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PEER-REVIEW REPORT

Name of journal: *World Journal of Orthopedics*

Manuscript NO: 72399

Title: Quantitative alpha-defensin testing: Is synovial fluid dilution important?

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 02713510
Position: Peer Reviewer
Academic degree: MD, MSc
Professional title: Doctor
Reviewer's Country/Territory: Netherlands
Author's Country/Territory: Brazil

Manuscript submission date: 2021-10-15
Reviewer chosen by: AI Technique
Reviewer accepted review: 2021-10-16 07:17
Reviewer performed review: 2021-10-21 20:22
Review time: 5 Days and 13 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer	Peer-Review: [ ] Anonymous [Y] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In general, when it is even mentioned, the dilution is very unclear, I agree. - Deirmengian 2015 (the AD test outperforms): "This optimization included dilution optimization of the synovial fluid to eliminate the effects of varying viscosity between samples" - Deirmengian 2014 (combined measurement): "The assays were optimized specifically for performance in synovial fluid by scientists with specific training in immunoassay development. This included dilution optimization of the synovial fluid for both assays. One purpose of the dilution optimization was to attain a synovial fluid dilution that eliminated the effect of fluid viscosity on immunoassay results, even for the samples with the highest viscosity" Most other studies seem to send it to a laboratory and just get a result. If hospitals want to use the kit themselves, and have a spectrometer, it seems the dilution indeed is necessary. In that sens, this study is useful. However, do you have any comments on the two quotes by Deirmengian that the viscosity needs to be addressed per sample? Also, you should probably comment on the methods these authors used, to show you've checked what others did, and also to underline why your study is useful for others. Regarding the definition, could you elaborate on the infected cases: i.e., how many had a sinus tract, how many had two or more positive samples, and how many just minor criteria, and which? Usually a table is helpful for this. This gives the reader an idea of the patient population. Another question about the population: the ratio of infected - non infected is relatively high, when considering that pain >3 months is an inclusion criterion; in my experience, many patients still have pain. Could you explain who made the choice to perform aspiration in those patients? Looking at the different studies written by Deirmengian et al, their alpha defensin mean seems to be around 60-80 mg/mL, probably twice the mean you seem to have found in

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the 1:5000 dilution; don't you think the effect wouldn't have been better even with 1:10000 dilution? You don't offer an explanation why the more diluted samples yield a higher concentration. Is this a matter of the spectrometer being more sensitive, a matter of calculation, or something else? Do you think 1:10000 dilution would result in concentrations ranging from 25-100 mg/L (double that of 1:5000)? I'm just a simple orthopedic surgeon I guess, but it seems strange that double dilution would result in double the concentration. Please elaborate. Overall, I think the English is pretty good, but for publication a readover by a native speaker may be useful.

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Reviewer's code: 05371577
Position: Peer Reviewer
Academic degree: BSc
Professional title: Research Assistant
Reviewer's Country/Territory: China
Author's Country/Territory: Brazil

Manuscript submission date: 2021-10-15

Reviewer chosen by: Xin Liu

Reviewer accepted review: 2021-12-27 07:10

Reviewer performed review: 2021-12-27 08:21

Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
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SPECIFIC COMMENTS TO AUTHORS

This study aimed to evaluate the potential influence of dilution on the quantitative alpha-defensin ELISA test for the diagnosis of PJI in the synovial fluid of patients with total knee arthroplasty (TKA). If there is a lack of relevant international experimental operation standards, this study has certain clinical significance. Tthere were any question: 1. Can you describe how to use Wilson Brown method to obtain diagnostic efficiency? Because the calculation of sensitivity and specificity requires a gold standard as a reference system. 2.Make sure the experimental operation is accurate?Does the solvent not add enough in the dilution process? From Fig.1, we can see an interesting phenomenon; with the increase of dilution ratio, the greater the dispersion of the results. 3.We can try to measure the same sample repeatedly at the dilution ratio of 1:1000 to obtain the coefficient of variation of the result, which is a supplement to whether the method is suitable for practical experimental operation. 4. Why should it be divided into affected and aseptic cases? Would it be better to use alpha defensin to distinguish between affected and aseptic cases? 5.The prozone phenomenon, also known as hook effect, this may be the reason why the dilution ratio needs to be increased.