List of ten best papers, highlighting the important discoveries/contributions described in them briefly

1. The research work entitled **"Assessment of clinically suspected tubercular lymphadenopathy by real time PCR compared to non-molecular methods on lymph node aspirates"**, published in Acta Cytologica 2017 contributed in clinical research in area of tuberculosis.

Tuberculosis (TB) is one of the greatest killers worldwide and continues to threaten the human race since time immemorial not only due to its effect as a medical problem, but also by its impact as a social and economic tragedy. TB accounts for 9.6 million new cases and 1.5 million deaths in 2014. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top 5 causes of death among women aged 15 to 44 years. The six countries that stand out as having the largest number of incident cases in 2014 were India, Indonesia, Nigeria, Pakistan, People's Republic of China and South Africa. India accounts for 23% of global tuberculosis incidence with an estimated 2.2 million cases reported in 2014.

TB can occur as pulmonary tuberculosis or extra- pulmonary tuberculosis (EPTB). EPTB tuberculosis comprise for one fifth of all the cases of tuberculosis and tubercular lymphadenitis (TBLN) accounts for 20-40 % cases of EPTB. TBLN is not always associated with the common presenting symptoms of TB like fever, cough, weight loss, fatigue and night sweats. Patients often present only with enlarged lymph node to the clinician wherein they are prescribed initial two weeks of antibiotic treatment. If the enlarged lymph node does not regress after treatment, there is suspicion of TBLN and such cases are referred to pathologist for investigations like Fine Needle Aspiration Cytology (FNAC) or biopsy for histopathology.

Cytology/Histopathology evidences of TBLN is taken from the stained smears or sections screened for epithelioid cell granuloma with or without multinucleated giant cell and

caseation necrosis. Conventional, diagnostic methods like Ziehl Neelsen (ZN) staining and culture for lymph node aspirates or biopsy can help to provide the evidence of tuberculosis by detecting Mycobacterium tuberculosis bacilli, but ZN staining lacks sensitivity and specificity, and culture is time consuming. The diagnostic difficulty is further enhanced as the TBLN samples are paucibacillary. The real time PCR for detection of Mycobacterium tuberculosis complex (MTBC) are rapid, sensitive and specific, and can be used on lymph node aspirates providing evidence of tubercular lymphadenitis.

The research work demonstrated comparison of diagnostic performance of cytology and PCR alone and in combination to culture as the gold standard is depicted in table 1.

Table 1: Concordance of Diagnostic Modalities with culture as Gold Standard

	Cytology	PCR	Any Diagnostic Modality	Combined Diagnostic Modality
Positive agreement	28%	40%	39%	20.6%
Negative agreement	38%	34%	36%	39.7%
Concordant	66%	74%	75%	60.3%
False Positive	10%	15%	13%	9.0%
False Negative	24%	11%	12%	30.7%
Pearson Chi-square	21.87	41.86	45.66	10.69
Phi	0.34	0.47	0.49	0.24

N=189; all Pearson Chi-square statistics are significant at $P \leq 0.0005$

Positive agreement in PCR was 40% and in cytology was 28%, PCR therefore diagnosed additional 12% of positive cases which were missed by cytology thus had better diagnostic power than that of cytology, overall.

The concordance rate was high for any one of the diagnostic modality either cytology or PCR being positive at 75%. It was lowest when cytology and PCR were combined with concordance of 60.3%. The false positive cases by PCR were 15% and reduced to 9% for combined category.

For the combined category, it implies that two of five TB patients undergoing FNAC and PCR could be placed on appropriate and immediate ATT. At the later stage of patient so medicated, two of the five would not be confirmed on traditional culture as harboring active mycobacterial infection.

For the category of any diagnostic modality being positive, the clinical implication is such that four of five TB patients undergoing FNAC or PCR could be placed on appropriate and immediate Anti tubercular treatment (ATT). At the later stage of patient so medicated, two of the five would not be confirmed on traditional culture as harboring active mycobacterial infection.

Pearson Chi-square test was highly significant at $P \leq 0.0005$ suggesting a strong relationship between culture and all the tested categories or detection of TBLN. Strong degree of positive association was observed between PCR and culture results (Phi 0.47) and PCR or cytology and culture results (Phi 0.49) indicating that both the categories measure the percent of patients tested having TBLN.

Real time PCR for MTBC on aspirates proved to be a useful molecular investigation in clinically suspected tubercular lymphadenopathy and offers a definitive and comparable diagnosis. This obviates the need for lymph node biopsy had PCR for MTBC been included in

diagnostic algorithm for the evaluation for TBLN and will reduce the burden of tuberculosis. Based on the findings, the diagnosis of TBLN cases also need a multidisciplinary approach involving Pathology, Microbiology, Molecular Biology and other clinical specialties for evaluating the lymph node.

2. The research work **"Diagnosis of Clinically Suspected and Unsuspected Tubercular Lymphadenopathy by Cytology, Culture and Smear microscopy"** published in Indian Journal of Tuberculosis 2017, aimed to study that Fine Needle Aspiration Cytology (FNAC) combined with Zeihl Neelsen stain and culture for Mycobacterium tuberculosis bacilli can improve the diagnostic accuracy in patients clinically suspected and unsuspected for tubercular lymphadenitis. The research demonstrated that cytology combined with culture improves the diagnostic accuracy in cases with enlarged lymph nodes suspected or unsuspected for tuberculosis.

3. Another research work **"Clinical and cytological features in diagnosis of peripheral tubercular lymphadenitis – a hospital based study from central India"**, published in Indian Journal of tuberculosis 2017, aimed to describe the symptoms, clinical characters and results of cytology analysis in tuberculous lymphadenitis patients to assess their diagnostic value in cohort of patients.

The research work demonstrated that constitutional symptoms, clinical and cytological features reported help in diagnosing peripheral tubercular lymphadenitis cases and also open new frontiers to further study the immune reactions taking place at cellular and molecular levels, affecting the nature of aspirate and cytomorphological spectrum seen as granuloma and caseous necrosis in these cases.

4. Research work **"Molecular Diagnosis of Tubercular Lymphadenopathy from Fine-Needle Aspirates in Pediatric Patients"**, published in Acta Cytologica 2017.

Peripheral tubercular lymphadenopathy (TBLN) in pediatric age group is often associated with non-specific symptoms and deceptive clinical signs. The research work "Molecular Diagnosis of Tubercular Lymphadenopathy on Fine Needle Aspirates in Pediatric patients", concluded that that PCR is useful molecular method for detection of MTBC in pediatric patients with high sensitivity, specificity and accuracy on aspirates of clinically suspected and unsuspected Tubercular Lymphadenitis cases. TBLN in pediatrics population is frequently missed by FNAC of lymph nodes, due to cell mediated immune response, PCR has proved to be highly effective in diagnosis of such cases.

5. Research work by Dr. Vivek Gupta entitled **"Reactive Lymphoid Hyperplasia or Tubercular Lymphadenitis: Can Real time PCR on Fine Needle Aspirates help Physician in concluding the diagnosis?",** made significant scientific contribution towards major health problem.

This research work focused on detection of tubercular lymphadenitis at an early stage. The research work included adult population. It concluded that the asymptomatic patients with lymphadenopathy on FNAC of lymph nodes frequently revealed cytomorphology of reactive lymphoid hyperplasia. The research study also concludes that interventive investigation of such cases by RT-PCR for Mycobacterium Tuberculosis on Fine Needle Aspiration is offering the definitive and comparable diagnosis of TBLN. This helps in arriving at a diagnostic conclusion.

The policy makers over the world especially the developing and third world countries may control TB had the RT-PCR for MTBC been included as primary investigation on the

aspirates by simple technique of FNAC that would help to contain the world vide problem of TB.

Research work made the following scientific contributions:

The research work numbered 4 and 5 published is the first search reports all over the world. Such research have never been done earlier by any researcher in any scientific community. The points below summarizes the scientific contribution and translatory component of this clinical research.

A. Lymphadenopathy is the most common Extra pulmonary tuberculosis (EPTB) manifestation in the children and the school health survey may endorse it in India. Lymphadenopathies that remain unsuspected of TB due to multiple factors at the level of family of the concerned and at the community level because of the lack of health education and paucity of health services, in children if surveyed by molecular diagnostic technique for TB would reduce the morbidity in paediatric as well as future adult TB burden in India. Therefore, lymphadenopathy in the children should be brought to this arena of work that would enable early case detection of EPTB and its treatment.

B. The cellular immunity in the extreme ages of life is physiologically poor, the granuloma formation is markedly absent, therefore TB PCR on aspirates of FNA would option for the diagnosis of TB in avoidance of further morbidity and complication.

C. The molecular epidemiology of the mycobacteria species is wide, the emergence of new strains in the species had also been noted worldwide by different studies conducted in different continents, an effort increasing sensitivity and specificity of TB PCR is needed. A technical consideration is comprehensively needed at probe making of mycobacterium tuberculosis detection. The results of present study through some cases are highly suggestive of some strains of mycobacterium species which elutes the detection of presently available TB PCR probes.

This research suggests that the important technical viewpoint is needed which would be adopted by molecular laboratories while making not the selective but comprehensive blending of nucleic acid sequences of mycobacterium tuberculosis to widen the scope in translatory research for the highest benefit of community.

D. The best way to treat EPTB is to prevent its occurrence, which if not possible, then early case detection of such cases in absence of laboratory and clinical evidence is must. TB PCR of EPTB especially including TBLN cases as inferred from the present research will solve the problem by early case detection of these cases. This will eventually be a step at killing TB; the medical community fears most.

6. The research work by Dr. Vivek entitled **"Triple positive breast cancer: A case series",** published in European Journal of Molecular & Clinical Medicine in 2021, scientifically contributes towards a unique category of cancers. Breast cancer is the most common malignancy in women around the world, accounting for 25.1 % of all cancers among women. The molecular tumor profiling has enabled detailed classification based on gene expression, each category with a distinct management. There are five subtypes of breast cancer in molecular classification: Luminal A (hormone receptor positive, HER2-, lower proliferating index), Luminal B (lower level of positive estrogen or progesterone receptor negative, sometime with HER2+, higher proliferating index), HER2+, basal-like subtype (usually triple negative) and claudin-low subtype, less reproducible defined yet (usually triple negative BC). The standard treatment of Breast cancer differs in between the molecular subtypes. Luminal A and B subtype treatment is primarily by hormone-based therapy, that of Triple negative/Basal like is based on chemotherapy and that of HER2+ve is monoclonal antibody.

However, cases with all three receptor positive 'Triple Positive' are rare. The research work highlights patients diagnosed as invasive ductal carcinoma with Immunohistochemistry (IHC) positive for all three receptors (Hormone receptors and human epidermal growth factor receptor). Such cases were earlier never reported in Indian literature.

The research work contributes towards assessment of this new distinct subtype of triple positive breast cancer with HR+ and HER2+ that may raises a series of problem concerning its treatment. It is also discussed by his group that HER2 blocking agents are effective in patients with HER-2-positive disease, irrespective of HR status. Molecular classification plays an increasingly essential role in the personalized care of breast cancer, and the three key molecular determinants, HER2, ER and PR, needs to be evaluated in routine clinical practice.

7. Further the research work by Dr. Vivek "**Impact of RASSF1A gene methylation on clinic-pathological features of tumor and non-tumor tissue of breast cancer",** published in Annals of Diagnostic Pathology year 2021 vol 52, elucidated the mechanism how epigenetic changes cause breast cancer?

The research work contributed significantly in clinical research in area of breast cancer. Today breast cancer is the most common cancer in women and is of major concern in women health as it causes heavy mortality and morbidity and also poses a heavy burden on medical health. Yet mechanism and events leading to breast cancer are poorly understood.

Dr. Vivek established direct relationship of breast cancer with epigenetic changes in RASSF1A tumor suppressor genes. The RASSF1A tumor suppressor gene was hypermethylated at promoter regions in tumor tissues as compared to normal tissue in same patient. The methylation at promoter at this gene was associated with breast tumor size and grade. The research work enrolled patients in stage I to III of breast cancer with meaningful

outcome. Methylation at promoters increases with cancer staging and correlated with the histological grade.

This research was first of its nature published from India. The research contributed to clinical sciences in a way that could be useful in assessing cancer progression. It also provided insight for new biomarkers for early detection and/or disease monitoring thereby influencing therapeutic decisions. The research also paved pathway for exploring possibility of methylation in other tumor suppressor gene that may also be leading to breast cancer.

8. Another research work entitled **"Role of Ki 67 In Pathological Prognostic Staging of Breast Cancer",** done on breast carcinoma a leading malignancy around the world among women demonstrates the judicious use of biomarkers or combinations of biomarkers that plays important role in prognosis. Biomarkers are now believed to be essential in guiding therapy and providing personalized care to the patients. The work demonstrates Ki 67 is one of the markers which reflect the proliferative index of tumour cells and it also helps to determine response to chemotherapy. Ki67 can be a good prognostic marker as it reflects high proliferative potential in breast cancer. The study established the correlation between clinicopathological factors, expression of Ki67 with pathological staging in different molecular subtypes of breast cancer for its prognostication. The study described gross features of resected breast and histopathological section stained by H and E stain and immunohistochemical stain were also studied. It was found in study that Ki 67 expression is expected to be more in in highly metastasizing, in tumors of high grade and tumors of relatively large sizes. In conclusion the study will be helpful to establish the correlation between clinicopathological factors and expression of Ki67 in molecular subtypes of breast cancer for its prognostication.

9. The research work by Dr. Vivek entitled **"To assess the utility of Proliferative marker Ki-67 in surface epithelial ovarian tumor",** published in Journal of Datta Meghe Institute of Medical Sciences, 2019, contributed towards women health. Ovarian cancers is one of the most common cancer affecting around 30% of all gynecological cancers. It affects mostly women in reproductive age and elderly women. More than 70% of women diagnosed with ovarian carcinoma are in advanced stage due to asymptomatic nature and lack of reliable markers for early diagnosis. Cellular proliferation plays a significant role in clinical behavior and aggressiveness of ovarian tumor.

The research work explores the Ki-67 marker (an immunohistochemical marker) to be used as a cellular proliferative marker in ovarian cancer. Ki-67 is expressed in all active phases of cell cycles (G1, S, G2 and M phase) and not in resting cells (quiescent gaps). The research on 74 cases of ovarian cancer showed high proliferation of Ki-67 in resected specimens. A highly significant correlation also existed in between Ki-67 and CA-125 (an established marker for ovarian carcinoma) $r = 0.505$; $p = 0.008$. The benign tumors did not showed Ki-67 proliferation. Thus, the research work contributed in establishing Ki-67 expression in ovarian cancer cells that helps in diagnosis, prognosis and predicting response to treatment. The research further contributed towards understanding the biological behaviour of ovarian cancers which has implications in modifying treatment strategy.

10. Another two research work contributed immensely towards containment of COVID 19 pandemic. The research used molecular testing tools to combat COVID 19 pandemic. The first **"Asymptomatic reinfection in 2 health care workers from India with genetically distinct severe acute respiratory syndrome coronavirus 2",** published as correspondence in Clinical Infectious Diseases Journal 2021 were the first reported cases of reinfection of SARS

CoV 2 in Indian health care worker. Both individuals had a higher viral load in the second episode of reinfection (CT values were 36 and 16.6 for I1 and 28.16 and 16.92 for I2 for the first and second episodes, respectively). Analysis of the genomes revealed 9 and 10 unique variant differences between the virus isolates from the two episodes of infection for I1 and I2 respectively. It was also a noteworthy finding that a genetic variant 22882T>G (S: N440K) found during reinfection in I2 possibly confers resistance to neutralising antibodies.

These cases were diagnosed by molecular testing at the Institute by Dr. Vivek and taken up for genomic sequencing as a part of his ongoing collaborative project: 'Combined Digital Surveillance and Effective COVID19 Testing Frameworks and Tools', carried out between Government Institute of medical Sciences & Council for Scientific Indian Research-Institute of Genomics and Integrative Biology.

Based on his findings it was emphasized the importance of RT PCR based surveillance of healthcare workers who are at higher risk of infection and reinfection and a detailed workup of cases of reinfection required from across the country needs to be done.
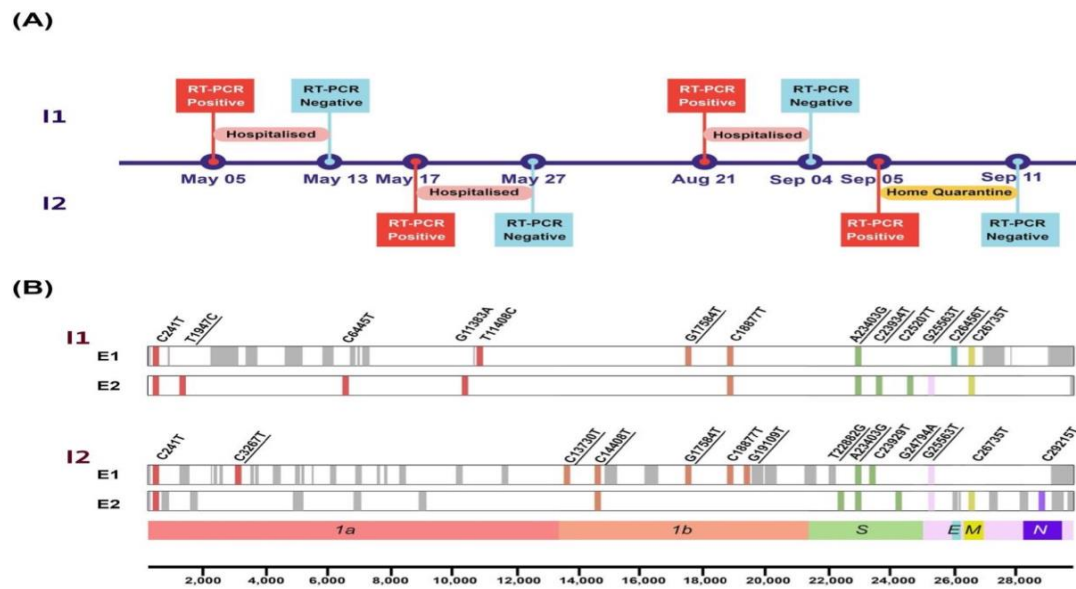
Figure 1. A. The timeline of SARS-CoV-2 infection in the two individuals I1 and I2. **B.** The genetic variants in isolates of the 2 episodes (E1 and E2) for individuals I1 and I2. Non-synonymous variants have been underlined and the gaps in the genome are marked in grey.

List 10 best publication

1. **Gupta V**, Bhake A. Assessment of Clinically Suspected Tubercular Lymphadenopathy by Real Time PCR Compared to Non-Molecular Methods on Lymph Node Aspirates. Acta Cytol. 2018;62(1):4-11. doi: 10.1159/000480064. Epub 2017 Sep 26. PubMed PMID: 28946148.

2. **Gupta V**, Bhake A. Diagnosis of clinically suspected and unsuspected tubercular lymphadenopathy by cytology, culture, and smear microscopy. Indian J Tuberc. 2017 Oct;64(4):314-317. doi:10.1016/j.ijtb.2016.11.014. Epub 2017 Feb 13. PubMed PMID: 28941855.

3. **Gupta V**, Bhake A. Clinical and cytological features in diagnosis of peripheral tubercular lymphadenitis - A hospital-based study from central India. Indian J Tuberc. 2017 Oct;64(4):309-313. doi: 10.1016/j.ijtb.2016.11.032. Epub 2017 Feb 13. PubMed PMID: 28941854

4. **Gupta V**, Bhake A. Molecular Diagnosis of Tubercular Lymphadenopathy from Fine-Needle Aspirates in Pediatric Patients. Acta Cytol. 2017;61(3):173-178. doi: 10.1159/000475832. Epub 2017 May 20. PubMed PMID: 28528339.

5. **Gupta V**, Bhake A. Reactive Lymphoid Hyperplasia or Tubercular Lymphadenitis: Can Real-Time PCR on Fine-Needle Aspirates Help Physicians in Concluding the Diagnosis? Acta Cytol. 2018;62(3):204-208. doi: 10.1159/000488871. Epub 2018 May 15. PubMed PMID: 29763927.

6. **Vivek Gupta,** Prerna Agarwal, Sunita Vagha. Triple Positive breast cancer: A case series. European Journal of Molecular & Clinical Medicine. 2021;08(04): 600-607.

7. **Gupta V.,** Agarwal P., Deshpande P. Impact of RASSF1A gene methylation on clinico-pathological features of tumor and non-tumor tissue of breast cancer. Annals of Diagnostic Pathology. 2021;52(151722): 1-5. DOI: 10.1016/j.anndiagpath.2021.151722. PMID: 33621744

8. Wankhade, R., **Gupta, V.,** Belsare, A. Role of Ki 67 in pathological prognostic staging of breast cancer. International Journal of Pharmaceutical Research. 2019, 11(3), pp. 1469–1473

9. Sheronica Laishram, **Vivek Gupta,** Arvind Bhake, Akanksha Wankhede, Deepika Agrawal. To Assess the Utility of Proliferative Marker Ki-67 in Surface Epithelial Ovarian Tumor. JDMIMS. 2019;14(1):6-10. Doi: 10.4103/jdmimsu.jdmimsu_71_18

10. **Vivek Gupta**, Rahul C. Bhoyar, Abhinav Jain, Saurabh Srivastava, Rashmi Upadhayay, Mohamed Imran. Asymptomatic reinfection in two healthcare workers from India with genetically distinct SARS-CoV-2. Clinical Infectious Diseases. PMID: 32964927. PMCID: PMC7543380 DOI: 10.1093/cid/ciaa1451/5910388