

Final report on Ring Trial Interphase FISH Tumour 2018

Summary: Ring trial for quality assurance in interphase FISH diagnostics in tumour cytogenetics 2018 (QUITZ)

Referral reason: Patient: Anna Auerstübchen; suspected AML M3, FISH diagnostics to clarify the presence or absence of a PML-RARA rearrangement t(15;17)(q24;q21)

Patient: Pia Promyelozyt; suspected AML M3, FISH diagnostics to clarify the presence or absence of a PML-RARA rearrangement t(15;17)(q24;q21)

Results:

- Patient: Anna Auerstübchen (AA); no evidence of a PML-RARA rearrangement
- Patient: Pia Promyelozyt (PP); evidence of a PML-RARA rearrangement

Analysis criteria:

Correctness of the results:

- Patient: Anna Auerstübchen (AA); no evidence of a PML-RARA rearrangement
- Patient: Pia Promyelozyt (PP); evidence of a PML-RARA rearrangement

All laboratories correctly reported that AA did not have a PML-RARA rearrangement and that PP did have a PML-RARA rearrangement.

Only one laboratory was outside the defined range of 85%–100% in the PML-RARA-positive case.

Another laboratory reported an atypical signal constellation in PML-RARA, with only one PML-RARA fusion signal when using a Dual Color, Dual Fusion probe.

Committee for Quality Assurance

Elected members:
Prof. Dr. rer. nat. Jürgen Kunz (chairman)
Dipl.-Biol. Susanne Anders
PD Dr. rer. nat. Barbara Fritz
PD Dr. rer. nat./med. habil. Thomas Liehr
Dr. rer. nat. Anja Weise

Ring Trial Managers:
Dr. rer. nat. Sebastian Eck
Dr. rer. nat. Eveline Fiedler
Prof. Dr. med. Claudia Haferlach
Sarah Matos Meder, M. Sc.
Prof. Dr. med. Harald Rieder

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And one laboratory used a RARA break-apart probe.

Interpretation in relation to the referral reason:

The interpretation should contain:

Anna Auerstäbchen

- No indication of the existence of APL with PML-RARA rearrangement, or no evidence of the existence of a PML-RARA rearrangement
- Recommendation of further investigations:
 - Chromosome analysis (optional)
 - PML-RARA-specific RT-PCR, because submicroscopic insertions could be present, which cannot be detected using FISH (optional)
 - Molecular genetic analysis, e.g. mutations in the genes NPM1, CEBPA, RUNX1, FLT3, TP53 and ASXL1 (optional)

Pia Promyelozyt:

- Evidence of a PML-RARA rearrangement: APL / M3 or M3v is thus present
- Recommendation of molecular genetic analysis for the course of a quantitative PML-RARA-specific PCR. Before the start of therapy with planned monitoring, a determination of the fusion transcript type using PCR (optional)
- APL with PML-RARA rearrangement have a very good prognosis, provided they are treated in compliance with APL-specific therapy protocols, which typically include ATRA and/or arsenic trioxide (optional)

Error on the part of the ring trial organisers: Discrepancy in the dates of birth provided for the two patients between the sample tubes and the delivery notes. Both dates of birth were accepted, and were not included in the evaluation. All participants were informed of the error via email by Ms Brandt (coordination office).

As already announced last year, a new evaluation category has been introduced regarding the consistency of the test result. Point 4. Consistency of report.

Because last year there were discrepancies within the test result, e.g. between the ISCN formula and the text: e.g. in the text the percentages for biallelic and monoallelic deletions had been mixed up, but were correctly specified in the ISCN formula, or the results in the evaluation table were correct, but were then mixed up in the ISCN and in the expert report.

Provided it is apparent who is responsible for the test result, point 10 was marked as correct, even if signatures were absent.

The description of the DNA probes (point 11) was marked as correct, provided that related specifications were present in the test result, e.g. including in the ISCN formula.

The specifications according to ISCN (point 11) needed to be fully correct, and were assessed against ISCN 2016:

ISCN 2016 (page 108):

Like last year, in some cases cells were specified at the cut-off in the ISCN formula

→ nuc ish 15q24(PMLx2),17q21(RARAx2)[97/100]

This was marked as incorrect in the evaluation. According to ISCN 2016, page 108, it is only permitted for abnormal cells to be listed “when normal and abnormal cells are found, the number of abnormal cells is listed over the total number of cells scored for each abnormal locus...”

The long written version is permitted -> ISCN 2016 (page 106) "ISCN(1995) provided for the use of a band designation in interphase FISH. This is considered an optional detailed form to be used at the discretion of the investigator or laboratory director."

In some cases, a comma was incorrectly placed between the 2nd and 3rd brackets:

nuc ish 15q24(PMLx3),17q21(RARAx3).(PML con RARAx2)[186/200] -> points deducted

Comments from the assessment committee

The duration of the processing time between the receipt of samples and the date of the test result was in some cases very long. In cases of suspected AML M3, the investigation for a PML-RARA rearrangement should be performed very promptly (within 24 hours).

Unfortunately, neither the WHO classification 2016 nor the Onkopedia Guidelines 2018 always specify the correct cytogenetic localisation of the breakpoints for the PML-RARA translocation: In acute promyelocytic leukaemia (APL) with PML-RARA, a t(15;17)(q24.1;q21.2) translocation is present. The providers of the FISH probes are now specifying the correct breakpoints.

Where a PML-RARA rearrangement is present, prognostic specifications without reference to therapy protocols could be misunderstood. The assessors therefore recommend that in cases of a confirmed PML-RARA rearrangement, specifications regarding the prognosis should be described by reference to the associated therapeutic approach (with reference to corresponding studies where appropriate).

Formulations such as "APL can be excluded" should not be used, because e.g. submicroscopic insertions causing a PML-RARA rearrangement on a molecular level (known as 'cryptic rearrangements') cannot reliably be excluded by means of a PML-RARA translocation probe. More suitable formulations include, "No indication of the existence of a PML-RARA rearrangement was found. If the existence of APL continues to be suspected, a supplementary PML-RARA-specific RT-PCR should be performed."

Only recommendations relating to the referral reason or the FISH results should be made. Pre-written text blocks should be adapted depending on the test result.

Best regards,



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Ring Trial Supervisor



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Prof. Dr. Jürgen Kunz
Chairman of Committee QA