ORIGINAL ARTICLE

# Impact of Capping Agent Incorporation on pH Stability of Silver Diamine Fluoride: An in Vitro Analysis

Mehmood Asghar[1], Ros Anita Omar[2*], Adrian UJin Yap[3], Nida Zehra Bano[1], Rosiyah Yahya[4], Ahmad Danial Azzahari[5]

## ABSTRACT

**Objective:** To determine the chemical stability by evaluating time-dependent pH changes in 38% silver diamine fluoride with or without incorporating different concentrations of tannic acid and glutathione as capping agents.

**Study Design:** Experimental, in vitro study.

**Place and Duration of Study:** The study was conducted at the Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia from January 2021 to April 2022.

**Methods:** Tannic acid and Glutathione-modified silver diamine fluoride solutions were prepared by incorporating tannic acid or Glutathione at different concentrations (5,10,15% w/v) into silver diamine fluoride. pH changes in the solutions were measured at different time points: from 1 hour after the bottles were opened to day 90.

**Results:** The pH of 38% silver diamine fluoride from baseline till day-90 ranged between 7.73±0.02 and 7.69±0.01. The incorporation of tannic acid and glutathione resulted in a significant increase in the pH of the silver diamine fluoride solution. In tannic acid-modified silver diamine fluoride, the highest 90-day pH was shown by silver diamine fluoride + 5% tannic acid (w/v) (9.00±0.01), while in the glutathione-modified silver diamine fluoride, silver diamine fluoride + 15% Glutathione (w/v) had the highest 90-day pH (9.80±0.02). Repeated measures analysis of variance showed a statistically significant difference between the groups' pH values. A pairwise comparison showed significant differences in the pH of all the possible pairs at all-time points ($P<0.05$), except the pairs silver diamine fluoride + 15% tannic acid (w/v) and silver diamine fluoride + 15% glutathione (w/v) after the 1st hour and day 1 ($P > 0.05$).

**Conclusion:** Silver diamine fluoride and tannic acid/glutathione-modified silver diamine fluoride solutions remain stable for up to 90 days. Capping agent incorporation significantly increases the pH of silver diamine fluoride, thereby enhancing its long-term stability and shelf life.

**Keywords:** *pH, Silver Diamine Fluoride, Tooth Remineralization.*

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## Introduction

Silver diamine fluoride (SDF) is a colorless solution of silver ($Ag^+$) and Fluoride ($F^-$) ions, stabilized with ammonia.[1,2] SDF is regarded as a cost-effective, efficacious, and safe option for the topical management of dental caries in children and adults.[3] The $Ag^+$ ions provide an antibacterial effect while the $F^-$ ions remineralize the dental tissues. A study by Zhang et al. showed that the combination of SDF annual application of 38% SDF combined with oral hygiene education was the most effective option for root caries prevention among other professionally

*[1]Department of Basic Dental Sciences*
*Army Medical College, Rawalpindi, Pakistan*
*[2]Department of Restorative Dentistry/Chemistry[4]/*
*Centre for Foundation Studies in Science[5]*
*University of Malaya, Kuala Lumpur, Malaysia*
*[3]Department of Dentistry*
*Ng Teng Fong General Hospital and Faculty of Dentistry*
*National University Health System, Singapore*

*Correspondence:*
*Dr. Ros Anita Omar*
*Department of Restorative Dentistry*
*Faculty of Dentistry, Universality of Malaya, Kuala Lumpur, Malaysia*
*E-mail: anitaomar@um.edu.my*

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applied topical remineralization agents, such as sodium fluoride (NaF) and acidulated calcium phosphate (ACP).[4] Similarly, a systematic review by Duangthip et al. showed that SDF was superior to NaF varnish in preventing occlusal surface caries of the primary teeth.[5]

Despite the efficacy of SDF, its clinical use is limited owing to its potential to stain demineralized dental tissues due to Ag deposition. Various approaches have been utilized to overcome the staining potential of SDF, such as the incorporation of glutathione (G) in SDF, or the application of potassium iodide (KI) following SDF treatment. However, these studies revealed varying results and did not eliminate the issue.

Despite the potential of CAs to minimize SDF-mediated dentine staining, their effect on the chemical properties of SDF is not clearly known. Previously, the successful capping effect of TA, GA, CMC, and G on SDF through FTIR and UV-Vis spectroscopy. However, it is also pertinent to consider the effect of CA-incorporation on the pH of the SDF, as it has been shown that the SDF solution only remains stable in an alkaline environment.[6,7] Previous studies have shown that the critical pH for SDF, above which it remains chemically stable, is 6.7.[8,9] It is probable that the incorporation of CAs may induce a pH-change that may affect its stability, shelf-life, and chemical efficacy.

Mei et al. measured the pH values of three commercially available SDF products and showed that all the solutions remained stable after 28 days with minimal pH variations.[10] Similarly, Crystal et al. showed that 38% SDF could remain stable in terms of pH for up to 28 days.[11] Yan et al. evaluated the pH values of five commercially available SDF products for up to 180 days and showed that the solutions exhibited minimal time-dependent pH changes.[12]

While there is scientific evidence currently available that demonstrates the long-term stability of SDF, information regarding the effect of such modifications on the long-term stability of SDF is still lacking. According to Yan et al. SDF continues to be used clinically for up to 6 months after opening the bottle.[12] Hence, a similar shelf life should be expected from any SDF modifications. Therefore, it is pertinent to determine whether CA incorporation will affect SDF chemically, as it is an indirect indicator of shelf-life and clinical efficacy.

The aim of this study was to determine the long-term pH changes in SDF following the incorporation of various capping agents. Based on the findings of our previous studies, TA and G were the most effective CA in terms of dentine stain minimization associated with SDF. Hence, these CA agents were selected for subsequent testing. Different concentrations (5, 10, 15% w/v) of TA and G were incorporated in 38% SDF, and the time-dependent pH changes were measured for up to 90 days. The Null Hypothesis was that there would be no effect of CA-incorporation on the long-term pH of SDF.

## Methods

The study was conducted at the Faculty of Dentistry, University of Malaya Kuala Lumpur, Malaysia from January 2021 to April 2022 after taking approval from the Institutional Review Board (IRB) of the university prior to the commencement of the study vide letter no: IRB No. DF RD 1930/0093 (L), held on dated: 07th October 2019.

In the current study, 38% SDF (FAgamin, Argentina) was used as a control. The procedure for the preparation of the experimental studies has been discussed in detail in our previous studies. Briefly, CA-modified SDF solutions were prepared by incorporating TA and G into SDF in different concentrations (5, 10, and 15% w/v). The CAs were weighed in a measuring balance (Sartorius, CPA 4235, Göttingen, Germany) with an accuracy of up to four decimal places and incorporated in SDF, according to their respective concentrations. The control and experimental groups, and the amount of capping agent needed for preparing the solutions, are shown in table-1.

Since small volumes of the solutions were sufficient for application to the dentine specimens, 1 mL solutions were prepared for the capping agent-modified SDF. The weighed amount of the

capping agents were transferred to a 2 mL Eppendorf tube. Subsequently, a magnetic stirrer bar having a diameter of 3 mm and a length of 5 mm was placed inside the Eppendorf tube. A micropipette (Eppendorf, Hamburg, Germany) having a maximum capacity of 1 mL was used to extract 1 mL SDF solution from the bottle and deposit it in the

**Table-1: The control and experimental groups used in the study**

| Type of Solution | Chemical Composition | | Control and Experimental Groups | Required volume of 38% SDF (mL) | Required weight of capping agent (g) |
|---|---|---|---|---|---|
| Control | 38% SDF | | SDF | 1 | |
| | Capping agent and respective concentration | | Group Name | | |
| Experimental | SDF + TA | 5% TA | TA5 | | 0.05 |
| | | 10% TA | TA10 | | 0.1 |
| | | 15% TA | TA15 | | 0.15 |
| | SDF + G | 5% G | G5 | | 0.05 |
| | | 10% G | G10 | | 0.1 |
| | | 15% G | G15 | | 0.15 |

Eppendorf tube. The Eppendorf tube was then immediately wrapped with aluminum foil to prevent the passage of light and then placed on a magnetic stirrer in an upright position using a beaker clamp. (Figure.1).

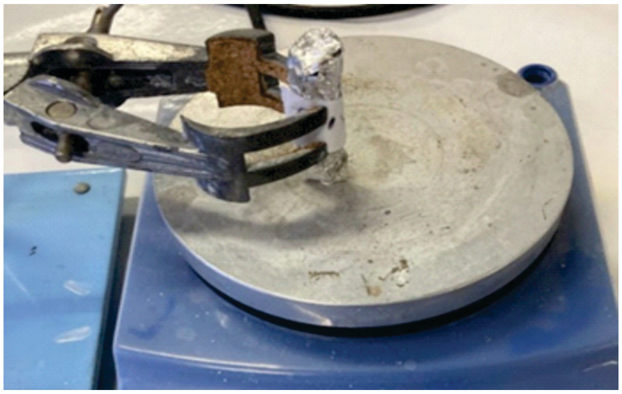

**Fig.1: Apparatus used for the preparation of the experimental solutions. The Eppendorf tubes were covered in aluminum foil to prevent light-induced Ag+ reduction**

The solutions were mixed vigorously with a magnetic stirrer at 600 rpm at 25 $^{0}$C until homogenous. The prepared solutions were then stored in light-proof cabinets at 25 °C to prevent the $Ag^+$ ions from undergoing light-induced reduction until further use.

Eppendorf tubes were used for solution preparation and storage in this study because of the small volume required for specimen treatment. It has been shown that 1 drop of SDF (25 µL) is sufficient for treating all the surfaces of 5 teeth.[13] Hence, 1 mL of the capping agent modified SDF solutions were deemed sufficient for application on all the prepared dentine specimens. Besides, the polypropylene-based Eppendorf tubes have been shown to be resistant to a wide range of organic and inorganic solvents, acids, and bases, and it is biologically inert.[14] Glass bottles were not used for storage of capping agent modified SDF solutions because the high concentration of $F^-$ ions tends to etch the glass, causing leaching of substances from the glass and altering the $F^-$ ion concentrations of the solutions.[15]

Time-dependent pH changes in the SDF, TA-, and G-modified SDF solutions were measured using a pH meter (pH700, Eutech Instruments, Singapore). Due to the narrow diameter of the Eppendorf tubes, which did not permit the penetration of the pH meter probe, separate solutions were prepared in medical-grade, light-proof, polyethylene bottles to ensure sufficient penetration of the pH probe in the bottles for obtaining accurate readings. 5 mL of each experimental solution was prepared. The bottles were kept in a light-proof cabinet at 25 °C to prevent the reduction of the $Ag^+$ ions. Before pH measurement at each time point, the meter was calibrated with reference solutions of three different pH values: pH = 4, 7, and 10. Three readings were recorded for each experimental solution at every time point, and means ± standard deviations were recorded. Each measurement was taken at ambient room temperature.

Percent changes in the SDF, TA- and G-modified SDF solutions from the 1st hour till day-90 were calculated using the following equation:

$$\frac{(pH_{initial} - pH_{final})}{(pH_{initial})} \times 100$$

where pH initial is the solutions' initial pH at the first hour, while pH final is the solutions' pH at day 90.

## Results

The time-dependent pH changes in the SDF solution up to day 90 are shown in figure.2. At the first hour of measurement, the pH for SDF was 7.73 ± 0.02, while the mean pH of the SDF solution at day 90 was 7.70 (± 0.01).

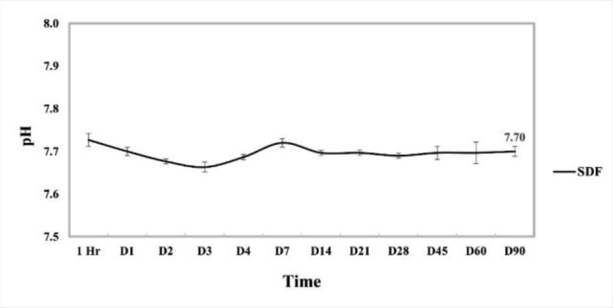

**Fig.2: Time-dependent pH changes in SDF solutions till day-90**

However, following the incorporation of different concentrations of TA and G, an increase in the pH was noted (figure.3 and 4), which was more prominent for TA-modified SDF. At subsequent time points, the pH of SDF remains consistent (figure.2), while a decrease in pH was observed for TA-modified SDF (figure.3) and an increase for G-modified SDF (figure.4) to 60 days. However, no further change in pH was observed after the 60th day. Among the G-modified SDF solutions, TA5 showed the highest pH at day 90 (9.06 ± 0.01). On the other hand, G15 showed the highest 90-day pH among the G-modified solutions (9.80 ± 0.02).

The time-dependent pH changes in TA- and G-modified SDF solution are shown in figure.3 and 4, respectively.

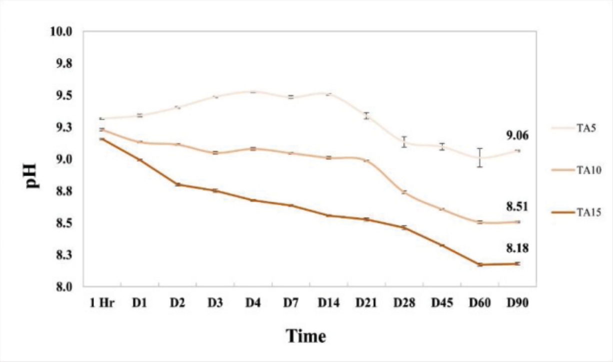

**Fig.3: Time-dependent pH changes in TA-modified SDF solutions till day 90**

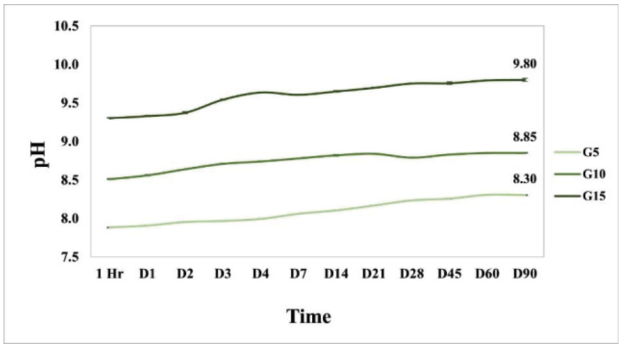

**Fig.4: Time-dependent pH changes in G-modified SDF solutions till day 90**

Figure.5 shows the percentage changes in the pH of SDF, TA—and G-modified SDF solutions from the first hour to day 90.

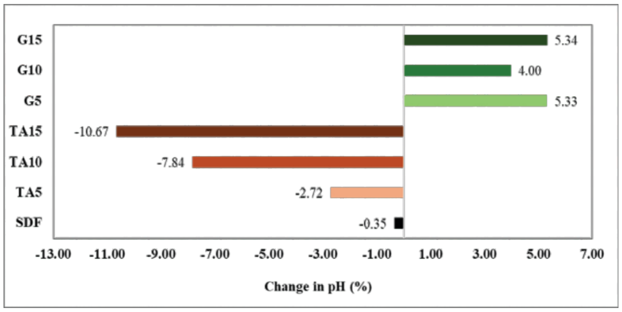

**Fig.5: Changes in pH (%) of SDF, TA-, and G-modified SDF solutions from the 1st hour till day-90**

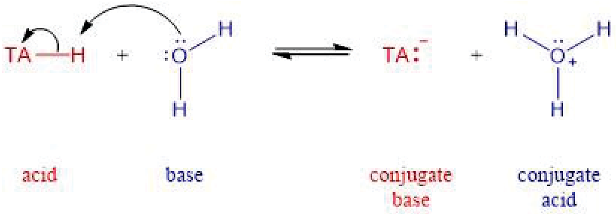

**Fig.6: Schematic representation of the proposed time-dependent pH changes taking place in TA-modified SDF**

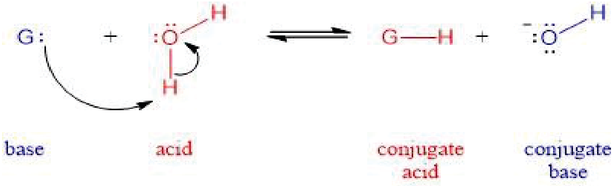

**Fig.7: Schematic representation of the proposed time-dependent pH changes taking place in G-modified SDF**

Repeated measures ANOVA analysis showed a significant difference between the pH values of SDF, TA-, and G-modified SDF at all time points. Similarly, the pairwise comparison showed that all possible treatment pairs were significantly different from each other, except the TA5-G15 pair at hour 1 and day 1. (Additional File.1).

## Discussion

Silver diamine fluoride (SDF) is a topical solution of $Ag^+$ and F- ions, which has shown excellent potential in arresting and preventing dental caries.[16] Despite its efficacy, information regarding its long-term stability is not entirely available. The long-term stability of SDF could directly impact its clinical efficacy, as pH changes in the solution may lead to increased or decreased caries preventive or arrest efficacy. In the current study, time-dependent pH changes were measured as an indicator of chemical stability because slight pH changes can have a considerable effect on the stability of metal ions and nanoparticles.[17] Moreover, it has been shown that the pH of SDF must be alkaline to ensure its stability.[7]

Hence, it is imperative to understand the long-term pH changes occurring in SDF and various capping agent-modified SDF solutions.

In the current study, the long-term chemical stability of SDF was evaluated following the incorporation of TA and G as capping agents. Capping agents are amphiphilic molecules comprising a polar head and a non-polar hydrocarbon tail.[18,19] In the case of SDF, the polar head of TA and G interacts with Ag+ ions, thereby reducing the chances of agglomeration. So, the incorporation of a capping agent could potentially enhance the chemical stability of SDF.

The pH of 38% SDF was shown to be slightly basic (pH = 7.73 ± 0.02) at the first hour of measurement, while it was 7.69 ± 0.01 at the 90th day, indicating minimal time-dependent physical or chemical changes. (Figure.2). The pH values of SDF observed in the current study are consistent with those of Patel et al. who showed that the pH of FAgamin SDF was neutral (pH = 6.99).[20] Similar results were shown by Rossi et al. who showed that the baseline pH of FAGAmin was 6.9 and at day-30 was 7.6.8.[7] The difference in baseline pH measurement compared to our study may have occurred due to the difference in the measurement instruments and the storage conditions.

However, the other brand used by Rossi et al. Fluorosilver had a baseline pH of 11.5 and a 30-day pH of 11.3, respectively.[7] Similarly, Crystal et al. measured the pH changes in a commercial SDF (Advantage Arrest, USA) solution obtained from three different lots (clear solution and blue-tinted) up to 28 days, which ranged between 9.91 (0.01) and 10.05 (0.04) at day-28.[11]

Hence, it can be seen that the baseline pH values of SDF may vary based on the commercial product. In the current study, the pH of SDF remained consistent up to 90 days, in contrast to the other studies that demonstrated an increase in the pH of SDF with time.[7,11]

This difference may have occurred due to the difference in the measurement instruments and the storage conditions. One possibility could be the exposure of SDF to light during pH measurement, which may have led to $Ag^+$ ion reduction, triggering a pH change. In the current study, the solutions were stored in light-proof bottles, and pH measurements were performed using the same containers. Hence, light-induced Ag reduction and subsequent pH change were unlikely.

Interestingly, a significant increase in the pH of all TA- and G-modified SDF solutions was observed one hour after incorporating these capping agents. At this point, the pH of SDF was slightly basic (7.73 ± 0.02). In aqueous form, the $NH_3$ moieties in SDF, which function as a simple capping agent for the SDF sphere, may become loose and form bonds with $H^+$ ions in water. Since this phenomenon results in reduced free $H^+$ ions, an increase in the pH was observed for both TA- and G-modified SDF.

Furthermore, both TA and G contain many coordination sites that can extract $H^+$ ions from their surroundings, resulting in an immediate pH increase following incorporation in SDF. However, with time, the pH decreased for TA-modified SDF while in G-modified SDF, the pH increased until a plateau was achieved around day 60. (Figure.3).

In the case of TA-modified SDF, this can be explained based on the pH-dependent solution chemistry and acid dissociation constant (pKa) of TA, which ranges between 2.2 and 8.5, depending on the pH and ionic strength. The pKa of TA is essential in estimating the net charge and hence, its potential to deprotonate.[21] Dultz et al. studied the effect of pH modification on the surface charge of TA molecules in aqueous form.[22] Their results showed that at lower pH, the protonation of the TA functional groups did not lead to a significant change in the surface charge, remaining close to 0. However, between pH 5 and 10, the surface charge on TA became negative due to deprotonation and an abundance of acidic functional groups. As a result, TA serves as an acid in alkaline conditions.[21,23] Hence, the time-dependent pH decreases in SDF-modified solutions, which occur due to the concentration-dependent increase in the release of H+, result-in the formation of a conjugate base. (Figure.6). It is proposed that the incorporation of SDF in TA initiates a ligand exchange reaction where the $Ag^+$ ions in SDF react with the phenolic hydroxyl groups of TA. This affects the $NH_3$ dissociation equilibrium, leading to its partial dissolution and subsequent release of $H^+$ ions, further lowering the pH of the solution.

The reduction in pH associated with TA-modified SDF solutions should not be interpreted as a negative aspect. Instead, it simply implies that the conjugate base formed from the TA is a stable structure, and it has more available sites to form coordination bonds to the SDF sphere instead of forming coordination bonds to its original H atom. This also explains the strong capping effect of TA that positively influenced the color change and remineralization properties of the specimens treated with TA-modified SDF solutions, as demonstrated in our previous studies. Furthermore, higher alkalinity of SDF is a desirable property as it promotes remineralization and inactivates the enzymatic degradation of the collagen matrix.[20,24]

As per the findings from the previous studies, an overall negative surface charge is expected on the $Ag^+$ ions capped with TA.[25,26] This explains the minimal color changes and the drastic reduction in the antibacterial properties of SDF following TA incorporation. In addition to pH, the surface charge on TA molecules is also dependent on their concentration. As TA concentration is increased from 5% to 15%, a subsequent increase in negative surface charge of the TA-modified SDF solutions can also be expected. The strong electronegative repulsion between the TA molecules prevents the $Ag^+$ ions from clumping together or forming Ag compounds that cause dentinal surface staining.

On the other hand, an increase in the pH was observed with time in the G-modified solutions, which became more prominent with increased G concentrations. G, being inherently basic, resulted in an increase in the pH of SDF with time. Again, this must also not be considered as a disadvantage as it merely shows that G has many lone pairs available to form coordination bonds (Figure.7).

It was shown by Krezel and Bal that G biomolecule possesses as many as eight coordination sites: one thiol, two carboxyl, one amino, and two carbonyl and amide donor pairs within two peptide bonds that allow activity at multiple pH ranges.[27] Among these, four atoms can protonate or deprotonate in water, including two carboxyl acids, amine, and thiol groups. The presence of various functional groups in G, such as carboxyl (-COOH) and amino ($NH_2$), provides exceptional metal coordination capabilities to the biomolecule.[28] This results in a strong capping effect on the $Ag^+$ ions in SDF through metal complex formation.[29]

The strong capping effect of G can be attributed to a strong interaction of the thiol (SH) group and a weaker interaction between the NH and OH groups in G with the $Ag^+$, resulting in capping, which prevents the $Ag^+$ ions from aggregation. In metal complexes formed with G, it has been shown that a higher pH favors interaction coordination with the SH groups, resulting in the formation of a stable complex.

It must be noted that in all SDF, TA-, and G-modified SDF solutions, the pH never fell below 7 or reached the critical pH of 6.7, which leads to hydroxyapatite (HA) dissolution in root dentine and cementum, leading to demineralization. Hence, being basic, all these solutions are expected to promote dentinal remineralization.[30]

## Conclusion

Within the limitations of this study, it can be concluded that silver diamine fluoride remains stable for up to 90 days. Furthermore, the incorporation of tannic acid and glutathione in silver diamine fluoride results in a significant rise in the solution's pH, which plateaus around day 60. Hence, capping agent modification of silver diamine fluoride to prevent silver-mediated dentine staining is a viable method as it does not have a significant effect on silver diamine fluoride's chemical properties, stability, and shelf-life. Moreover, clinicians can safely store and use SDF for up to 90 days without risk of degradation, provided the solution is kept away from direct light. Further studies should focus on testing the chemical stability of silver diamine fluoride for longer durations.

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**Conflict of Interest**: The authors declare no conflict of interest

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## Author Contributions

**MA:** Manuscript writing for methodology design and investigation  
**RAO:** Writing the original draft, proofreading, and approval for final submission  
**AUY:** Validation of data, interpretation, and write-up of results  
**NZB:** Revising, editing, and supervising for intellectual content  
**RY:** Conception and design of the work  
**ADA:** Data acquisition, curation, and statistical analysis

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