Personalized Therapeutic Report- Focused

Test Description

It is an integrated report of multiplatform testing including comprehensive genomic profiling (56 gene panel) and tumor mutation burden (TMB) using next generation sequencing (NGS), microsatellite instability (MSI) using molecular beacon probe-based multiplex polymerase chain reaction (7 biomarkers) and PD-L1 expression using immunohistochemistry.

Patient Demographic

Name: Tejram Mathur
Sex: Male
Date of Birth/Age: 65 years
Disease: Signet ring cell carcinoma, Carcinoma Colon

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Max Hospital
Pathologist: Not Provided

Specimen

Booking ID: 011911070060
Site: Rectal Hemicolectomy
Sample Type: FFPE block (1), SB 2598/18
Date of Collection: 07-11-2019
Date of Booking: 07-11-2019

CLINICAL SYNOPSIS

Signet ring cell carcinoma of caecum [as per the histopathology report dated 05.09.2018]. The tumor was identifiable in the block [SB 2598/18] and it was adequate for further analysis.

RECOMMENDATION & REPORT INTERPRETATION

Clinically relevant mutations were identified in the *KRAS* gene with MSI-High. Resistance to anti-EGFR treatment may be predicted. Immunotherapy (Check point inhibitors) may be useful.

Disclaimer: *Report interpretation & recommendation(s) should not be considered as final; and should be used at the discretion of the treating Physician or the molecular tumor board. The report interpretation & recommendation(s) does not bear any medical, legal, ethical & moral responsibilities, and liabilities.*

BIOMARKERS

Targeted Therapy

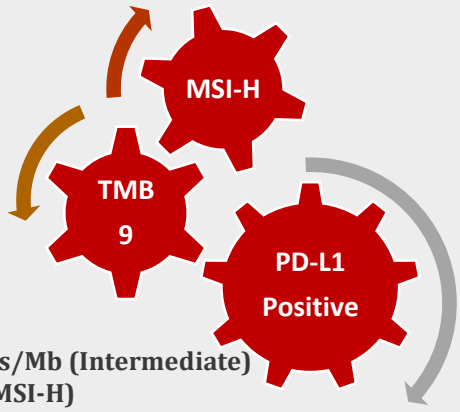
Genomic Findings

Clinically Significant

KRAS ***p.Gly12Asp,*** Exon 2
TP53 ***p.Arg213Ter,*** Exon 6; ***p.Arg248Trp,*** Exon 7
PTEN ***p.Arg130Gln,*** Exon 5; ***p.Glu91Ter,*** Exon 5

Variant of Unknown Significance
KRAS ***p.Leu120Ser,*** Exon 5
PIK3CA ***p.Arg88Gln,*** Exon 2
ATM ***p.Arg2719His,*** Exon 56

Immunotherapy



TMB **9 muts/Mb (Intermediate)**
MSI-High **(MSI-H)**
PD-L1 **Positive**

TABLE 1: GENOMIC ALTERATIONS THAT CAN BE TARGETED WITH APPROVED DRUGS IN THE SUBJECT'S TUMOR TYPE

Gene (Transcript) #	Location	Variant	Overall Depth	Mutant Allele Percentage	Function of the Gene in Cancer
KRAS (ENST00000256078.4)	Exon 2	c.35G>A (p.Gly12Asp)	7092X	3.9%	Oncogene

TABLE 2: NON-DRUGGABLE/DRUGGABLE CLINICALLY SIGNIFICANT GENOMIC ALTERATIONS INDICATED IN OTHER TUMORS

Gene (Transcript) #	Location	Variant	Overall Depth	Mutant Allele Percentage	Function of the Gene in Cancer
TP53 (ENST00000269305.4)	Exon 6	c.637C>T (p.Arg213Ter)	17254X	1.5%	Tumor Suppressor
TP53 (ENST00000269305.4)	Exon 7	c.742C>T (p.Arg248Trp)	88190X	2.8%	Tumor Suppressor
PTEN (ENST00000371953.3)	Exon 5	c.389G>A (p.Arg130Gln)	7186X	4.7%	Tumor Suppressor
PTEN (ENST00000371953.3)	Exon 5	c.271G>T (p.Glu91Ter)	3112X	1.6%	Tumor Suppressor

TABLE 3: VARIANTS OF UNKNOWN SIGNIFICANCE

Gene (Transcript) #	Location	Variant	Overall Depth	Mutant Allele Percentage	Function of the Gene in Cancer
KRAS (ENST00000256078.4)	Exon 4	c.359T>C (p.Leu120Ser)	9552X	4.2%	Oncogene
PIK3CA (ENST00000263967.3)	Exon 2	c.263G>A (p.Arg88Gln)	8305X	1.7%	Oncogene
ATM (ENST00000278616.4)	Exon 56	c.8156G>A (p.Arg2719His)	1021X	7.4%	Tumor Suppressor

CLINICAL CORRELATION AND VARIANT INTERPRETATION

KRAS **p.Gly12Asp** **c.35G>A** **Oncogene**
Overall depth: 7092X **Mutant Allele Percentage: 3.9%**

KRAS gene, a guanine nucleotide (GDP/GTP) binding protein, is a member of the human *ras* family required for various cellular process including normal development and growth. In many cancers, somatic mutations in KRAS gene lead to its constitutive activation. A missense variation (**chr12:g.25398284C>T; c.35G>A**) that leads to an amino acid substitution at codon 12 (**p.Gly12Asp**) was detected in the *KRAS* gene of this subject. This variant has been reported in ExAC database with MAF of 0.002% and not reported in 1000 genomes database.

The frequency of *KRAS* mutations in colorectal cancer (CRC) is 36-40% [15, 16] and the frequency of this particular variant in *KRAS* mutated colorectal cancers is 7.9%[1]. Retrospective and prospective analysis of two-Phase III trials (PRIME and FIRE) established the role of extended RAS testing (includes screening for HOTSPOT mutations in *KRAS* exon 2, 3, 4 and *NRAS* exons 2, 3, 4) and its clinical significance as a predictive tool for decisions related to anti-EGFR therapy in patients with metastatic CRC[2-4]. Presence of somatic activating mutations in *KRAS* gene predicts lack of response to anti-EGFR therapy in metastatic CRC patients[5,6]. In a study on 2559 patients enrolled in the PETACC-8 trial, tumor blocks of resected stage III colon adenocarcinoma showed *KRAS* mutation frequency of 33.1% (588 of 1776) [23]. *KRAS* mutation was significantly associated with shorter disease-free survival (DFS; P<0.001) and overall survival (OS; P=0.008) [23]. The subgroup analysis showed in patients with microsatellite-stable tumors that KRAS (P<0.001 for DFS; P=0.002 for OS) was independently associated with worse clinical outcomes[7]. Kindly correlate clinically.

TP53 **p.Arg213Ter** **c.637C>T** **Tumor Suppressor Gene**
Overall depth: 1725X **Mutant Allele Percentage: 1.5%**

TP53 gene encodes P53 tumor suppressor protein which is involved in DNA damage repair and regulation of cell division and apoptosis. Loss of function (LOF) mutations in P53 has been reported in >50% of human cancers. In addition to LOF, gain of function (GOF) mutations endow cancer cells with more malignant properties.

A nonsense variant (**chr17:g.7578212G>A; c.637C>T**) that leads to termination at codon 213 (**p.Arg213Ter**) and a missense variant (**chr17:g.7577539G>A;c.742C>T**) that leads to amino acid substitution at codon 248 (**p.Arg248Trp**) were detected in the *TP53* gene of this subject and both the variants are loss of function mutation. These variants are reported in the ExAC database with MAF of 0.0008% and are not reported in 1000 genomes and databases.

TP53 mutations are one of the commonly occurring events in the classical chromosomal instability pathway of colorectal carcinogenesis that involves activation of *RAS* oncogene and inactivation of suppressors *TP53* and *APC*[8]. CRC patients with mutant *TP53* tend to have more chemo-resistance and poorer prognosis than those with wild-type *TP53*[9,10].

A combined analysis of the QUASAR 2 (A phase 3 clinical trial) and Australian cohorts (n=807) with stage II or stage III colorectal cancer, showed that mutations in *KRAS*, *BRAF*, and *TP53*, and lower mutation burden were all independently associated with poor prognosis, whereas micro satellite instability was not[11]. Please correlate clinically.

PTEN **p.Arg130Gln** **c.389G>A** **Tumor Suppressor Gene**
Overall depth: 7186X **Mutant Allele Percentage: 4.7%**

This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded by this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. *PTEN* is a multi-functional tumor suppressor that is very commonly lost in human cancer. Observed in prostate cancer, glioblastoma, endometrial, lung and breast cancer to varying degrees. Up to 70% of prostate cancer patients have been observed to have loss of expression of the gene. It is a part of the PI3K/AKT/mTOR pathway and mTOR inhibitors have been relatively ineffective in treating patients with *PTEN* loss.

A missense variant (**chr10:g.89692905G>A; c.389G>A**) that results in amino acid substitution at codon 130 (**p.Arg130Gln**) and a nonsense variant (**chr10:g.89692787G>T;c.271G>T**) that resulted in protein truncation at codon 91 (**p.Glu91Ter**)

were detected in the *PTEN* gene of this subject. These are loss of function mutations. This variant has not been reported in ExAC and 1000 genomes databases.

PTEN mutations constitutes 5-14% of colorectal cancers and inactivating mutations are known confer reduced response to anti-EGFR therapy[12]. Kindly correlate clinically.

Note: These particular mutations have been detected at a mutant allele percentage below the limit of detection of this assay for the SNPs or missense mutations. Please correlate with other clinical findings.

ADDITIONAL FINDINGS

Variants of unknown significance (VUS) in genes relevant in cancer

Table 3 provides a list of VUS in genes known to function as oncogene or tumor suppressor or relevant in epigenetic regulation in human cancers. These variants specifically detected in this tumor have not been characterized sufficiently in biochemical assays and therefore their impact in this cancer remains speculative.

Note:

- The ***KRAS*** gene variant **p.Leu120Ser** has not been reported in ExAC and 1000 genomes databases. This variant is predicted to be damaging by SIFT, LRT and probably damaging by Polyphen2 prediction tools.
- The ***PIK3CA*** gene variant **p.Arg88Gln** has not been reported in ExAC and 1000 genomes databases. This variant is predicted to be damaging by LRT and probably damaging by Polyphen2 prediction tools.
- The ***ATM*** gene variant **p.Arg2719His** has been reported in ExAC and 1000 genomes databases with MAF of 0.017% and 0.04% respectively. This variant is predicted to be damaging by SIFT, LRT and probably damaging by Polyphen2 prediction tools.

The functional impact and clinical significance of the above-mentioned variants in the tumor type of the subject under investigation has not been not well documented in medical literature. Hence, these variants have been included in the Table 3 as VUS. Please correlate clinically.

Note: These particular mutations have been detected at a mutant allele percentage below the limit of detection of this assay for the SNPs or missense mutations. Please correlate with other clinical findings.

RECOMMENDATIONS

Correlation of the genetic findings with the clinical condition of the patient is required to arrive at accurate diagnosis, prognosis or for therapeutic decisions.

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APPENDIX 1: TEST METHODOLOGY

Background

The next-generation sequencing based multi-gene analysis, allows us to sequence and identify variants associated with multiple genes with diagnostic, prognostic and therapeutic implications in different cancer types. This tumor somatic panel in investigation, has been designed to screen for somatic mutations in 56 cancer related genes associated with tumorigenesis, prognostication and predictive value for chemotherapy and targeted therapy drugs in different tumor types. Targeted sequencing represents a cost-effective approach with the ability to detect specific variants causing protein-coding changes in individual human genomes. These multi-gene, affordable tests will enable personalized treatment by matching the patient's tumor with the appropriate drug, based on the mutational findings.

Method

Tumor genomic DNA isolated from FFPE was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced with a panel mean coverage depth of **35311X**, on Illumina sequencing platform. The sequences obtained were aligned to human reference genome (GRCh37/hg19) using BWA program[1,2]. Somatic mutations were identified using LoFreq (version 2) variant caller[3,4]. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. The mutations were annotated using our in-house annotation pipeline (VariMAT). Gene annotation of the variants was performed using VeP program[5] against the Ensembl release 90 human gene Model[6]. Clinically relevant mutations were annotated using published literature, databases and in-house propriety databases. The common variants were filtered for reporting based on the presence in various population databases (1000G, ExAC, EVS, 1000Japanese, dbSNP, UK10K[7-12]. Reportable mutations are prioritized and prepared based AMP-ASCO-CAP guidelines[13] based on annotation metrics from OncoMD[14], reference lab's curated somatic database which includes somatic mutations from TCGA. Possibility of false negative or false positive below the limit of detection of this assay cannot be ruled out.

The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 90 human gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.

"This test was developed, and its performance characteristics determined by Reference lab".

DISCLAIMER

- The classification of variants of unknown significance can change over time. Please contact MolQ laboratory at a later date for any change.
- The scope of this assay limits to SNPs, Short Indels (in DNA) and gene fusions and splice variants (in RNA)
- Intronic variants are not assessed using this method.
- Large deletions of more than 20 bp or copy number variations / rearrangements cannot be assessed using this method.
- This panel is intended to screen for hotspot mutations only.
- The mutations have not been validated by Sanger sequencing.
- This NGS panel is not intended to report germline variants.
- This NGS test used does not allow definitive differentiation between germline and somatic variants
- TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION.
- A false negative result for any variant below the LOD, i.e., 5% for SNVs and 10% for short indels, cannot be ruled out.

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APPENDIX 2: GENE LIST WITH COVERAGE

S. No.	Gene	Exon Number**	Coverage (%)
1.	*EGFR*	EX 3; EX 7; EX 15; EX 18: G719X; EX 19: DELETIONS; EXON 20: S768I, INSERTIONS, T790M; EX 21: L858R, L861Q	100.00
2.	*KRAS*	EX 2, 3, 4 [CODONS 12, 13, 61, 117, 146]	100.00
3.	*NRAS*	EX 2,3,4 [CODONS 12, 13, 61, 117, 146]	100.00
4.	*HRAS*	EX 2, 3 [CODONS 12, 13, 61]	100.00
5.	*BRAF*	EX 15/L597R/Q/S/V/V600E/K/L/R: K601E: G466V (EX 11)	100.00
6.	*MAP2K1/ MEK1*	EX 2,3,6,7,11 [F53L, I111S, C121S, P124S, P124L, E203K, P264S, N382H, Q56P, K57N, D67N]	100.00
7.	*PIK3CA*	EX 2; EX 5; EX 7; EX 8; EX 10: [E542X,E545X,Q546X,D549N]; EX 14; EX 19; EX 21 [H1047L/R]	100.00
8.	*AKT1*	EX3/E17K	100.00
9.	*PTEN*	EX 1, 2, 3, 4, 6, 7, 8, 9/EX 5: [R130G/*/Q/FS*4,R159S] EX 7: [R233*, K267FS*, P248FS*5], EX 8: [N323FS*2, N323FS*21]	100.00
10.	*KIT*	EX 2, 10, 15; EX 9: A493, Y 494;	100.00
	KIT	EX 11: W557R, V559A/D, V560D, L576P; EX 13: K642E,V654A; EX 14: T670I; EX17: D816H/V, D820X, N822X, Y823D,	100.00
	KIT	EX 18 [A829P]	100.00
11.	*PDGFRA*	EX 12: V561D; EX 14: N659K; EX 15; EX 18 WT	100.00
		EX 18: D846V	100.00
12.	*DDR2*	EX 18: S768R	100.00
13.	*ERBB2*	EXON 20 INSERTIONS (778-780 INS)	100.00
	ERBB2	EX 8(G309A); EX 19(L755S); EX 19 755 TO 759 DELETIONS; EX 19(D769H/Y); EX 20(V777L); EX 21(V842I);	100.00
14.	*IDH1*	EX 4 (R132X)	100.00
15.	*IDH2*	EX 4 (R172X), R140X	100.00
16.	*ALK*	EX 23 AND EX 25/F1174C/R1275Q/Y1278S	100.00
17.	*GNA11*	EX 4, 5 [R183C, Q209L/P]	100.00
18.	*GNAQ*	EX 4, 5 [R183Q, Q209L/P/R]	100.00
19.	*TSC1*	EX 15: E636FS	100.00
20.	*RET*	EX 10, 11(C634W/R/Y), 13,15, 16(M918T)	100.00
21.	*TP53*	ALL EXONS	100.00
22.	*VHL*	EX 1; EX 2; EX 3	100.00
23.	*APC*	EX 16	100.00
24.	*ATM*	EX 8,9,12,17,26,34,35,36,39,50,54,55,56,59,61,63	100.00
25.	*CDH1*	EX 3, 8, 9	100.00
26.	*CDKN2A*	EX 2	100.00
27.	*CSF1R*	EX 7, 22	100.00
28.	*CTNNB1*	EX 3/S37F/Y; S45P/F/Y	100.00
29.	*DNMT3A*	EX 23	100.00
30.	*ERBB4*	EX 3, 4, 6, 7, 8, 9, 15, 23	100.00
31.	*FBXW7*	EX 5, 8, 9, 10, 11	100.00
32.	*FGFR1*	EX 4, 7	100.00
33.	*FGFR2*	EX 5, 7, 10	100.00
34.	*FGFR3*	EX 7, 9, 14, 16, 18	100.00
35.	*FOXL2*	EX 1	100.00
36.	*GNAS*	EX 8 , EX 9	100.00

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37.	*HNF1A*	EX 3, 4	100.00
38.	*KDR*	EX 6, 7, 11, 19, 21, 26, 27, 30	100.00
39.	*MET*	EX 2, 11, 14, 16, 19	100.00
40.	*MLH1*	EX 12	100.00
41.	*MSH6*	EX 4	100.00
42.	*PTPN11*	EX 3, 13	100.00
43.	*RB1*	EX 4, 6, 8, 10, 11, 14, 17, 18, 20, 21, 22, 23	100.00
44.	*STK11*	EX 1, 4, 6, 8	100.00
45.	*SMAD4*	EX 3,4,5,6,8,9,10,11,12	100.00
46.	*SMARCB1*	EX 2, 4, 5, 9	100.00
47.	*SMO*	EX 3, 5	100.00
48.	*SRC*	EX 14	100.00
49.	*ABL1*	EX 4, 5, 6, 7	100.00
50.	*EZH2*	EX 16	100.00
51.	*FLT3*	EX 11, 14, 16, 20	100.00
52.	*JAK2*	EX 14, 16	100.00
53.	*JAK3*	EX 10, 13, 14	100.00
54.	*MPL*	EX 10	100.00
55.	*NOTCH1*	EX 26, 27, 34	100.00
56.	*NPM1*	EX 11	100.00

iMSI Rapid™ Assay

MSI testing is used for Hereditary Cancer screening (Hereditary Non-Polyposis Colorectal Cancer -HNPCC or Lynch syndrome); As a biomarker (Prognostic and predictive biomarker for the response of Immunotherapy)

Result — Microsatellite - High (MSI-H)

BIOMARKER FINDINGS

ACVR2A	Mutation detected
BTBD7	No mutation detected
DIDO1	Mutation detected
MRE11	No mutation detected
RYR3	No mutation detected
SEC13A	No mutation detected
SULF2	No mutation detected

INTERPRETATION

*MSS	<2 of the 7 markers demonstrate instability
#MSI-H	≥2 of the 7 markers demonstrate instability

*Microsatellite stable

Microsatellite Instability-High

For valid batch test results specific controls are being run with every batch.

METHODOLOGY

Multiplex detection of seven mononucleotide repeats using molecular beacon probe-based polymerase chain reaction followed by high resolution melt-curve analysis. The assay uses seven novel biomarkers *ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR3*, *SEC31A* and *SULF2* as this set of biomarkers is stable over different cancer types and ethnicities and show high performance than other known assays like *Bethesda Panel.* This test is carried out on Idylla platform using the MSI/1.0 Cartridge based kit which is CE IVD approved.

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Programmed Death Ligand 1 (PD-L1) Immunohistochemistry

Test Description

This test is useful for identification of neoplasms expressing programmed cell death 1-ligand 1 (clone SP263). PD-L1 also known as B7 homolog 1 (B7-H1) or CD274, is a transmembrane protein involved in the regulation of cell-mediated immune responses through interaction with the receptor programmed death protein-1 (PD-1). PD-L1 has been identified as both a prognostic and theranostic marker in a variety of neoplasms. Overexpression of PD-L1 has been observed in carcinomas of the urinary bladder, lung, thymus, colon, pancreas, ovary, breast, kidney, and in melanoma and glioblastoma.

Specimen

Sample Type: FFPE Block SB 2598/19
Site: Rectum Hemicolectomy
Pathology ID: MOLQ/IHC-42112019
Disease: Metastatic Carcinoma Colon

Interpretation

The scoring system is based on type and origin of tumor. If additional interpretation or analysis is needed, send request for Pathology Consultation.

Methodology

Immunostaining for PD-L1 protein was done using Ventana Rabbit Anti-Human PD-L1/CD274 Monoclonal Antibody (Clone SP-263) on Ventana Autostainer.
Positive PD-L1 staining/expression is defined as complete and/or partial, circumferential or linear plasma membrane or cytoplasmic staining at any intensity that can be differentiated from background.

Note

Preclinical studies suggest that positive programmed cell death 1-ligand 1 (PD-L1) immunohistochemistry in tumor cells may predict tumor response to therapy with immune checkpoint inhibitors. This result should not be used as the sole factor in determining treatment, as other factors (eg, tumor mutation burden and microsatellite instability) have also been studied as predictive markers.

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Programmed Death Ligand 1 (PD-L1): Negative

Microscopy Evaluation

HE Staining (Figure 1)
Tumor cells: 25%
Immune cells: 30%

Tumor cells positive for PD-L1: 10 % (Moderate immunostaining)
Immune cells positive for PD-L1: 04% (Mild Immunostaining)

HE Stained Section

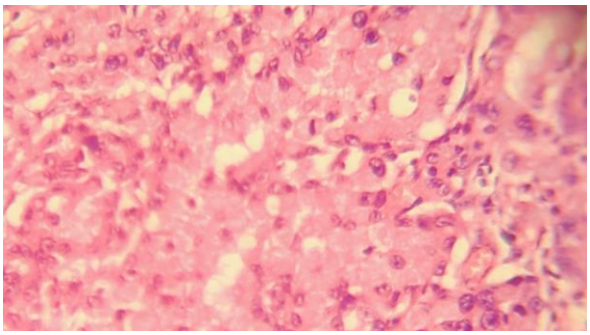

Figure 1

PD-L1 IHC- Tumor Cells

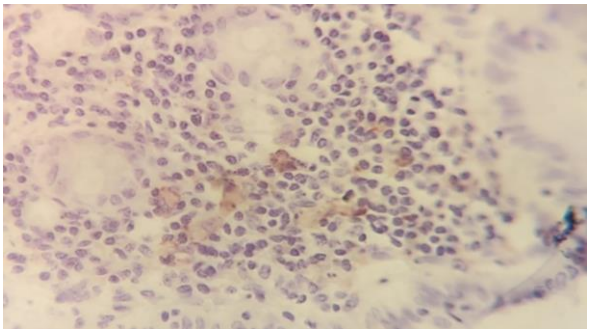

Figure 2

PD-L1 IHC- Immune Cells

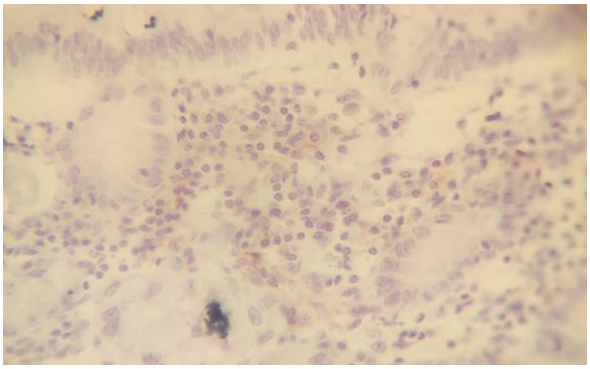

Figure 3