CANADIAN BIOMARKER QUALITY ASSURANCE
PROGRAMME CANADIEN D'ASSURANCE
QUALITÉ DES BIOMARQUEURS

CBQA Prostate Cancer – Somatic Tissue FFPE *BRCA* Gene Testing (2025)
Scheme Report
January 9, 2026

Background

Genetic testing in prostate cancer is now standard of care in Canada, in order to provide important information for clinical management of patients and to help assess familial cancer risk. Patients with metastatic castration-resistant prostate cancer (mCRPC) who have progressed following prior treatment, and whose tumors harbour *BRCA1* or *BRCA2* deleterious or suspected deleterious somatic or germline variants, are eligible for treatment with PARP inhibitor therapy in Canada[1]. As a result, many Canadian clinical laboratories are validating or performing routine clinical NGS testing of FFPE tumor tissue for somatic variants in *BRCA1* and *BRCA2* genes. Canadian guidelines for genetic testing in prostate cancer have also been published with evidence-based recommendations for somatic and germline testing[2].

The specific aims of the CBQA Prostate Cancer – Somatic Tissue FFPE *BRCA* Gene Testing scheme were:

1. To ensure appropriate performance of NGS testing for *BRCA1* and *BRCA2* deleterious or suspected deleterious variants on DNA extracted from FFPE tissue from mCRPC tumors;
2. To provide a source of tissue with known *BRCA* variants for test improvement or validation exercises in Canadian laboratories.

In addition, the overall aim of all CBQA schemes are to educate Canadian labs on current practices, and to facilitate the raising of standards.

Participating Laboratories

Enrollment was open to any Canadian clinical laboratory performing *BRCA1* and *BRCA2* testing by NGS on FFPE tissue, for using in decisions regarding PARP inhibitor therapy for prostate cancer. A total of 11 laboratories enrolled, with 6 laboratories from Ontario and the rest from other provinces (New Brunswick, Nova Scotia, Quebec, Manitoba, Saskatchewan).

Samples and Reporting

- 10 cases were sent to participating laboratories
- For each of the 10 cases, labs received 3 FFPE sections of 7 microns each, which were prepared on uncoated slides and air dried
- An H&E image with the tumor region circled was provided as an aid to macrodissection
- All samples had been pre-tested for *BRCA1* and *BRCA2* on two NGS platforms - Illumina NGS (hybridization capture library) and ThermoFisher NGS (amplicon library)
- Results were requested as anonymized clinical reports for Cases 1-3, and as genotyping information only for Cases 4-10 without clinical reports
- Clinical vignettes were provided for Cases 1-3 for use in drafting clinical reports

- Tissue cellularity estimates were provided for all samples

Expected Results

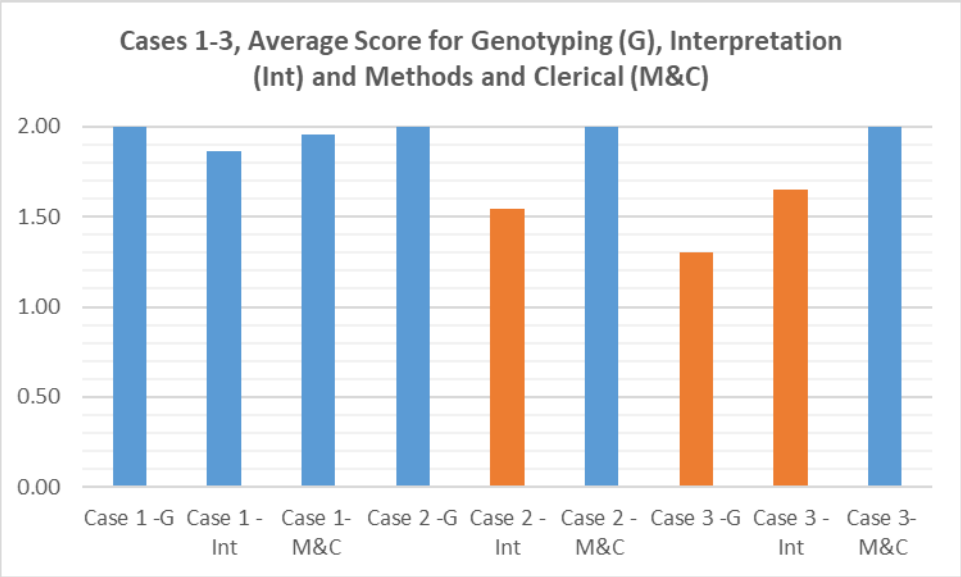
Expected genotype results for each of the 10 cases are shown in the table below. Lab reports for each of Cases 1-3 were evaluated for accuracy of genotyping (2 marks), interpretation of results (2 marks), and method details and clerical accuracy (2 marks). For Cases 4-10, where labs submitted only genotype results without clinical reports, only the accuracy of the genotype was evaluated (2 marks each case).

Case Identifier	Expected Genotype - *BRCA1* and *BRCA2* Genes
Case 1	*BRCA2* (NM_000059.3):c.2957_2958insG p.(Asn986Lysfs*2) *BRCA2* (NM_000059.3):c.9382C>T p.(Arg3128*)
Case 2	No variants in *BRCA1* or *BRCA2*
Case 3	*BRCA2* (NM_000059.3): full gene deletion *BRCA1* (NM_007294.3):c.60A>C p.(Lys20Asn)
Case 4	*BRCA2* (NM_000059.3):c.9380G>A p.(Trp3127*)
Case 5	*BRCA2* (NM_000059.3):c.7617+2T>G p.(?)
Case 6	No variants in *BRCA1* or *BRCA2*
Case 7	*BRCA2* (NM_000059.3):c.2269_2270insG p.(Lys757Argfs*6)
Case 8	No variants in *BRCA1* or *BRCA2*
Case 9	*BRCA2* (NM_000059.3): full gene deletion
Case 10	*BRCA1* (NM_007294.3):c.3436_3439delTGTT p.(Cys1146Leufs*8)

Scheme Results

Of the 11 enrolled laboratories, all submitted clinical reports for Cases 1 and 2. Ten labs submitted a clinical report for Case 3, while testing failed in one lab for Case 3. 10 labs submitted results for the genotyping-only Cases 4-10 (one lab did not test Cases 4-10 by choice). Overall test success rate was very high, with only 1 sample failing in one lab (Case 3).

Results from all labs with the average scores for each component of Cases 1-3 are shown in the graph below.



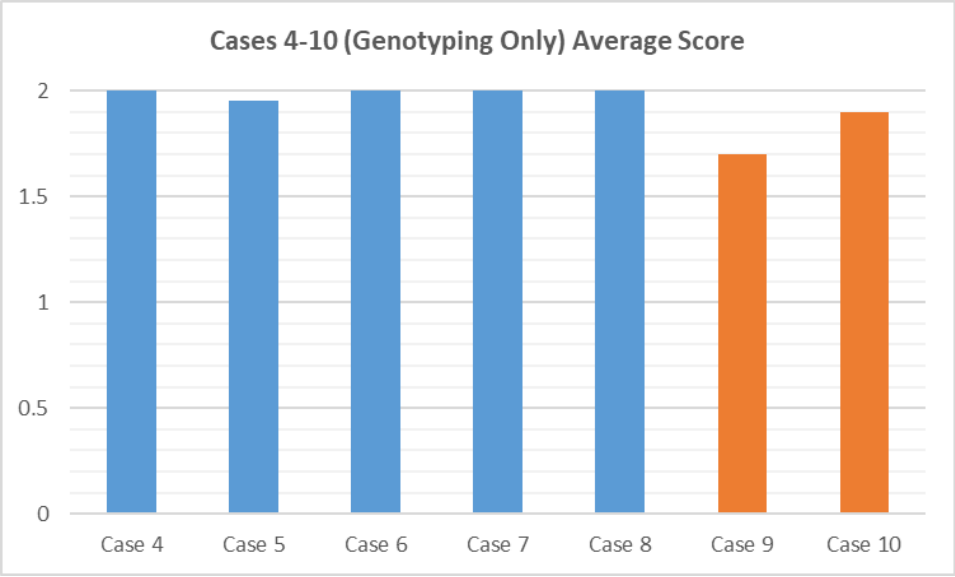
The most significant Genotyping finding was in Case 3, which contained a *BRCA2* full gene deletion and a *BRCA1* missense Tier III variant (variant of uncertain significance). The method limitations as stated on the clinical reports submitted by each lab were taken into account during scheme assessment to determine if results were correct within assay limitations, when one or both of these variants were not reported, as follows:

- Relevant to the *BRCA2* full gene deletion copy number variant (CNV), two labs indicated on the report they did not report CNVs, and two labs only report CNVs when the cellularity is greater than 80% (tissue cellularity provided in the clinical vignette for Case 3 was >50%)
- Relevant to the *BRCA1* missense Tier III variant, two labs noted that they only report Tier I and II variants

While taking into account these test limitations, only 3 of 10 labs obtained full marks for genotyping in Case 3. The remaining 7 labs either did not detect the CNV or the missense variant, or both, although by methods stated in the lab reports for Case 3 these variants would be detected. Labs are advised to review their results for this case, their report comments on the limitations of the methods with respect to detection of CNVs, and report statements regarding what variant tiers are reported.

For Interpretation, the most significant finding was in Case 2 regarding comments related to PARP inhibitor therapy. Although Case 2 did not have variants in either *BRCA1* or *BRCA2*, the clinical vignette reason for testing was for eligibility for PARP inhibitor therapy, and so a statement to the effect that the patient may be less likely to respond to PARP inhibitor therapy as no variants in *BRCA1* or *BRCA2* were identified would be appropriate. This same issue also caused lower scores for the interpretation in Case 3, for those labs who reported Case 3 as negative due to use of methods that did not detect CNVs.

Results for Cases 4-10 , where only genotype results for *BRCA1* and *BRCA2* were requested, are shown in the graph below.



Case 9 had a *BRCA2* full gene deletion CNV. Scheme assessment took into account the test method information provided in Case 3 as it pertained to CNVs testing when reviewing Case 9 results, in order to determine if Case 9 results were correct within the limitations of the assay used. Three labs did not detect the *BRCA2* CNV. Two of these labs also did not detect the Case 3 CNV (i.e. did not detect either

CNV in this scheme), although their methods stated CNVs would be detected. Labs are encouraged to review their methods related to CNV detection, and report comments about method limitations as required.

Case 10 had a 4 base pair deletion detected by all labs, however two labs did not correctly use HGVS cDNA nomenclature for this variant, specifically the 3' position rule. HGVS guidance states that for deletions, duplications and insertions, the most 3' position possible of the reference sequence is arbitrarily assigned to have been changed.

Other minor errors across Cases 1-10 included minor nomenclature errors, or clerical issues when reporting name or MRNs.

Survey results

As part of the scheme all 11 labs also completed a survey on additional lab practices related to analysis of FFPE tissue for somatic *BRCA1*/*BRCA2* variants in prostate cancer. An overview of the questions and responses are shown below.

Question	Response
Given the multifocal nature of prostate cancer, does your lab use scrolls, or do you perform macrodissection or coring of the FFPE blocks to enrich for the area with the highest tumor cellularity/highest Gleason score?	Scrolls – 4 labs Macrodissection on slides – 4 labs Coring of blocks – 3 labs *(3 labs noted that more than one approach may be used)*
Is your NGS assay and analysis pipeline optimized for copy number calling?	Yes – 7 labs No – 4 labs
Do you incorporate the tumor cellularity from the tissue samples to calculate copy numbers?	Yes – 5 labs No – 6 labs
If you report CNVs, do you distinguish monoallelic CNVs versus biallelic/deep deletions?	Yes – 3 labs No – 8 labs
If you report CNVs, do you distinguish focal gene deletions versus segmental alterations?	No – 11 labs

Responses demonstrate different practices between Canadian labs for *BRCA1* and *BRCA2* testing for prostate cancer, particularly for CNV testing and reporting. Similar to the discussion of Case 3 and 9 results, if labs are not testing for CNVs or have limitations in when CNVs would be reported, they are encouraged to make this information clear on reports so that referring health care providers are aware of the test limitations.

Final comments

CBQA would like to thank all participating labs for their hard work and co-operation during this scheme. We would also like to thank our pharmaceutical industry partners for support of this scheme, and the assessment team for their time and effort to mark the results for this scheme.

CBQA Authorization

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References:

1. pCODR Expert Review Committee. Final recommendation for olaparib (lynparza) metastatic castration-resistant prostate cancer. pCODR, April 21, 2021.
2. Rendon RA, Selvarajah S, Wyatt AW, Kolinsky M, Schrader KA, Fleshner NE, Kinnaird A, Merrimen J, Niazi T, Saad F, Shayegan B, Wood L, Chi KN, Black P, Sridhar S, Yip S. 2023 Canadian Urological Association guideline: Genetic testing in prostate cancer. Can Urol Assoc J, 2023, 17:314–25