein contamination rate (OD < 1.8). In these cases, it related to a shorter Proteinase K exposure time, which cannot be modified in-house. Better tissue lysis might induce a lower protein contamination rate.

One original aspect that could be useful in daily practice concerns the correlation obtained in this study between DNA concentration and the clinicopathological parameters of CRCs. Patient age and gender did not influence the DNA concentration, as well as most of the tumor parameters (localization, macroscopic features, pT and pN stage, and lymph node ratio).

We successfully proved that the highest concentration of DNA can be obtained from FFPE well-differentiated CRCs with a polypoid aspect, irrespective of their localization. As ulcero-infiltrative tumors are usually associated with a higher grade of macroscopic lysis, this parameter can influence DNA parameters.

Tumor dedifferentiation might be associated with a high cell division rate<sup>[9,10]</sup>, which could lead to a lower rate of successful DNA lysis.

There are several commercial kits available that include manual and automated isolation procedures. However, although time-consuming, nucleic acid isolation can be done by in-house preparation of all the buffers and solutions necessary for extraction<sup>[9,10]</sup>. The method used should take into account the quantity of DNA needed [e.g., for adductomics studies or polymerase chain reaction (PCR)] but also the human component, as manual systems need to be managed by well-prepared technicians or biologists.

Fully automated methods can be used successfully for PCR reactions. Although the DNA concentration obtained is lower than by manual methods, it is sufficient for PCR. The costs are higher than for the manual methods.

All of the probes from this study were successfully amplified for real-time PCR reactions. The literature data show that both DNA and RNA can be isolated by automated methods from FFPEs<sup>[11-13]</sup>. The authors applied a fully automated xylene-free isolation with iron oxide beads coated with a nanolayer of silica<sup>[11-13]</sup>.

An important step in performing DNA isolation from FFPEs is the pre-isolation protocol. Deparaffinization can be performed in tubes (such as in this study) or using slide-digestion (overnight or 72 h) based on in-house protocols. Both methods can be successfully adopted. DNA concentration obtained after 72 h on slide-deparaffinization can be over 500 ng/ $\mu\text{L}$ <sup>[11-13]</sup>.

In 2015, Kocjan *et al*<sup>[14]</sup> compared 69 commercially available DNA extraction kits from 43 companies. They showed that deparaffinization and supplementary lysis can induce a lower DNA concentration<sup>[14]</sup>. In this study, we have shown that a lower amount of Proteinase K with longer tissue exposure (which is possible for manual kits) leads to a higher concentration of DNA. Although manual extraction confers a higher yield and DNA concentration, automated isolation will replace it in short time, when the costs decrease significantly<sup>[15]</sup>.

The unresolved issue refers to the imbalance between concentration and quality. We have obtained a reverse correlation between concentration and OD value, which could help researchers in their decisions regarding the most appropriate methods

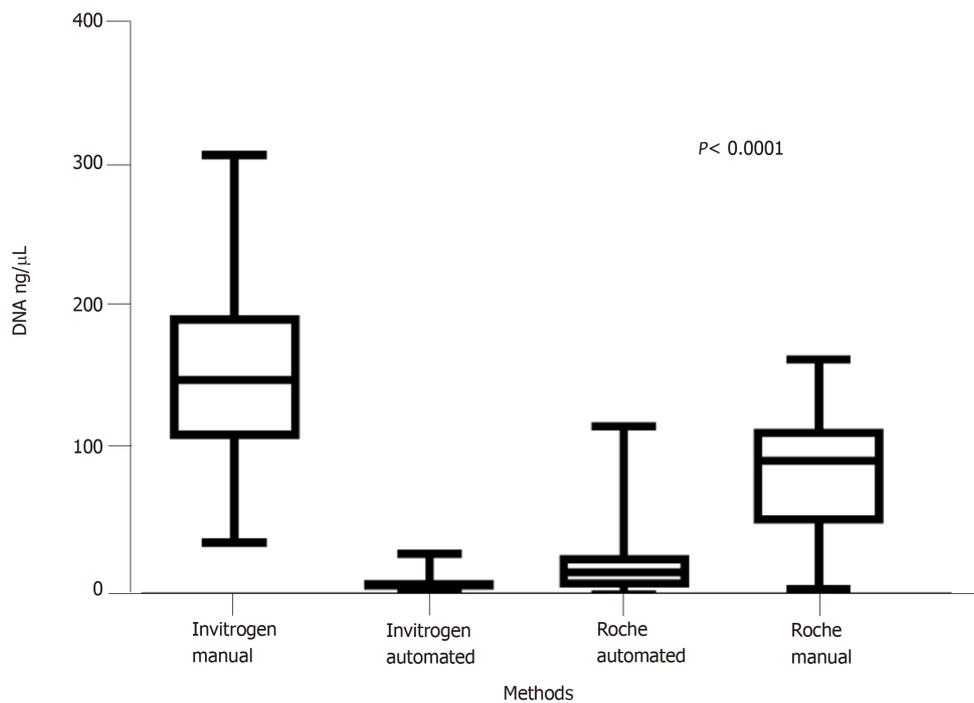


Figure 1 Comparison of four commercially kits show that a higher DNA concentration was obtained with manual, compared with automated methods.

(manual *vs* automatic) and provide explanations for the understandable problems encountered daily in the laboratory. Similar to our findings, it was previously demonstrated that DNA integrity is higher with manual purification, for both tissues and whole blood<sup>[16]</sup>.

The present study has some limitations. Firstly, the number of included cases is small and originates from a single department, with the same techniques used for tissue preparation. Secondly, only CRC samples were used. The above-mentioned aspects should be investigated in larger cohorts with sample size calculations.

In conclusion, the results of this single-center study highlight the importance of the quality of nucleic acid isolation techniques. Manual methods proved to be more controllable and permit in-house adaptation of the protocol, while the obtained DNA concentrations and purity were higher. On the other hand, automated methods are a time-saving option for PCR and real-time quantitative PCR reactions. For CRC samples, it is expected that a higher DNA concentration would be obtained from differentiated polypoid carcinomas.

**Table 4 Correlation between DNA concentration<sup>1</sup> and clinicopathological aspects of colorectal cancer**

Characteristics		Number	DNA concentration PureLink Genomic DNA mini kit (manual)		P value
			≤ 150 ng/μL	> 150 ng/μL	
			Age	≤ 60	
	> 60	26	11	15	
Gender	Male	26	15	11	0.37 <sup>3</sup>
	Female	20	8	12	
Macroscopic aspect	Polypoid	10	4	15	0.0010 <sup>3</sup>
	Ulceroinfiltrative	36	19	8	
Microscopic aspect	G1	13	3	10	0.04 <sup>3</sup>
	G2 + G3	33	20	13	
Localization	Proximal colon	8	5	3	0.89 <sup>2</sup>
	Distal colon	21	11	10	
	Rectum	17	7	10	
Depth of infiltration (pT stage)	T2-T3 T4	40 6	20 3	20 3	> 0.99 <sup>2</sup>
Lymph node metastasis (pN stage)	Present (pN1-3)	17	13	4	0.06 <sup>3</sup>
	Absent (pN0)	29	10	19	
Lymph node ratio	< 0.15	36	17	19	0.72 <sup>3</sup>
	≥ 0.15	10	6	4	

<sup>1</sup>Manual PureLink Genomic DNA mini kit-Invitrogen.<sup>2</sup>Chi square test.<sup>3</sup>Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.**Table 5 Correlation between DNA concentration<sup>1</sup> and clinicopathological aspects of colorectal cancer**

Characteristics		Number	iPrep genomic DNA kit (magnetic beads)		P value
			≤ 10 ng/μL	> 10 ng/μL	
			Age	≤ 60	
	> 60	26	22	4	
Gender	Male	26	19	7	0.99 <sup>3</sup>
	Female	20	15	5	
Macroscopic aspect	Polypoid	10	3	7	0.0004 <sup>3</sup>
	Ulceroinfiltrative	36	31	5	
Microscopic aspect	G1	13	4	9	< 0.0001 <sup>3</sup>
	G2 + G3	33	30	3	
Localization	Proximal colon	8	6	2	0.52 <sup>2</sup>
	Distal colon	21	17	4	
	Rectum	17	11	6	
Depth of infiltration (pT stage)	T2-T3 T4	40 6	28 6	12 0	0.11 <sup>2</sup>
Lymph node metastasis (pN stage)	Present (pN1-3)	17	14	3	0.31 <sup>3</sup>
	Absent (pN0)	29	20	9	
Lymph node ratio	< 0.15	36	25	11	0.25 <sup>3</sup>
	≥ 0.15	10	9	1	

<sup>1</sup>Automated iPrep genomic DNA kit-Invitrogen.<sup>2</sup>Chi square test.<sup>3</sup>Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.

**Table 6 Correlation between DNA concentration<sup>1</sup> and clinicopathological aspects of colorectal cancer**

Characteristics		Number	High Pure FFPE DNA isolation kit (manual)		P value
			< 100 ng/μL	≥ 100 ng/μL	
Age	≤ 60	20	12	8	0.99 <sup>3</sup>
	> 60	26	15	11	
Gender	Male	26	15	11	0.99 <sup>3</sup>
	Female	20	12	8	
Macroscopic aspect	Polypoid	10	8	17	0.12 <sup>3</sup>
	Ulceroinfiltrative	36	19	2	
Microscopic aspect	G1	13	3	10	0.002 <sup>3</sup>
	G2 + G3	33	24	9	
Localization	Proximal colon	8	5	3	0.96 <sup>2</sup>
	Distal colon	21	12	9	
	Rectum	17	10	7	
Depth of infiltration (pT stage)	T2-T3 T4	40 6	24 3	16 3	0.64 <sup>2</sup>
Lymph node metastasis (pN stage)	Present (pN1-3)	17	8	9	0.35 <sup>3</sup>
	Absent (pN0)	29	19	10	
Lymph node ratio	< 0.15	36	23	13	0.27 <sup>3</sup>
	≥ 0.15	10	4	6	

<sup>1</sup>Manual High Pure FFPE DNA isolation kit-Roche.<sup>2</sup>Chi square test.<sup>3</sup>Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.**Table 7 Correlation between DNA concentration<sup>1</sup> and clinicopathological aspects of colorectal cancer**

Characteristics		P value	MagnaPure LC DNA Isolation kit (magnetic beads)		P value
			< 20 ng/μL	≥ 20 ng/μL	
Age	≤ 60	20	12	8	0.38 <sup>3</sup>
	> 60	26	12	14	
Gender	Male	26	12	14	0.38 <sup>3</sup>
	Female	20	12	8	
Macroscopic aspect	Polypoid	10	8	2	0.04 <sup>3</sup>
	Ulceroinfiltrative	36	16	20	
Microscopic aspect	G1	13	4	9	0.02 <sup>3</sup>
	G2 + G3	33	23	10	
Localization	Proximal colon	8	3	5	0.60 <sup>2</sup>
	Distal colon	21	11	10	
	Rectum	17	10	7	
Depth of infiltration (pT stage)	T2-T3	40	20	20	0.44 <sup>2</sup>
	T4	6	4	2	
Lymph node metastasis (pN stage)	Present (pN1-3)	17	5	12	0.03 <sup>3</sup>
	Absent (pN0)	29	19	10	
Lymph node ratio	< 0.15	36	18	18	0.72 <sup>3</sup>
	≥ 0.15	10	6	4	

<sup>1</sup>Automated MagnaPure LC DNA Isolation kit-Roche.<sup>2</sup>Chi square test.<sup>3</sup>Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.

## ARTICLE HIGHLIGHTS

### Research background

Nucleic acid isolation from formalin-fixed, paraffin-embedded tissue (FFPET) samples is a daily routine in molecular pathology laboratories, but extraction from FFPET is not always easily achieved. Choosing the right extraction technique is key for further examinations. Several commercial kits are available on the molecular biology market, including both manual isolation procedures and automated extraction. When choosing the right method for isolation, consideration must be given to the aspects of time, precision, downstream applications and price. Choosing the right technique is key for success in molecular biology, because nucleic acid isolation is always the first step in molecular biology and molecular pathology.

### Research motivation

The aim of this paper was to compare the advantages and disadvantages of four DNA extraction kits used in daily practice for DNA isolation from formalin-fixed, paraffin-embedded (FFPE) colorectal adenocarcinoma (CRC) surgical specimens: two manual and two automated magnetic bead kits. A correlation of the results with the clinicopathological features of CRCs was also performed.

### Research objectives

By comparing the advantages and disadvantages of nucleic acid isolation techniques used in daily routines, precise decisions can be made regarding the most suitable DNA extraction approach for molecular applications.

### Research methods

DNA was extracted from FFPE-CRCs. The selection of tumor area was based on the presence of tumor cells in over 80% of the marked tissue, without necroses, hemorrhages, inflammatory, or highly fibrotic stroma. For manual DNA isolation, two commercially available kits were used: The PureLink Genomic DNA Mini Kit from Invitrogen Carlsbad, CA 92008, United States and the High Pure FFPE DNA Isolation Kit from Roche GmbH, Mannheim, Germany. For automated DNA isolation, two commercially available automated magnetic bead kits were used: The iPrep Genomic DNA Kit from Invitrogen and the MagnaPure LC DNA Isolation Kit from Roche. DNA parameters (concentration and quality) were determined using a Nanodrop machine (ThermoScientific, United States). Readings were taken at wavelengths of 260 nm and 280 nm. The optical density (OD) ratio (A260/A280) was automatically calculated, before being correlated with tumor localization, macroscopic and microscopic features, the depth of infiltration, the lymph node ratio, and tumor stage, which were determined according to the latest classification rules.

### Research results

DNA concentration was influenced by the macroscopic features and grade of differentiation. A higher DNA concentration was obtained for polypoid compared with ulcero-infiltrative carcinomas, with both Roche systems and using the automated system from Invitrogen. The manual kit from Invitrogen allowed good concentrations to be extracted, but in half of the cases (23 of 46 cases) a value below 150 ng/μL was obtained. For this reason, the *P* value was considered to be at the limit of statistical significance.

### Research conclusions

Manual methods of DNA extraction are more controllable and allow the in-house adaptation of the protocol. The obtained DNA concentrations and purity are higher. Automated methods are a time-saving option for polymerase chain reaction (PCR) and real-time quantitative PCR reactions. For CRC samples, a higher DNA concentration is expected to be obtained from differentiated polypoid carcinomas.

### Research perspectives

DNA integrity is higher when manual purification is performed, for both tissues and whole blood. The unresolved issue refers to the imbalance between concentration and quality. The above-mentioned aspects should be investigated in larger cohorts with sample size calculations.

## REFERENCES

- 1 **Dahm R.** Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. *Hum Genet* 2008; **122**: 565-581 [PMID: 17901982 DOI: 10.1007/s00439-007-0433-0]
- 2 **Miller SA, Dykes DD, Polesky HF.** A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215 [PMID: 3344216 DOI: 10.1093/nar/16.3.1215]
- 3 **Lahiri DK, Nurnberger JI.** A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**: 5444 [PMID: 1681511 DOI: 10.1093/nar/19.19.5444]
- 4 **Goelz SE, Hamilton SR, Vogelstein B.** Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem Biophys Res Commun* 1985; **130**: 118-126 [PMID: 2992457 DOI: 10.1016/0006-291X(85)90390-0]
- 5 **Ludyga N, Grünwald B, Azimzadeh O, Englert S, Höfler H, Tapio S, Aubele M.** Nucleic acids from long-term preserved FFPE tissues are suitable for downstream analyses. *Virchows Arch* 2012; **460**: 131-140 [PMID: 22270699 DOI: 10.1007/s00428-011-1184-9]
- 6 **Barresi V, Cinnirella G, Valenti G, Spampinato G, Musso N, Castorina S, Condorelli DF.** Gene

- expression profiles in genome instability-based classes of colorectal cancer. *BMC Cancer* 2018; **18**: 1265 [PMID: 30563495 DOI: 10.1186/s12885-018-5174-z]
- 7 **Smith C**, Otto P, Bitner R, Shiels G. A silica membrane-based method for the isolation of genomic DNA from tissues and cultured cells. *CSH Protoc* 2006; **2006** [PMID: 22485501 DOI: 10.1101/pdb.prot4097]
- 8 **Fulop ZZ**, Gurzu S, Bara T, Dragus E, Bara T, Voidazan S, Banias L, Jung I. Lymph node ratio, an independent prognostic factor for patients with stage II-III rectal carcinoma. *Pathol Res Pract* 2019; **215**: 152384 [PMID: 30910253 DOI: 10.1016/j.prp.2019.03.013]
- 9 **Jordan P**. Colorectal Cancer Subtypes - The Current Portrait. *Adv Exp Med Biol* 2018; **1110**: 1-6 [PMID: 30623362 DOI: 10.1007/978-3-030-02771-1\_1]
- 10 **Tan SC**, Yiap BC. DNA, RNA, and protein extraction: the past and the present. *J Biomed Biotechnol* 2009; **2009**: 574398 [PMID: 20011662 DOI: 10.1155/2009/574398]
- 11 **Hennig G**, Gehrman M, Stropp U, Brauch H, Fritz P, Eichelbaum M, Schwab M, Schroth W. 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. *Clin Chem* 2010; **56**: 1845-1853 [PMID: 20947696 DOI: 10.1373/clinchem.2010.151233]
- 12 **Bohmann K**, Hennig G, Rogel U, Poremba C, Mueller BM, Fritz P, Stoerkel S, Schaefer KL. RNA extraction from archival formalin-fixed paraffin-embedded tissue: a comparison of manual, semiautomated, and fully automated purification methods. *Clin Chem* 2009; **55**: 1719-1727 [PMID: 19617290 DOI: 10.1373/clinchem.2008.122572]
- 13 **Sengüven B**, Baris E, Oygur T, Berktaş M. Comparison of methods for the extraction of DNA from formalin-fixed, paraffin-embedded archival tissues. *Int J Med Sci* 2014; **11**: 494-499 [PMID: 24688314 DOI: 10.7150/ijms.8842]
- 14 **Kocjan BJ**, Hošnjak L, Poljak M. Commercially available kits for manual and automatic extraction of nucleic acids from formalin-fixed, paraffin-embedded (FFPE) tissues. *Acta Dermatovenerol Alp Pannonica Adriat* 2015; **24**: 47-53 [DOI: 10.15570/actaapa.2015.12]
- 15 **Kalmár A**, Péterfia B, Wichmann B, Patai ÁV, Barták BK, Nagy ZB, Furi I, Tulassay Z, Molnár B. Comparison of Automated and Manual DNA Isolation Methods for DNA Methylation Analysis of Biopsy, Fresh Frozen, and Formalin-Fixed, Paraffin-Embedded Colorectal Cancer Samples. *J Lab Autom* 2015; **20**: 642-651 [PMID: 25576093 DOI: 10.1177/2211068214565903]
- 16 **Riemann K**, Adamzik M, Frauenrath S, Egensperger R, Schmid KW, Brockmeyer NH, Siffert W. Comparison of manual and automated nucleic acid extraction from whole-blood samples. *J Clin Lab Anal* 2007; **21**: 244-248 [PMID: 17621359 DOI: 10.1002/jcla.20174]



Published By Baishideng Publishing Group Inc  
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA  
Telephone: +1-925-2238242  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <https://www.f6publishing.com/helpdesk>  
<https://www.wjgnet.com>

