

Format for ANSWERING REVIEWERS



June 10, 2019

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: reviewed manuscript-47274.doc).

Title: DNA extraction from paraffin embedded colorectal carcinoma samples: A comparison study of manual versus automated methods, using four commercial kits

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Name of Journal: *World Journal of Clinical Oncology*

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Reviewer`s 1 opinion

The present study was undertaken to compare the performance of four commercial kits used for DNA extraction from formalin-fixed paraffin-embedded CRC tissues in routine practice. The authors have compared DNA concentration and quality among the four systems, two manual and two automated, and the results obtained have been subjected to correlation with the various clinicopathological aspects of CRC cases. They have found that manual systems provided DNA of higher concentration, although all four kits may be used in daily routine. The work is very interesting, since DNA extraction is the first step in molecular diagnosis or general molecular studies in cancer. However, the m/s cannot be accepted for publication in its present form, since there are many points requiring correction or clarification.

A. Major points:

1. Lines 277-279: Table 4 gives different information than that described. The problem seems to be mainly with the automated method the results of which are presented in Table 5, where 34 of 46 cases gave DNA of concentration less than 10 ng/mL.
2. Lines 298-299: The explanation seems to be incorrect, since the use of different proteinase K amounts is related with the company providing the kits.

B. Minor points

1. Line 241: Give additional information in "in only four of the 46 cases". All kits provided pure DNA from the same 4 samples? Or, only 4 samples, but different in each one of the methods applied, gave pure DNA?
2. Lines 242, 244, 245, 264, 302: It is not simply OD measurement, it is OD ratio.
3. Lines 244-247 & 264-265: It is not included in the table the number of cases providing DNA with lower than 1.8 or higher than 2.0 OD ratio (OD[260nm]/OD[280nm]).
4. Grammar/typo errors Line 113, 271: statistically Line 123: commercially Lines 146, 147: chloroform Lines 156, 225: Xylene Line 168: Technology Line 202: lose Line 216: takes Line 261: automated Line 284, 330: deparaffinization Line 305: regarding Line 311: replace "form" with "from" or "of" Line 315: successful Line 317: nucleic acid isolation Line 319: replace "fro" with "for" Line 322: successfully Line 341: refer Line 355: replace "abd" with "and"

Authors' answers

- A. Thank you for all your precious suggestions.
1. Lines 277-279 were reformulated, in order to be unanimous with the Tabel 4, mentioned at this section.
 2. Lines 298-299 Explanation of the use of different proteinase K amount was reformulated, because proteinase K quantities does not influence the DNA purity.
- B. Thank you for the minor points, too.
1. Line 241 was reformulated.
 2. We have modified.
 3. It is included separately in each table of each of the used DNA isolation method.
 4. Grammar/typo mistakes were corrected by a professional language service. A certificate was attached.

Reviewer's 2 opinion

Well written manuscript. I have some suggestions.

- 1- What is the statistical method?
- 2- "Endoscopy may help at differential diagnosis of CRC" (Turkiye Klinikleri J Gen Surg-Special Topics. 2018;11(2):112-49 and (<https://doi.org/10.1016/j.ijssu.2018.04.037>) I suggest both of these up-to-date studies for the references.

Authors' answers

1. Thank you for reviewing our manuscript. The used statistical method was Chi square test and Fisher's exact test analyzed with Graph Pad Prism 8 software. We have added this information in the main text.
2. The two articles you have suggested for reference are about thyroid pathology and pilonidal sinus and abscess and are not related to the present paper.

Reviewer's 3 opinion

Dear Authors, I read with care the manuscript titled: "DNA extraction from paraffin embedded colorectal carcinoma samples: A comparison study of manual versus automated methods, using four commercial kits". My greatest objection is that no sample size calculation was performed to ensure that negative results could be interpreted as negative.

Authors' answers

Thank you for your time reviewing our manuscript. As we described at the limitations of the study, we used a small sample size in order to see if it is reasonable to do further testing on these population. After this study we have decided to do a larger cohort study with colorectal cancer, comparing the same DNA isolation methods, and doing further molecular analysis on these biological samples. We have added in the main text that this is the limitation of the study, which is based on a small sample size.

Thank you again for publishing our manuscript in the *World Journal of Clinical Oncology*

Sincerely yours,

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