The potential of salivary biomarkers of nutritional status and dietary intake: A Systematic Review

1   **Title:** The potential of salivary biomarkers of nutritional status and dietary intake: A Systematic

2   Review

3   **Abstract**

4   **Objectives:** To explore whether nutritional salivary biomarkers could be used to aid nutritional

5   status assessment and/or support traditional dietary assessment methods for patients.

6

7   **Data and Sources:** Searches were performed using four electronic databases; MEDLINE,

8   EMBASE, Scopus and Web of Science. Trial registers (i.e. Cochrane), grey literature and

9   reference lists were searched.

10

11   **Study Selection:** Studies which measured nutritional salivary biomarkers related to nutritional

12   status and/or dietary intake outcome were included. No restrictions on participants' age, study

13   design, publication date, setting or health status. Animal studies, non-English language studies,

14   commentaries, and conference abstracts were excluded.

15

16   **Results:** Study titles and abstracts were screened (n=7982), full-texts assessed (n=176) and 85

17   studies included in a narrative synthesis. The most promising salivary biomarkers for nutritional

18   status included: glucose, where saliva and serum levels were positively correlated in those with

19   type 2 diabetes (T2D), higher salivary calcium levels in post-menopausal women in general and

20   specifically those with lower bone mineral density (BMD), and salivary vitamin D to assess

21   vitamin D status in healthy volunteers. Higher salivary total antioxidant capacity (TAC),

22   nitrate/nitrite and fluoride were observed with increased antioxidant, nitrate/nitrite and fluoride

23   dietary intake, respectively. A meta-analysis found significantly higher mean salivary glucose

24   (n=12) in T2D compared with healthy controls, but there was substantial heterogeneity (I^2=94%)

25   and evidence of publication bias.

26

27   **Conclusions:** The most promising salivary biomarkers identified were, glucose, vitamin D,

28   calcium, TAC, nitrate/nitrite and fluoride. However, this was based on a small number of studies

29   of varying quality, with many lacking a salivary assay performance assessment.

30

31   **Clinical Significance:** At present, nutritional salivary biomarkers cannot be used alone to assess

32   nutritional status or dietary intake. Further research into the most promising nutritional salivary

33   biomarkers is required.

34

35   <u>**Introduction**</u>

36   Nutritional status is defined as the state of an individual's health, in terms of the intake and

37   utilisation of nutrients [1]. The traditional assessment of nutritional status is complex, as it

38   typically involves a combination of different measures including, anthropometry, clinical

39   assessment of disease state, dietary intake assessment, a blood test interpreted alongside a clinical

40   examination and environmental assessment relating to factors affecting eating behaviours [2].

41   Individuals with a combination of unsatisfactory assessment results such as, blood results outside

42   of normal ranges or inadequate dietary intake, may be at risk of for example, malnutrition, iron-

43   deficiency anaemia (IDA), osteoporosis or T2D, if nutritional intervention does not take place.

44   Dietary data collection as part of a nutritional status assessment and additionally the individual

45   collection of dietary data to assess dietary intake, is not without challenge. This is because the

46   usual methods of dietary data collection including, a food frequency questionnaire (FFQ), 24-hour

47   recall or food diary, are subjective, at risk of recall bias and are prone to under- and over-reporting

48   due to difficultly associated with reporting portion sizes [3,4]. Therefore, one method to help

49   overcome these limitations, is using dietary biomarkers to objectively support traditional dietary

50   assessment methods and aid nutritional status assessment [4]. Dietary biomarkers can more

51   accurately associate dietary intake with disease risk and nutritional status, in comparison to self-

52   reported methods [4]. Similarly, biomarkers can help detect, monitor and diagnose conditions [5].

53   Blood is generally considered the best body fluid to evaluate biomarkers of systemic health and

54   dietary intake, however it is not without its limitations, as collection is inherently invasive, it can

55   cause discomfort and induce patient anxiety from a fear of needles, and a risk of infection exists

for both the patient and practitioner [6]. Blood collection in older adults can also prove difficult due to poor venous access, while it also tends to be less favoured amongst children in research [6]. Therefore, the process of assessing nutritional status is currently complex and invasive, creating a need for a non-invasive, timely and cost-effective method to provide a biological sample which could help assess nutritional status [7,8]. Similarly, the use of a quick method to support traditional dietary assessment methods would be helpful in dietary research.

This is where the use of saliva has come to attention as it is often referred to as a "window on health status" or a "mirror of the body" [9]. Saliva mainly consists of water (99%), but the remaining 1% consists of various inorganic compounds, organic compounds and proteins/ polypeptide compounds [10]. Collection of saliva is considered the least invasive of all biological samples [11] and may offer a credible means to evaluate health status [12]. Saliva collection is relatively simple with specialised training not required and a biological sample produced which is easier to ship and store, compared to serum samples, which need processed relatively immediately after collection [7]. One previous systematic review [13] plus narrative literature reviews [9,14–17] have suggested the possible clinical usefulness of different salivary biomarkers in the early diagnosis and treatment of various oral or systemic disorders including, dental caries, cancer and cardiovascular disease. Previous research also suggested that saliva may be a source of nutritional biomarkers, as nutritional deficiencies may affect salivary function and composition [18,19].

To date, however, no systematic review has investigated the available range of potential nutritional salivary biomarkers in relation to different nutritional states and dietary intake. The aim of this systematic review was to explore whether nutritional salivary biomarkers in humans of any age could aid nutritional status assessment and/or support traditional dietary assessment methods in comparison to corresponding nutritional serum biomarkers or control groups (where applicable).

Methods

Protocol and registration

This systematic review was conducted and reported according to the Preferred Reporting Items for

83	Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20] (Supplementary File 1). The
84	review protocol was prospectively registered with PROSPERO: International Prospective Register
85	of Systematic Reviews (CRD42018107667).

Eligibility criteria

87	Broad criteria were predefined to select papers for inclusion, using the participants, interventions,
88	outcomes and study design reporting system (PICOS). The predefined list of inclusion and
89	exclusion criteria used for this systematic review are detailed in Table 1.

Information sources

91	Four electronic bibliographic databases (MEDLINE, Web of Science, Scopus and EMBASE) were
92	searched by the principal author (DL) to identify eligible studies. Trial registers (i.e. Cochrane) and
93	other grey literature sources (open grey, google and google scholar) were searched using the key
94	words, as well as relevant reference lists. The literature search was updated on January 9, 2021.

Search strategy

96	The literature was initially scoped in order to develop the search strategy. The search terms
97	employed were either Medical Subject Headings (MeSH) terms or key words classified under
98	general (all fields) category. The search terms were then combined with an "OR", and PICO
99	categories combined using "AND" to produce the search query. Appropriate adaptations were
100	made to allow search strategies to be carried out on all the databases (Table 2). All searches were
101	limited to include published, English and human studies and the library at Queen's University
102	Belfast retrieved any full-texts of identified articles that were not able to be accessed online.

Study selection

104	Study titles and abstracts were screened against the eligibility criteria. Duplicates were excluded
105	automatically on an online programme (Covidence, Veritas Health Innovation Ltd, Australia) and
106	manually checked. Those not meeting the inclusion criteria were removed and reasons noted. Full
107	texts were retrieved if studies were considered potentially eligible. Studies meeting inclusion
108	criteria were included in the narrative synthesis. If multiple studies measured the same salivary
109	biomarker and nutritional state, with the same study design and outcome, they were considered

| 110 | appropriate for a meta-analysis. Reviewer disagreements relating to study selection were resolved |
| 111 | through discussion. |

Data collection process and data items

113	Data extraction was carried out independently by two researchers (DL and SW) for those studies
114	meeting inclusion criteria. The following data were extracted including: first author's name, year
115	of publication, study design, country, study population, demographics (sample size, age range and
116	gender), salivary collection methods and analysis, primary outcome, as well as the comparator (i.e.
117	serum) if available, covariates and main conclusions. Any disagreements between reviewers during
118	data extraction were resolved through discussion and a consensus reached prior to finalising
119	extracted data. Data items extracted from the included studies are detailed in Supplementary File 2.

Missing data

| 121 | As this review included studies which either had the identification and/or level of nutritional |
| 122 | salivary biomarkers, corresponding authors were not contacted for missing data. |

Risk of bias and quality assessment of included studies

124	Risk of bias was assessed by the principal author (DL) according to individual study design. The
125	Newcastle-Ottawa Scale (NOS) was used to assess the quality of non-randomised studies [21].
126	However, adaptations ensured responses were suitable in relation to non-response rate and
127	ascertainment of exposure (Supplementary File 3). For a good quality case-control study, 7-9 stars
128	were required, 5-7 stars for a fair quality study and anything lower suggested a poor quality study.
129	For cross-sectional studies an existing adapted NOS version was used [22]. This was further
130	adapted with the same changes as case-control studies. A maximum of 9 stars were given, where 0-
131	3 was unsatisfactory, 4-5 was satisfactory, 6-7 was good and 8-9 was very good [22]. Randomised
132	controlled trials (RCT) used the Cochrane risk of bias tool [23] and randomised cross-over trials
133	used an existing adapted Cochrane risk of bias tool [24]. For before and after studies with no
134	control group, the National Institute of Health quality assessment tool was used [25].

Summary measures

Outcome measures

| 137 | The primary outcomes in this review were the identification and/or level of nutritional salivary |

138   biomarkers in relation to nutritional status or dietary intake outcomes. Secondary outcomes were

139   the comparison of the corresponding serum biomarkers to the salivary biomarkers if available.

140   **Synthesis of results**

141   Salivary glucose was the only biomarker appropriate for a meta-analysis to identify any glucose

142   differences in those with and without T2D. This was presented as a cumulative forest plot with

143   standardised mean differences and 95% confidence intervals (CI), using a random-effects model.

144   Statistical heterogeneity was quantified using I-squared (I^2) statistics from the RevMan meta-

145   analysis (Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration,

146   2014). The other nutritional salivary biomarkers were not suitable for meta-analyses due to data

147   heterogeneity, thus a narrative synthesis was carried out for these. A league table was also devised

148   to determine which salivary biomarkers were the most promising and could be explored further in

149   additional future research (Supplementary File 5).

150   **Risk of publication bias across studies and additional analyses**

151   Publication bias across the meta-analysis studies was assessed visually using a funnel plot of

152   salivary glucose levels in those with and without T2D. No additional analyses was conducted.

153

154   **<u>Results</u>**

155   **Study selection**

156   The initial search retrieved 6585 possible studies and, following the removal of duplicates, 4836

157   studies were identified and screened. Of these, 134 full texts were assessed and 64 studies were

158   eligible. The search was updated in March 2020 and this identified a further 1906 studies, of which

159   16 studies met the inclusion criteria after full text review. A third search update was conducted in

160   January 2021 and a further 1240 studies were identified, of which five met the inclusion criteria

161   after full text review, leaving a total of 85 studies for inclusion in the review. Full details of the

162   process are presented in the PRISMA Flowchart including reasons for exclusions (Figure 1).

163   **Study characteristics**

164   ***Studies included for narrative analysis***

165   The data from each included study (n=85) were summarised in Supplementary File 2. 63 studies

identified salivary biomarkers relating to nutritional status and 22 related to dietary intake. Of the

85 included studies, 53 were case-control studies, 20 cross-sectional, six randomised controlled

crossover trials, four before and after studies (with no control group), one RCT, and one study which

included aspects of a cross-sectional study and a before-after study (with no control group). Studies

were conducted across different continents including; 35 in Asia, 19 in Europe, 14 in South

America, eight in North America, five in Africa, three in Australia and one in Oceania. The total

sample size was 16486 participants, ranging from six up to 8317 participants. Participants' age

varied from six weeks to >65 year olds. Studies were published between 1956 up until 2020.

Studies included for meta-analysis

Of the salivary biomarkers identified, glucose had the greatest number of studies with the same

study design and outcome measure, compared to other biomarkers. A total of 12 case-control

studies were identified as eligible for a salivary glucose meta-analysis [26,27,36,37,28–35]. These

studies measured glucose in saliva of children and adolescents [37], adults [26–30,32,36],

adolescents, adults and older adults [35], adults and older adults [31,34] and one study the age was

not stated [33]. These studies ranged in sample size from 40 [30] up to 127 participants [31].

Risk of bias within studies

Risk of bias within each study was summarised according to study design (Supplementary File 4).

The quality of case-control studies varied, ranging from one to eight and a 4.28 average out of nine

(poor quality). This average is reflected by only 10 studies having a consecutive or representative

set of cases [29,30,38–45]. The quality of cross-sectional studies ranged between two and five,

with a 3.86 average out of nine (unsatisfactory). This average is reflected by only nine studies

having a truly representative sample [73–81]. All case-control and cross-sectional studies lacked at

least one or more of the ascertainment of exposure criteria. The one RCT was of fair quality due to

two unclear criteria [93]. Of the six randomised controlled crossover trials, three were poor [94–

96], one fair [97] and two good [98,99] quality. Of the five before and after studies, two studies

achieved six [85,100], two achieved seven [101,102] and one achieved nine [103] out of 12.

Synthesis of results

193   *Meta-analysis of the included studies: salivary glucose levels in all ages*

194   A meta-analysis was performed for salivary glucose levels in those with T2D and controls

195   [26,27,36,37,28–35]. The random-effects model using standardised mean difference revealed that

196   the mean glucose levels were significantly higher in individuals with T2D by on average 1.26

197   standard deviations (SD) compared to controls (p<0.0001) (Figure 2). However, substantial

198   heterogeneity existed as the I^2 statistic was 94% (Figure 2). According to the funnel plot analysis,

199   potential publication bias was illustrated by the non-symmetrical funnel plot (Figure 3).

200   *Narrative synthesis of nutritional salivary biomarkers*

201   This systematic review identified 34 nutritional salivary biomarkers in relation to nutritional status

202   and/or dietary intake, as summarised in the league table (Supplementary File 5). The results for the

203   most promising biomarkers including, glucose, vitamin D, TAC, calcium, fluoride and

204   nitrate/nitrite are presented in this section. A summary of the results for the less promising salivary

205   biomarkers is included in Supplementary File 6.

206   *Glucose*

207   In addition to the glucose meta-analysis, ten studies out of 17 reported on the correlation between

208   salivary glucose and serum glucose; a positive correlation was found in six studies including, adults

209   with T2D and controls [26,29,32] and in healthy men [89]. However, salivary and serum glucose

210   only correlated amongst those with T2D, as salivary glucose was not detected in controls [50], whilst

211   a correlation only existed between salivary glucose and fasting plasma glucose in those with T2D

212   [33]. However, three studies found no significant correlation between salivary and serum glucose in

213   adults with T2D [31,36] or in 19-72 year olds [78] and no significant correlation between salivary

214   glucose and serum fasting plasma glucose in older adults with T2D and controls [30].

215   *Vitamin D*

216   Two studies investigated the relationship between salivary and serum 25-hydroxyvitamin D_3

217   [25(OH)D_3] in adults. One study found a significant positive linear relationship between salivary

218   and serum 25(OH)D_3, as well as an increase in salivary 25(OH)D_3 levels after taking 10 days of

219   25(OH)D_3 supplementation [85]. Similarly, a positive correlation was found between salivary and

220 serum 25(OH)D$_3$ after adjusting for salivary flow rate in adults [102].

221 *Total antioxidant capacity*

222 An increasing trend in salivary TAC was found with an increased dietary antioxidant intake in

223 adults [93] and a positive association and an inverse association between salivary TAC and simple

224 carbohydrate and complex carbohydrate, respectively [74]. However, salivary TAC was not

225 associated with micronutrient or macronutrient intake during pregnancy [61].

226 *Calcium*

227 Two studies found that post-menopausal women with a lower BMD had higher salivary calcium

228 than post-menopausal women without bone mineral changes [88] or pre-menopausal or pregnant

229 women [45]. Long-term supplementation of vitamin D combined with calcium, did not

230 significantly affect salivary calcium in osteoporotic women [40].

231 *Fluoride*

232 Three studies investigated dietary fluoride in relation to salivary fluoride, with salivary fluoride

233 levels significantly increasing after inclusion of a sodium fluoride supplement in children [52], a

234 significant correlation in young children [86], but not repeated in adolescents [75].

235 *Nitrate/Nitrite*

236 Two studies reported salivary nitrate/nitrite in relation to diet. One study showed that adults had

237 significantly higher salivary and plasma nitrate and nitrite levels after consuming a high nitrate diet,

238 compared to a low-nitrate diet [97]. The other study found that salivary nitrate and nitrite levels

239 returned to baseline levels seven days after cessation of the high-nitrate diet [103].

240

241 **Discussion**

242 This systematic review explored the potential of nutritional salivary biomarkers to aid nutritional

243 status assessment and/or support traditional dietary assessment methods for patients. The results for

244 the six most promising biomarkers, based on consistent evidence and biological plausibility, are

245 discussed in this section. For nutritional status this included; glucose for T2D, vitamin D for

246 vitamin D status and calcium related to BMD. For dietary intake this included; TAC, nitrate/nitrite

247   and fluoride. This evidence, even when considered promising, was based on a small number of

248   studies of varying quality. Current literature, however, does not suggest a role for a large number of

249   nutritional salivary biomarkers identified in the scoping exercise for this review, due to the

250   inconsistent direction of significant associations across studies or the lack of evidence to inform a

251   conclusion (see Supplementary Files 5 and 6).

252

253   ***Salivary biomarkers and nutritional status***

254   One of the most promising biomarkers identified was salivary glucose in relation to T2D. The data

255   generated in the meta-analysis, supports previous evidence which suggested that salivary glucose

256   levels are significantly elevated in patients with T2D [112]. The positive correlation between

257   salivary and serum glucose mirrors the increased serum glucose in T2D [26,29,32,33,50,89].

258   Increases in salivary glucose may reflect microvascular structural changes within the salivary

259   glands which increase the glucose diffusion rate from the blood to oral cavity [112]. However, the

260   meta-analysis was subject to publication bias as shown in Figure 3 and substantial heterogeneity (I^2

261   =94%) as the absolute values varied between studies. High heterogeneity may be due to the

262   different age groups including, adults and children [26,27,36,37,28–35]. All studies in the meta-

263   analysis analysed unstimulated saliva using colorimetric glucose kits, but one study was not

264   included as glucose was only detected in adults with T2D [50]. The reason is unclear but perhaps

265   levels were below the limit of detection in controls. Overall, the collection and analyses of glucose

266   in unstimulated saliva is promising for T2D diagnoses and glucose monitoring. However, this was

267   based on poor and fair quality studies and should be interpreted with caution. Higher quality studies

268   are needed to confirm the use of salivary glucose to monitor and diagnose T2D.

269

270   Despite limited evidence, vitamin D ($25(OH)D_3$) in unstimulated saliva, positively correlated with

271   serum $25(OH)D_3$ in healthy adults [85] and after adjusting for salivary flow rate [102]. This

272   corresponds to low serum $25(OH)D_3$ in those with a vitamin D deficiency [113]. Therefore, the

273   current analysis suggests that reduced salivary $25(OH)D_3$ may also indicate this deficiency. This is

274   important as measuring serum $25(OH)D_3$ is expensive and universal screening is not supported

275   [113]. However, saliva was collected on three consecutive days which may restrict community use

276   [102]. The results may not be representative due to small study samples sizes [85,102]. Larger

277   studies are required to confirm the use of salivary 25(OH)D$_3$ for assessing vitamin D status.

278

279   Limited, but promising, evidence was reported for salivary calcium and BMD in post-menopausal

280   women, as those with lower a BMD had higher unstimulated calcium levels compared to pre-

281   menopausal or pregnant women [45] or post-menopausal women without bone mineral changes

282   [88]. This suggests that salivary calcium may be a useful screening tool for bone mineral changes

283   and has the potential for early diagnoses of osteoporosis [88]. Unfortunately, as the included

284   studies did not assess serum calcium levels, the relationship between salivary and serum calcium is

285   currently unclear. However, recent research found that adults in Sweden with elevated serum

286   calcium at baseline developed osteoporosis more often than controls [114]. This finding

287   corresponds with increased salivary calcium in those with a low BMD. Longitudinal studies with

288   larger sample sizes are required, to confirm the use of salivary calcium in relation to BMD.

289

290   ***Salivary biomarkers and dietary intake***

291   Despite limited evidence, salivary TAC was promising, as a RCT showed an increasing trend in

292   salivary and plasma TAC amongst intervention adults, who increased fruit and vegetable intake

293   compared to controls [93]. Samples were analysed using the Trolox Equivalent Antioxidant

294   Capacity (TEAC) assay [93]. Results suggested that both salivary and plasma TAC respond to an

295   increased antioxidant intake similarly. However, no differences in salivary TAC [93] or nutrient

296   intake during pregnancy [61] were detected using the ferric reducing antioxidant power (FRAP)

297   assay. Therefore, the TEAC assay may be more sensitive than the FRAP assay in detecting salivary

298   TAC changes. It is unclear which saliva collection is most appropriate, as this information was not

299   included [93]. Cross-sectionally, a higher intake of simple carbohydrate was associated with higher

300   unstimulated TAC in adults, using the Oxygen Radical Absorbance Capacity (ORAC) assay [74].

301   Results may differ if diet was recorded for longer, instead of 24 hours [61,74] or three days [93].

302   Further evidence is required to confirm the use of salivary TAC and the best assay.

303 Limited, yet promising evidence, suggested that salivary and plasma nitrate and nitrite respond in

304 the same way to an increased dietary intake, as levels increased amongst those with a greater

305 consumption of spinach and green leafy vegetables in adults and older adults compared to those on

306 a low-nitrate diet [97]. Salivary nitrate and nitrite levels also returned to baseline levels seven days

307 after cessation of the high-nitrate diet [103]. This increase in salivary nitrate and nitrite may be

308 explained by dietary intake, along with nitrate produced from NO, both entering the enterosalivary

309 nitrate-nitrite-NO-pathway, and absorbed from plasma into the salivary glands [97]. It was unclear

310 how saliva was collected, but gas chromatography mass-spectrometry was used to analyse saliva

311 samples and a food diary used to record diet [97,103]. Based on good quality evidence, salivary

312 nitrate and nitrite was successful in validating dietary intake, but further studies are required.

313

314 The use of salivary fluoride to reflect fluoride intake was also promising, as unstimulated fluoride

315 levels increased with higher fluoride intake in children living in higher fluoride areas, via food

316 (moderate correlation) [86] or sodium fluoride supplementation over a 12 month period [52],

317 compared to those living in lower fluoride areas. Similar results were achieved using a fluoride

318 selective electrode [52] and acid diffusion [86]. Salivary and plasma fluoride levels may respond in

319 the same way, as a strong positive correlation existed between plasma fluoride and total daily

320 fluoride intake [86]. However, stimulated fluoride levels did not correlate with dietary fluoride

321 intake in adolescents using a fluoride selective electrode [75]. Therefore, 24-hour dietary recalls

322 may not be long enough to reflect salivary fluoride [75]. Further higher quality studies are needed

323 to confirm the use of salivary fluoride in reflecting dietary fluoride intake.

324

325 **Strengths and limitations**

326 This is the first systematic review to investigate a comprehensive range of nutritional salivary

327 biomarkers in relation to nutritional status and dietary intake. Broad inclusion criteria meant that all

328 age groups, publication years, study design (with or without controls), and settings (any health

329 condition), maximised the number of studies. Data extraction involved two independent reviewers,

330 although only one reviewer conducted the risk of bias assessment. Six salivary biomarkers were

331   promising based on consistent significant evidence and biological plausibility. However, this

332   review was limited by the quality of the evidence, as the majority of studies lacked an assessment

333   of salivary assay performance, so results may be based upon unreliable methods. Comparisons

334   were also limited due to high variability in salivary collection and analysis methods. Many studies had

335   small sample sizes which may have lacked power to detect significant findings. A meta-analysis was

336   only possible for salivary glucose, but substantial heterogeneity and publication bias existed.

337

338   **Conclusions**

339   Saliva represents a biological matrix that is easily accessible, considered the least invasive of all

340   biological samples and its collection does not require specialised training. It can also be collected

341   by patients themselves, thus offering an alternative to invasive blood tests in an era of limited

342   access to healthcare facilities. At present, the evidence is generally limited, inconclusive and

343   inconsistent as the change in the direction of significant associations was not always consistent.

344   Despite this, some results were judged as promising, with the potential use of salivary glucose for

345   monitoring and diagnosing T2D, vitamin D for assessing vitamin D status, calcium levels related to

346   BMD, TAC for assessing dietary antioxidant intake, nitrate/nitrite for assessing dietary

347   nitrate/nitrite intake and fluoride for assessing dietary fluoride intake. In general, these promising

348   salivary biomarkers responded to nutritional state or dietary intake in the same way as their

349   corresponding serum/plasma biomarker. Therefore, using nutritional salivary biomarkers is still

350   largely uncertain and requires further research. At present they should not be used alone to assess

351   nutritional status or reflect dietary intake. Future studies should include salivary assay performance

352   to ensure reliable results and explore the methodology of commercially available serum kits for

353   detecting nutritional salivary biomarkers. More specific and sensitive methods may be required to

354   evaluate potential salivary biomarkers as indicators of nutritional status or dietary intake.

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