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REVIEW

New challenges for *BRCA* testing: a view from the diagnostic laboratory

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Increased demand for *BRCA* testing is placing pressures on diagnostic laboratories to raise their mutation screening capacity and handle the challenges associated with classifying *BRCA* sequence variants for clinical significance, for example interpretation of pathogenic mutations or variants of unknown significance, accurate determination of large genomic rearrangements and detection of somatic mutations in DNA extracted from formalin-fixed, paraffin-embedded tumour samples. Many diagnostic laboratories are adopting next-generation sequencing (NGS) technology to increase their screening capacity and reduce processing time and unit costs. However, migration to NGS introduces complexities arising from choice of components of the *BRCA* testing workflow, such as NGS platform, enrichment method and bioinformatics analysis process. An efficient, cost-effective accurate mutation detection strategy and a standardised, systematic approach to the reporting of *BRCA* test results is imperative for diagnostic laboratories. This review covers the challenges of *BRCA* testing from the perspective of a diagnostics laboratory.

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INTRODUCTION

The demand for *BRCA* testing has steadily increased since the discovery that women carrying pathogenic *BRCA* mutations have elevated lifetime ovarian and breast cancer risks.[1–3] In a meta-analysis of pathogenic *BRCA* mutation penetrance, carriers of *BRCA1* and *BRCA2* pathogenic mutations were shown to have a cumulative risk of 57 and 49%, respectively, for developing breast cancer and 40 and 18%, respectively, for developing ovarian cancer by 70 years of age.[1] In support of this observation, results from a prospective epidemiological study (EMBRACE) showed carriers of *BRCA1* and *BRCA2* pathogenic mutations have a cumulative risk of 60 and 55%, respectively, for developing breast cancer and 59 and 17%, respectively, for developing ovarian cancer by 70 years of age.[2]

A contributory factor to the demand for *BRCA* testing has been heightened public awareness of the consequences, costs and prophylactic options surrounding *BRCA* testing, an issue highlighted by celebrity publicity.[3,4] For women carrying pathogenic *BRCA* mutations, routine surveillance for breast cancer is recommended from 25 years of age and prophylactic salpingo-oophorectomy is recommended after 35 years or once childbearing is complete.[5,6] Prophylactic oophorectomies and mastectomies have been shown to reduce cancer incidence compared with chemoprevention or surveillance.[7]

The increasing demand for *BRCA* testing is placing a strain on diagnostic laboratories, particularly in those offering rapid genetic testing at the point of diagnosis. For instance, the UK's National Institute for Health and Care Excellence recommends fast-track genetic testing as part of a clinical trial within 4 weeks of a diagnosis of breast cancer.[8] Against this backdrop of rising demand, more diagnostic laboratories are adopting next-generation sequencing (NGS) technology for *BRCA* testing, which offers the potential of fast, scalable, cost-efficient and comprehensive sequencing. In the 2014 *BRCA* scheme report from the European Molecular Genetics Quality Network (EMQN), 19% of laboratories were using NGS for *BRCA* testing, an increase from 6% of laboratories from the previous year's scheme (Dr S Patton, EMQN Director, personal communication). The same EQA scheme reports also indicated a reduction in the use of Sanger sequencing alone for *BRCA* testing: from 83% down to 75% of laboratories.

Adopting NGS in the diagnostic laboratory setting is not straightforward, as the technology is not simple or homogeneous and many potential configurations are possible. Transitioning to NGS also imposes a significant validation overhead for clinical laboratories, as they are compelled to demonstrate that a new assay is sensitive, specific and fit for purpose prior to adoption. This review covers key considerations with respect to NGS and the specific challenges relating to *BRCA* testing, such as difficulties in interpreting complex *BRCA*-sequencing data and the issues of testing tumour samples.

BRCA TESTING: AN OVERVIEW

Genetic testing is undertaken in many countries to detect *BRCA1* and *BRCA2* sequence variants.[6] The selection of candidates appropriate for testing is typically based on national guidelines or by larger international societies.[5,8] A blood sample is typically used for these tests; however, other sample types can be used, for example, buccal scrape.[5,6] Written informed consent should be obtained from all patients prior to storage or analysis of their sample, and genetic counselling is standard practice both prior to the decision to test and at the time results are given to the patient.

Sequence variants in *BRCA1* and *BRCA2* can be subdivided into three broad classes: single-nucleotide changes, small insertion or deletion events (indels) and large genomic rearrangements (LGRs). Pathogenic *BRCA* single-nucleotide mutations and small indels are found widely distributed throughout the coding sequence and conserved intronic sequences of both genes. Typically, a very broad spread

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of pathogenic mutations is present in populations; however, founder pathogenic mutations are present at high frequency in some populations. For example, in the Ashkenazi Jewish population three founder pathogenic mutations (*BRCA1* NM_007294.3: c.68_69 delAG p.(Glu23Valfs*17), *BRCA1* NM_007294.3: c.5266dupC p.(Gln1756Profs*74) and *BRCA2* NM_000059.3: c.5946delT p.(Ser1982Argfs*22)) account for the overwhelming majority of clinically relevant pathogenic mutations and are observed at relatively high frequency (~2% in total).[9,10] In addition, in Polish breast and breast-ovarian cancer families, three pathogenic mutations in *BRCA1* (NM_007294.3: c.5266dupC, NM_007294.3: c.181T>G p.(Cys61Gly) and NM_007294.3: c.4034delA) were found to account for the majority of pathogenic *BRCA* mutations.[11] More recently, three further pathogenic founder *BRCA1* mutations have been observed in a study of 1164 Polish women with unselected breast cancer (NM_007294.3: c.3700_3704del p.(Val1234Glnfs*8), NM_007294.3: c.68_69delAG p.(Glu23Valfs*17) and NM_007294.3: c.5251C>T p.(Arg1751*)).[12]

BRCA1 contains a number of *Alu* sequences,[13] which are known to mediate the occurrence of LGRs. In *BRCA1*, LGRs have been detected with varying frequencies among patients with breast or ovarian cancer.[14–24] In a study of 805 Dutch families with a known predisposition for breast and/or ovarian cancer, those without identified pathogenic *BRCA1* or *BRCA2* mutations by conventional mutation screening methods (661) were assessed for *BRCA1* germline LGRs.[23] A total of 33 families with a deletion or duplication event in *BRCA1* were identified, representing 27% of the total 121 pathogenic *BRCA1* mutations. In a separate study of unrelated individuals ($n = 3580$) with a family history of breast and ovarian cancer or those with early onset disease ($n = 934$), a 6 kb *BRCA1* exon 13 duplication event was identified in 11 families with ancestry links to Northern Britain.[22] *BRCA2* contains fewer *Alu* sequences,[25] which may explain why fewer rearrangements have been reported for this gene. Examples of *BRCA2* LGRs include deletion of exon 2 and an *Alu* insertion in exon 3, which occurs with relatively high frequency in those patients from North and Central Portugal.[15,26,27]

The interpretation of the results of *BRCA1* and *BRCA2* screening is made complex by the significant numbers of patients with variants of unknown clinical significance (VUS). VUS are alterations in the DNA sequence of a gene that have an unknown effect on the function of the gene product or on the risk of disease.[28] They can include variants in promoter regions, intronic nucleotide changes close to the exon boundary, small in-frame insertions/deletions and missense/synonymous substitutions where there is no firm evidence for a deleterious effect on RNA processing, or protein structure and function. In an analysis of *BRCA1* and *BRCA2* sequences from 10 000 individuals, 13% were observed to harbour a VUS.[29] However, the frequency of VUS should be lower in well-characterised populations, as databases of pathogenic *BRCA* mutations are updated, allowing reclassification of previous VUS. The finding of a potential VUS during screening requires detailed expert interpretation and as many sources of evidence about a variant should be collated and assessed as possible before coming to a conclusion that is clinically reported.

BRCA testing is commonly performed by direct (Sanger) DNA sequencing. This method is considered the 'gold standard' of DNA sequencing; technologically reliable, widely available and a relatively simple workflow. The drawbacks of Sanger sequencing are limited throughput and lower cost-effectiveness compared with NGS. In addition, Sanger sequencing cannot detect LGRs, which require alternative polymerase chain reaction (PCR)-based techniques for analysis. Quantitative PCR is a viable technique for detecting LGRs;[24] however, this is labour intensive for analysis of all *BRCA1* and *BRCA2* exons. Instead, the most commonly used method for analysing LGRs is multiplex ligation-dependent probe amplification (MLPA), a technique in which pairs of oligonucleotide probes able to ligate to each other, bind to adjacent positions at the genomic area of interest and amplify only if both probes are bound and ligated in a semi-quantitative manner. Examples of commercially available, research-use-only (RUO) *BRCA* testing MLPA probes are P002 for *BRCA1* and P045 for *BRCA2* (MRC-Holland, Amsterdam, The Netherlands). Alternatively, some laboratories have developed custom probes analogous to the MLPA technique,[30] or customised PCR assays to detect specific LGRs.[27]

BRCA testing with NGS technology offers many advantages over Sanger sequencing, including the potential to detect LGRs in a single workflow, although NGS LGR detection has not been fully established in the diagnostic setting. Some of the key advantages and disadvantages associated with NGS are summarised in Table 1.

The following section describes the key considerations with respect to adopting NGS in the diagnostic laboratory.

Table 1 Advantages and disadvantages of next-generation sequencing[31–35]

Advantages	*Disadvantages*
High throughput	Higher start-up cost
Able to multiplex	Mutation-positive test may require Sanger sequencing for confirmation
Lower cost	Complex workflow
Automated analysis	Dedicated data storage and analysis required
Uses less DNA	Reduced sensitivity for large insertions/deletions >20 base pairs
Can run in parallel with other genetic tests	

NEXT-GENERATION SEQUENCING: CHOICE AND COMPLEXITY

The rapid evolution of massively parallel sequencing technology and NGS platforms is revolutionising the management of inherited diseases, where traditionally molecular diagnostics have been under-used due to the issues of cost, time, labour and availability of services. A number of early clinical studies in *BRCA* testing have shown that NGS offers high sensitivity, specificity and cost-effectiveness compared with current approaches.[35–41]

Recently, NGS benchtop platforms have made available gigabase-scale DNA sequencing with relatively short run times (<24 h), for example, MiSeq (Illumina, San Diego, CA, USA) and the Ion Torrent Personal Genome Machine (PGM; Life Technologies, Carlsbad, CA, USA) (refer to Table 2 for examples of NGS platforms). The potential of high-volume analytical throughput makes NGS platforms an increasingly attractive investment for diagnostic laboratories in the clinical setting. However, the choice of NGS platform is only one factor to consider in the total NGS workflow, which is also dependent on other components including enrichment methods, sequencing chemistries and analytical procedures (Figure 1).

The following section describes some of the key considerations with respect to NGS platforms, chemistries and analysis of data.

PLATFORMS

The MiSeq (Illumina) and Ion Torrent PGM (Life Technologies) are examples of benchtop NGS platforms. Both operate on the principle of sequencing-by-synthesis, in which the addition of nucleotide triphosphates to primed clonal DNA templates are measured. In the

Table 2 Examples of next-generation sequencing platforms as of May 2014

Platform	*Read length (bp) and mode*	*Run time[a] (hours)*	*Number of reads (single or paired; millions)*	*Data yield (Gb per run)*
Illumina MiSeq v2 chemistry[b]	2×150 paired	~24	24–30 (paired)	4.5–5.1
Illumina MiSeq v3 chemistry[b]	2×75 paired	~21	44–50 (paired)	3.3–3.8
Illumina MiSeq v3 chemistry[b]	2×300 paired	~56	44–50 (paired)	13.2–15.0
Ion Torrent PGM, 316 Chip v2[c]	Up to 200 single	3	2–3 (single)	0.3–0.5
Ion Torrent PGM, 318 Chip v2[c]	Up to 200 single	4.4	4.0–5.5 (single)	0.6–1.0
Ion Torrent PGM, 318 Chip v2[c]	Up to 400 single	7.3	4.0–5.5 (single)	1.2–2.0
Ion Torrent Proton with PI Chip[c]	Up to 200 single	2–4	60–80 (single)	Up to 10
Illumina NextSeq 500 (mid output)[b]	2×150 paired	26	Up to 260 (paired)	32.5–39.0
Illumina HiSeq 2500 (rapid run single flow cell)[b]	2×150 paired	40[d]	Up to 300 (paired)	75–90

[a]Upstream preparatory work not taken into consideration.
[b]www.illumina.com
[c]www.lifetechnologies.com
[d]Run time based on dual flow cell.

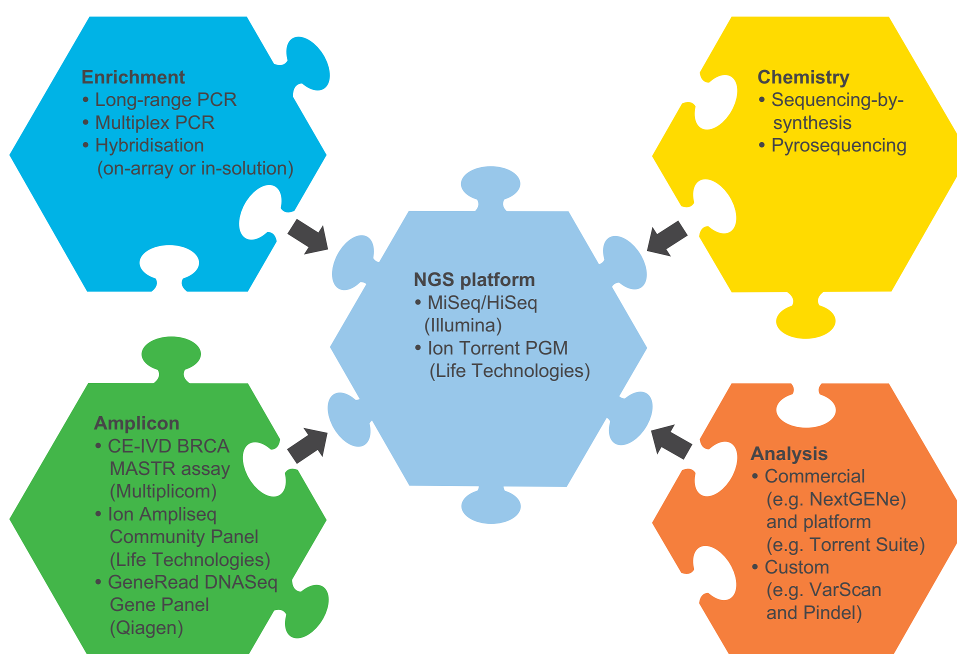

Figure 1 Components/complexities to consider in the NGS workflow, including the NGS platform, enrichment methods, sequencing chemistries and analytical procedures.

case of the MiSeq, these are fluorescently labelled reversible deoxyribonucleoside triphosphates (dNTPs) and sequences are optically read by fluorescence imaging.[42] Conversely, the Ion Torrent reads sequences non-optically through the use of semiconductor sequencing technology (Thermo Fisher, Waltham, MA, USA), where pH changes resulting from the addition of dNTPs to the nascent strand are recorded as voltage changes.[43] The rapid pace of NGS platform development renders any detailed review out-of-date by the time of publication. Given this, up-to-date online reviews such as the *NGS Field Guide* from The Molecular Ecologist are important references when choosing an NGS platform.[44]

ENRICHMENT METHODS

In the clinical setting, NGS is typically used for sequencing specific genes, such as *BRCA1* and *BRCA2*, or panels of genes rather than for sequencing entire genomes, as it is far more cost-effective and time-efficient to target, capture and sequence only the genomic regions of interest. This has led to the development of numerous enrichment methods, most commonly based on PCR or hybridisation approaches (Figure 2).[45] It is possible that future decreases in the overall cost for gene sequencing may result in exome sequencing, with a virtual panel being a cost-effective mode of delivery.[46]

PCR is a well-established pre-sequencing enrichment technique, particularly for use with Sanger sequencing. In the case of NGS, laboratory-developed long-range PCR methods have been used successfully with *BRCA* testing;[47] however, to make full use of high-throughput NGS, a large number of amplicons must be prepared separately and then combined and sequenced together. This has led to the development of commercially available multiplex PCR kits that enrich for a specific gene or panel of genes in a small number of PCR amplifications (Figure 2a). For instance, the CE-IVD BRCA MASTR assay (Multiplicom, Niel, Belgium) amplifies the coding regions of *BRCA1* and *BRCA2* in 93 amplicons in five multiplex PCR reactions. Other RUO multiplex kits are available for *BRCA1* and *BRCA2,* such as the Ion Ampliseq Community Panel (Thermo Fisher) and Gene-Read DNASeq Gene Panel (Qiagen, Hilden, Germany).

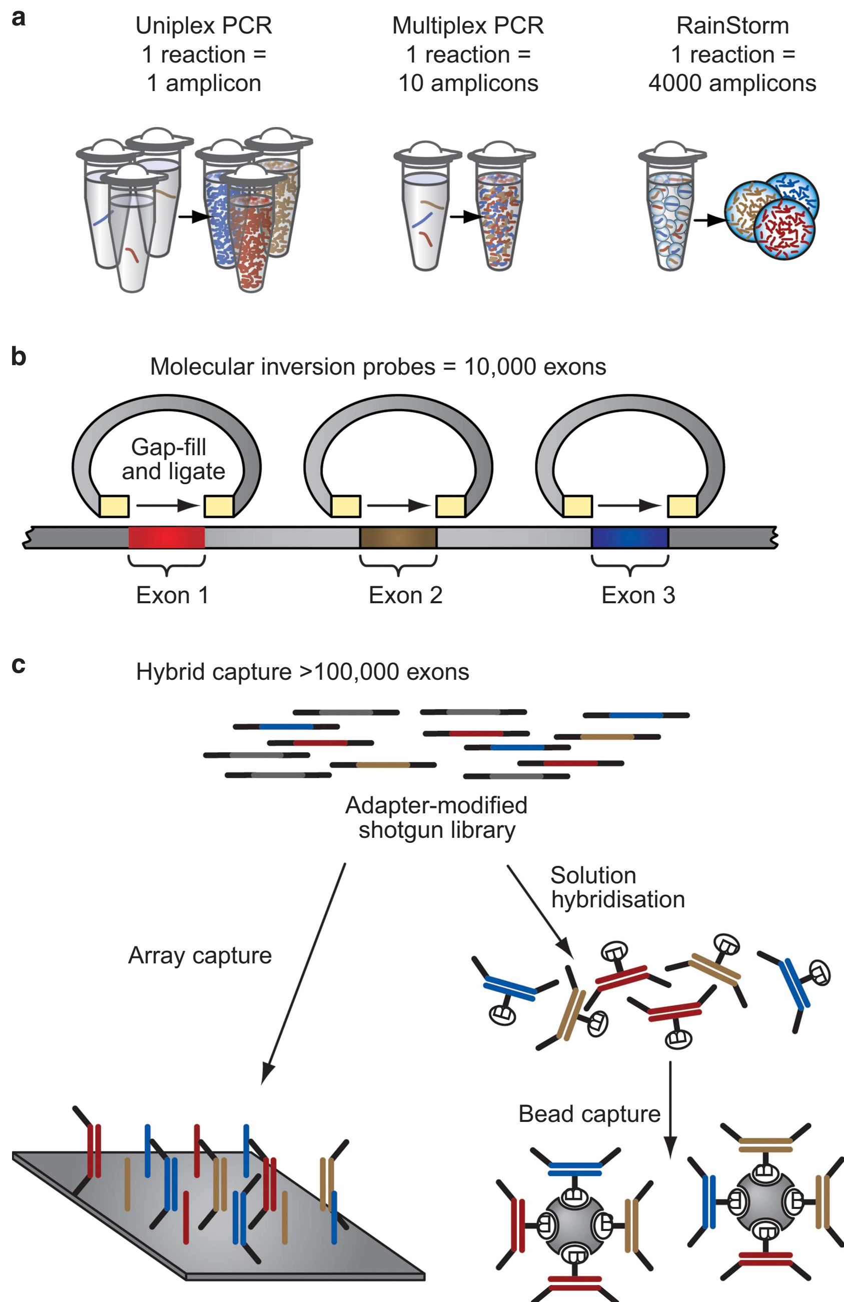

Figure 2 Enrichment methods. (**a**) PCR-based approach. Multiplex PCR kits enrich for a specific gene or panel of genes in a small number of PCR amplifications. (**b**) Molecular inversion probes consist of amplicons containing a universal spacer region flanked by target-specific sequences. Genomic DNA is digested, and the target DNA is PCR-amplified and sequenced. (**c**) Hybridisation enrichment methods work on the principle of selection using probes complementary to DNA in the genomic area of interest either by surface microarray or in solution with labelled beads.[45] Reprinted with permission from Mamanova *et al.*[45]

Molecular inversion probe (MIP)-based enrichment approaches can provide greater specificity over standard PCR-based approaches.[45] MIPs consist of a universal spacer region flanked by sequences specific to either side of the target region (Figure 2b). Once the MIP anneals to the target, the gap between the sequences is filled by a DNA polymerase and ligase. Genomic DNA is digested, and the target DNA is PCR-amplified and sequenced. Although PCR as an NGS enrichment method is highly sensitive, specific and reproducible,[45] it is inefficient for NGS of larger genomic targets where other approaches to target enrichment should be considered.

Hybridisation enrichment methods work on the principle of selection using probes complementary to DNA in the genomic area of interest (Figure 2c). On-array capture uses high-density microarrays containing complementary probes, whereas in-solution capture uses complementary probes that are then purified using labelled beads (Figure 2). The in-solution approach has the advantage of being highly scalable and does not require additional equipment associated with processing microarrays. Hybridisation kits are available that target *BRCA1* and *BRCA2* specifically, such as RUO, in-solution capture kits, HaloPlex and SureSelect (Agilent, Santa Clara, CA, USA). In addition,

hybridisation kits are available that include *BRCA1* and *BRCA2* as part of a larger panel of genes associated with cancer, such as RUO TruSight Cancer Sequencing Panel (Illumina), which targets 94 genes, or NimbleGen Comprehensive Cancer Design (Roche NimbleGen, Madison, WI, USA), which targets 578 genes. It should be noted that hybridisation methods, particularly on-array capture methods, can add additional cost, time and, if the panel of genes is extensive, incidental findings to the overall NGS process.

BIOINFORMATICS ANALYSIS AND LABORATORY INFORMATION MANAGEMENT SYSTEMS

Even benchtop NGS platforms produce large amounts of sequence data that require bioinformatics analysis to align, variant call and filter the data to make them accessible, coherent and comprehensible. Many options are available for a diagnostic laboratory for analysing NGS data; however, choosing appropriate software and thorough validation is crucial in order to obtain accurate and reliable results suitable for clinical application. Commercially available software include NextGENe, CLC Workbench (Qiagen) and those linked to specific platforms, such as the Torrent Suite Software Plugins (Life Technologies). Publicly available and open-source bioinformatics software can also be used to build a custom bioinformatics pipeline. Examples include Pindel, VarScan, GATK-lite and Samtools. It should be noted that input from bioinformaticians into an NGS-based diagnosis is an important consideration for reaching a quality level required for medical analysis.

Tracking and managing clinical samples through a high-throughput NGS workflow requires a laboratory information management system (LIMS) that can support NGS, is configurable and customisable to suit diagnostic laboratories' needs and yet flexible to accommodate changes in testing practice. Numerous commercial systems are available, some of which are tailored to managing NGS workflows in clinical laboratories, such as Exemplar LIMS (Sapio Sciences, Baltimore, MD, USA), Clarity LIMS (GenoLogics, Victoria, BC, Canada) and Sequencing LIMS (Edinburgh Genomics, Edinburgh, UK). Commercial systems tend to involve high setup costs and can require extensive configuration and customisation to address specific laboratory needs. Open-source solutions are also available, such as Galaxy LIMS (Tron, Mainz, Germany).

NGS FOR DETECTING LARGE GENOMIC REARRANGEMENTS

An NGS-based strategy offers the potential to screen for point mutations and LGRs on a single platform and workflow. NGS has been applied to detection of LGRs in DNA from cancers using depth of coverage and a paired-end mapping whole-genome sequencing approach.[48,49]

The ability to detect LGRs in NGS data can be limited by the use of an enrichment method. PCR enrichment methods are currently unsuitable for reliable measurement of copy-number variants (CNVs). Hybridisation NGS enrichment methods do offer the potential to detect CNVs affecting targeted regions. However, this requires specialist bioinformatics, and particular problems are encountered in detection of smaller CNVs; <200 bp where sensitivity is low.[50,51] Extensive site-specific validation in the clinical setting is required before NGS can be routinely used for comprehensive CNV detection in a clinical setting for *BRCA* analysis.

BRCA TESTING FROM TUMOUR SAMPLES

Significant frequencies of somatic *BRCA1* and *BRCA2* pathogenic mutations have been observed in patients with ovarian cancer.[52] There is growing evidence that tumours with somatically acquired *BRCA1* or *BRCA2* pathogenic mutations will respond to drugs that inhibit poly (ADP-ribose) polymerase (PARP).[53–56] Tumour samples, as part of standard pathology practice, are routinely processed and stored as formalin-fixed paraffin-embedded (FFPE) blocks; this presents a number of challenges to the diagnostic laboratory. FFPE samples are typically a variable mix of neoplastic and normal cell tissue (stroma) and the DNA extracted is often limited in quantity, fragmented and of poor quality. In addition, DNA extracted from FFPE samples may contain artefactual sequence alterations arising from formalin cross-linking and deamination of cytosine nucleotides. These problems can be mitigated by the use of shorter amplicons, de-crosslinking steps and treatment with uracil-DNA glycosylase, a DNA repair enzyme, which has been shown to markedly reduce the number of sequence artefacts in damaged FFPE DNA when used prior to PCR amplification.[57,58]

The challenges in sequencing FFPE tumour samples are observed in NGS analysis. In a large-scale, prospective, cohort study of the incidence of cancer in a population in Victoria, Australia, an initial pilot phase for the first 488 patients established the feasibility of NGS for profiling mutations in tumours.[59] Disproportionate levels of C > T/G > A changes were displayed in the 1–10% allele frequency range, whereas artefacts were less apparent in the 10–25% allele frequency range. Importantly, an example of the dangers of mutational artefacts was shown in one sample, where an activating NRAS p.Gly12Asp mutation was discovered on first screening but not confirmed in the same DNA specimen by subsequent sequencing of uracil-DNA glycosylase-treated FFPE DNA or repeat NGS. This highlights the utility of replicate analysis to confirm the identification of mutations.

Despite the limitations imposed by DNA extracted from FFPE samples, successful sequencing with NGS has been shown.[57,60,61] In an NGS study of ovarian ($n=68$) and breast ($n=30$) FFPE samples, DNA was amplified using a GeneRead DNAseq Targeted Exon Enrichment Breast Panel (Qiagen) and sequenced using a 2×150 bp analysis on a MiSeq platform (Illumina).[62] The majority of samples with low DNA yields produced adequate PCR products and sequencing data without any significant deterioration in coverage or read depth until <1 ng of amplifiable DNA was added per primer pool (Figure 3). Among 75 samples with less than optimal DNA input, 32 samples still generated the maximum possible coverage of ~97%, and a further 20 samples generated a coverage of $>95\%$ at a minimum read depth of $100\times$.[62] As the input quantity of DNA diminished, there were still many samples with $>90\%$ coverage depth at $100\times$, although the frequency of samples with low percentage coverage at $100\times$ increased. Significant variants were confirmed by Sanger sequencing. A small number of variants were identified as potential artefacts common to FFPE-extracted DNA; however, the majority of these were in the poor-quality low-input DNA samples with lower coverage depth and artefacts could be identified as such by replicate analysis. The conclusion of this study is that routine analysis of *BRCA1* or *BRCA2* sequences from FFPE breast and ovarian tumours is feasible. However, it is important that tumour *BRCA* screening should not be substituted for germline *BRCA* screening in patient groups at high risk of carrying an inherited pathogenic mutation unless proven to be at least as sensitive as germline screening at detecting the full range of inherited pathogenic *BRCA1* and *BRCA2* mutations.

INTERPRETING *BRCA* TEST RESULTS: DEALING WITH VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE

Because of the size of the *BRCA1* and *BRCA2* genes and the large number of screens carried out by diagnostic laboratories, many *BRCA* gene VUS have been identified. One study reported a VUS frequency rate for *BRCA1* and *BRCA2* of 13% for 10 000 consecutive

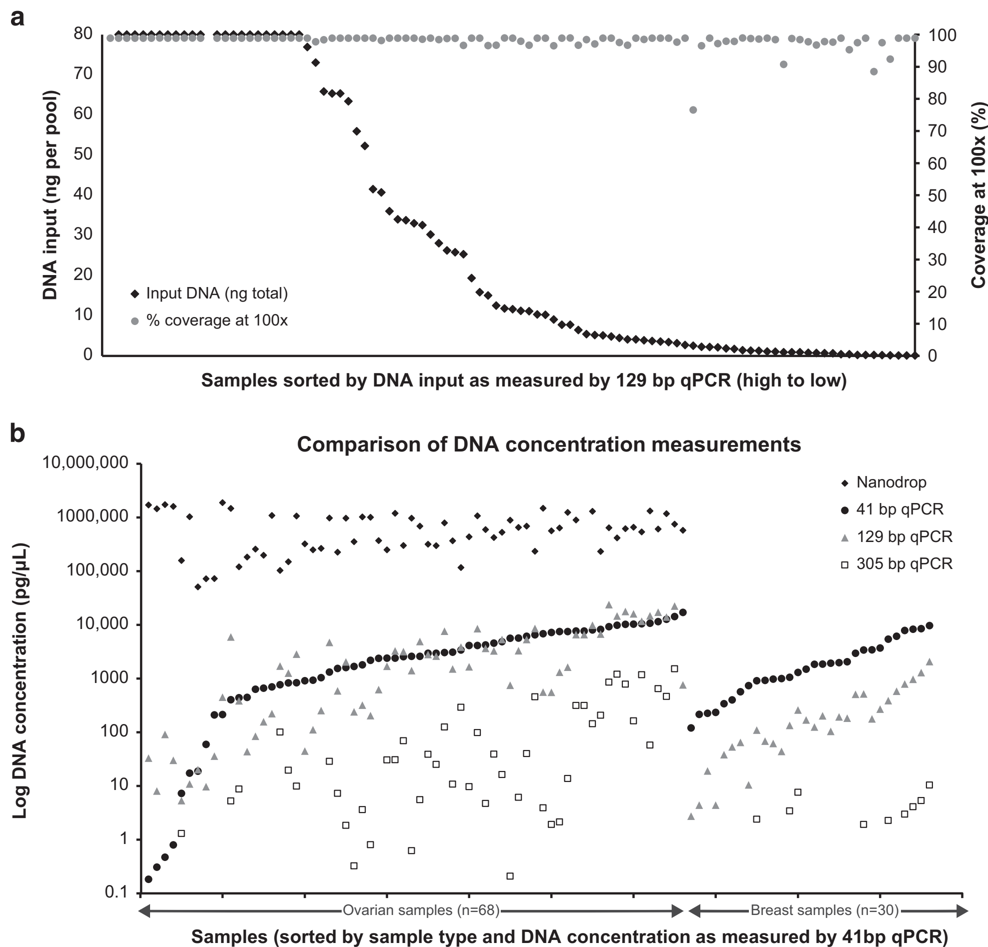

Figure 3 (**a**) NGS percentage 100× coverage *vs* DNA input from 98 breast and ovarian cancer FFPE DNA samples. (**b**) Comparison of DNA concentration measurements using a hgDNA Quantification and QC Kit (KapaBiosystems, Anachem, Luton, UK) and three different amplicon sizes. The ovarian DNA samples were also quantified using a Nanodrop (Thermo Fisher). The 129 bp product was selected to determine the amount of DNA to add into the *BRCA* panel, as it was the closest measure to the mean amplicon size of all methods being evaluated (GeneRead (Qiagen) V.1: 155 bp (estimated), V.2: 153 bp, Ion AmpliSeq (Life Technologies) ~ 197 bp). Figure reproduced under the terms of the Creative Commons Attribution License from Ellison *et al.*[62]

individuals.[29] In certain populations, a higher VUS frequency has been reported, such as 21% of alterations reported in patients with African–American ancestry.[63] However, initiatives to reclassify *BRCA* VUS are likely to reduce this number.[64–66] In our experience (St Mary's Hospital, Manchester, UK) the rate of *BRCA* VUS is ~ 8%.

Assessing a *BRCA* VUS is a complex task (Figure 4), but one that is greatly assisted by pooling of genetic, clinical and histopathological information from a world-wide network of laboratories. There are many data-sharing initiatives aimed at developing sequencing methods and resources to facilitate *BRCA1* and *BRCA2* variant classification. Examples include the BRCA Challenge (a joint initiative of the Global Alliance for Genomics and Health and the Human Variome Project) and the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA).[64,67] There has also been a large-scale collaboration of the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) to develop, test and apply a standardised classification scheme for mismatch repair gene variants in the setting of Lynch Syndrome.[68] The guidance from ENIGMA, along with peer-reviewed publications, *in silico* assessment and information from mutation databases, including BIC (research.nhgri.nih.gov/bic/), UMD-BE (www.umd.be), DMudB (www.dmudb.net) and HGMD (www.hgmd.org), assist a diagnostic laboratory to establish the likely pathogenicity of a *BRCA* VUS. Caution should be applied when using information from public mutation databases because of the varying levels of curation. After reviewing all of the evidence on variant classification, clinical laboratories are required to provide an opinion for the purpose of clinical decision-making.

As part of the assessment of a *BRCA* VUS, it is essential to clearly and systematically categorise a VUS as to whether it is pathogenic, neutral or of unknown status. This is a critical task, as the risk of miscomprehension is high among VUS uneducated genetic counsellors. In a recent survey exploring genetic counsellors' information preferences on VUS laboratory reports, a minority of respondents expressed concerns about awareness of VUS and appropriate medical recommendations among other health-care professionals.[69] The survey also highlighted that the majority of respondents (243/267 (91%)) reported too little information provided on laboratory VUS reports, and that additional information would help contextualise the VUS result for patients. This is supported by a previous survey of breast cancer genetic counselling practices, where only 63% of genetic counsellors felt their patients understood the meaning of a VUS finding.[70] It should be noted however, that a minority of genetic counsellors believe interpretation of additional information to guide

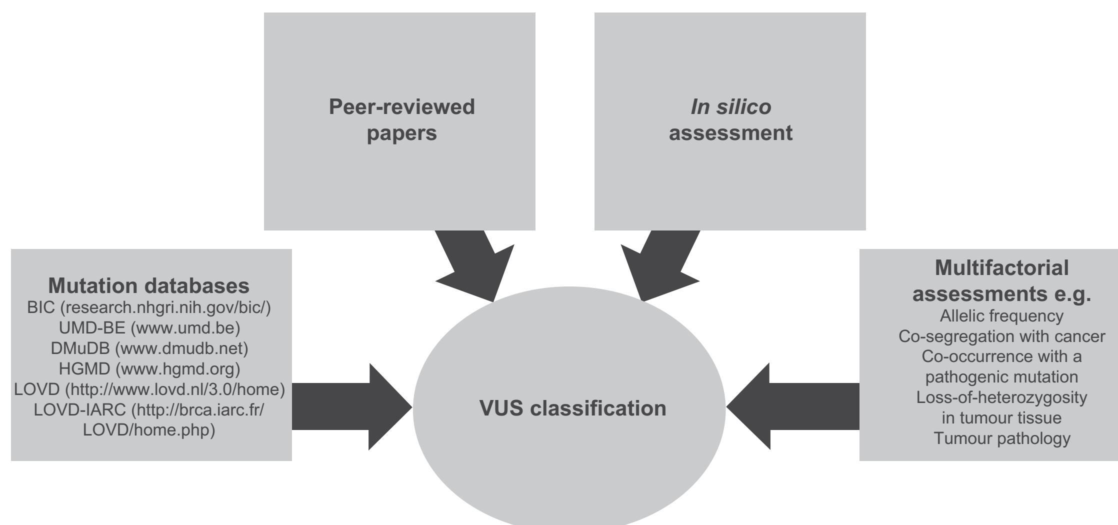

Figure 4 VUS classification for *BRCA*: a complex task involving many elements.

Table 3 Five-class system for VUS[71]

Class	*Description*	*Likelihood*	*Clinical management*
5	Definitely pathogenic	>0.99	Test at-risk relatives for variant, full high-risk surveillance guidelines
4	Likely pathogenic	0.95–0.99	Test at-risk relatives for variant[a], full high-risk surveillance guidelines
3	Uncertain	0.05–0.949	Do not use for predictive testing in at-risk relatives[a]
2	Likely not pathogenic or of little clinical significance	0.001–0.049	Do not use for predictive testing in at-risk relatives[a]
1	Not pathogenic or of no clinical significance	<0.001	Do not use for predictive testing in at-risk relatives[a]

[a]Recommend continuing to test proband for any additional testing modalities available for the disorder in question, for example, rearrangement testing.

patient medical management could be problematic, as interpretation was the responsibility of the laboratory conducting the test.[69]

In 2008, Plon *et al*[71] devised a system of five classes of variants based on the degree of likelihood of pathogenicity, alongside recommendations for clinical management (Table 3). In contrast to an earlier classification system,[72] this system subdivided the VUS category by the addition of 'likely not pathogenic' or 'likely pathogenic'. The five-class system provides health-care professionals with consistent classification information and clinical recommendations for each variant class. In order to assist with the application of this system, standardised data collection forms for VUS assessment are used by diagnostic laboratories.

CONCLUSIONS

As demand for *BRCA* testing increases, diagnostic laboratories will need to adapt their testing strategies and technologies to deal with the increased sequencing demand. Adoption of NGS can help meet this high demand and reduce the overall unit cost and staff time required for analysis; however, the choice and complexity in adopting NGS requires considerable thought and coordination of multiple-interdependent elements. Interpretation of screening results, particularly those of VUS, requires a thorough, systematic assessment, and consistent, clear reporting is essential for mainstream (non-genetic) disciplines. It is also important for the diagnostic laboratory to be aware of the significant challenges involved with screening tumour DNA, particularly in view of the potential utility of PARP inhibitors in tumours with somatic pathogenic *BRCA* mutations.

CONFLICT OF INTEREST

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