Original Article

Spike Protein Mutations in *SARS-CoV-2* at Rajasthan, India in December 2020

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ABSTRACT

Introduction: Many mutations have been reported in *Severe Acute Respiratory Syndrome Coronavirus-2* (*SARS-CoV-2*) since its first identification. Some are variants of concern (VOC) as they have higher transmission rates. Mutations in the spike protein region are of main concern as they affect transmission rates and can affect efficacy of the vaccines. The objective of present study was to look for spike protein mutations in *SARS-CoV-2* using Next Generation Sequencing (NGS) in representative samples received in December 2020 at Rajasthan.

Methodology: Ten nasopharyngeal/throat swab specimens from known COVID-19 positive patients were processed for RNA extraction, library preparation, and sequencing was done using specific *SARS-CoV-2* Ion-Ampliseq panel by Ion torrent S5 system.

Results: Only seven samples gave high quality data. All the isolates belonged to clade GH. We found 11 different mutations in the spike protein region most common were Q1201K, Q677R, D614G, L18F. Some novel mutations were found like S689I, Q23R, D1146E, and M153K. D614G mutation was present in all the samples which are known to promote transmissibility of the *SARS-CoV-2* virus.

Conclusion: We did not find any VOC but one sample had N440K mutation which is reported to escape immune response, was found to be prevalent in some other parts of India also, and was considered epidemiologically important at the beginning of second wave. There is a need to carry out sequencing on regular basis to check for emerging mutants and monitor their effect on vaccine efficacy.

Keywords: NGS, *SARS-CoV-2*, spike protein mutation.

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 (*SARS-CoV-2*) was first reported in December 2019 from China.[1] Rapidly the virus spread all over the world and World Health Organization declared the outbreak a global pandemic in March 2020. The virus has been reported to constantly develop new mutations. Different mutations are prevalent in different regions of the world and evolve over a period of time too. Fatality rates also vary worldwide due to number and type of mutations in *SARS-CoV-2*, depending on the virulence of the strains.[2] Various clades of *SARS-CoV-2* have been identified in Global Initiative on Sharing All Influenza Data (GISAID)[3], clade O, clade L, and clade S (variant ORF8-L84S) were prevalent during January and February 2020, subsequently, other clades appeared; clade V (a variant of the ORF3a coding protein NS3-G251), clade G (variant of the spike protein S-D614G) and various derivatives of clade G like GH, GV, GR, etc.[4] The cause for concern in December 2020 and January 2021 were the new mutants in spike protein which had emerged in UK (501Y.V1), South Africa (501Y.V2), Brazil (501Y.V3), California (B.1.427 and B.1.429), etc. These variants have been reported to be highly transmissible and pose the risk of increase in number of the hospitalizations and deaths.[5] Moreover the spike protein mutations can also affect efficacy of the vaccines.[6] Now with emergence of the new variants and of the number of vaccines on the horizon it's important to carry out whole genome sequencing of the virus to not only monitor transmission dynamics, but look for development of mutants, their effect on transmission of the virus, and efficacy of the vaccines. The objective of the present study was to look for spike protein mutations on *SARS-CoV-2* using NGS in representative samples received in December 2020 at Rajasthan.

METHODS

Nasopharyngeal/throat swab samples were collected in

viral transport medium (VTM) from patients and sent to the lab. RNA was extracted on automated nucleic acid extraction system, Nucliescens Easy MAg (Biomeurex). The samples which were reported positive for *SARS-CoV-2* using ICMR NIV real time reverse transcriptase PCR (RT-PCR) kits were shortlisted. Ten random samples of symptomatic patients with high viral load (cycle threshold <25 for E and ORF gene) detected positive in last week of December were selected for NGS analysis among the 909 detected as positive in the last week of December 2020.

Quantification of RNA was done on Qubit HS RNA kit (Life Technologies, USA), cDNA was then prepared using Superscript VILO reverse transcriptase kit (Invitrogen, USA). The libraries were prepared by using Ion Ampli Seq library kit plus (Life Technologies) and the Ion Ampli Seq *SARS-CoV-2* research panel (Life Technologies) following the protocol of the kit. Ion OT2 kit was used for preparing the template and enrichment and process was performed on the Ion One Touch 2 and Ion One Touch ES systems (Life Technologies). The prepared libraries were loaded on Ion 530 chip and sequencing was done on Ion torrent S5 system. The FASTA files were downloaded from the Torrent Suite software version 5.0.5 and aligned using Clustal-W software with whole genome sequences of *SARS-CoV-2* taken from GISAID (Wuhan-Hu-1 isolate Gene Bank accession number NC 045512.2 and sequences from UK, India, USA, etc). Nextstrain and GISAID tool were used for mutation analysis. Phylogenetic tree was constructed using the Neighbour joining algorithm in MEGA7 software.

Table: Spike and NSP protein mutations in SARS-CoV-2 samples from Rajasthan

S. No.	Accession ID no. (GSAID)	On-target read (%)	Average read length (bp)	Mean depth (100x–1000x)	Mutations in spike protein	Mutations in NSPs protein
1	EPI_ISL_891152	99.50	204	7,245	Q1201K, Q677R, D614G, L18F	N_S194L, NS8_S67, NSP8_A1, NSP12_P323L, NS3_Q57H, NSP2_V381A,NSP3_D165G, N_R203K, NSP3_L1802F
2	EPI_ISL_891207	99.92	205	9,529	D614G, M153K, N440K*	N_S194L, NSP3_A260V, NSP12_P323L, NSP3_P874S,
3	EPI_ISL_891210	99.36	191	7,545	Q677H, D614G	N_S194L, NSP14_A323S, NSP12_P323L,NS3_Q57H, NSP3_V1229F,NSP2_V381A, NS3_D155Y, NSP14_R163C NSP3_L1328F, NSP6_L37F
4	EPI_ISL_891211	99.97	203	30,921	Q677H, D614G	N_S194L, NSP14_A323S, NSP12_P323L, NS3_Q57H, NSP3_V1229F, NSP2_V381A, NS3_D155Y, NSP14_R163C NSP3_L1328F, NSP6_L37F
5	EPI_ISL_891213	99.68	206	17,270	S689I D614G	N_S194L, NSP6_A161S, NSP12_P323L, NS3_Q57H, NSP16Q218K, NSP2_V381A, M_L29F
6	EPI_ISL_891214	96.66	194	7,824	Q23R, D614G	D1146E N_S194L, NSP12_P323L, NS3_Q57H, NSP6_Q208R, NSP3_V238L, NSP2_V381A, N_D377Y, NSP7_L76M, NS3_T223I,M_K15R
7	EPI_ISL_891219	99.89	173	1526	D614G	N_S194L, NSP3_A1179V, NSP12_P323L, NS3_Q57H, NSP6_Q208R, NSP3_V238L, NSP2_V381A, N_D377Y, NS3_T223I,

* Found in one *SARS-COV-2* positive patient in Rajasthan.

RESULTS

Ten samples were processed for NGS, out of which only seven samples gave high quality data. These isolates belonged to clade GH. Our genome sequences were compared to the complete genome of the *SARS-CoV-2* Wuhan-Hu-1 isolate using the Beta-coronavirus BLAST tool and showed 99.8% similarity. The mean coverage depth, total reads and spike protein mutations are elaborated in table. Two strains had 97% similarity and 100% similar mutation patterns. On analysis of spike protein mutations, we found 11 different mutations in spike region, in which most common and previously reported mutations were Q1201K, Q677R, D614G, L18F (Table). Some novel mutations were found like S689I, Q23R, D1146E, and M153K. Spike mutation N440K related with high transmission rate was present in one sample.

DISCUSSION

In the present study, all samples belonged to GH clade, derivative of G clade characterized by mutation ORF3a: Q57H. G and G-derived clades reached North America and Asia in March 2020 and were the fastest growing viral sub population worldwide in 2020. On analysis of downloaded sequences, the initial isolates from Rajasthan belonged to clade O (ESI_ISL_454830) and to clade G during March 2020[7]; the sequences of the present study belonged to clade GH (ESI_ISL_891211, ESI_ISL_891210, ESI_ISL_891152, ESI_ISL_891213, ESI_ISL_891219, ESI_ISL_891214, ESI_ISL_891207) which is in accordance with a previous study which reports GR (31.93%) and GH (31.33%) to be the predominant clades in Asia and have the highest mutation rates.[8] Similarly, clade GH was prevalent in Delhi and Gujarat also (Figure).

In the present study, D614G mutation was present in all the samples which is known to promote transmissibility of the *SARS-CoV-2* virus. The D614G mutation was also reported in strains from other parts of our country.[9] Other mutations of spike region found in the present study have different effects; L18F is reported synomorphic variant. Q1210 variant do not affect the structure, while Q677R variant occur near S1/S2 cleavage site and affect the entry process of virus and play a significant role in evolution selection process.

None of our samples in December 2020 from Rajasthan had the variant of concern (VOC) though Indian *SARS-CoV-2* Consortium on Genomics (INSACOG) by early

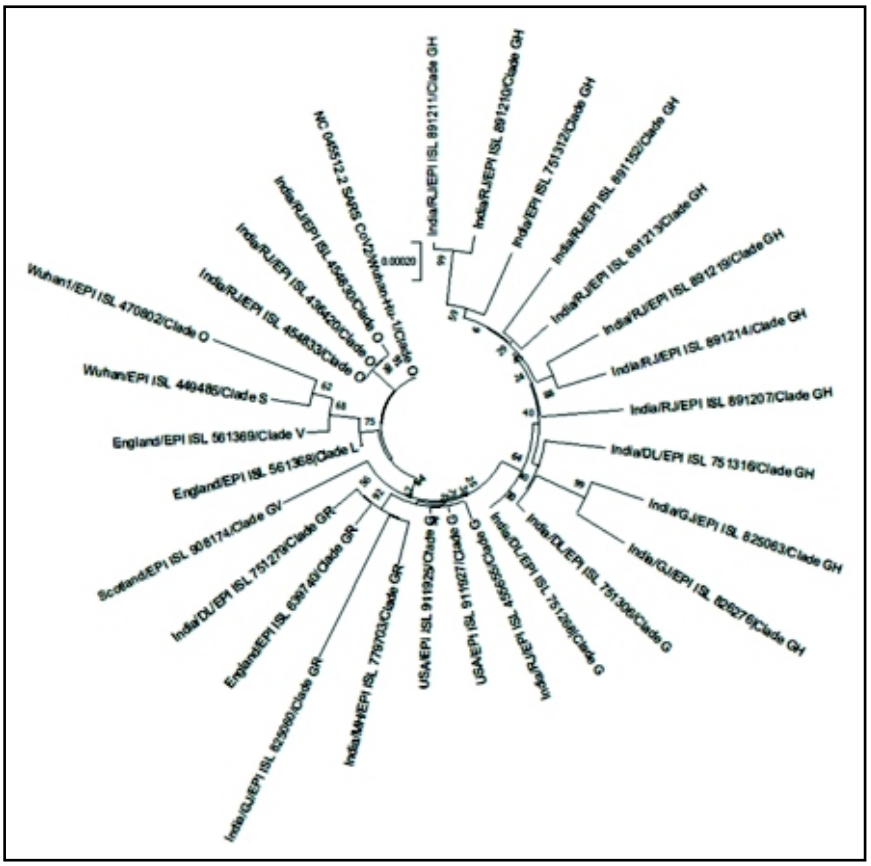

Figure: Phylogenetic circular tree of *SARS-CoV-2* sequences analyzed.

2021 had reported 771 VOC (in 10787 positive samples) from India; 736 being UK (B.1.1.7) lineage, 34 South African (B.1.351) lineage and 1 was Brazilian (P.1) lineage.[10] The most important finding of current study was presence of N440K variant in one sample from Jaipur, Rajasthan. Many studies have reported that this variant was spreading in southern states of India and was reported in 2.1% of samples from all over India, 33.8% from Andhra Pradesh[11], and 50.9% from Telangana.[10] In a genomic analysis of *SARS-CoV-2* from different parts of India, N440K mutation was reported in samples from Maharashtra as early as March 2020 and from south India in May 2020 samples.[9] The N440K is reported to exhibit complete loss of binding to C135, which results in higher transmission rate. The N440K mutation was cause for concern of all public health officials as it was associated with immune escape and was present in many cases in beginning of the second wave of COVID-19 in India.[10,11] Strict vigilance was done by the Government of India to restrict international travel and test and isolate all international travellers to minimize spread of variants in the country. Development of mutations and emergence of newer VOC or strain is a constant process with the *SARS-CoV-2* virus. During March 2021 to July 2021, India faced the second wave due to the Delta virus and now the Omicron variant has also emerged in December 2021[12,13] and caused the third wave. Limitation of our study is that the findings are from old samples but does convey the importance of carrying out genomic surveillance to control the spread of the virus.

CONCLUSION

The study concludes that, N440K variant was found in one *SARS-CoV-2* positive patient in Rajasthan, India. All seven samples belonged to GH clade. D614G mutation was present in all the samples which are known to promote transmissibility of the *SARS-CoV-2* virus. Some novel mutations were found like S689I, Q23R, D1146E, and M153K. Variant of concern; UK (501Y.V1), South Africa (501Y.V2), Brazil (501Y.V3), California (B.1.427 and B.1.429) were not found in our study.

Declaration of competing interest: The authors have no conflict of interests.

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Appendix 1

Virus Name	Accession No.	Collected	Originating Lab	Submitting Lab	Authors
hCoV-19/India/DL-IGIB11305082/2020	EPI_ISL_751272	2020-08	Devki Devi Foundation, a unit of Max Healthcare	CSIR-IGIB/Max	Rajesh Pandey#, Samreen Siddiqui, Janani Srinivasa Vasudevan, AkshayKanakan, Ranjeet Maurya, Uzma Shamim, BansidharTarai, Akansha Tyagi, Mitali Mukerji, Poonam Das, Sujeet Jha, Mohammed Faruq, Anurag Agrawal
hCoV-19/England/GSTT-6697/2020	EPI_ISL_561369	2020-03-29	Centre for Clinical Infection and Diagnostics Research and Genomics Innovation Unit, Guy's and St. Thomas' NHS Trust	Centre for Clinical Infection and Diagnostics Research and Genomics Innovation Unit, Guy's and St. Thomas' NHS Trust	Chloe Fisher, Luke Snell, Rahul Batra, Jonathan Edgeworth, Ali Raza Awan
hCoV-19/Wuhan/Tongji-04-2/2020	EPI_ISL_470802	2020-04-20	State Key Laboratory of Agriculture Microbiology	State Key Laboratory of Agriculture Microbiology	Zhong Zou
hCoV-19/India/RJ-SMSCOV109/2020	EPI_ISL_454830	2020-04-23	SMS Medical College, Jaipur	CSIR Institute of Genomics and Integrative Biology	Sudhir Bhandari, Rahul Bhoyar, Mohammed Imran, Mohit Divakar, Disha Sharma, Anshul Kumar, Bani Jolly, Rahul Sahlot, Abhinav Jain, Paras Sehgal, Gyan Ranjan, Vinod Scaria, Sridhar Sivasubbu, Sandeep K Mathur
hCoV-19/India/RJ-SMSCOV175/2020	EPI_ISL_454833	2020-04-27	SMS Medical College, Jaipur	CSIR Institute of Genomics and Integrative Biology	Sudhir Bhandari, Rahul Bhoyar, Mohammed Imran, Mohit Divakar, Disha Sharma, Anshul Kumar, Bani Jolly, Rahul Sahlot, Abhinav Jain, Paras Sehgal, Gyan Ranjan, Vinod Scaria, Sridhar Sivasubbu, Sandeep K Mathur
hCoV-19/India/DL-IGIB11301421/2020	EPI_ISL_751268	2020-06	Devki Devi Foundation, a unit of Max Healthcare	CSIR-IGIB/Max	Rajesh Pandey#, Samreen Siddiqui, Janani Srinivasa Vasudevan, AkshayKanakan, Ranjeet Maurya, Uzma Shamim, BansidharTarai, Akansha Tyagi, Mitali Mukerji, Poonam Das, Sujeet Jha, Mohammed Faruq, Anurag Agrawal

Virus Name	Accession No.	Collected	Originating Lab	Submitting Lab	Authors
hCoV-19/India/GJ-GBRC-460-R2b/2020	EPI_ISL_826276	2020-12-13	Sterling Hospital, Memnagar, Ahmedabad	Gujarat Biotechnology Research Centre	Dinesh Kumar, Zuber Saiyed, Labdhi Pandya, Afzal Ansari, Nikha Trivedi, Apurvasinh Puvar, Ramesh Pandit, Janvi Raval, Zarna Patel, Nitin Savaliya, Atul K Patel, Naman Shashtri, Chaitanya Joshi, Madhvi Joshi
hCoV-19/India/GJ-GBRC-459/2020	EPI_ISL_825063	2020-12-30	NHL Municipal Medical College, Ahmedbad	Gujarat Biotechnology Research Centre	Janvi Raval, Zarna Patel, Nitin Savaliya, Dinesh Kumar, Zuber Saiyed, Afzal Ansari, Nikha Trivedi, ApurvasinhPuvar, Ramesh Pandit, JayshriPethani, MonilaPatel, Atit Shah, NM Shaikh, Bimal Chauhan, Tanmay Mehta, Bhavin Prajapati, Chaitanya Joshi, Madhvi Joshi
hCoV-19/India/DL-IGIB11305043/2020	EPI_ISL_751316	2020-08	Devki Devi Foundation, a unit of Max Healthcare	CSIR-IGIB/Max	Rajesh Pandey#, Samreen Siddiqui, Janani Srinivasa Vasudevan, AkshayKanakan, Ranjeet Maurya, Uzma Shamim, BansidharTarai, Akansha Tyagi, Mitali Mukerji, Poonam Das, Sujeet Jha, Mohammed Faruq, Anurag Agrawal
hCoV-19/India/DL-IGIB11304857/2020	EPI_ISL_751306	2020-07	Devki Devi Foundation, a unit of Max Healthcare	CSIR-IGIB/Max	Rajesh Pandey#, Samreen Siddiqui, Janani Srinivasa Vasudevan, AkshayKanakan, Ranjeet Maurya, Uzma Shamim, BansidharTarai, Akansha Tyagi, Mitali Mukerji, Poonam Das, Sujeet Jha, Mohammed Faruq, Anurag Agrawal
hCoV-19/India/DL-IGIB11305615/2020	EPI_ISL_751312	2020-08	Devki Devi Foundation, a unit of Max Healthcare	CSIR-IGIB/Max	Rajesh Pandey#, Samreen Siddiqui, Janani Srinivasa Vasudevan, AkshayKanakan, Ranjeet Maurya, Uzma Shamim, BansidharTarai, Akansha Tyagi, Mitali Mukerji, Poonam Das, Sujeet Jha, Mohammed Faruq, Anurag Agrawal
hCoV-19/ Scotland/ QEUH-1067295/ 2021	EPI_ISL_908174	2021-01-17	Lighthouse Lab in Glasgow	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	Harper VanSteenhouse, Yumi Kasai, David Gray, Carol Clugston, Anna Dominiczak and Alex Alderton, Roberto Amato, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team
hCoV-19/India/AP-CS0998/2020	EPI_ISL_862495	2020-08-03	Kurnool Medical College (KMC)	CSIR Institute of Genomics and Integrative Biology	PallavaliRoja Rani, Mohamed Imran, J. Vijaya Lakshmi, Bani Jolly, S. Afsar, Abhinav Jain, Mohit Kumar Divakar, Panyam Suresh,

Virus Name	Accession No.	Collected	Originating Lab	Submitting Lab	Authors
					Disha Sharma, Nambi Rajesh, Rahul C Bhoyar, DasariAnkaiah, Sanaga Shanthi Kumari, Gyan Ranjan, ValluriAnitha Lavanya, Mercy Rophina, S. Umadevi, Paras Sehgal, Avula Renuka Devi, A. Surekha, Pulala Chandra, Rajamadugu Hymavathy, P R Vanaja, Vinod Scaria, Sridhar Sivasubbu
hCoV-19/USA/IN-Lilly-IPB0173-14bc03/2020	EPI_ISL_911927	2020-03-26	Clinical Diagnostics Laboratory, Diagnostic & Experimental Pathology, Lilly Research Laboratories	Clinical Diagnostics Laboratory, Diagnostic & Experimental Pathology, Lilly Research Laboratories	Tim Holzer, Mayuri Vaidya, Angie Fulford, Sam McNeely, Rachael Redmond, Phil Ebert, John Calley, Leslie ONeill Reising, Pat Finnegan, Erin Wray, John McElwee, Jeff Fill, Joe Oakley, Andrew Schade
hCoV-19/India/RJ-S25/2020	EPI_ISL_455655	2020-04-30	ICMR-National Institute of Cholera and Enteric Diseases	National Institute of Biomedical Genomics	Arindam Maitra, Mamta Chawla Sarkar, Sreedhar Chinnaswamy, Hasina Banu, Ananya Chatterjee, Shanta Dutta, Saumitra Das
hCoV-19/India/AP-CS0830/2020	EPI_ISL_862501	2020-07-30	Kurnool Medical College (KMC)	CSIR Institute of Genomics and Integrative Biology	Pallavali Roja Rani, Mohamed Imran, J. Vijaya Lakshmi, Bani Jolly, S. Afsar, Abhinav Jain, Mohit Kumar Divakar, Panyam Suresh, Disha Sharma, Nambi Rajesh, Rahul C Bhoyar, Dasari Ankaiah, Sanaga Shanthi Kumari, Gyan Ranjan, Valluri Anitha Lavanya, Mercy Rophina, S. Umadevi, Paras Sehgal, Avula Renuka Devi, A. Surekha, Pulala Chandra, RajamaduguHymavathy, P R Vanaja, Vinod Scaria, Sridhar Sivasubbu
hCoV-19/India/DL-IGIB11304579/2020	EPI_ISL_751279	2020-07	Devki Devi Foundation, a unit of Max Healthcare	CSIR-IGIB/Max	Rajesh Pandey#, Samreen Siddiqui, Janani Srinivasa Vasudevan, AkshayKanakan, Ranjeet Maurya, Uzma Shamim, BansidharTarai, Akansha Tyagi, Mitali Mukerji, Poonam Das, Sujeet Jha, Mohammed Faruq, Anurag Agrawal
hCoV-19/England/Kunming_kms-3/2020	EPI_ISL_639740	2020-03-29	Respiratory virus Laboratory, Chinese Academy of Medical Science	Respiratory virus Laboratory, Chinese Academy of Medical Science	Li,J., Zhen,H., Chen,Y. and Liu,L.
hCoV-19/India/GJ-GBRC-457/2020	EPI_ISL_825060	2020-12-26	NHL Municipal Medical College, Ahmedbad	Gujarat Biotechnology Research Centre	Nikha Trivedi, ApurvasinhPuvar, Ramesh Pandit, JanviRaval, Zarna Patel, Nitin Savaliya, Dinesh Kumar, Zuber Saiyed, Afzal Ansari, JayshriPethani, Monila Patel, Atit Shah, NM Shaikh, Bimal Chauhan,

Virus Name	Accession No.	Collected	Originating Lab	Submitting Lab	Authors
					Tanmay Mehta, Bhavin Prajapati, Chaitanya Joshi, Madhvi Joshi
hCoV-19/India/MH-CMP-19/2020	EPI_ISL_779703	2020-08-24	The Foundation for Medical Research	The Foundation for Medical Research	Ayan Mandal, Kayzad Nilgiriwala, Kalpana Sriraman, Ambreen Shaikh, Grishma Patel, TejalMestry, Smriti Vaswani, Swapneil Parikh, Shreevatsa Udupa, Nirjhar Chatterjee, Jayanthi Shastri, Nerges Mistry
hCoV-19/USA/IL-CDC-9ME8-8816/2021	EPI_ISL_911923 EPI_ISL_911925	2021-01-04	IL Department of Public Health Chicago Laboratory/ Tempus Labs	Pathogen Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention	Ying Tao, Yan Li, Jing Zhang, Krista Queen, Anna Uehara, Peter Cook, Clinton R. Paden, Haibin Wang, Suxiang Tong
hCoV-19/USA/IN-Lilly-IPB0173-13bc26/2020		2020-03-26	Clinical Diagnostics Laboratory, Diagnostic & Experimental Pathology, Lilly Research Laboratories	Clinical Diagnostics Laboratory, Diagnostic & Experimental Pathology, Lilly Research Laboratories	Tim Holzer, Mayuri Vaidya, Angie Fulford, Sam Mc Neely, Rachael Redmond, Phil Ebert, John Calley, Leslie ONeillReising, Pat Finnegan, Erin Wray, John McElwee, Jeff Fill, Joe Oakley, Andrew Schade