

P2.121: Comparative Evaluation of PAXgene® and RNAlater® Fixation for DNA Sequencing in Lung Biopsies



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INTRODUCTION

Clinical relevance: Molecular profiling is essential for treatment selection in lung cancer, but bronchoscopic biopsies provide limited tissue that must support both histopathological diagnosis and molecular testing.

FFPE Limitations: Formalin-fixed paraffin-embedded (FFPE) tissue remains the clinical standard, but formalin-induced crosslinking and DNA fragmentation can compromise downstream molecular analysis.

Alternative fixatives: PAXgene® and RNAlater® may better preserve nucleic acids, although evidence for targeted DNA sequencing in small bronchoscopic lung biopsies remains limited.

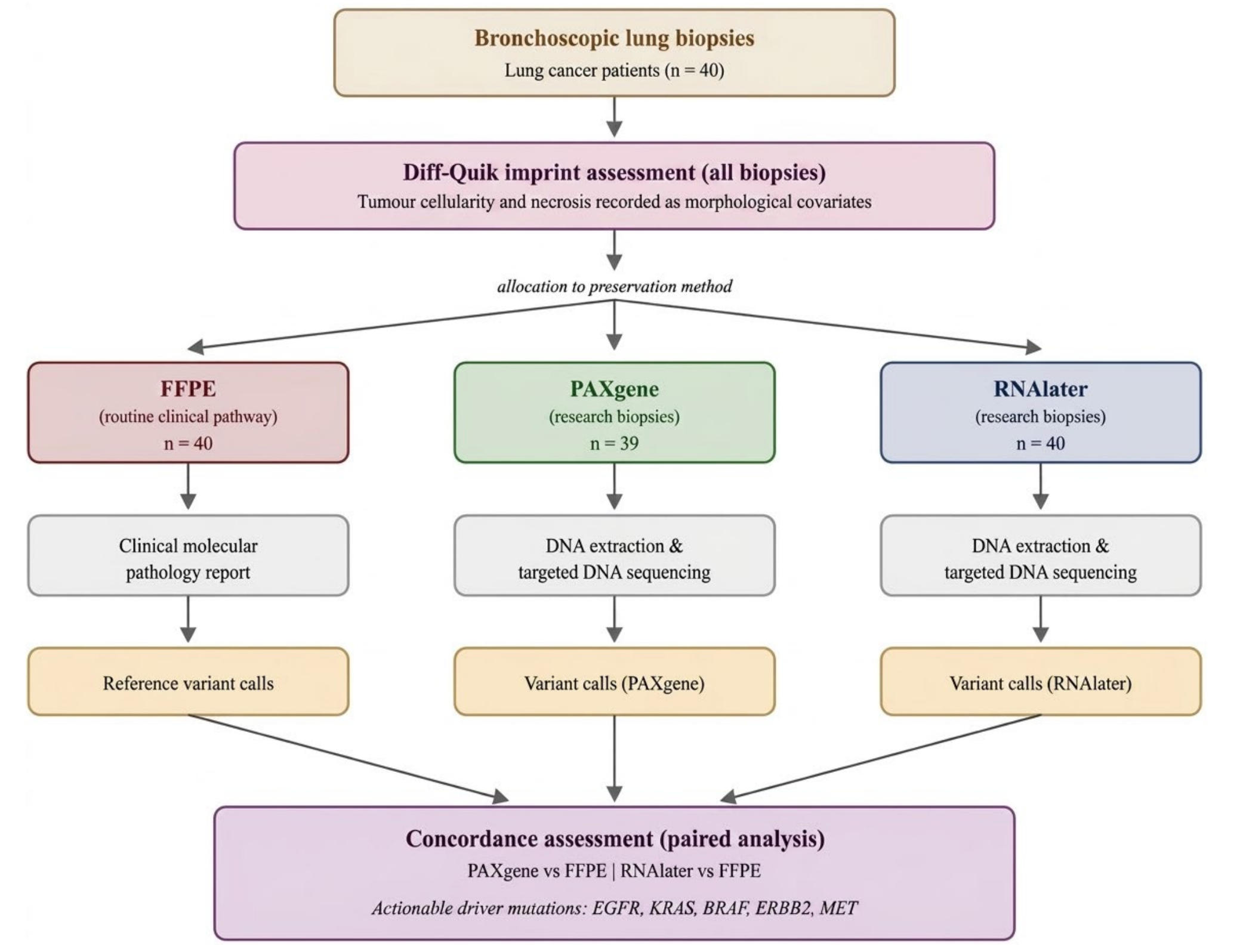
Study aim: This study evaluated PAXgene- and RNAlater-preserved bronchoscopic biopsies against participant-matched clinical FFPE biopsies and investigated whether biopsy-level touch imprint cytology could predict sequencing success.

METHODS

Study design: Bronchoscopic lung biopsies from 40 participants were preserved as single research biopsies in PAXgene® (n=39) or RNAlater® (n=40) and benchmarked against participant-matched clinical FFPE reference samples, routinely derived from pooled multiple biopsies.

Molecular analysis: Biopsies underwent touch imprint cytology followed by targeted DNA sequencing using the accredited AmpliSeq panel targeting EGFR, KRAS, BRAF, ERBB2 and MET.

Analysis: Variant concordance was assessed independently against FFPE, and associations between imprint cytology and sequencing outcomes were analysed using Fisher's exact test.



DISCUSSION AND CONCLUSION

Fixation performance: PAXgene® and RNAlater® were feasible non-crosslinking approaches for targeted DNA sequencing of single bronchoscopic lung biopsies, with PAXgene showing marginally higher diagnostic performance.

Discordance: Most discordant outcomes likely reflected limited tumour representation and inter-biopsy heterogeneity rather than fixation-related analytical error. Biopsy adequacy: Malignant touch-imprint cytology was significantly associated with successful variant detection across both preservation methods.

Actionable Take Away Message
PAXgene® and RNAlater® provide feasible non-crosslinking alternatives for molecular testing of small bronchoscopic lung biopsies, with specimen adequacy remaining a key determinant of sequencing success.

Future Direction for Research

- Validate this approach prospectively in larger, histologically characterised cohorts and establish reproducible biopsy-adequacy criteria.
- Determine whether imprint-guided biopsy selection can reduce sequencing failure, repeat biopsy procedures and delays to molecularly guided treatment.

REFERENCES & ACKNOWLEDGEMENTS

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Artificial Intelligence (AI) Use Disclaimer

Tool	Use	Prompt(s)
Claude	Data visualisation assistance	Generate a Sankey diagram illustrating relationships between imprint cytological parameters and variant detection outcomes

RESULTS

1. Study Cohort: Forty participants with histopathologically confirmed lung cancer were included: 23 mutation-positive (MT) and 17 wild-type (WT). MT: ≥1 reportable pathogenic variant in EGFR, KRAS, BRAF, ERBB2 or MET; WT: no reportable pathogenic variant across these five genes.

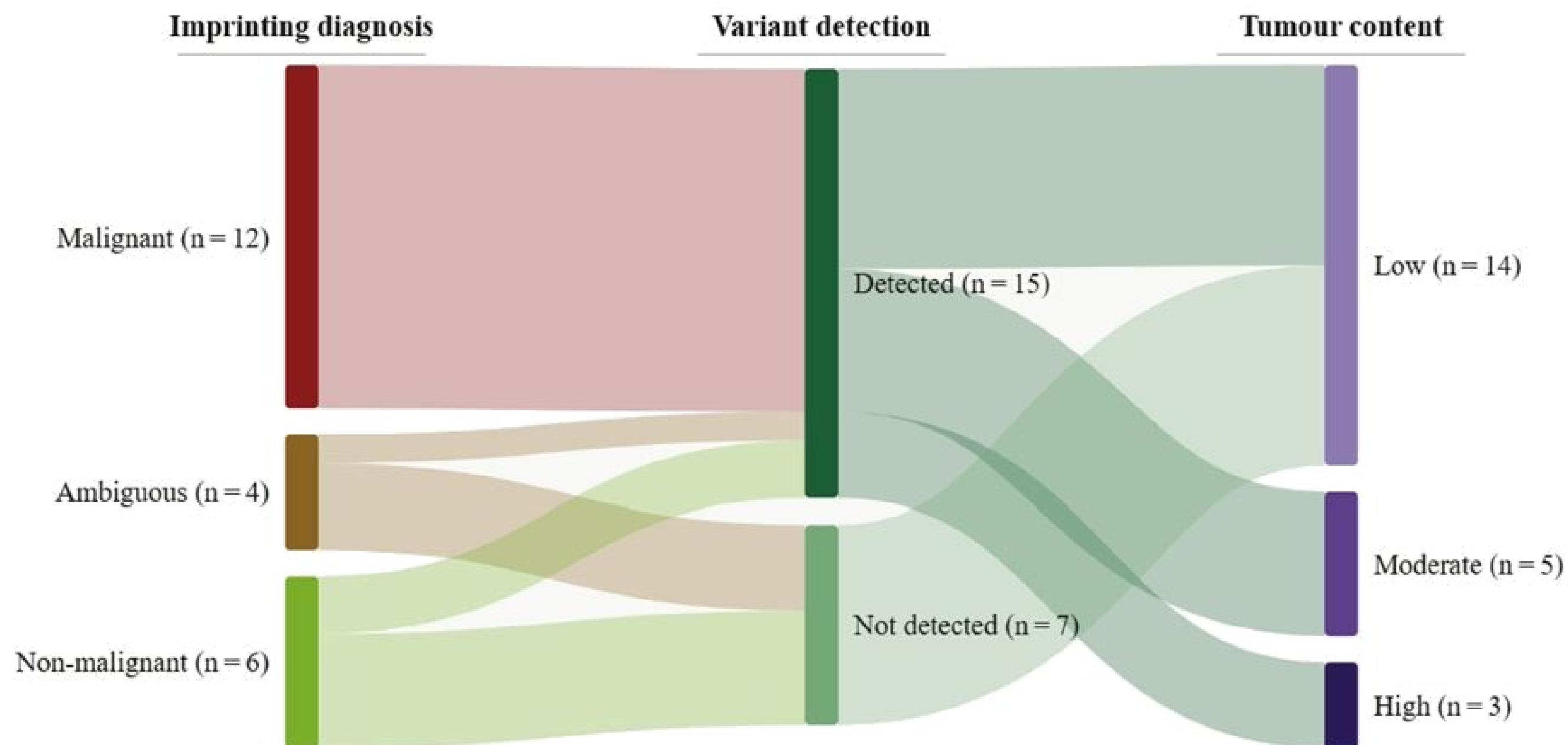
2. Variant Concordance with Clinical FFPE
Finding: PAXgene® and RNAlater® showed comparable agreement with clinical FFPE. PAXgene® achieved slightly higher positive percent agreement (PPA), negative percent agreement (NPA), and overall concordance than RNAlater®. No pathogenic variant miscalls occurred in WT cases. DNA yield: Median 133.6ng (PAXgene®) vs 201.4ng (RNAlater®), p=0.0181; matched FFPE yield unavailable.

Table 1: Concordance of PAXgene®- and RNAlater®-fixed biopsies against clinical FFPE reference testing.

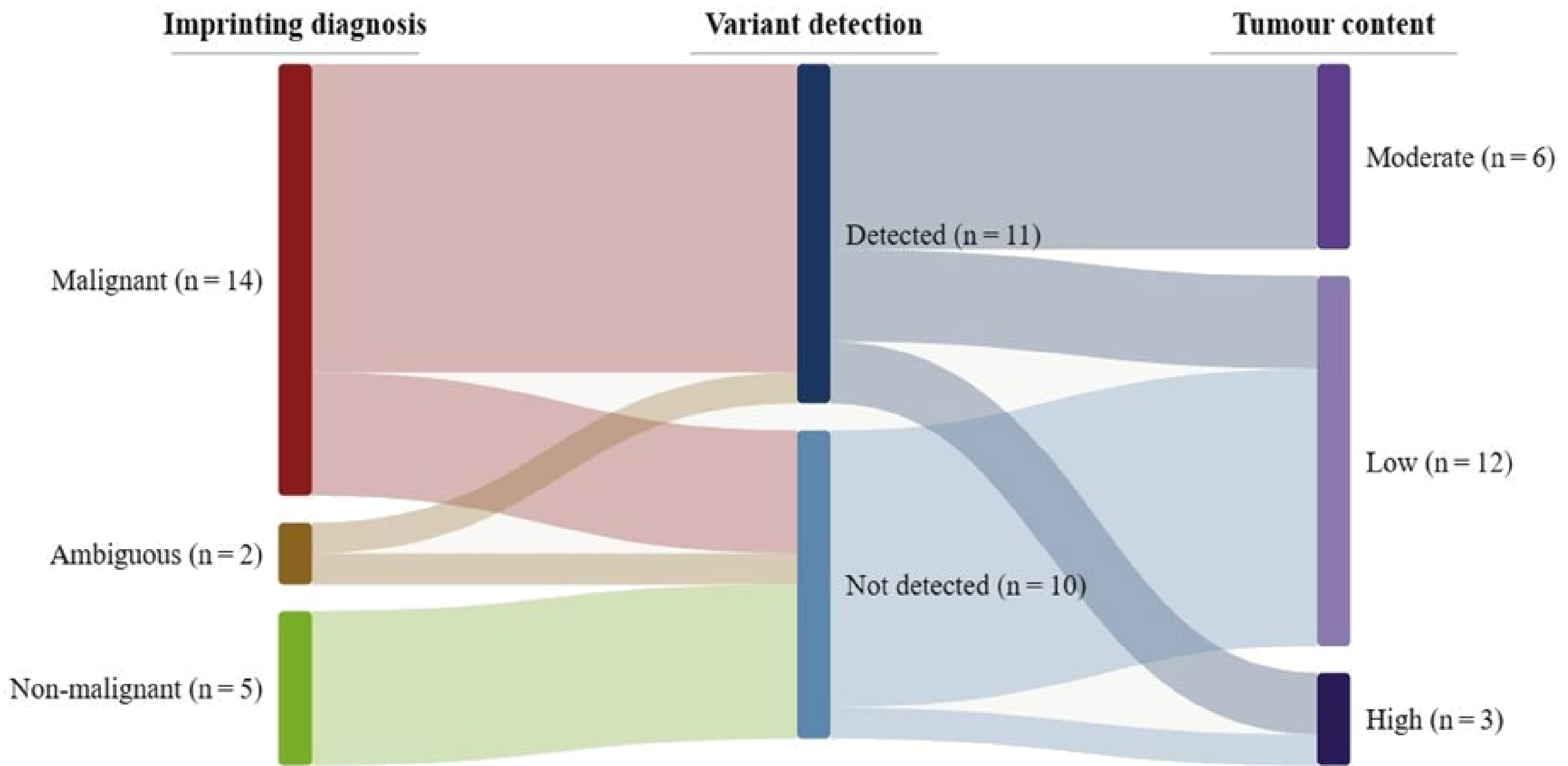
Clinical FFPE Reference	PAXgene® Positive	PAXgene® Negative/Fail	RNAlater® Positive	RNAlater® Negative/Fail
Reference Positive	12 TP	10 FN	11 TP	10 FN
Reference Negative	3 FP*	10 TN	4 FP*	10 TN
Positive Percent Agreement (PPA)	54.5% (12/22)		52.4% (11/21)	
Negative Percent Agreement (NPA)	76.9% (10/13)		71.4% (10/14)	
Overall Concordance	62.9% (22/35)		60.0% (21/35)	

*FP outcomes represent sequencing failures in clinically WT cases; not pathogenic variant miscalls.

(A) PAXgene®-fixed biopsies:



(B) RNAlater®-fixed biopsies:



3. Imprint Cytology and Sequencing Success

Finding: Variant detection was significantly higher in biopsies with malignant versus non-malignant/ambiguous imprints for both PAXgene® (100% vs 30%; p=0.002) and RNAlater® (71.4% vs 14.3%; p=0.023).