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Multiplex immunoassay for Inflammatory Proteins quantification in saliva– a methodologic approach

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Introduction

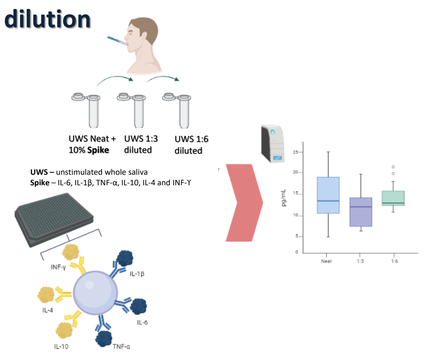
The identification of inflammatory proteins in saliva has potential applications in different fields, including dentistry and medicine, to diagnose and monitor periodontal disease and systemic inflammatory conditions, respectively (Song et al., 2023). Several inflammatory proteins have been identified in saliva, including cytokines, chemokines, growth factors and other molecules that are involved in the immune response and inflammation (Szabo and Slavish, 2021).

Among the techniques used to identify inflammatory proteins in saliva, Multiplex immunoassay has been attracting attention due to its advantage in the detection and quantification of multiple proteins in a single sample using a low volume with high accuracy. This methodology has already been used in the inflammatory protein's quantification in saliva, however few reports were published on the methodological limitations and results reproducibility. This issue becomes more critical when examining analyte concentrations in biological fluids with particular matrix characteristics (e.g., saliva) that could impact the assay performance (Browne et al., 2013; Szabo and Slavish, 2021).

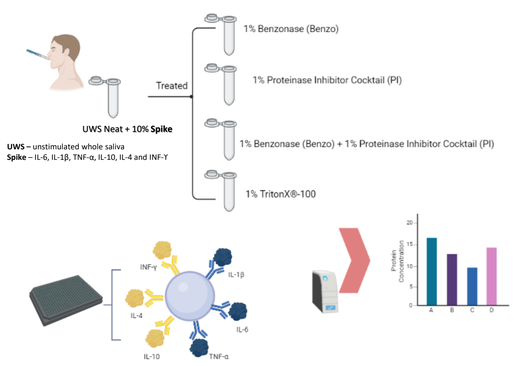
This work reports a methodological study using Multiplex technique with a panel of inflammatory proteins not previously validated in saliva, where the effect of saliva dilution spiked with inflammatory proteins and the addition of additives (benzonase, TritonX®-100 and Proteinase Inhibitor Cocktail) were evaluated.

Methods

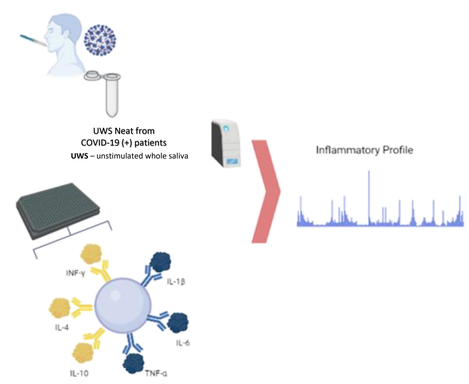
Effect of saliva dilution



Effect of Additives



Inflammatory Profile of COVID-19 (+) patients

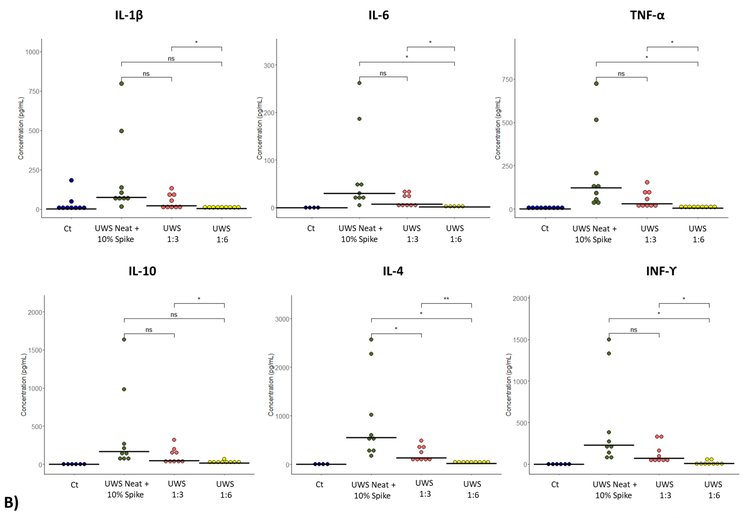


Results

Effect of saliva dilution

- Multiplex Immunoassay allowed the quantification of salivary inflammatory proteins with high percentages of recovery and high reproducibility
- No benefits was achieved in inflammatory proteins detection with diluted saliva samples.

A)



% Recovery			
	UWS Neat + 10% Spike	UWS 1:3	UWS 1:6
IL-1β	21.74	6.23	0.98
IL-6	304.48	70.41	8.45
TNF-α	30.13	7.94	1.12

% Recovery			
	UWS Neat + 10% Spike	UWS 1:3	UWS 1:6
IL-10	19.46	5.31	0.80
IL-4	2.38	0.50	0.03
INF-Y	40.08	11.26	2.11

B)

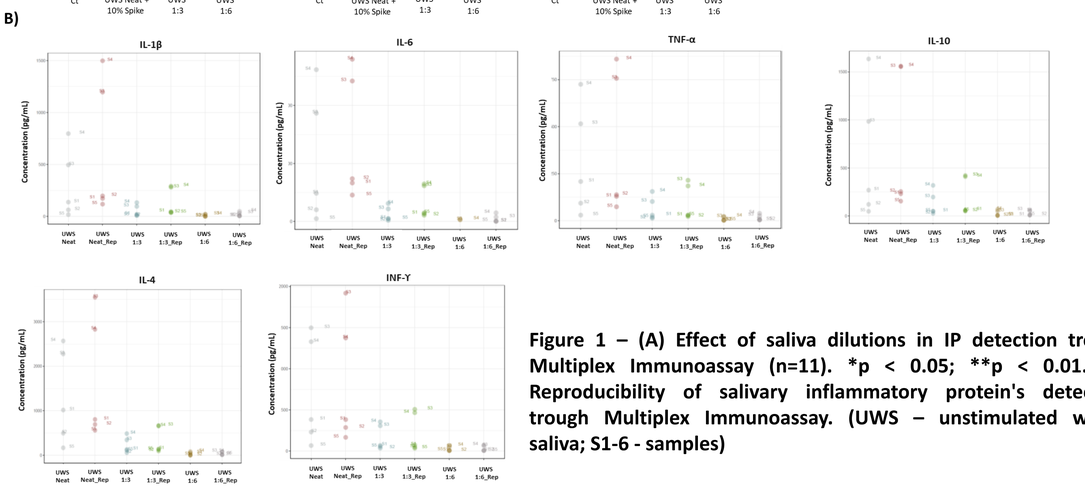


Figure 1 – (A) Effect of saliva dilutions in IP detection trough Multiplex Immunoassay (n=11). *p < 0.05; **p < 0.01. (B) Reproducibility of salivary inflammatory protein's detection trough Multiplex Immunoassay. (UWS – unstimulated whole saliva; S1-6 - samples)

Effect of Additives

- No significant differences was achieved in inflammatory proteins detection after treatment saliva samples with benzonase, Proteinase Inhibitor Cocktail and TritonX®-100

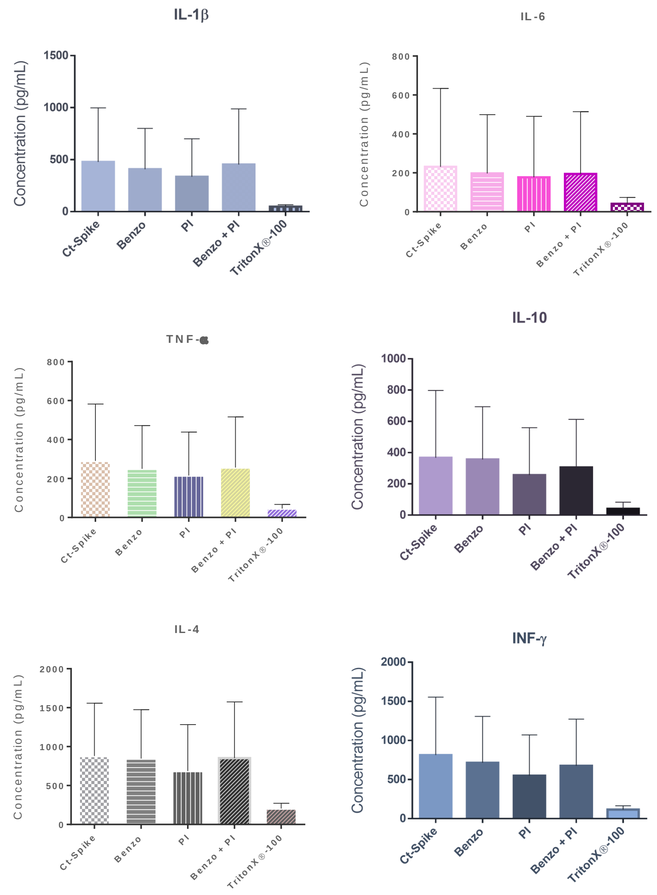


Figure 2 – Effect of the treatment of saliva samples with benzonase (Benzo), Proteinase Inhibitor Cocktail (PI) and TritonX®-100 on the inflammatory protein's detection by Multiplex Immunoassay (n= 5).

Inflammatory Profile of COVID-19 (+) patients

- Patients recently diagnosed with COVID-19 had salivary pro-inflammatory IL-1β and TNF-α increased

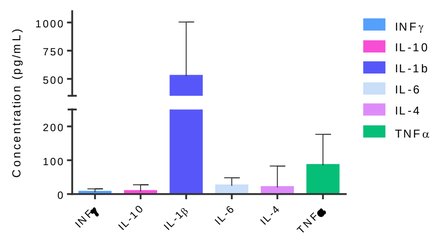


Figure 3 – IP detection in saliva samples of COVID-19 (+) patients' trough Multiplex Immunoassay (n= 24).

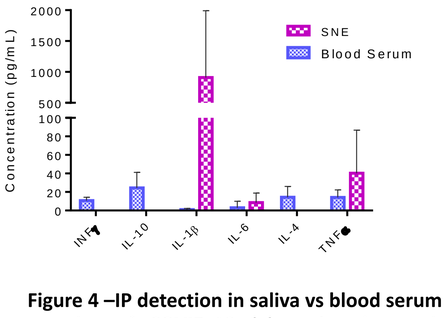


Figure 4 –IP detection in saliva vs blood serum samples of COVID-19 (+) patients' trough Multiplex Immunoassay (n=6)

Conclusion

Multiplex Immunoassay is a powerful tool for the identification and quantification of salivary inflammatory proteins. With our results its possible to stablished a guideline for quantification of salivary inflammatory proteins achieving high percentages of recovery and high reproducibility in non-diluted saliva samples. No improvement in the results was observed after treatment of saliva samples with benzonase, Proteinase Inhibitor Cocktail and TritonX®-100. This approach was used to quantify inflammatory proteins in saliva samples of COVID-19 patients recently diagnosed, where it was found that pro-inflammatory IL-1β and TNF-α were increased.

References

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Ethical declaration

The ethical aspects of the present study were reviewed and approved by the Ethics Committee for Health at Centro Hospitalar Tondela Viseu approved in 28/05/2020.

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