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Common causes of genotyping error; Trends over time

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Introduction

EMQN believes that every patient should have the most accurate genomic test results possible, providing external quality assessments (EQAs) to laboratories across the globe. Despite our aspirations, errors still occur in diagnostic testing, with the most severe genotyping errors having the potential to cause harm and negatively impact a patient's quality of life.

EMQN asks each laboratory that experiences poor performance during an EQA to complete an optional root cause analysis (RCA) form to identify the source of these errors, and whether they have the potential to have impacted patients. Although the data provided is optional, it can provide an insight into the cause of errors in diagnostic settings.

Using this data, we identified trends for the most prevalent causes of genotyping error in the hopes that a deeper understanding will lead to improved patient care in the future.

Aims

- To determine the most common underlying root causes of critical genotyping errors found through the external quality assessment process between 2019 and 2023, for both germline and molecular pathology schemes.
- To identify trends in the root causes for critical genotyping errors over time.

Methodology

A total of 359 error reporting forms were completed over a five-year period. The annual distribution of forms is shown in the table below. Those with genotyping errors due to result non-submission were excluded from the analysis.

Year	Number of forms included in analysis	Number of forms excluded (non-submission of results)
2019	51	1
2020	67	4
2021	54	1
2022	92	7
2023	94	4
TOTAL	359	17

For each year, forms were systematically reviewed to identify the primary root cause of each error. Categories were converted into percentages to enable direct comparison across years.

To evaluate trends over time:

- A line of best fit was plotted for each root cause.
- A Cochrane's Q test was performed to determine significance of error type over the five-year period.

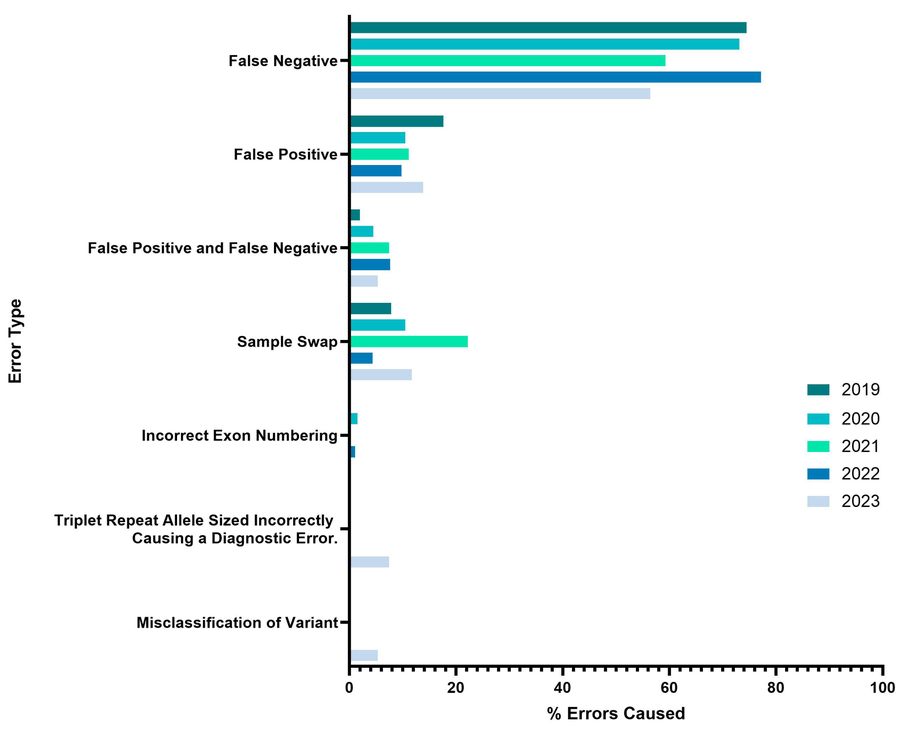
Additionally, errors were categorised into five types:

- False positive
- False negative
- False positive & false negative
- Incorrect exon numbering
- Sample swap

Trends in root causes within each error type were tracked over time. Cochrane's Q tests were applied to assess statistical significance of temporal changes.

Results

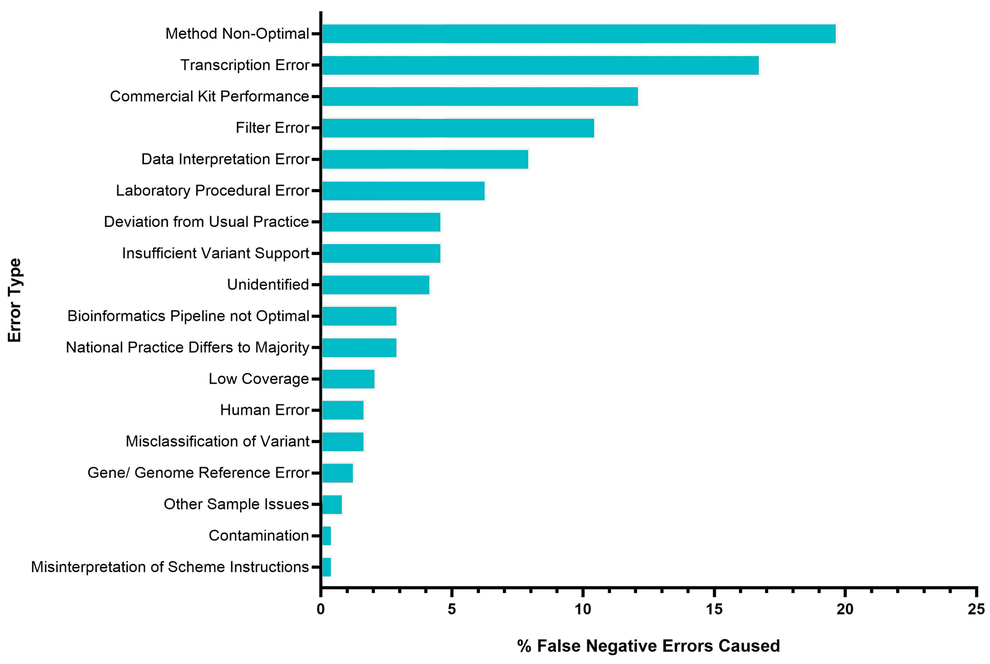
Figure 1. Changes in Proportion of Genotyping ErrorType from 2019-2023



False negatives were the most common error type, accounting for 71.3% of all reported genotyping errors *(Figure 1)*. These showed a significant downward trend in frequency over the five-year period.

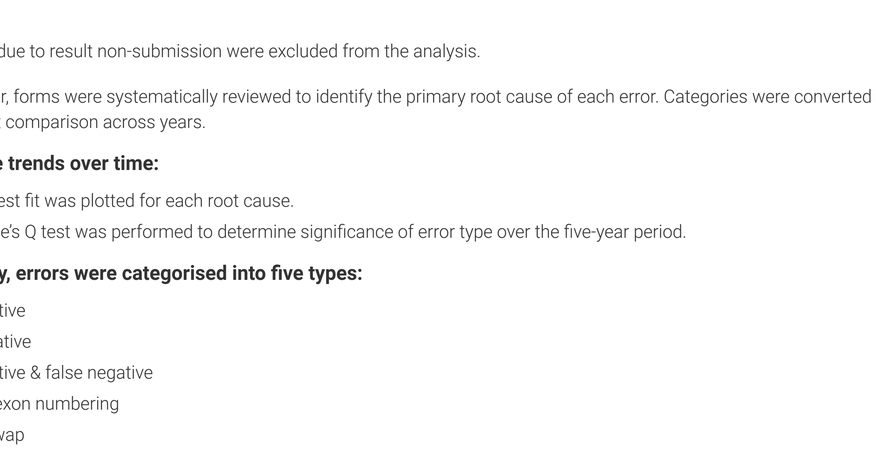
Sample swaps and false positives were the next most frequent, contributing 10.3% and 8.8% of total errors, respectively.

Figure 2. Root Cause of Falso Negative Errors



- The most frequent root causes of false negatives were:
- Method Non-Optimal: defined as deviations from standard procedures, limitations of test not specified, or faulty method design.
- Transcription error: Typographical and copy/paste mistakes *(Figure 2)*.

Figure 3. Root Cause of False Positive Errors

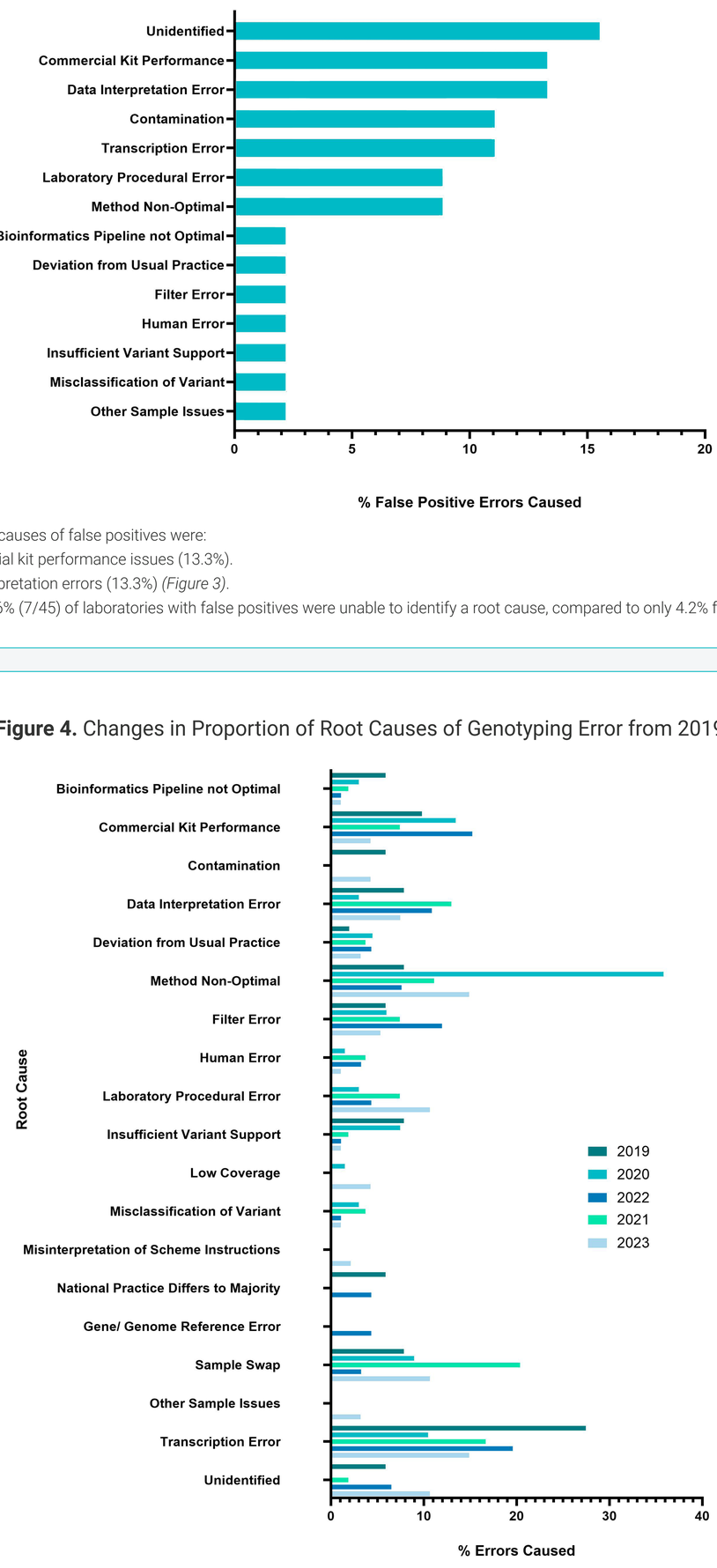


The leading causes of false positives were:

- Commercial kit performance issues (13.3%).
- Data interpretation errors (13.3%) *(Figure 3)*.

Notably, 15.6% (7/45) of laboratories with false positives were unable to identify a root cause, compared to only 4.2% for false negatives.

Figure 4. Changes in Proportion of Root Causes of Genotyping Error from 2019-2023



Across all error types, transcription errors emerged as a persistent contributor:

- Responsible for at least 10% of genotyping errors each year.
- The most prevalent root cause in 2019, 2022, and 2023.
- Method nonoptimal was the leading cause in 2020, and sample swaps in 2021 *(Figure 4)*.

Discussion

Overall, the number of RCA forms submitted increased over the five-year period, though it is important to note that RCA submissions are optional, with responses containing varying levels of detail and clarity. Additionally, some laboratories that received genotyping errors did not submit an RCA, introduce a degree of bias into the analysis.

False negatives were the most common error type, the majority of which were from lack of follow up or referral for further testing, leaving certain mandatory testing areas untested, or an inaccurate description of their methods and limitations.

The root cause was deemed 'Unidentified' in many cases where laboratories listed multiple potential causes (up to 12 root causes for a singular genotyping error in some cases) without identifying a primary contributor, or where they stated that no definitive cause could be determined. This is concerning, as it limits the ability to address and reduce false positive results effectively.

Several RCAs, particularly from 2020 to 2022, cited the COVID-19 pandemic as a contributing factor, highlighting issues such as staff shortages and prioritisation of COVID-related testing over routine genotyping.

Over the five years, NGS-related errors, such as a 'Bioinformatics pipeline not optimal' and 'insufficient variant support' decreased, likely due to improved bioinformatics pipelines. Filter errors remained largely unchanged, with a peak in 2022, and of the germline schemes, were mainly found in Hereditary Breast and Ovarian Cancer, and Hereditary Hearing Loss.

Conclusions

The most frequent poor performances were False Negatives – accounting for 71.3% of all errors – followed by False Positives and Sample Swaps at 8.8% and 10.3% respectively. Laboratories more frequently identified causes of False Negatives than Positives, with nonoptimal methods and transcription errors accounting for over 15% of False Negatives each.

Laboratories aiming to decrease likelihood of genotyping errors should participate in EQA to ensure procedures are optimal, maintain practices such as witness checks, and ensure filtering parameters are optimal before use. EMQN urges participants to conduct a RCA when a critical error is identified during EQA to improve accuracy for future, real-life patient cases.