

Quality assurance and quality control processes

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DOI:

[10.1007/s11306-017-1188-9](https://doi.org/10.1007/s11306-017-1188-9)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Dunn, WB, Broadhurst, DI, Edison, A, Guillou, C, Viant, MR, Bearden, DW & Beger, RD 2017, 'Quality assurance and quality control processes: summary of a metabolomics community questionnaire', *Metabolomics*, vol. 13, no. 5, 50. <https://doi.org/10.1007/s11306-017-1188-9>

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Checked for eligibility: 24/05/2017.

The final publication is available at Springer via <http://dx.doi.org/10.1007/s11306-017-1188-9>.

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1 **Quality assurance and quality control processes: Summary of a**
2 **metabolomics community questionnaire**

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41 23
42 24
43 25 Keywords: metabolomics, quality assurance, quality control, questionnaire,
44 26 Metabolomics Society

28 **Abstract**

1 29

2 30 *Introduction*

3 31 The Metabolomics Society Data Quality Task Group (DQTG) developed a
4 32 questionnaire regarding quality assurance (QA) and quality control (QC) to
5 33 provide baseline information about current QA and QC practices applied in the
6 34 international metabolomics community.

7 35

8 36 *Objectives*

9 37 The DQTG has a long-term goal of promoting robust QA and QC in the
10 38 metabolomics community through increased awareness via communication,
11 39 outreach and education, and through the promotion of best working practices.
12 40 An assessment of current QA and QC practices will serve as a foundation for
13 41 future activities and development of appropriate guidelines.

14 42

15 43 *Method*

16 44 QA was defined as the set of procedures that are performed in advance of
17 45 analysis of samples and that are used to improve data quality. QC was defined as
18 46 the set of activities that a laboratory does during or immediately after analysis
19 47 that are applied to demonstrate the quality of project data. A questionnaire was
20 48 developed that included 70 questions covering demographic information, QA
21 49 approaches and QC approaches and allowed all respondents to answer a subset
22 50 or all of the questions.

23 51

24 52 *Result*

25 53 The DQTG questionnaire received 97 individual responses from 84 institutions
26 54 in all fields of metabolomics covering NMR, LC-MS, GC-MS, and other analytical
27 55 technologies.

28 56

29 57 *Conclusion*

30 58 There was a vast range of responses concerning the use of QA and QC
31 59 approaches that indicated the limited availability of suitable training, lack of
32 60 Standard Operating Procedures (SOPs) to review and make decisions on quality,
33 61 and limited use of standard reference materials (SRMs) as QC materials. The
34 62 DQTG QA/QC questionnaire has for the first time demonstrated that QA and QC
35 63 usage is not uniform across metabolomics laboratories. Here we present
36 64 recommendations on how to address the issues concerning QA and QC
37 65 measurements and reporting in metabolomics.

38 66

67 **Introduction**

68
69 Metabolomics is a scientific approach applied to the systems analysis of
70 metabolism [Dunn 2011] operating in microbes, plants and animals [Furusawa
71 2013; Kusano 2015; Cheng 2015]. The discipline of metabolomics is less than 20
72 years of age [Oliver 1998] although the roots are much older [Pauling 1971].
73 Metabolomics studies typically use a pipeline from experimental design through
74 analytical measurements (sample preparation and data acquisition) to
75 bioinformatics processing (data processing and statistical analysis) [Brown
76 2005]. The validity of and confidence in the biological conclusions resulting from
77 this pipeline are highly dependent on the quality of the procedures applied
78 during the metabolomics study. The appropriate application of quality assurance
79 (QA) and quality control (QC) processes are important but are often overlooked
80 in metabolomics. In targeted metabolite studies, guidelines are available to guide
81 the scientist in some aspects of the process including the most frequently applied
82 Food and Drug Administration (FDA) guidelines for bioanalytical method
83 validation [<http://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf>.]
84 as well as other materials [Garfield 2000; Hibbert 2007; Westgard 2008; Booth
85 2015]. However, there are currently no clear guidelines for untargeted
86 metabolomic studies.

87
88 The Metabolomics Society’s mission includes “To promote the growth and
89 development of the field of metabolomics internationally” [Metabolomics Society
90 website]. To address this mission, several scientific task groups have been
91 established to act for the community in areas requiring international community
92 consensus. One of these is the Data Quality Task Group (DQTG) chaired by Drs.
93 Daniel Bearden and Richard Beger. The DQTG promotes robust QA and QC in the
94 metabolomics community through increased awareness via communication,
95 outreach and education, and through the promotion of best working practices
96 [Bearden 2014; Metabolomics Society task group website]. One objective of this
97 task group is to define the current application levels of QA and QC processes in
98 both targeted and untargeted studies across all applications in metabolomics. To
99 complete this objective, the task group operated a questionnaire for 2 months
100 (August – September 2015) via the SurveyMonkey website
101 (<https://www.surveymonkey.com>), which was advertised via e-mail alerts,
102 Metabolomics Society web pages, Twitter and MetaboNews newsletters. The
103 questionnaire included 70 questions covering demographic information, QA
104 approaches and QC approaches and allowed all respondents to answer a subset
105 or all of the questions. All responses are available in the supplementary
106 information and on the Metabolomics Society website [13]. Here we will
107 summarize the most important information and facts derived from the
108 questionnaire and a number of important recommendations.

109
110 **The respondents**

- 111 • 97 respondents
- 112 • 36 % were principal investigators (PIs) or group leaders, 14 % were staff
113 scientists, 20 % were post-doctoral researchers and 19 % were PhD
114 students.

- 115 • 11 % of respondents had less than 2 years of experience in metabolomics
116 with 31 % having greater than 8 years experience.
117 • The respondents applied metabolomics in a diversity of different
118 applications and many respondents worked across multiple disciplines
119 including clinical sciences (65 %), toxicology (35 %) and systems biology
120 (45 %).
121 • 70 % responded as working in a combination of a biological/chemical
122 laboratory and data processing/bioinformatics.
123 • Greater than 70 % of respondents worked with cells, biofluids and tissues
124 and investigated microbes (42 %), plant (34 %), mammals (62 %) and
125 humans (76 %). 73 % and 88 % of respondents applied targeted and
126 untargeted assays, respectively, with 34 % applying NMR spectroscopy in
127 their studies, 83 % applying liquid chromatography-mass spectrometry
128 and 50 % applying gas chromatography-mass spectrometry.
129 • 74 % of respondents investigated less than 200 samples in a typical
130 biological study and 63 % studied less than 5000 total biological samples
131 each year.

132 ***Training***

134 Quality processes include training and competence assessment to ensure a
135 minimum quality-level is associated with processes involving staff. 65 % of 94
136 responses defined that they operate in an environment with no in-house training
137 program and 74 % were not required to be involved in ongoing continuous
138 professional education. In environments where training was conducted (33
139 responses), professional staff (49 %) and post-doctoral/graduate staff (36 %)
140 were the major providers of training. Where training is provided, only 21 % of
141 instrument operators have to pass a certification test after training, with 57 %
142 applying professional staff to perform the assessment. 79 % of 85 responses do
143 not operate in an environment where there was a requirement to pass a
144 certification test after training. 73 % of 33 responses applied periodic checks of
145 professional practice with 58 % of checks performed by professional staff as
146 indicated by 33 responses.

147 ***Standard Operating Procedures***

149 The mistakes that can be introduced into metabolomics experiments through
150 improper or inconsistent pre-analytical or analytical procedures may cause the
151 data to be inaccurate or invalid, and this may lead to erroneous findings and
152 conclusions. For examples see [Gika 2008; Bernini 2011; Kamlage 2014; Dunn
153 2012]. Consistent procedures as simple as pipetting, balance usage, sample
154 cross-contamination control, proper preparation of solvents and sample
155 extraction techniques all contribute to the veracity of the analytical
156 measurements and should be thoroughly documented in Standard Operating
157 Procedures (SOPs) and enforced in training programs. For long-term studies or
158 interlaboratory studies, SOPs are essential for communicating well and ensuring
159 the consistency of the data.

160
161 Eighty-seven respondents answered questions related to SOPs. SOPs were
162 available in the laboratories of 76 % of respondents with 58 % developed in-
163 house and a further 37 % developed from in-house and published methods.

164 When investigated in more detail, 90 % of respondents had access to SOPs for
165 sample extraction, 53 % for sample storage, 75 % for analytical instruments,
166 52 % for assessment of data from QC samples and 33 % for deciding when QC
167 data from instrumental analysis has failed and defining how to correct the
168 instrumental data. As a matter of concern and shown for 84 responses was that
169 70 % of respondents did not have access to a protocol for independent review of
170 quality-related results (Figure 1A) and 80 % did not have access to a written
171 protocol of QA review criteria (Figure 1B).

172 173 ***Sample measurement validation***

174 The majority of respondents (82 responses) validate sample measurements with
175 73 % using repeat sample extractions and analyses, 87 % performing repeated
176 analysis of the same sample and 54 % analyzing a historical sample periodically
177 (Figure 2). 88 % of 80 responses analyze a blank sample with extraction
178 performed as for biological samples. Blank samples were analyzed either at the
179 start and end of the analytical batch (28 %), at regular intervals (44 %) or
180 randomly (21 %) as defined in 68 responses. 78 % of respondents operated a
181 process to reduce carryover (80 responses) and 91 % randomize the order of
182 sample analysis (80 responses). 94 % operated instrument condition checks and
183 79 % of 80 responses did not apply standard reference materials (SRMs); when
184 applicable, 47 % applied a SRM once or less than once a day and 16 % greater
185 than once per day. Methods for reporting of QC data were variable in the 80
186 responses collected; 34 % reported precision measurements for each metabolite,
187 45 % report a single range of precisions for all metabolites, 24 % report QC data
188 on a boxplot, 56 % visualize QC samples on a PCA scores plot and 56 % provide a
189 descriptive statement of the QC results.

190 191 ***QC samples***

192 Of 80 responses, 83 % of respondents applied pooled project materials and 48 %
193 applied standard reference materials (SRMs) as QC materials. This contradicts
194 the results for SRM use as defined above in the sample measurement validation
195 section. Figure 3 illustrates how often QC samples were applied for different
196 processes including the assessment of consistency in sample preparation (80 %)
197 and chromatography column integrity (76 %). Importantly, 59 % of respondents
198 applied replicate extractions and 69 % applied replicate analytical
199 measurements with 85 % analyzing individual samples and 15 % analyzing a
200 single pooled sample.

201 202 ***Data storage***

203 Of 84 responses, 89 % store data in an archive, with 95 % of data storage being
204 performed in an in-house archive. A lower percentage (73 %) archived QA/QC
205 data.

206 207 ***Inter-laboratory comparisons***

208 Of 82 responses, 33 % had participated in an inter-laboratory comparison study
209 and 48 % were interested in participating in a future inter-laboratory
210 comparison.

211 212 ***Laboratory accreditation***

213 Of 85 responses, 89 % were not required to meet laboratory accreditation and
214 74 % were not voluntarily attempting to meet any accreditation.

215

216

217

218 ***Biggest issues in QA and QC implementation and processes***

219 The most frequent comments related to the currently regarded biggest issues in
220 QA and QC are detailed below:

221 • Training including staff turnover and lack of training available outside the
222 organization

223 • SOP formalization, consistency and maintenance including reported
224 changes to published methods (for example papers published in *Nature*
225 *Protocols*)

226 • Ensuring routine compliance to SOPs and QA processes

227 • Insufficient control over sample collection and sampling consistency

228 • Inadequate availability of reference standards, isotopically labeled
229 compounds, QC samples and SRMs

230 • Providing a balance between QA/QC and sample throughput

231 • QC does not contribute to assessment of output by the wider community
232 and there is a need for true standards across the community

233 • A global strategy for QA/QC and its review is required

234 • Establishment of QC acceptance criteria as currently there is a lack of
235 reported QC results and acceptance criteria

236 • Additional measures beyond pooled QC samples

237

238 **Key conclusions and recommendations**

239 1. The level of training, both in-house and external to the organization, is low;
240 65 % of responses replied that they operate in an environment with no in-house
241 training program. 74 % of responses were not required to be involved in ongoing
242 continuous professional education.

243 *Recommendation: Enhance training focused on QA and QC available as online and*
244 *face-to-face courses (for example, the Birmingham Metabolomics Training Centre*
245 *operates a 2-day course focused on QA and QC processes).*

246

247 2. 76 % of respondents applied SOPs. However, 70 % of respondents did not
248 have access to a protocol for review of quality and 80 % did not have access to
249 protocols focused on a review of quality processes.

250 *Recommendation: Appropriate agencies and the Metabolomics Society should*
251 *provide guidance on quality assurance processes and their review; develop*
252 *consensus processes through specialist meetings and reports.*

253

254 3. The majority of respondents validate sample measurements, apply sample
255 blanks, apply protocols to minimize sample carryover and randomize the
256 analysis order of biological samples.

257 *Recommendation: To provide education to the metabolomics community, with an*
258 *emphasis on early career scientists, on sample measurement validation, and to*
259 *provide continuing education to ensure these good practices continue.*

260

261 4. 83 % of respondents applied pooled project materials and 48 % applied
262 standard reference materials (SRMs) as QC materials. 59 % of respondents
263 applied replicate extractions and 69 % applied replicate analytical
264 measurements.

265 *Recommendation: To provide education to the metabolomics community, with an*
266 *emphasis on early career scientists, on usage of quality materials, and to provide*
267 *continuing education to ensure these good practices continue.*

268
269 5. 79 % of respondents did not access SRM materials.

270 *Recommendation: To communicate with the metabolomics community to define*
271 *the types and volumes of SRMs required.*

272
273 6. 33 % had participated in an inter-laboratory comparison study and 48% were
274 interested in participating in a future inter-laboratory comparison.

275 *Recommendation: To communicate with the metabolomics community to define*
276 *the types and frequency of inter-laboratory comparison exercises and encourage*
277 *independent agencies to support inter-laboratory exercises.*

278
279 7. 89 % of respondents were not required to meet laboratory accreditation and
280 74 % were not voluntarily attempting to meet any accreditation.

281 *Recommendation: To investigate the requirement for laboratory accreditation*
282 *with the regulatory agencies, funding bodies, the Metabolomics Society and the*
283 *metabolomics community.*

284
285 8. There is little incentive for laboratories to improve their QA/QC practices,
286 especially given the non-trivial costs associated with a thorough QA/QC
287 program.

288 *Recommendation: Recognizing the need to provide further incentive for*
289 *laboratories to improve overall QA/QC practices, expert panels should be convened*
290 *to develop workable, practical QA/QC recommendations and guidelines. One*
291 *possible mechanism is a workshop currently being planned for later in 2017 that*
292 *will define appropriate QA/QC frameworks that may be adopted widely in*
293 *laboratories and, possibly, by funders, data repositories and scientific publishers.*

294

295

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306 CFR 200.113).

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309 **Compliance with Ethical Standards**

1 310 The authors have defined that there are no potential conflicts of interest. All data
2 311 is anonymised and meets with appropriate ethical standards for this type of
3 312 community questionnaire.
4 313

5 314

6 315

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401 **Figure Captions**

1 402

2 403 **Figure 1.** A) Responses to “Do you have a protocol for independent review of
3 404 quality-related results?”; B) Responses to “Do you have a written protocol for QA
4 405 review criteria?”

5 406

6 407 **Figure 2.** Average response to “Do you validate your project sample
7 408 measurements with: (Check all that apply)?”

8 409

9 410 **Figure 3.** Average responses to “What types of QC materials do you routinely use
10 411 in analytical measurements for metabolomics projects? (Check all that apply)?”
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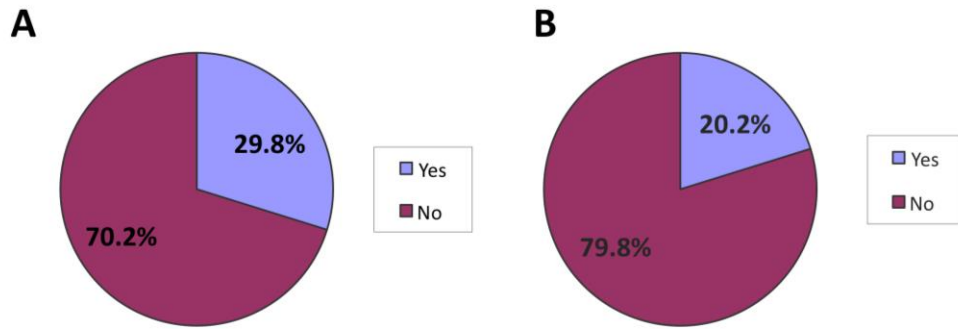
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413 **Figures**

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415 **Figure 1**

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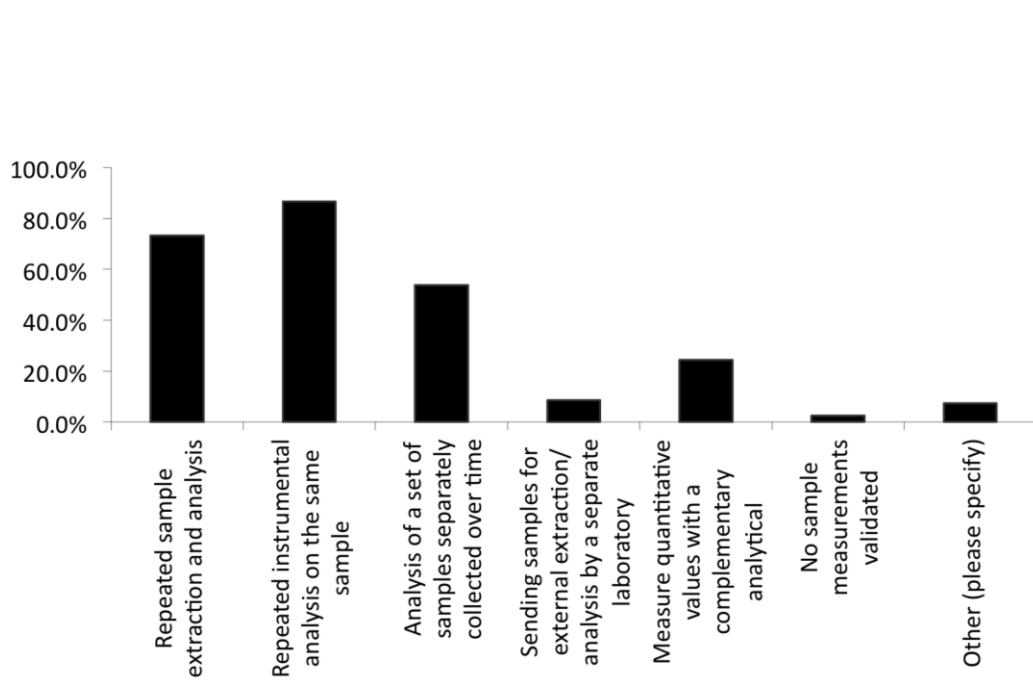


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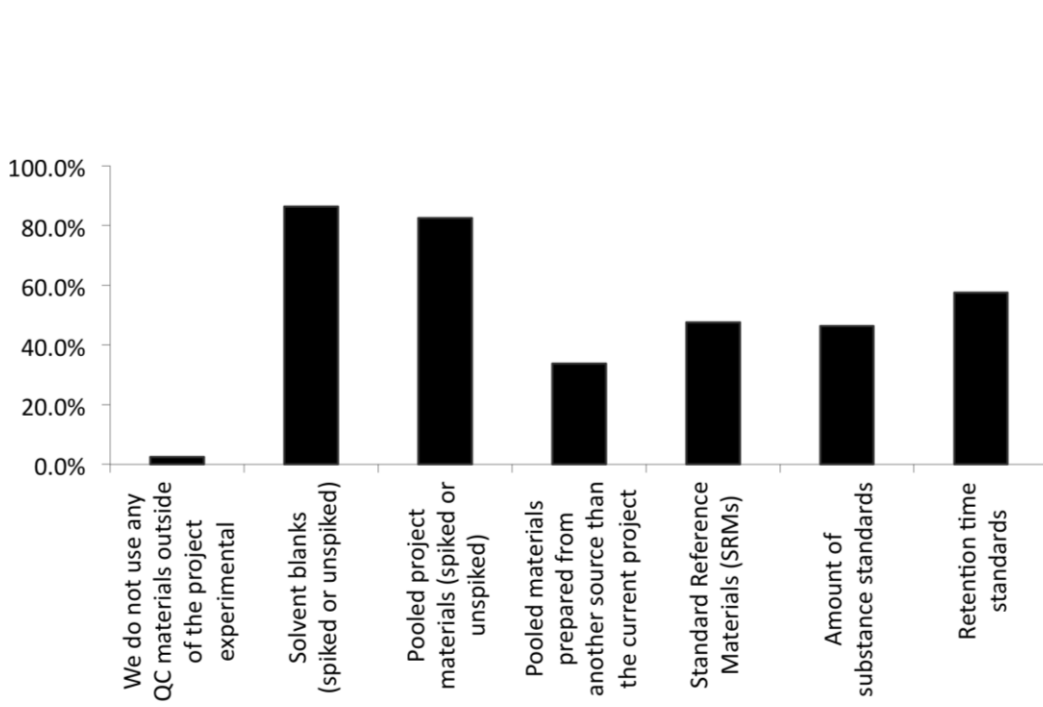
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420 **Figure 2**



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422 **Figure 3**



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