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Current mismatch repair deficiency tumor testing practices and capabilities: A Survey of Australian pathology providers

Short Title: Australian MMR deficiency tumor testing

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ABSTRACT

Aim & Methods: An electronic survey of the Royal College of Pathologists of Australasia accredited pathology services was conducted to assess Lynch Syndrome tumor screening practices and identify barriers and capabilities to screening newly diagnosed colorectal and endometrial tumors in Australia.

Results: Australia lacks a national policy for universal dMMR testing of incident colorectal and endometrial tumors cases. Routine Lynch Syndrome tumor screening program for colorectal and/or endometrial tumors was applied by 95% (37/39) of laboratories. Tumor dMMR screening methods varied; MMR protein immunohistochemistry (IHC) alone was undertaken by 77% of 39 laboratories, 18% performed both IHC and microsatellite instability testing, 5% did not have the capacity to perform in-house testing. For colorectal tumors 47% (17/36) reported following a universal approach without age limit, 30% (11/36) tested only "red flag" cases; 6% (3/36) on clinician request only. For endometrial tumors, 37% (12/33) reported clinician request generated testing, 27% (9/33) were screening only "red flag" cases, and 12% (4/33) carried out universal screening without an age criteria. *BRAF* V600E mutation testing of colorectal tumors demonstrating aberrant *MLH1* protein expression by IHC was the most common secondary tumor test, with 53% of laboratories performing the test; 15% of laboratories also applied the *BRAF* V600E test to endometrial tumors with aberrant *MLH1* expression despite no evidence for its utility. Tumor testing for *MLH1* promoter methylation was performed by <15% laboratories.

Conclusion: Although use of tumor screening for evidence of dMMR is widely available, protocols for its use in Australia vary widely. This national survey provides a snapshot of the current availability and practice of tumor dMMR screening and identifies the need for a uniform national testing policy.

Key Words: Hereditary Non-polyposis Colorectal Cancer, Immunohistochemistry, Lynch Syndrome, Microsatellite Instability, Screening

INTRODUCTION

Lynch Syndrome (LS), or hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant condition caused by germline mutations in the DNA mismatch repair (MMR) genes, *MLH1, MSH2, MSH6* and *PMS2*. It is the most common form of inherited predisposition to colorectal (CRC) and endometrial (EC) cancer, with 1-3% of both tumor types attributable to LS [1-7], although the proportion may be higher [8-10] . Diagnosis of LS in people with MMR-deficient (dMMR) CRCs can potentially alter clinical care by influencing both the extent of colorectal resection and adjuvant chemotherapy treatment decisions [14-16]. Identifying dMMR CRCs, regardless of germline or somatic causes, may become more important in future if programmed cell death ligand (PD-L1) inhibitors live up to their initial promise in the treatment of tumors displaying dMMR [17]. In addition to impacting immediate cancer management, identifying LS brings additional benefit in screening for secondary cancers and cancer risk management of family members subsequently found to have LS [2, 19]

dMMR can be detected in tumors by: (i) Microsatellite instability (MSI) testing through molecular comparison of tumor and normal tissue DNA; (ii) Immunohistochemistry (IHC) tumor testing for lack of expression of the corresponding MMR protein. The pattern of IHC protein expression determines which gene is likely mutated, guiding germline DNA testing[2], potentially providing a more efficient approach to germline testing than MSI alone. As MMR IHC does not require a molecular laboratory or micro-dissection of tumor for DNA extraction it is usually more feasible as a routine screening test than MSI analysis. Testing options for dMMR in tumors have been reviewed in detail [18].

The simple presence or absence of dMMR is sufficient to guide decisions regarding adjuvant systemic therapy for patients with dMMR tumors. However, the full clinical benefit of tumor dMMR screening depends on appropriately identifying individuals with a likely somatic etiology for their tumor dMMR profile and then offering germline genetic testing only to those without a likely somatic etiology. Approximately 15% of all CRCs and ECs will demonstrate dMMR, but approximately 75% of these will be due to somatic inactivation of *MLH1* promoter gene, which is rarely seen in LS-associated germline MMR mutations [20,21]. Therefore, tumors showing a loss of *MLH1* protein expression or MSI high tumors without a contemporaneous MMR IHC test to indicate the MMR protein affected, a secondary tumor test is required to find tumors likely to have *MLH1* promoter inactivation as a cause of their dMMR phenotype. For CRCs this secondary test can take the form of direct tumor *MLH1* promoter methylation testing, or testing the tumor for the presence of somatic *BRAF* V600E mutation as these are rarely seen in the presence of a germline *MLH1* mutation [20]. Somatic hypermethylation of the *MLH1* promoter is observed in up to 89% of ECs that demonstrate loss of *MLH1* protein expression [22,23], but in contrast to the CRC setting, ECs rarely acquire *BRAF* V600E mutations [24,25] – thus only the *MLH1* promoter methylation test is an appropriate secondary test to triage patients with EC for germline testing.

Reduction in mortality resulting from active risk management of LS patients , and potential therapeutic benefits of determining a tumor's dMMR status, have led to the call for routine dMMR testing of all CRC and EC at the time of diagnosis without a clinician request. Such "universal testing" overcomes the poor historic performance of triage algorithms for LS ascertainment using clinical or tumor histological characteristics [2, 3, 7, 26-29]. Cost-effectiveness studies support implementation of systematic universal screening of all newly diagnosed CRCs and ECs [18,30] and detects twice as many cases of LS as targeting younger patients alone [31-34].

Western Australia implemented state-wide IHC and/or MSI screening in all newly diagnosed CRC occurring <60 years of age and annual LS cases detected increased fourfold [35]. In South Eastern Sydney, all incident CRCs, regardless of patient age, were tested using MMR IHC supported by MSI and *BRAF* mutation testing. Germline mutations were identified in ~7% of the incident cases after they were referred for germline testing by the treating surgeons [9]. Another study in NSW (10) screening 1426 consecutive unselected colorectal carcinomas for dMMR reported 6.6% of these tumors demonstrated dMMR and no evidence of MLH1 Promoter methylation by BRAF V600E IHC; no germline mutation screening was

undertaken as part of this study. MMR IHC tumor testing of two CRC cohorts from Victoria demonstrated a combined prevalence of MMR gene mutation carriers of 2.7% where all carriers were diagnosed < 70 years of age [6]

Despite evidence of cost-effectiveness, there is no national Australian policy for universal dMMR tumor testing of all incident CRC or EC cases. The Inherited Cancer Connect (ICCon) Partnership (http://www.iccon.org.au), in collaboration with the Familial Cancer Group of the Clinical Oncology Society of Australia (COSA), is engaging specialist and consumer groups with the aim of developing such a policy. As part of that initiative, the ICCon Partnership has undertaken a survey to understand current capability of dMMR screening in diagnostic pathology laboratories, identifying potential enablers and barriers to universal tumor screening in the Australian environment.

METHODOLOGY

Sample & Procedures

An online survey administered via Limesurvey® was emailed to all Royal College of Pathologists Australasia (RCPA)-accredited laboratories in Australia. The survey questionnaire is included under Appendix I.

The Head of the laboratory or their nominated delegate(s) were invited to complete the survey. Telephone contact was made after a second email reminder to non-responders, with the option to complete the survey by telephone. An additional reminder was also posted in the March 2015 edition of the RCPA newsletter "Pathology Today", open to all RCPA members. The survey was open from 08th January 2015 through 1st December 2015.

The study was approved by Peter MacCallum Cancer Centre Human Research Ethics Committee.

Data Measures

The survey consisted of 21 multiple choice questions and open field to describe the "other" option. More than one response was allowed for most questions. Response was mandatory on MMR IHC/MSI testing capabilities and several open-ended questions allowed individuals to describe their views on development of a national policy for universal screening of dMMR. The questions aimed at identifying triggers for tumor MMR assessment at the point of CRC and EC diagnosis in the local centers.

Data Analysis

Descriptive analyses were performed using STATA & Microsoft Excel to assess the most common practices of LS screening. Chi square tests performed in STATA compared LS testing strategies between private and public laboratories.

RESULTS

Of 53 Australian invited histopathology and genetic pathology laboratories, representatives from 4 laboratories did not handle anatomical pathology specimens and/or were associated with other laboratories already participating and therefore were excluded from the study. Forty-four individuals responded, representing 39 laboratories from 6 states, giving a response rate of 80% (39/49 laboratories) (Table 1). The remaining laboratories who did not respond to this surveywere mostly privately-owned laboratories 7/10, (70%). Data is presented by laboratory rather than participant; if more than one representative responded for a particular laboratory, the most common response was used, except for items which invited a personal view or comment. Laboratories with multiple sites but having common practices were treated as single participant; if different sites of the same laboratory service reported different practices the sites were contacted for clarification and responses reported separately if required. The 39 participating laboratories were from the following health sectors and majority 30/39(77%) respondent laboratories were urban services:

- public laboratories associated with a public hospital(19/39, 49%)
- private pathology providers (17/39, 43%)
- private laboratories contracted to a public hospital(3/39, 8%).

Table 1:

Capability for dMMR screening

Ninety-five percent (37/39) of laboratories undertook dMMR tumor screening by IHC, MSI or both (Figure 1). The remaining 5% (2/39) were either a regional pathology service (both private & public) orreported low case volume and lack of funds/expertise to carry out tumor testing in-house.

Figure 1:

MMR IHC and/or MSI testing in other tumor types

Laboratories also reported activity for MMR IHC and/or MSI in other tumor types. The majority reported testing sebaceous neoplasms (17/39, 44%) with other types less commonly tested: small bowel (7/39, 18%), gastric (6/39, 15%), ovarian (4/39,10%), pancreas (4/39, 10%), prostate (1/39, 3%) and none on breast tumors. Ten reported testing the non-colon/non-endometrial tumor tissues on clinician request but not as a matter of routine practice.

Colorectal and Endometrial Cancer Screening practices and barriers to IHC/MSI testing

Tumor screening is practiced more routinely in CRC and EC than in other tumor types, with the latter occurring more often by clinician request.

dMMR testing was carried out in 36 laboratories who receive CRC specimens and in 33 who receive EC specimens. Selection criteria to initiate MMR IHC testing on CRC and EC are summarized in Figure 2.

Figure 2:

CRC is more commonly screened using a "truly universal" approach than EC, where testing prompted by the treating clinician is most common practice.
Table 2 details the "red flag" criteria which trigger dMMR testing in laboratories. When routinely testing on "red flag" cases, the most common eligibility criteria applied were age < 50 years (7/17,41.1%), pedigree features indicated on the pathology requisitions ~ 33% and tumor characteristics (~ 50%). In 58% (10/17) of the times for CRC and 67% (13/17) of the times for EC where the "red flag" approach was followed, laboratories were associated with a Familial Cancer Centre.

Table 2:

The reported barriers to dMMR testing by IHC and/or MSI were low volume of cases (3/39, 8%) and lack of specific technical expertise (13/39, 33%), funding constraints (12/39, 31%) being the main concerns identified by laboratories.

MMR IHC Tumor Testing Strategy

Of the 37 laboratories who undertook MMR IHC testing, 32 (87%) reported testing CRC and EC, 4 (11%) CRC only, 1 (2%) EC only. Tumor testing strategy varied between and within

laboratories. The majority of laboratories (30/37, 81%) reported IHC testing for all 4 MMR proteins (*MLH1*, *MSH2*, *MSH6*, *PMS2*) while a few (4/37, 11%) employed an initial MMR IHC screen of *PMS2* and *MSH2*, followed by testing for the remaining proteins (*MLH1* or *MSH2*) if loss of one of the paired proteins occurred. Two (5%) laboratories indicated variable practices as their standard was to screen *PMS2* and *MSH2* initially but with some pathologists favoring the 4 MMR protein screen, unknown (1/37, 3%).

Tumor MSI testing strategy

Of the 7 laboratories who reported MSI testing, 3 (43%) tested tumors for MSI subsequent to MMR IHC testing when there was loss of MMR protein expression. One laboratory reported MSI testing only when there was a request from the clinician/genetics services to test for MMR deficiency independent of the order of IHC/MSI (14%). A single laboratory reported testing for IHC and MSI routinely at the time of initial screen in all EC and CRC cases (14%), unknown (2/7, 29%).

Tumor *MLH1* promoter methylation & *BRAF*V600E somatic mutation testing as a secondary test in the setting of MSI high or loss of *MLH1* protein expression by IHC

Out of the 37 laboratories, 10 (27%) did not have capacity to offer secondary test and reported lack of expertise (7/10, 70%), equipment (6/10, 60%), evidence to support routine use (1/10) and cost (1/10) as a barrier; (7/10, 70%) laboratories confirmed that cases were referred to a reference laboratory for testing of which one laboratory had not validated the test in-house and therefore sent such cases to a sister laboratory.

BRAF V600E mutation testing of CRCs was the favored approach over *MLH1* methylation testing for secondary tumor testing. Five of 37 laboratories (14%) reported testing both CRC and EC for the *BRAF* V600E mutation. *MLH1* promoter methylation testing was much less commonly available (Figure 1) across Australia.

Funding sources

The majority of respondents indicated that dMMR IHC tumor screening was billed through Medicare (22/33, 67%). Six respondents stated funding was included in the hospital budget (6/33,18%) and one billed privately (1/33,3%). Some laboratories (3/33, 9%) indicated that for public patients testing is included in the hospital departmental budget and private patients were either billed through Medicare or charged to their Private Health Insurance. One respondent (1/33, 3%) also indicated that anything above Medicare is covered by the research budget.

In laboratories who responded to a universal screening approach for CRCs, (10/17, 59%) the funding source was Medicare.

Capacity for Tumor or germline gene sequencing

For the 26 laboratories who responded to this question, the majority (18/37, 48.6%) did not have the capacity for tumor and/or germline DNA sequencing. However, 6 of these laboratories, could either perform it at a reference laboratory if required (2/18, 11%) or sent these cases to their sister lab (4/18, 22%). Very few reported having the resources and expertise to perform DNA sequencing in both tissue types (4/37, 10.8%). Four laboratories stated having the capacity for targeted re-sequencing of tumor DNA only (4/37, 10.8%). Lack of equipment (11/18, 61%) & expertise (11/18, 61%), low volume of cases (1/11, 9%), no request (1/11, 9%) and cost (2/11, 18%) were reported as testing barriers (more than one response allowed)

Private v/s Public

There was no statistically significant difference in the approach to dMMR testing between private and public pathology services once a specimen was selected for testing (Table 3).

Table 3:

Views on implementation of universal screening approach

Nine of the thirteen (69%) respondents who answered this question felt the need to have a common approach to dMMR testing, including uniform guidelines and process of investigations, and were also supportive of the idea of developing a definitive Australian national statement for dMMR tumor testing. One respondent detailed that implementation of a national policy will require stakeholder involvement and specific government funding. Four (31%) others thought that a national unified approach was not the best use of limited health resources with one respondent comment that "local practices developed were more effective than imposed policies". One respondent also stated that the ethical considerations around tumor testing that could indicate an inherited predisposition could limit pathologist-driven testing.

If funding were not a constraint, almost half of the respondents (20/42, 47.6%) would like to perform dMMR testing for all incident CRCs and/or ECs. Age-based criteria were still preferred by others - <50 year (3/42, 7.1%), <60 years (1/42, 2.4%), <70 years (2/42, 4.8%). Some respondents would still prefer receiving a specific case-by-case clinician request (4/42, 9.5%). One respondent was concerned about a low yield from universal versus targeted dMMR testing and felt that the pathologist would be liable for following up an abnormal dMMR result that had not been specifically ordered by the clinician. The remaining 3/42 (7.1%) would prefer to base dMMR testing on Bethesda/Amsterdam clinical criteria.

DISCUSSION

This is the first national Australian survey of current practice and capacity for screening tumors for dMMR to identify families with LS and/or guide adjuvant chemotherapy choices. It is very encouraging to find that 95% of laboratories have the capability and do undertake some form of dMMR screening, and that almost half already undertake universal screening for incident colorectal cancers (Fig 2). These data will help guide the continuing development of a clear national policy for systematic dMMR screening of CRC and EC. Current guidelines from the National Health and Research Medical Council (all CRCs under age 70 years should be tested) and the Royal College of Pathologists of Australasia (all CRCs under age 50 years should be tested) are not aligned (table 4). Furthermore, even the more recent NHMRC guidelines for dMMR screening in CRC (all tumors under age 70 years) are not aligned with the Lynch Syndrome guidelines (universal testing of all CRC tumors regardless of age). The new NHMRC guidelines are a useful step forward raising dMMR testing of tumors from a very discretionary test to a more routinely performed test, but the NHMRC guidelines need to be consistent with each other and also be widened to include recommendations around dMMR testing in endometrial cancer.

The most common tumor screening process within this group was MMR IHC. The majority of laboratories (76%) offered subsequent secondary tumor testing in CRCs and/or ECs that showed loss of *MLH1* and *PMS2* expression by IHC. This was usually testing for the *BRAF* V600E somatic mutation rather than *MLH1* promoter methylation testing. The preference for *BRAF* V600E tumor testing is despite evidence from CRC studies demonstrating that the presence of *MLH1* promoter methylation is a better negative predictor than *BRAF* V600E mutation for a germline *MLH1* mutation [6, 20] and probably relates to the existing capability of laboratories which are likely to have the *BRAF* V600E test available for other therapeutic reasons. Importantly, the *BRAF* V600E somatic mutation is rarely detected in EC [24] leaving only *MLH1* promoter methylation testing as a viable secondary test in ECs demonstrating loss of *MLH1* and *PMS2* protein expression. We have previously shown *MLH1* promoter methylation to be an effective negative predictor of *MLH1* germline mutation status in EC [7]. Despite this, *MLH1* promoter methylation testing was offered as a secondary test by only 13% of laboratories. Barriers to implementing *MLH1* promoter methylation testing were mainly related to this being a molecular rather than an IHC-based test and included issues around resources, expertise and evidence to support its routine use. It is not surprising that few laboratories had the specialist resources for specific molecular analyses which are generally the work of molecular diagnostic laboratories rather than histopathological laboratories. Certainly the approach of undertaking MMR IHC, followed by secondary testing to identify tumors likely to have a somatic *MLH1*-based etiology is endorsed by evidence of cost-effectiveness [1, 2, 31, 32, 36]. Although there is strong published evidence to suggest that *BRAF* V600E in EC is not useful in determining a potentially somatic etiology [24, 25], there were a few laboratories who reported ordering *BRAF* V600E testing on EC (Fig 3). This suggests a need for education and guidelines to help optimize the use of dMMR testing in Australia.

The practices identified here were largely similar to the findings from a recent US study looking at CRC dMMR testing in National Cancer Institutes and Community Hospitals [37] Although US sites tended to favor a combination of MMR IHC and MSI whereas in Australia MMR IHC-only approach is favored. Both the US and our Australian survey have demonstrated that there are different algorithms practiced within each country which indicates that more uniformity in testing processes is desirable to maximize the benefits and minimize costs of a universal testing policy.

A survey of US-based genetic counsellors identified an improvement in the prevalence of universal dMMR tumor screening following the statement issued by EGAAP (Evaluation of Genomic Applications in Practice and Prevention) in support of screening all patients newly diagnosed with CRC to identify at-risk families [38]. The same EGAAP recommendations excluded EC, but the NSGC (National Society of Genetic Counsellors) and Collaborative Group of the Americas on Inherited Colorectal Cancers published a joint statement advocating the addition of universal dMMR tumor screening of newly diagnosed EC to identify new families with LS [39] . The recently published Australian National Framework for Gynecological Cancer Control indicated that IHC for MMR proteins is "an appropriate tumor test for women diagnosed with endometrial cancer under 60 years of age" but did not specifically state that it should be performed in all cases diagnosed under 60 years of age[40].

Our survey identified that there is capacity and interest for routine tumor dMMR testing in Australia, but also highlighted areas that need to be addressed before such a policy can be implemented:

1. Development of a clear policy outlining tumor dMMR testing endorsed by professional bodies; in particular whether this will be universal versus "triggered" (e.g. an age-threshold) approach, what secondary tests are preferred and the process to access germline testing as appropriate.
2. Education of professionals and the public that tumor testing is not germline genetic testing and therefore formal genetic counselling is not required ahead of the tumor test, although patients should be informed about the existence of the test and its possible germline implications by their treating specialists
3. Education of professionals about the testing process, including the lack of utility of *BRAF* V600E testing as a secondary test in EC
4. Funding of tumor dMMR testing needs to be clarified including the permitted use of existing MBS billing codes for appropriate tests (eg MBS billing for IHC for MMR proteins in inpatients having surgery) and identification of billing codes or development of new billing codes for secondary tests as needed. The apparent use of needs further investigation. Currently BRAF & MSI tests are not covered by MBS while IHC is if ordered by treating practitioner, which can clearly affect what tests are ordered by pathologists.
5. Development of expertise and resources to support wider adoption of *MLH1* promoter methylation testing as a secondary tumor test and negative predictor of germline mutation status.

6. Clear pathways to access to tumor and germline sequence testing as needed. Most responding laboratories (18/26) stated they could not provide this test but only 6 of these provided an explanation as to how they would access these tests if needed. It is not known whether the remaining laboratories currently have access to these tests (within their laboratory network or externally) if needed.

There is limited evidence on barriers for the successful implementation of a tumor screening program for LS outside the Australian environment. From expert commentaries, inferences of specific barriers vary with location but include lack of clinical resources, financial limitations, and clinical expertise gaps [41]. Others suggest lack of infrastructure, lack of provider knowledge and the need to establish a reporting protocol for tumor screening as potential challenges in implementing a national screening policy [42,43]. Our survey data support all of these areas as issues in the Australian setting, and indicate that endorsement of a national policy by all Australian professional bodies will be essential in overcoming most of these.

This main strength of this study is that it is the first to survey the practice around and capacity for undertaking dMMR assessment of colorectal and endometrial cancers in public and private laboratories accredited by the Royal College of Pathologists of Australasia. However, our results may be reflective of the healthcare system only in the more populous Australian States only, as there was no representation from laboratories in ACT & NT. Furthermore, the consolidated nature of laboratories servicing multiple clinical locations providing surgical services may obscure local variations in clinical practice in terms of selection for dMMR screening. In contrast to the rate of responses to specific questions in the survey about current practice, we had fewer descriptive responses from pathologists regarding their views and barriers around having a national policy for tumor dMMR screening; these views may be better studied through a qualitative research approach with individual interviews if needed.

Our survey focused on the availability and practice of tumor dMMR screening, but it is clear overall cost-effectiveness of universal tumor dMMR screening for ascertaining new families with LS is highly dependent on subsequent uptake of germline mutation testing by the affected individual and their relatives when a germline mutation is identified. Ward et al [9] and Schofield et al [35] demonstrated less-than-optimal uptake of germline testing by patients after dMMR was identified in their tumor, therefore further research is required in identifying barriers and factors in uptake of germline testing. The WA group suggests that a regional coordinator could prove helpful in following up LS cases for mutation testing, which would also allay fears some respondents had of a laboratory's responsibility for the follow up of a patient with a dMMR tumor. After implementation of a national policy of routine dMMR screening, an evaluation of outcomes of newly identified individuals and families with LS would be needed. Protocols on patient follow up and compliance rates will also add value to successful implementation of a universal tumor dMMR screening program in Australia.

AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

None of the author(s) reported any potential conflict of interest.

AUTHOR CONTRIBUTIONS/ACKNOWLEDGEMENT

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Table 1: Response rates by State/Territory

State/Territory	Number of laboratories responding			Total laboratories invited	Remoteness*		% responses
	Private	Public	Total		Urban	Regional	
NSW	7	7	14	15	12	3	93
VIC	8	8	16	17	14	3	94
QLD	2	0	2	4	4	0	50

WA	1	1	2	4	4	0	50
SA	1	1	2	3	3	0	66
TAS	1	2	3	3	0	3	100
ACT	0	0	0	2	1	0	0
NT	0	0	0	1	0	1	0

†*NSW – New South Wales, VIC – Victoria, QLD – Queensland, WA - Western Australia, SA - South Australia, TAS – Tasmania, ACT – Australian Capital Territory, NT – Northern Territory.*

** http://www.doctorconnect.gov.au/internet/otd/Publishing.nsf/Content/locator*

Table 2: "Red flag" dMMR tumor testing Criteria in Colorectal/Endometrial tumors*

	Colorectal Cancer		**Endometrial Cancer**	
	N = 17	**%**	**N =19**	**%**
Age				
- <50	7	41.1	5	35.7
- <55	2	11.7	1	7.1
- <60	5	29.4	4	28.6
- <70	2	11.7	4	28.6
Pedigree Features				
Family History	5	29.4	6	42.8
Bethesda/Amsterdam criteria	4	23.5	1	7.1
Colorectal Cancer or Endometrial Cancer in the presence of other Lynch – related cancer diagnoses in patient	8	47.1	8	57.1
Tumor features				
Morphological/ Histological tumor features	8	47.1	5	35.7

Tumor location CRC- right sided, proximal EC-lower uterine segment/isthmus	9	52.9	3	21.4

** More than one response was allowed for this question*

Table 3: dMMR testing approach and use of secondary tests in private & public laboratories

	Private (n=19)	**Public (n=18)**	***p-value**
Modality used for dMMR testing			*0.376*
IHC	*84.2% (16)*	*72.2% (13)*	
IHC+MSI	*15.8% (3)*	*27.8% (5)*	
Use of *MLH1* promoter methylation			*0.927*
CRC only	*5.3% (1)*	*11.1% (2)*	
CRC+EC	*5.3% (1)*	*5.6 % (1)*	
No	*73.6% (14)*	*66.7% (12)*	
Unknown	*15.8% (3)*	*16.6% (3)*	
Use of *BRAF* V600E			*0.952*
CRC only	*36.8% (7)*	*39% (7)*	
CRC+EC	*10.5% (2)*	*16.6% (3)*	
No	*31.6% (6)*	*27.8% (5)*	
Unknown	*21.1% (4)*	*16.6% (3)*	
Capacity for tumor and/or germline DNA testing			*0.858*
Yes	*21% (4)*	*22.2% (4)*	
No	*52.6% (10)*	*44.4% (8)*	
Unknown	*26.3% (5)*	*33.3% (6)*	

Table 4: Current Australian recommendations for dMMR testing of Colorectal Cancer

Institution	Date	Recommendation	Link
Royal College of Pathologists of Australasia	May 2016, 3rd Edition (Version 3.0).	For the purposes of detecting individuals with Lynch syndrome (HNPCC), MMR testing is currently recommended as the initial screening procedure. At a minimum all cases of colorectal cancer arising in individuals less than 50 years of age should be tested. In addition, all cases meeting the revised Bethesda guidelines should be tested.	https://www.rcpa.edu.au/getattachment/3942f934-6d8c-4a84-a43c-476d0e8c3d0e/Protocol-colorectal-cancer.aspx
NHMRC/Cancer Council Australia - Colorectal cancer	Nov-17	Immunohistochemical testing for the four MMR proteins (MLH1, MSH2, MSH6 and PMS2) is now widely available, and universal testing of colorectal cancers (or at least in patients under the age of 70) has been recommended for the detection of Lynch syndrome.	http://wiki.cancer.org.au/australiawiki/index.php?oldid=173069, cited 2017 Nov 15
NHMRC/Cancer Council Australia - Lynch Syndrome	Nov-17	All colorectal cancers should be tested for mismatch repair deficiency as a means to subsequently identify Lynch syndrome	http://wiki.cancer.org.au/australiawiki/index.php?oldid=173055, cited 2017 Nov 15

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ACT	0	0	0	2	1	0	0
NT	0	0	0	1	0	1	0

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