October 2022

Important Note #2

therascreen® FGFR RGQ RT-PCR Kit (24), REF 874721; Confirmatory retest of positive results might be required

Dear *therascreen* FGFR RGQ RT-PCR Kit customer,

QIAGEN has identified an increased occurrence rate of false positive results obtained with the *therascreen* FGFR RGQ RT-PCR Kit. For a proportion of samples with the Overall sample result "FGFR Alteration Detected", the result could be a false positive result.

For the continued use of REF 874721 *therascreen* FGFR RGQ RT-PCR Kit with LOT number 172033373 or higher, use the following criteria for the interpretation of results:

- Review this notice with your laboratory/medical director.
- Forward this information to all individuals and departments within your organization who are using REF 874721 therascreen FGFR RGQ RT-PCR Kit.
- In addition to the "Analysis Report", use the following criteria for the interpretation of results for "FGFR Alteration Detected" results:
    - All "FGFR Alteration Detected" results aside from "S249C Mutation Detected" (*i.e.*, detected targets other than p.S249C (c746C>G on Exon 7) should be disregarded and a retest for confirmatory positive result should be performed using the extracted RNA if available, or re-extracted if it is not. An overview of the affected results is provided in Table 1.

Table 1. Overview of affected results

Overall sample result	Individual target result	Affected by the issue	Action to be taken by customer
No Alteration Detected	n/a	no	n/a (correct result)
FGFR Alteration Detected	S249C Mutation Detected	no	n/a (correct result)
FGFR Alteration Detected	S249C Mutation Detected and additional individual target results	no	n/a (correct result)
FGFR Alteration Detected	One or more individual target results that don't include S249C	yes	Disregard result for this sample. Retest for confirmatory positive result

- The following results should be assigned to the affected samples after the retest:
  - **Retest result is positive for the same target(s)/ alteration(s)**: Regard the result for the corresponding sample as positive.
  - **Retest result is positive for different target(s)/ alteration(s) in the retest compared to the initial result**: Regard the result as indeterminate.
  - **Retest is negative**: The result for the corresponding sample should be regarded as negative
  - **Retested sample is within an invalid run**: Repeat the run.
  - **Result for individual sample is invalid in the retest**: Regard the result as indeterminate.
  - An overview of the interpretation of retest results is provided in Table 2.

Table 2. Overview of interpretation of retest results

Retest result	Individual target result in retest	Result to be assigned after retest
FGFR Alteration Detected	Same target(s)/ alteration(s) as within initial test	FGFR Alteration Detected, "positive result"
FGFR Alteration Detected	Different target(s)/ alteration(s) as within initial test	Indeterminate result
No Alteration Detected	n/a	No Alteration Detected
Invalid run	n/a	n/a, repeat the run
Invalid individual sample result	n/a	Indeterminate result

- Samples with the following results are not affected and thus no further actions required for such samples:
  - Overall sample result “No Alteration Detected”
  - Overall sample result “FGFR Alteration Detected” results with Individual target result S249C Mutation Detected. This includes samples with additional alterations detected.
- An overview of the affected results is provided in Table 1.

Detailed description of the issue

In the affected runs, the Run Controls pass correctly (leading to a valid result for the sample), while an artefact in one or more of the mutation assays might cause an incorrect valid mutation positive result for individual samples. The software for the interpretation of all mutations claimed in the assay does not distinguish such artefacts from a real amplification obtained with a valid mutation positive sample.

The investigation at QIAGEN demonstrated that all targets/Amino acid variants/Fusion IDs are affected, except for Amino acid variant p.S249C (c.746C>G on Exon 7).

As an interim solution, the newly manufactured batches with LOT number 172033373 and higher will undergo additional testing (until the issue is resolved or the investigation is completed) to ensure a minimum specificity of 95%. If a positive sample result is obtained, a retest is required as described in this Important Note in order to mitigate the risk of a false positive result. Upon retest, the minimum specificity increases to 99%.

Potential risks associated with this issue

The issue can potentially lead to a false positive sample result that could subsequently be incorrectly reported from the laboratory. A false positive result could lead to the risk of the patient being exposed to an inappropriate or suboptimal anti-cancer treatment and/or unnecessarily experiencing side effects.

Actions taken by QIAGEN

QIAGEN is working on identifying and correcting the root cause of false positivity. Any update on mitigation measures will be promptly communicated. In the interim, we are providing kits with this Important Note.

If you have any questions or concerns, please contact your local QIAGEN Technical Service Department through any of the following:

Telephone: 800 362 7737
Email: **TechService-NA@qiagen.com**

We sincerely apologize for any inconvenience this may cause and thank you in advance for your cooperation.

With kind regards,

QIAGEN
www.qiagen.com