



Title	Authors' response
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1 Authors' response

2 We sincerely appreciate the interest in our
3 recent article published in *Gut*¹ and the
4 comments raised. The comments by
5 Heneghan *et al* raised some important ques-
6 tions concerning the emerging circulating
7 microRNA (miRNA) aspects of cancer diag-
8 nostics. These comments include: (1) the
9 choice of circulating medium; (2) the choice of
10 endogenous control; (3) premature for colo-
11 rectal cancer (CRC) screening; and (4) whether
12 elevated miRNAs in plasma reflect a general
13 cancer phenomenon, or a true CRC occurrence.

14 In response to comment 1, based on our
15 experience and commercial kit recommenda-
16 tion, total RNA <50 ng is recommended for
17 quantitative PCR (qPCR) of miRNA. A large
18 amount of RNA cannot improve qPCR
19 results and so is unnecessary. Although we
20 agree with the authors that total RNA
21 extracted from whole blood generates a
22 higher yield than that from plasma or serum
23 because a high percentage of RNA/miRNA is
24 derived from the cellular portion in whole
25 blood, one concern about using whole blood
26 for cancer diagnosis is whether the elevated
27 miRNAs identified are primarily derived from
28 the tumour itself or are simply a secondary
29 response of blood cells during tumouri-
30 genesis. If the elevated miRNAs are mainly
31 due to the response of blood cells, those
32 miRNAs may not reflect the patient's cancer
33 phenomenon and so lower the testing accu-
34 racy. Heneghan *et al* recently showed that
35 miR-195 and let-7a are elevated in blood
36 from patients with breast cancer. However,
37 a previous study by the same group of authors
38 demonstrated that let-7a is suitable as an
39 endogenous control for qPCR in breast cancer.²
40 So, this raises the issue that let-7a elevation
41 in blood is probably due to a secondary
phenomenon such as inflammation from
blood cells. Accordingly, using whole blood for
this diagnostic purpose is questionable.

In response to comment 2, ideally an
absolute quantitation approach with stan-
dard curve calibration is recommended to be
used for qPCR in the field of diagnostics.
For relative quantitation, there is still no
consensus on the use of an internal normal-
isation control in plasma. Downregulation of
miR-16 has been reported in several cancers
including leukaemia, pituitary adenomas,
prostate carcinoma and lung cancer.^{3–5} In
our laboratory, we also found that miR-16 in
plasma was aberrantly expressed in patients
with breast cancer (unpublished data). Thus,
the use of miR-16 as an internal normal-
isation control in whole blood is still ques-
tionable. Furthermore, it was surprising
that the same group of authors previously
recommended let-7a as one reliable endoge-
nous control in breast cancer.² Accordingly,
let-7a is not likely to be breast cancer specific
and so it raises the issue as to whether let-7a
should be used as an endogenous control or
diagnostic marker for breast cancer. Thus, an
internal normalisation control is still a crit-
ical issue for debate. From our point of view,
we should eventually switch to an absolute
quantitation approach to eliminate the use
of an endogenous control.

With regard to comment 3, we agree with
the authors that it is premature to apply
plasma miR-92 for CRC screening. Larger
scale validations are underway, as mentioned
in the Discussion section of our original
paper.

In response to comment 4, in our paper
we showed that elevation of plasma miR-92
and miR-17-3p levels is likely to be derived
from CRC. First, miR-92 and miR-17-3p had
been selected for further marker validation
because of their elevated levels in both plasma
and corresponding tumour of patients with
CRC. Secondly, their plasma levels were
significantly reduced after surgical removal
of the tumours. Thirdly, elevation of these
miRNAs in plasma due to inflammation,

such as inflammatory bowel disease, has been
ruled out. Finally, our recent data showed that
plasma levels did not increase in other cancer
types including breast and gastric cancer.
Collectively, miR-92 and miR-17-3p are very
likely to be CRC specific.

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