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Analytical techniques for gingival crevicular fluid mediators

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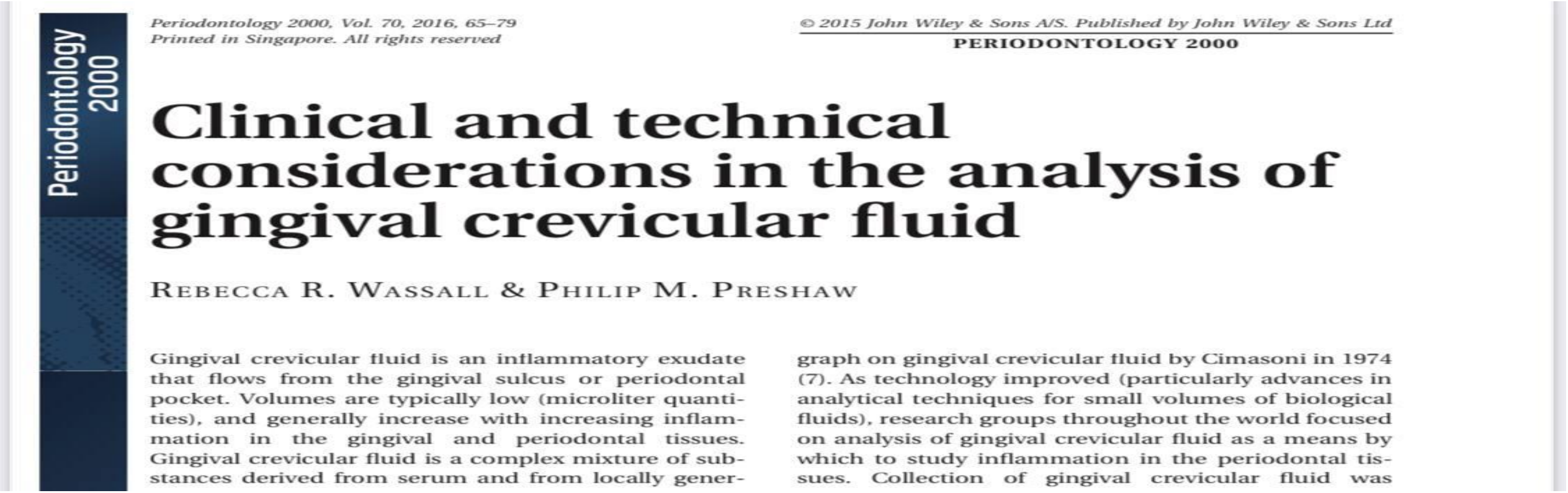
The aim of this topic is to focus on GCF sampling, analytical methods, it also attempts to explore the main reasons for differences in biomarker quantification among the investigators.



Periodontology 2000, Vol. 70, 2016, 65–79
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PERIODONTOLOGY 2000

Periodontology 2000

Clinical and technical considerations in the analysis of gingival crevicular fluid

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Gingival crevicular fluid is an inflammatory exudate that flows from the gingival sulcus or periodontal pocket. Volumes are typically low (microliter quantities), and generally increase with increasing inflammation in the gingival and periodontal tissues. Gingival crevicular fluid is a complex mixture of substances derived from serum and from locally generated

graph on gingival crevicular fluid by Cimasoni in 1974 (7). As technology improved (particularly advances in analytical techniques for small volumes of biological fluids), research groups throughout the world focused on analysis of gingival crevicular fluid as a means by which to study inflammation in the periodontal tissues. Collection of gingival crevicular fluid was

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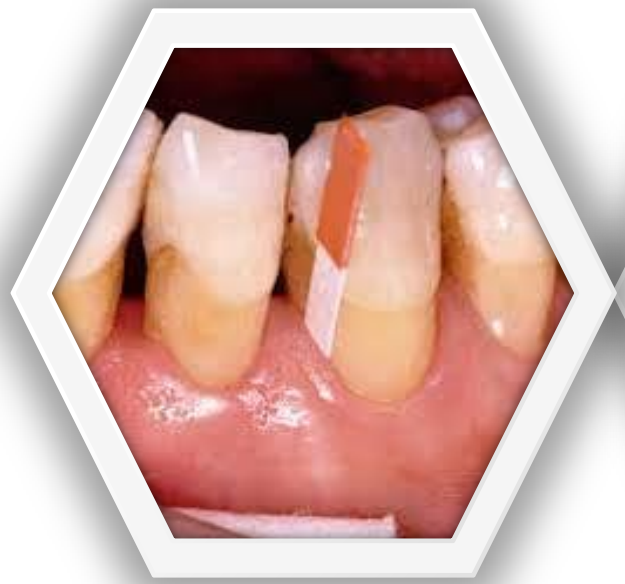
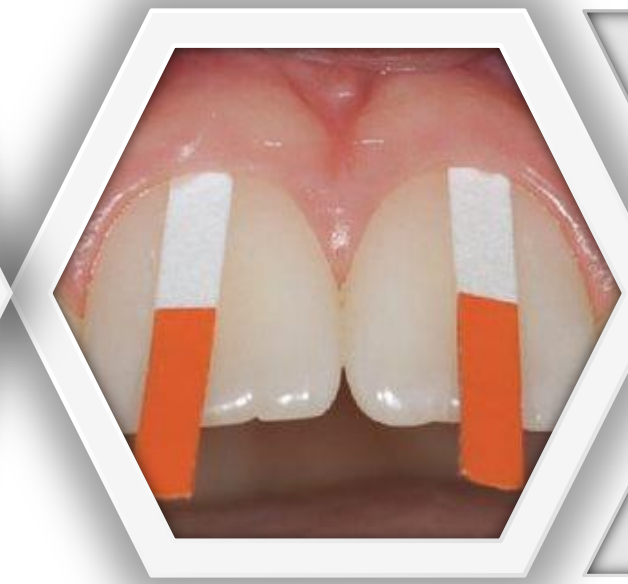




- **Absorbing paper strips**

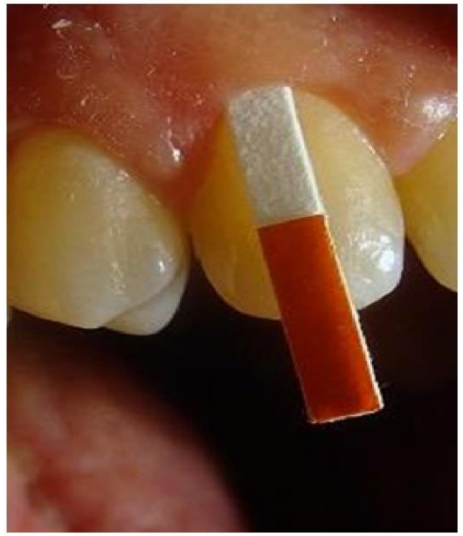
The gingival crevicular fluid volumes obtained from patients are small and that low cytokine levels within samples usually preclude serial dilutions, then typically only a single ELISA can be used for assessing a single gingival crevicular fluid sample.

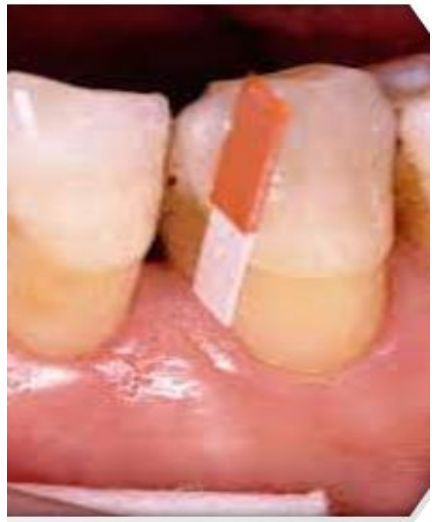
Sites of GCF collections

1. MB sites of 4 teeth, in PPD 4-6 mm Eltas & Orbak, 2012

2. Upper anterior teeth only Toker et al., 2012

3. Two single-rooted teeth with probing depth >5 mm Biyikoglu et al., 2013





4. Four sites per subject Fiorini et al.,2013

5. Mesiobuccal aspect of each tooth Kinney et al., 2014

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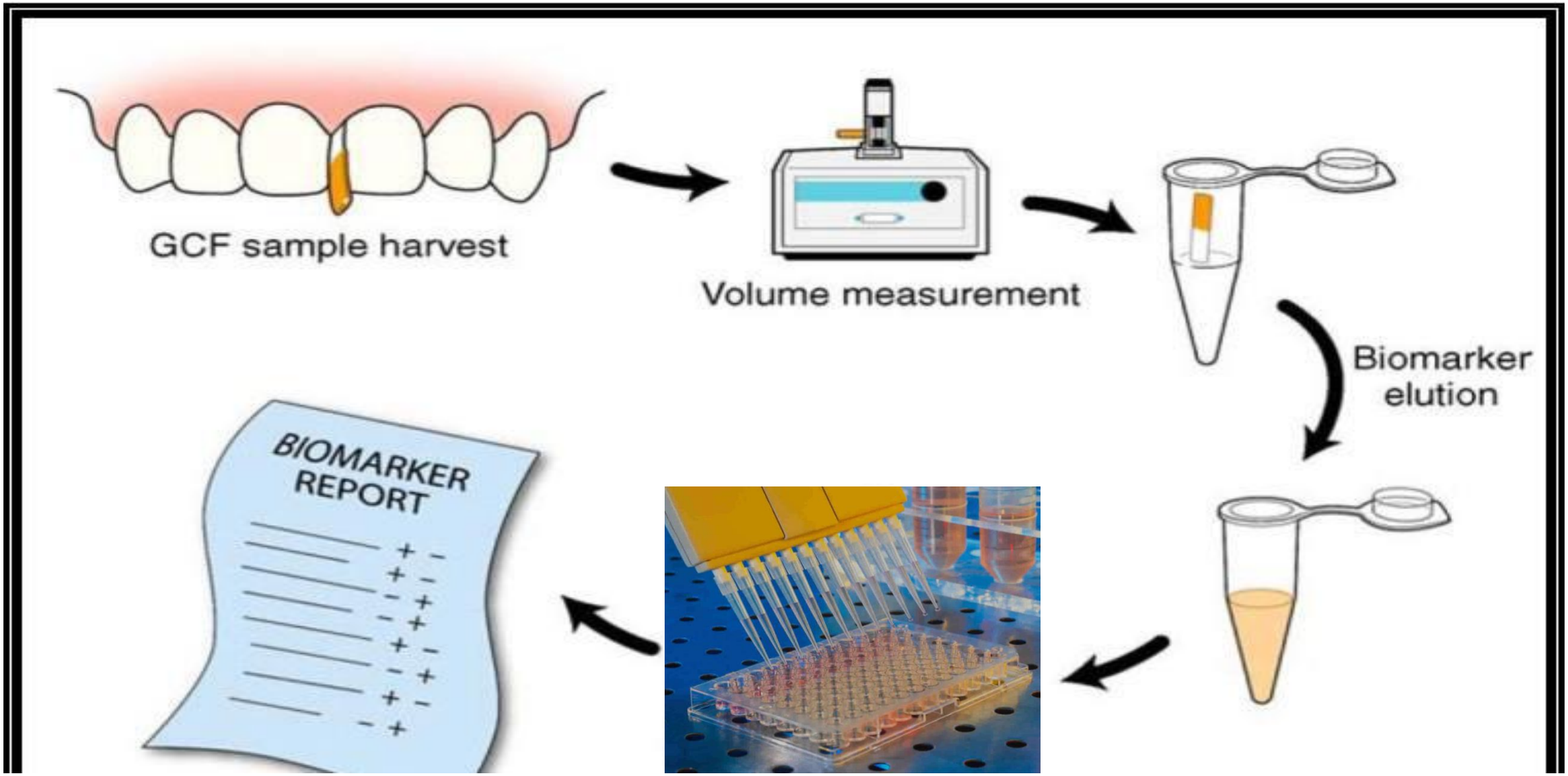
GCF sample harvest
Volume measurement
Biomarker elution
BIOMARKER REPORT

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How to convert ELISA output to the concentration in the original gingival crevicular fluid sample

The samples were eluted in 200 microleter of ELISA buffer

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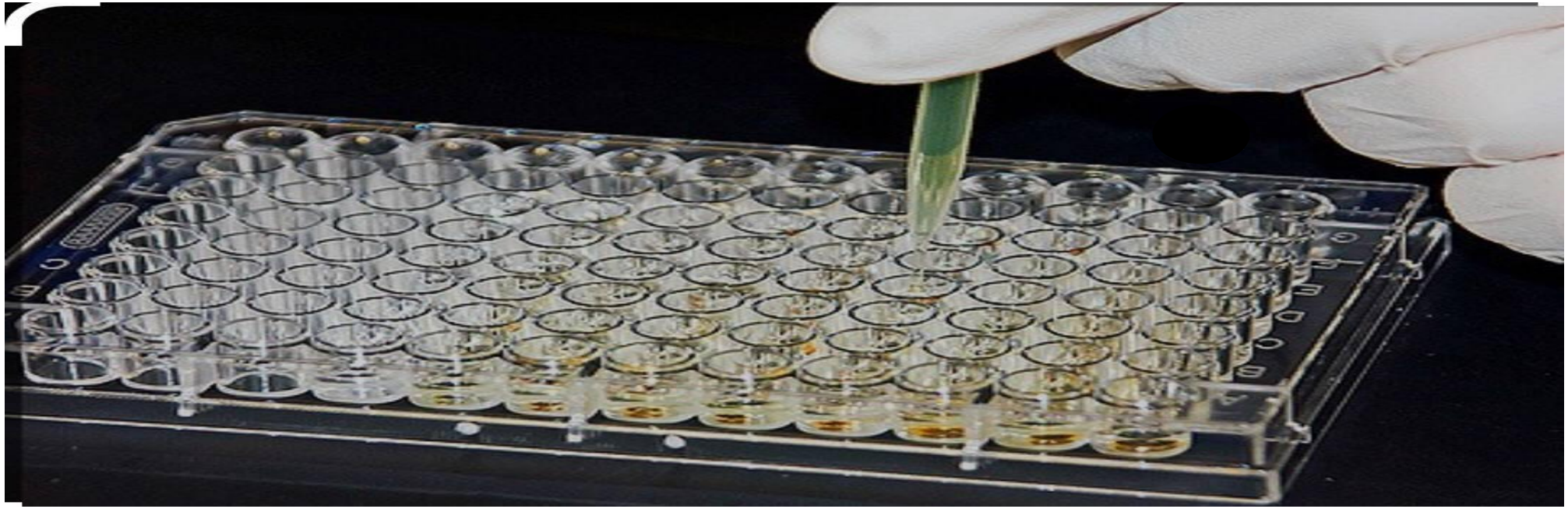
The information that we have available at this stage is:

(i) the ELISA concentration (in pg/ml)
(ii) the gingival crevicular fluid volume (in microleter).



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1. *mediator content per 30-s gingival crevicular fluid in pg/ml*

2. *mediator content in pg per 30-s gingival crevicular fluid sample*

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1. sample comes from the original gingival crevicular fluid sample eluted in 200 microleter.

2. If the output from the ELISA revealed that the concentration was X pg/ml of a particular mediator, therefore, in that 200 microleter, there must be X/5 or, to put it another way,

Conce of GCF in pg per sample = ELISA * 0.2

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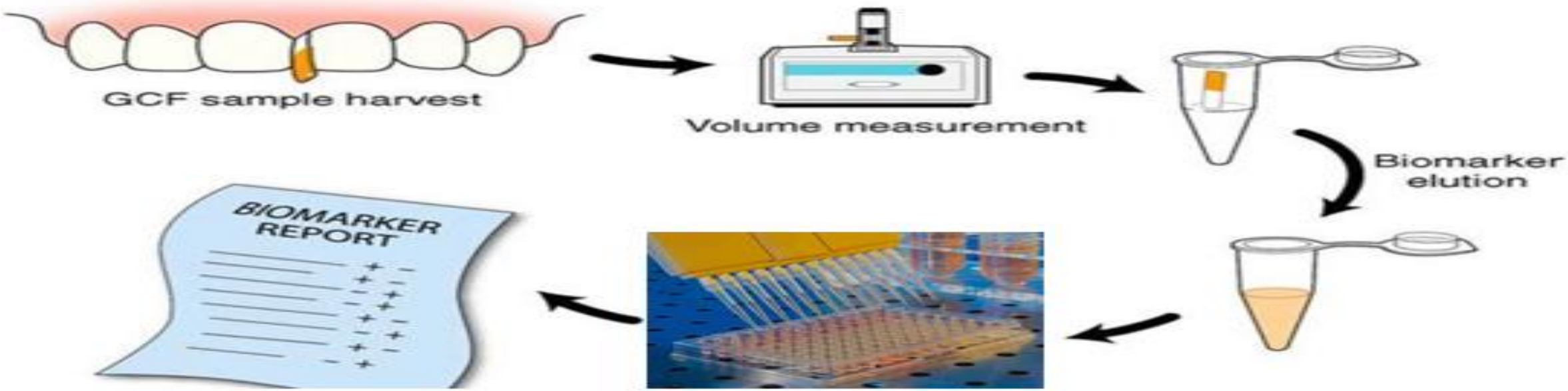
3. However, in this case, we wish to express the mediator concentration as a concentration in terms of the original gingival crevicular fluid volume that was measured using the Periotron. Gingival crevicular fluid volumes are measured in microleter.



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Therefore, the concentration of the mediator in the original gingival crevicular fluid sample = Total amount of mediator in 200 / Gingival crevicular fluid volume

The final formula is calculates the mediator concentration in the original gingival crevicular fluid sample (expressed as pg/microleter)= ELISA * 0.2 /GCF (pg/microleter)

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Worked example of conversion of ELISA output to concentration in the original gingival crevicular fluid sample

Periopaper that contains 0.5 micoleter of gingival crevicular fluid.
the concentration of mediator ‘Q’ in that sample calculated from the ELISA was 2,000 pg/ml.

2,000*0.2 = 400 pg of the mediator Q in the 200 microleter of sample + buffer

400/ 0.5 = 800 pg/microleter of gingival crevicular fluid.

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Thus, we could report the mediator data for this sample in any of three different scenarios:

1. if the sample was collected using a 30-s sampling protocol, we could report a mediator concentration based on the ELISA output of 2,000 pg/ml per 30-s sample.
2. Alternatively, and again If the sample was collected using a 30-s sampling protocol, we could report a mediator content of 400 pg per 30-s sample.

3 Finally, we could calculate the concentration according to the original gingival crevicular fluid volume, in which case we would report a mediator concentration of 800 pg/microleter of gingival crevicular fluid. (volume is considered)

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Clearly, very different numerical values are produced in each scenario, and while this may not be a problem within the context of a single research study (provided that the results are reported thoroughly), it certainly makes it extremely difficult to compare the results between studies that used different methods. A further source of problems if reporting according to scenario 3 is that when gingival crevicular fluid volumes are very low (e.g. in the case of healthy gingival tissues, or following successful periodontal therapy), then calculated gingival crevicular fluid concentrations of mediators can become artificially inflated (particularly toward the lower limits of detection of the Periotron), resulting in difficulty when interpreting results.

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pilot studies must be performed to determine the optimal method of analysis. If the decision is made to correct the data for original gingival crevicular fluid volume, then it is recommended to present both the total mediator content per 30-s sample and concentration in gingival crevicular fluid, together with gingival crevicular fluid volume data, so that a full picture is presented to the reader

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ANY QUESTION