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# 1 **Quality management for the collection of** 2 **biological samples in multicentre studies**

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## 10 **Abstract**

11 Large scale international multicentre studies require sophisticated quality management  
12 for the collection, processing and logistics of biological samples to ensure a maximum  
13 degree of standardisation across different environmental conditions and settings. This  
14 paper describes a quality management system for the collection of biological samples  
15 (QMS-BS) which was applied during IDEFICS, a large European multicentre study.  
16 The application was evaluated by several criteria like response rates for the different  
17 types of biological samples, measures of sample quality, compliance with the QMS-BS  
18 and efficiency of the document and sample control and of the quality assurance system.  
19 Response rates varied from 56.6% for venous blood collection to 90.1% for saliva  
20 collection. All sample types were associated with problems of sample quality (e.g.  
21 haemolysis of blood samples, lack of cooling for urine samples or desiccation of saliva  
22 samples). Overall compliance with the QMS-BS was good, with some exceptions  
23 mainly related to sample control. In conclusion the QMS-BS is a valuable tool for the  
24 management of biological sample collection in epidemiological multicentre studies.

## 25 **Keywords**

26 Quality management, Survey, Multicentre study, Cohort study, Biological specimen

27 bank, Sample database

28

## 29 **Abbreviations**

30 ADR Agreement concerning the International Carriage of Dangerous

31 Goods by Road

32 DNA Deoxyribonucleic acid

33 FA Fatty acid

34 GEP Good epidemiological practice

35 IATA International Air Transport Association

36 ICAO International Civil Aviation Organization

37 IDEFICS Identification and prevention of Dietary – and lifestyle – induced

38 health Effects in Children and infantS

39 IEA International Epidemiological Association

40 INRS Institut National de Recherche et de Securite

41 ISO International Organization for Standardization

42 QMS Quality Management System

43 QMS-BS Quality Management System for biological samples

44 RFID Radio-frequency identification

45 RNA Ribonucleic acid

46 SOP Standard operation procedure

47

## 48 **Introduction**

49 In epidemiological studies, biological samples have become an indispensable source of  
50 information [1]. Different sample types are used to measure a broad range of  
51 biomarkers, for example hormones, lipids, glucose, protein, bulk and trace elements or  
52 genetic factors. In epidemiology these are used to determine exposure, susceptibility or  
53 effects [2].

54 Several factors influence the observed concentration of a biomarker in the human body,  
55 some are inherent like age or sex, but others are controllable. These can affect the  
56 concentration of a biomarker either before, during or after sample collection. Before  
57 sample collection, fasting status, medication, drug intake, physical activity and diurnal  
58 and seasonal variation play an important role. For example glucose and fatty acids are  
59 known to be closely related to food intake and therefore fasting measurements are  
60 mostly used in epidemiological studies. The levels of most biomarkers vary during the  
61 day; some can even change considerably within minutes. Salivary morning cortisol for  
62 instance was found to change by approx. 10% within 30 minutes in adolescents [3].  
63 Deacon et al. [4] reported the effects of posture on melatonin concentrations in plasma  
64 and saliva. After sample collection, other variables affect the stability of a biomarker in  
65 vitro, e.g. temperature, time until processing or freezing and additives like  
66 anticoagulants or stabilising agents. As one example, stability tests for fatty acids  
67 showed that these are very unstable at room temperature without any treatment [5]. All  
68 of these factors are potential sources of bias, and thus require to be standardised. The  
69 most important influences on biological samples are summarised in Table 1. A  
70 comprehensive summary of factors influencing the quality of biological samples in  
71 molecular epidemiological studies is given in a review by Holland et al. [6].

72 The standardised collection of biological samples in epidemiological studies is a  
73 challenging task, especially in international multicentre studies. Environmental  
74 conditions, such as ambient temperature, distance between field study centres and  
75 laboratories, may largely vary thus affecting the quality of the biological samples.  
76 Nevertheless, if data ought to be suitable for joint data analysis, standardised collection,  
77 processing, shipment, storage and analyses of biological samples is indispensable. A  
78 reliable quality management system can ensure standardisation across different  
79 environmental conditions and settings.

80 The International Organization for Standardization (ISO) created their quality  
81 management system (QMS) standards in 1987. These are applicable in different types of  
82 industries, for different types of activities or processes, e.g. design, production or  
83 service delivery. The ISO QMS standards certify processes, not the product itself. The  
84 standards are regularly reviewed by the ISO, the last revision was done in 2008 and the  
85 series was called ISO 9001:2008 series [7].

86 Since the 1990s, the International Epidemiological Association (IEA) and many  
87 national associations have discussed quality criteria for epidemiological research. They  
88 have agreed on guidelines for Good Epidemiological Practice (GEP) which can be  
89 found on their respective websites [8]. All of these guidelines promote the idea of  
90 quality assurance in epidemiological studies, which was elaborated by Rajaraman and  
91 Samet [9]. A working group of the Department of Epidemiology at the INRS in France  
92 successfully implemented the ISO 9002 system in their department [10]. Their certified  
93 quality system includes procedures specific to the conduct of epidemiological studies in  
94 occupational epidemiology but detailed aspects of biomarker collection are not  
95 addressed by their quality system. An overview of quality aspects in molecular  
96 epidemiology was provided by Holland et al. [11].

97 Considering the work of Moulin et al. [10] and Holland et al. [11], we present a newly  
98 developed quality management system designed for the collection of biological samples  
99 in epidemiological studies (QMS-BS). The applicability of the QMS-BS was evaluated  
100 in the context of the IDEFICS study, a large European multi-centre study on childhood  
101 obesity, where several types of biological samples were collected. To evaluate the  
102 application of the QMS-BS, the following criteria were applied: sample response rates,  
103 measures of sample quality, compliance with the QMS-BS and efficiency of the  
104 document and sample control and of the quality assurance system.

105

## 106 **Methods**

### 107 ***Quality management system for biological samples (QMS-BS)***

108 The conduct of an epidemiological study is suitable for the implementation of an ISO  
109 standard as shown by Moulin et al. [10]. Table 2 shows the embedding of the newly  
110 developed quality management system for the collection of biological samples (QMS-  
111 BS) into the ISO system, in analogy to the quality system for occupational  
112 epidemiology by Moulin. The key elements of the QMS-BS are described below.

113

#### 114 **Standard operating procedures**

115 In clinical research, SOPs are defined as "detailed written instructions to achieve  
116 uniformity of the performance of a specific function" [12]. The QMS-BS foresees  
117 SOPs that are study-specific and have to be defined for each type of biological sample  
118 on the following aspects:

- 119 • Collection: consent, time of collection, materials, devices, temperatures, and  
120 prerequisites like e.g. fasting status
- 121 • Processing: laboratory devices, calibration procedures, time and temperature  
122 ranges allowed for processing steps
- 123 • Shipping: conditions, intervals
- 124 • Storage: temperatures, sorting conditions

125 ISO 15189:2007 specifies in detail the QMS requirements for medical laboratories and  
126 ensures both regular internal and external quality audits and participation in  
127 interlaboratory comparisons [13]. Since ISO 15189:2007 accredited laboratories have  
128 SOPs for all their procedures, the QMS-BS does not include SOPs on laboratory  
129 analyses.

130 For the QMS-BS an SOP template was developed (see Figure 1) which also includes a  
131 document log to record changes to previous versions. SOPs have to be accessible to  
132 study personnel at any time during their work. In a multicentre study, if the study  
133 personnel do not have sufficient skill of English language, all SOPs must be translated  
134 to the native languages. Possible errors introduced by translation are minimised by back  
135 translation.

136

## 137 **Document and sample control**

138 Identification and tracing of biological samples requires appropriate labelling of each  
139 sample aliquot. ID-numbers given to study subjects and samples should be  
140 pseudonymised in a way that does not allow for the identification of the donor but still  
141 clearly defines the sample and enables its retrieval at any time.

142 Depending on the storage needs, ID-labels should be frost-resistant for down to - 80°C  
143 or even suitable for storage in liquid nitrogen. An SOP has to address how labels are  
144 correctly attached to the biosample tubes, as samples with missing ID-labels will  
145 usually be excluded from the study. Coding of IDs in barcodes minimises reading  
146 mistakes and simplifies tracing of samples with the help of scanners. There are 1D, 2D  
147 or 3D barcode systems and radio-frequency identification (RFID) tags. The choice  
148 should depend on the amount of information that needs to be coded and the space  
149 available on the ID-stickers. 3D-labels are smallest and contain most information. RFID  
150 tags have read and write capabilities and can store up to 2 KB. If biological specimens  
151 are stored in a biobank for later analysis, a biosample database should be used for  
152 efficient retrieval and documentation of storage conditions. If all samples are used up  
153 for laboratory analysis, it might be more economic to use simple delivery notes.

154

## 155 **Purchases and subcontracting**

156 Uniform sampling equipment and consumables are important means of standardisation  
157 in a multicentre study. Use of standard materials can be assured by central purchasing,  
158 but additional management and shipping costs have to be accounted for. Alternatively,  
159 all materials have to be clearly defined for local purchase. In this case, feedback on the  
160 realisation of the requirements should be collected prior to study commencement.

161 Several components of biological sample collection can be subject to subcontract, either  
162 if they cannot be accomplished by own personnel or as a means of standardisation to  
163 improve quality. Possible candidates for subcontracting are sample collection,  
164 processing or sample analysis. It has to be kept in mind that study elements which are  
165 subcontracted are usually more difficult to control by the study management concerning  
166 adherence to defined SOPs.

167

### 168 **Process control**

169 Before the beginning of the study, biological samples of interest must be identified  
170 along with all the steps for their collection, processing, storage, shipment and analyses.  
171 In a multicentre study there are two main options for sample processing: it can either be  
172 done locally, followed by freezing and shipping on dry ice or centrally, after shipping  
173 on wet ice. To evaluate the best option several parameters have to be weighed against  
174 each other: shipping times and costs (if processing is done centrally, daily shipping is  
175 necessary) and the higher need for quality assurance if several local laboratories are  
176 involved. Either way a lot of conditions need to be defined including minimum and  
177 maximum resting times before centrifugation, analysis or freezing and temperatures at  
178 which samples should be kept at each stage.

179 Provisions should be taken for each sample type regarding packaging, shipping intervals  
180 and delivery documentation. Shipment of biological samples requires a tracking system  
181 to follow packages and a notification by the sender before shipping to ensure that the  
182 recipient can accept the package and properly store the samples upon receipt. In the  
183 European Community, human biosamples are not generally considered as potentially  
184 infectious material any more [14, 15]. Dry ice used for shipping however is subject to

185 the dangerous goods regulations of the International Air Transport Association (IATA),  
186 which implies e.g. that only cargo planes are used for transportation.

187 SOPs for long-term storage of biological samples define storage temperatures, a system  
188 for sample retrieval, a surveillance system to detect equipment failures, an emergency  
189 plan, regular quality checks, and sample inventories.

190 Many laboratories have a QMS accredited according to ISO 15189. It is advisable to  
191 choose an accredited central laboratory to guarantee for uniform sample analysis. If  
192 shipment of samples from the survey centres to a central laboratory cannot be achieved  
193 within 48h, transportation, even on dry ice, is not a safe option and decentral sample  
194 analysis should be preferred. Depending on the stability of the markers of interest there  
195 might be a need to analyse certain parameters directly upon collection (point-of-care  
196 analysis).

197

## 198 **Quality assurance**

199 According to ISO standards a set of activities should be introduced to ensure that the  
200 defined SOPs are implemented appropriately: This includes training activities and  
201 pretesting of all procedures. Internal and external quality audits should be conducted to  
202 verify adherence to the defined SOPs. All means and results of quality assurance have  
203 to be documented and should be comprehensible for the study personnel.

204

## 205 ***Application of the QMS-BS in the IDEFICS-study***

206 IDEFICS is an Integrated Project in the 6<sup>th</sup> Framework Programme of the European  
207 Commission tackling the “Identification and prevention of dietary- and lifestyle-induced  
208 health effects in children and infants”. 16,188 pre-school and primary school children

209 from eight European countries were examined during the baseline survey in 2007/2008.  
210 One major objective of the IDEFICS study was to assess the distribution of diet- and  
211 lifestyle-related health problems and to understand the causal pathways leading there.  
212 The main emphasis of the study was put on three disorders: obesity/ overweight, insulin  
213 resistance and impaired bone health. Each of these health problems is associated with a  
214 set of biological markers or is even partly defined by them, as it is the case for insulin  
215 resistance. The background of the study, its research goals and instruments have been  
216 described elsewhere in detail [16, 17]

217 Sample collection in the IDEFICS study was conducted according to the newly  
218 introduced QMS-BS (see Table 3). Children in the baseline survey were asked to donate  
219 fasting venous blood (native and EDTA blood), morning urine and saliva samples. If  
220 venous blood could not be obtained, capillary blood was taken where possible.

221 A set of SOPs was developed and translated to all survey languages describing the  
222 collection, processing, storage and shipping of all types of biological samples in detail.

223 Barcoded labels were used for sample tracking. Each biological sample was labelled  
224 with an unambiguous 10 digit identification number (ID) with the last two digits clearly  
225 defining the type of aliquot. A biosample database was developed to record detailed  
226 information on the preanalytical conditions (collection, processing and storage) of each  
227 sample (e.g. cryotube or vial) and its storage location (down to the position in the  
228 cryobox) so that retrieval of samples for further use or withdrawal of samples can be  
229 done easily. The database facilitates shipping of samples to the central laboratories by  
230 an automated generation of delivery notes. A central biobank of remaining samples was  
231 built up for long-term storage.

232 All sampling kits and processing material were purchased centrally. Biochemical  
233 analyses were carried out in a central laboratory accredited according to ISO 15189.

234 DNA extraction, genotyping, RNA extraction and gene expression analysis were  
235 conducted at different central laboratories.

236 As blood lipids and blood glucose were markers of primary interest to assess the risk of  
237 study participants for metabolic syndrome, these were assessed on site by point-of-care  
238 analysis with the Cholestech LDX analyser [18]. The decision was made to use this  
239 relatively expensive method because it only uses one drop of venous or capillary blood;  
240 thus children who only agreed to give capillary blood could be included to maximize  
241 response rates for these key measures. The immediate feedback on these core variables  
242 also served as an incentive for the parents. Another drop of blood was sufficient for the  
243 fatty acid (FA) analysis of circulating lipids and was collected via a simple kit (Sigma-  
244 Aldrich cod. 11312-1KT). This method, developed by Marangoni et al. [19], avoids the  
245 complex procedures for collection, storage, shipment, and sample preparation involved  
246 with the conventional method of FA analysis.

247 Apart from point-of-care analyses, all biomarkers were analysed by central laboratories.  
248 Markers analysed in blood samples comprised insulin, CRP and HbA1c, as well as  
249 hormones of energy/fat metabolism and markers of bone metabolism. Markers analysed  
250 in morning urine included glucose, albumin, and creatinine, as well as several minerals  
251 and cortisol. An overview of all biological markers assessed during the IDEFICS study  
252 is given in Table 4.

253 PAXgene tubes containing an RNA stabilising agent were used to collect blood for gene  
254 expression analysis in a subsample of children [20]. In order to maximise response  
255 rates, sample collection of morning urine was performed at home by the parents, who  
256 received a collection kit and a detailed instruction sheet and DNA was obtained non-  
257 invasively from saliva samples. Collection procedures for saliva differed by the  
258 children's ability to spit the required amount of saliva: Oragene™ DNA collection kit

259 OG 250 with saliva sponges were used for younger children who were usually not yet  
260 able to spit, Oragene™ DNA Self-Collection Kit OG 300 were used otherwise. The kits  
261 are user-friendly and provide a high amount of good quality DNA [20]. Samples of all  
262 types were processed at the local survey centres and shipped to central laboratories at  
263 regular intervals.

264 All procedures were instructed at a central training which was mandatory for all survey  
265 centres. The whole set of instruments was tested during a pretest (Suling, in  
266 preparation). During the survey, a central telephone hotline was established for all  
267 questions regarding the biological samples. Survey site visits were conducted by a  
268 central quality control unit where the practical field work was inspected and deviations  
269 from SOPs were corrected directly if possible, or else remedial actions were initiated.

270

## 271 **Results**

### 272 ***Evaluation of applied QMS-BS during the IDEFICS baseline*** 273 ***survey***

274 Response rates for different biological sample types in the eight survey centres are listed  
275 in Table 5. Response rates varied for different types of biological samples with highest  
276 response rates for non-invasive sample types. Urine samples were obtained from 85.6%  
277 of the children in total, with the lowest response of 67.9% in centre 3. Saliva samples  
278 were collected from 90.2% of the children; again centre 3 had the lowest response rate  
279 of 76.7%. Two of the centres (centre 7 and 8) nearly reached completeness for this  
280 sample type. For blood sampling, it was attempted to collect venous blood; only  
281 children that were not willing to give venous blood were asked for capillary blood. In  
282 total, a remarkable 79.7% of the children gave capillary or venous blood and were thus

283 eligible for point-of-care analysis. Centre 4 obtained a substantially lower rate than the  
284 other centres (61.6%). This centre also had some initial problems in obtaining fasting  
285 blood (7.3% of non-fasting blood samples compared to an average of 1.4%). Venous  
286 blood could be collected from on average 56.6% of children across all countries.  
287 Response rates for venous blood ranged from 51% to 83% between the centres with the  
288 exception of centre 3 where venous blood was only collected from 7.7 % of the  
289 children. The site visit showed that the low response rate at centre 3 resulted from non-  
290 compliance with the study protocol: due to the specific situation in the study region  
291 children were primarily asked for capillary blood at the survey site and then given the  
292 additional opportunity for later venipuncture at another facility.

293 As part of process control various measures of sample quality were recorded  
294 accompanying the different steps of sample collection and a quality check was  
295 performed for each sample in the laboratory upon arrival (see Table 6). Collection of  
296 morning urine was documented by the parents on a collection sheet; this included  
297 recording of potential problems with sample quality. About 2.9% of the parents reported  
298 that the sample was not the first morning urine, 3.9% that the children went to the toilet  
299 at night and 6.9% stated that the urine was left uncooled for several hours. These rates  
300 varied among the centres: for 45% of the urine samples of centre 4 at least one of these  
301 problems was reported compared to only 0.3% of centre 3. The quality check of urine  
302 samples at the central laboratory on the other hand generally showed no problems (data  
303 not shown).

304 Venous blood samples were repeatedly associated with several problems as noted both  
305 by the study centres in the biosample database and by the central laboratory. According  
306 to the study centres an average of 5.4% of the samples were haemolytic with a  
307 maximum prevalence of 25.1% in centre 1. The central laboratory on the other hand  
308 categorised 10.3% of samples as haemolytic, where again centre 1 was of main concern

309 with 30.8% of samples classified as haemolytic. High amounts of haemolytic samples  
310 were also reported for centre 2, 4 and 8 (13.8%, 8.2% and 12.1% respectively). It can  
311 thus be concluded that the assessment of haemolytic samples by the study centres was  
312 not a suitable tool to detect problematic samples for laboratory analyses. Coagulation of  
313 EDTA samples occurred in 0.7% of the checked samples, most of which came from  
314 centre 4. All centres had occasional problems to reach the required filling quantity for  
315 the aliquots. In total 4% of the samples were considered to be 'short samples' by the  
316 central laboratory.

317 Saliva samples were least error-prone of all sample types; the small fraction of dry  
318 samples (0.7%) which arrived at the central laboratory (probably due to leakage) were  
319 re-hydrated and still extracted with a lower, but yet sufficient, yield of DNA.

320 Compliance with the QMS-BS varied between survey centres and components. All  
321 survey centres participated in the central training and conducted subsequent local  
322 trainings as foreseen in the quality assurance system. The survey centres implemented  
323 all standardised procedures according to SOPs as verified by the external quality audits  
324 during site visits. The only exception was the SOP for blood collection, where one centre  
325 (centre 3) did not follow the instructions to take venous blood preferentially as  
326 mentioned above. For some other components compliance was lower. Most notably,  
327 centres had difficulties using the biosample database. The software was considered to be  
328 too complex and data entry too time-consuming. This caused a delay in data entry  
329 which in turn resulted in problems in the central laboratory, where biochemical analyses  
330 could not be conducted for samples delivered without a corresponding database.  
331 Moreover, due to the delay it was not possible to carry out direct quality control of  
332 sample processing with the biosample database as initially planned. Variables recorded  
333 in the database like time intervals, e.g. between collection of blood samples and their  
334 centrifugation represent important measures of quality assurance. As depicted in

335 Table 6 especially one centre had problems to manage sample processing within the  
336 requested time span. 11.4% of the samples in centre 5 were centrifuged later than  
337 foreseen in the SOP, for all other centres this rate was below 1.5%.

338

## 339 **Discussion**

340 This paper introduces a quality management system for the collection of biological  
341 samples in epidemiological studies (QMS-BS). The QMS-BS was applied to a large  
342 multi-centre study, the IDEFICS-study with the goal to collect biological samples of a  
343 standardised quality across all study centres and to build up a large biobank of blood,  
344 saliva and urine samples from children from different European regions. Evaluation  
345 showed that high average response rates were reached for all sample types. Similar rates  
346 were achieved during the German KIGGS study, a health study on children and  
347 adolescents, where a response rate of 85% was reached for urine samples in the ‘Iodine  
348 Module’ of the study [22] and 52.6% for an environmental survey module which  
349 included venous blood sampling amongst others [23]. Measures of sample quality  
350 revealed several problems which mainly occurred during sample collection and  
351 processing. Central purchase of consumables guaranteed comparability but was  
352 accompanied by high shipping costs; depending on the monetary value of the respective  
353 consumables up to 10% were added to their costs. Overall compliance with the QMS-  
354 BS was good in the study centres, although some exceptions were noted. These were  
355 related primarily to sample control. Study centres complained about the complexity of  
356 the biosample database, leading to a considerable delay in data entry. The database  
357 could thus not be used for quality control during the ongoing survey, only  
358 retrospectively. Nevertheless biobanking and sample retrieval would not have been  
359 possible without the biosample database.

360 Generally, not all differences between centres could be avoided by the QMS-BS. In  
361 particular, blood response rates and sample quality differed substantially between  
362 centres. Different response rates for invasive sample types may be related to national  
363 characteristics, whereas differences in sample quality were probably rather based on  
364 different survey logistics, e.g. allocated manpower or geographical distances between  
365 sample collection and processing. The influence of sample quality on the analyses of  
366 biological markers in the IDEFICS-study will be the topic of future investigations.

367 It should be noted that the introduction of a quality management system is not able to  
368 solve all problems during sample collection in epidemiological studies. Another key  
369 aspect is the complexity of the applied procedures. Decisions made for user-friendly  
370 solutions within IDEFICS were generally expensive but very successful like e.g. point-  
371 of-care analysis of the main biological markers for metabolic syndrome (glucose, total  
372 cholesterol, HDL, triglycerides), DNA collection with saliva kits that didn't need to be  
373 processed or cooled, and FA test strips that only needed one drop of blood for the  
374 analysis of complete fatty acid profiles. These solutions generally reached high response  
375 rates and offered a standardised quality. For example, because point-of-care analysis  
376 was employed for blood glucose and lipid analyses, response rates for the respective  
377 markers were increased by about 23% (representing the children which only agreed to  
378 the collection of capillary blood).

379 Results of the evaluation will contribute to further optimisation of biological sample  
380 collection for the IDEFICS follow-up survey that starts in autumn 2009. The biosample  
381 database has been substantially simplified and a barcode scanner and laptop will be used  
382 for storage documentation. Moreover, procedures for serum collection will be changed  
383 to reduce the amount of haemolytic samples which was most likely caused by  
384 insufficient clotting times in combination with the completely native blood tubes that  
385 were used to allow for the removal of two drops of venous blood for point-of-care

386 analysis and FA analysis before clotting started. To enhance compliance with the QMS-  
387 BS in the IDEFICS follow-up survey different options are currently discussed.  
388 Krockenberger et al. [24] investigated the adherence to SOPs in the context of clinical  
389 trials. They found that, for questions about the daily work, the staff was more likely to  
390 ask a colleague rather than to read the corresponding SOP (18.4% versus 13.8%). The  
391 authors suggest a computer-based information retrieval system for SOPs to increase the  
392 ease-of-use and usefulness of SOPs which might also be an interesting option for the  
393 QMS-BS. Other options are centralised re-training sessions and/or special emphasis on  
394 selected aspects of biological samples collection during the external site visits.  
395 Practical aspects of field work and sample collection are often neglected in scientific  
396 exchange. The QMS-BS fills this gap and represents a systematic approach to sample  
397 collection for application in epidemiological studies. Over all its application in  
398 IDEFICS helped to obtain a high quality standard for the biological samples collection  
399 in this European multicentre study.

400

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407

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495 **Table 1: Major sources of bias for biomarkers before, during and after sample collection**

Before sample collection	During sample collection	After sample collection
<ul style="list-style-type: none"> <li>• Fasting status (food, drinking, medication, smoking)</li> <li>• Timing of collection (diurnal variation, seasonal variation)</li> <li>• Recent exercise</li> <li>• State of health (e.g. infections, fever, lipaemia, pregnancy)</li> </ul>	<ul style="list-style-type: none"> <li>• Posture of study subject</li> <li>• Sample type (e.g. venous blood vs. Capillary blood)</li> <li>• Collection materials (e.g. tourniquet, collection cups)</li> <li>• Use of additives (e.g. anticoagulating agents, stabilising agents)</li> <li>• Insufficient volume in tube</li> <li>• Order of draw</li> <li>• Sterility</li> </ul>	<ul style="list-style-type: none"> <li>• Centrifugation conditions</li> <li>• Haemolysis of blood samples</li> <li>• Storage temperature</li> <li>• UV light (e.g. direct sunlight)</li> <li>• Time before processing/ freezing</li> <li>• Shipping conditions (temperature and time)</li> </ul>

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498 **Table 2: Quality management system for the collection of biological samples (QMS-BS),**  
 499 **adapted from the quality system for occupational epidemiology (Moulin, 1998)**

ISO 9002 elements	QMS-BS
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Production procedures	<b>Standard procedures (SOPs) specific to the collection of biological samples</b>
Document and data control	<b>Document and sample control</b>
Purchasing	<b>Purchases and subcontracting</b>
Process control	<b>Process control for biological samples:</b> - collection - processing - shipping - storage - laboratory analyses
Inspection and testing	<b>Quality assurance for sample collection</b>

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502 **Table 3: Application of the QMS-BS to biological sample collection in IDEFICS**

<b>QMS-BS</b>	<b>Fasting blood</b>	<b>Morning urine</b>
<b>Standard procedures (SOPs) specific to the collection of biological samples</b>	10 specific SOPs	3 specific SOPs Information sheet on collection for parents
<b>Document and sample control</b>	Barcode IDs Sample management by biosample database	Barcode IDs Sample management by biosample database
<b>Purchases and subcontracting</b>	Central purchase of all collection and processing material Central laboratory for blood analyses Central laboratory for analyses of FA test strips Central laboratory for RNA analyses	Central purchase of collection kits and tubes Central laboratory
<b>Process control: Sample collection</b>	Collection of venous blood; alternatively capillary blood For venous blood: 11 ml native and 7 ml EDTA blood 2,5 ml into Paxgene tubes with RNA stabilising agent	Collection is done by parents home
<b>Process control: Processing at survey centre</b>	<b>Cholestech:</b> first drop of whole blood applied to test cassette <b>FA test strip:</b> second drop of whole blood applied to strip <b>Blood aliquots:</b> separation of serum, plasma, WBC, RBC <b>Paxgene tubes:</b> N/A	Aliquoting
<b>Process control: Shipping</b>	<b>Cholestech:</b> N/A <b>FA test strip:</b> at room temperature <b>Blood aliquots:</b> frozen on dry ice <b>Paxgene tubes:</b> frozen on dry ice	Frozen on dry ice
<b>Process control: Storage</b>	<b>Cholestech:</b> N/A <b>FA test strips:</b> long term at -20°C, short term at +4°C <b>Blood aliquots:</b> long term at -80°C <b>Paxgene tubes:</b> at -20°C for up to a year	Long term storage at -20°C
<b>Process control: Laboratory analyses</b>	<b>Cholestech:</b> on site analysis of glucose, cholesterol and triglycerides <b>FA test strips:</b> composition of circulating lipids by HPLC <b>Blood aliquots:</b> insulin, CRP, HbA1c, selected hormones of	Minerals as markers of dietary habits, proteins (creatinine, albumin), cortisol

	energy metabolism and markers of bone health <b>Paxgene tubes:</b> RNA extraction and gene expression analysis	
<b>Quality assurance for sample collection</b>	Quality check of samples at the laboratory	Quality check of samples at laboratory

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505 **Table 4: Overview of biological markers analysed in the IDEFICS-study**

Sample type	Biological marker	Exposure
Native blood	Point-of care analysis: Blood glucose, Total cholesterol, HDL cholesterol Triglycerides	Metabolic syndrome, Insulin resistance
	Fatty acid profiles (collection kit)	Dietary patterns
EDTA plasma RBC	Fatty acid profiles (conventional methodology for validating the collection kit)	Dietary patterns
EDTA blood	HbA1c	Diabetes
Serum	Insulin	Insulin resistance
	C-reactive protein (CRP)	Inflammation
	Leptin Adiponectin	Markers of energy metabolism
	Vit D Ca NTX-peptide	Bone metabolism
Whole blood collected in RNA-stabilising PAXgene tubes	Quantity of RNA for selected genes	Gene expression
Morning urine	Urinary glucose	Diabetes
	Urinary albumin	Metabolic syndrome
	Urinary creatinine	Reference marker
	Minerals (Na, K, Mg, P, Ca)	Dietary patterns
	Cortisol	Chronic stress
Saliva	Selected candidate genes (sequencing of tag-haplotypes)	Genetic risk factors

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508 **Table 5: Response rates for biological samples during the baseline survey of IDEFICS**

Study centre	Total blood	Venous blood	Capillary blood	Urine	Saliva	Subjects included*
<b>Centre 1</b>	1,812 80,53%	1,296 57,60%	516 22,93%	1,946 86,49%	1,986 88,27%	2,250 100,00%
<b>Centre 2</b>	1,325	882	443	1,419	1,552	1,719

	77,08%	51,31%	25,77%	82,55%	90,29%	100,00%
<b>Centre 3</b>	1,729	184	1,545	1,615	1,825	2,380
	72,65%	7,73%	64,92%	67,86%	76,68%	100,00%
<b>Centre 4</b>	1,184	996	188	1,498	1,640	1,923
	61,57%	51,79%	9,78%	77,90%	85,28%	100,00%
<b>Centre 5</b>	1,540	1,058	482	1,596	1,638	1,810
	85,08%	58,45%	26,63%	88,18%	90,50%	100,00%
<b>Centre 6</b>	1,609	1,413	196	1,858	1,975	2,066
	77,88%	68,39%	9,49%	89,93%	95,60%	100,00%
<b>Centre 7</b>	2,407	2,133	274	2,550	2,534	2,567
	93,77%	83,09%	10,67%	99,34%	98,71%	100,00%
<b>Centre 8</b>	1,327	1,221	106	1,405	1,481	1,507
	88,06%	81,02%	7,03%	93,23%	98,27%	100,00%
<b>Total</b>	12,933	9,183	3,750	13,887	14,631	16,222
	79,73%	56,61%	23,12%	85,61%	90,19%	100,00%

509 \*a study subject was included if at least data on age, sex, height and weight was collected

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511 **Table 6: Measures of samples quality during IDEFICS baseline survey**

Study centre	1	2	3	4	5	6	7	8	Total
<b>Morning urine</b>									
Samples collected	1,946	1,419	1,615	1,498	1,596	1,858	2,550	1,405	13,887
<b>Problems with sample quality</b>									
Any problem	499	12	5	679	60	176	37	221	1689
% of urine samples	25.64%	0.85%	0.31%	45.33%	3.76%	9.47%	1.45%	15.73%	12.16%
<b>Problems with collection</b>									
Not first morning urine	145	6	2	60	37	44	30	80	404
% of urine samples	7.45%	0.42%	0.12%	4.01%	2.32%	2.37%	1.18%	5.69%	2.91%
Child went to the toilet at night	95	2	2	202	15	123	2	97	538
% of urine samples	4.88%	0.14%	0.12%	13.48%	0.94%	6.62%	0.08%	6.90%	3.87%
<b>Problems with processing</b>									
Urine uncooled for several hours	326	4	1	522	8	12	8	77	958
% of urine samples	16.75%	0.28%	0.06%	34.85%	0.50%	0.65%	0.31%	5.48%	6.90%
<b>Problems with storage</b> n/a									
<b>Venous blood</b>									
Samples collected	1,296	882	184	996	1,058	1,413	2,133	1,221	9,183
<b>Problems with collection</b>									
Child not fasting for >8h	2	13	0	71	33	18	17	29	112
% of blood samples	0.15%	1.47%	0.00%	7.13%	3.12%	1.27%	0.80%	2.38%	1.22%
<b>Problems with processing</b>									
Samples haemolytic	399	122	3	82	61	35	94	148	944
% of blood samples	30.79%	13.83%	1.63%	8.23%	5.77%	2.48%	4.41%	12.12%	10.28%

Samples coagulated	0	0	0	41	5	1	12	6	65
<i>% of blood samples</i>	0.00%	0.00%	0.00%	4.12%	0.47%	0.07%	0.56%	0.49%	0.71%
Short samples	75	1	17	64	47	105	29	34	372
<i>% of blood samples</i>	5.79%	0.11%	9.24%	6.43%	4.44%	7.43%	1.36%	2.78%	4.05%
Permitted times exceeded	1	4	0	0	121	1	6	4	137
<i>% of blood samples</i>	0.08%	0.45%	0.00%	0.00%	11.44% <sup>x</sup>	0.07%	0.28%	0.33%	1.49%
<b>Problems with storage</b> n/a									
<b>Saliva</b>									
<b>Samples collected</b>	1,986	1,552	1,825	1,640	1,638	1,975	2,534	1,481	14,631
<b>Problems with collection</b> n/a									
<b>Problems with processing</b> n/a									
<b>Problems with storage</b>									
Dry samples	14	9	16	7	14	6	33	6	105
<i>% of saliva samples</i>	0.70%	0.58%	0.88%	0.43%	0.85%	0.30%	1.30%	0.41%	0.72%

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514 **Figure 1: SOP template developed for the QMS-BS**

Version	Date	Comment	Author

**1 Person responsible for SOP**

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**2 Name of the procedure**

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**3 Short description of the procedure**

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**4 Scope of application** (e.g.project or institute)

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**5 Glossary** (technical terms and abbreviations)

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**6 Detailed description of procedure** (on additional pages)

- What is analysed/ examined?
- Executing person (doctor, interviewer, nurse...?)
- Prerequisites (storage, stability, criteria for exclusion, examination only at special time of day?, etc.)
- General principle of analysis/ examination
- Equipment (including calibration instructions) and resources needed for the analysis/ examination
- Consumables needed for the analysis/ examination
- **Conduct of analysis/ examination step by step.**
- How and where are the analysis/ examination and its results documented?
- How are possible problems dealt with (FAQs)?
- Detection limits, precision, validity, reliability...

**7 Annex** (related SOPs, standards, laws, instruction manuals which are attached)

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