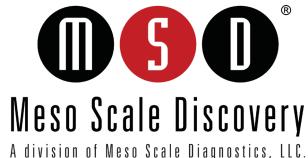
Development of a High-Throughput SARS-CoV-2 Strain-Typing Assay

November 2, 2020 Laure Moller



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Slide 1



Session Description and Objectives

 We have developed a highthroughput multiplexed SARS-CoV-2 single nucleotide polymorphism (SNP) panel using the MSD[®] N-PLEX platform

Learning Objectives

- To highlight the need for alternatives to traditional methods for the detection of SARS-CoV-2 SNPs
- To understand the basic principle of the MSD N-PLEX[®] platform for SNP detection in viral RNA
- To show the speed and ease of the development of singleplex and multiplex SARS-CoV-2 SNP assays on the N-PLEX platform





Biography and Contact Information

- Laure Moller is VP of Scientific Support at Meso Scale Discovery (MSD) where she manages a national team of field application scientists who provide training and support to MSD customers for the full suite of MSD assays and applications
- Laure has over 15 years of experience in development and implementation of a broad range of ligand binding assays including PK, immunogenicity and biomarker assays
- She obtained her Ph.D. in Biochemistry from the University of Cape Town, South Africa where she studied the impact of histone post-translational modifications on chromatin structure and function
- Contact info:

E-mail: <u>Imoller@meso-scale.com</u> Tel: +1-619-403-3340

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Background

- The genome of the virus responsible for the COVID-19 pandemic (SARS-CoV-2) is undergoing mutations, including many SNPs
- The ability to measure these mutations in a high-throughput and straightforward approach is necessary to assess viral pathogenesis, transmission patterns, and general straintyping
- We sought to develop a multiplex assay to quickly and easily assess 4 common SNPs associated with altered viral pathogenesis and/or transmission, see below

Nucleic Acid Change	Site in viral RNA	Functional Outcome
C>T	8782	One of two differentiating alleles for S strain type, less aggressive than L strain type
G>T	11083	Associated with asymptomatic infections, included in V clade
A>G	23403	Higher transmission rate and more pathogenic, included in G clade
T>C	28144	One of two differentiating alleles for S strain type, less aggressive than L strain type

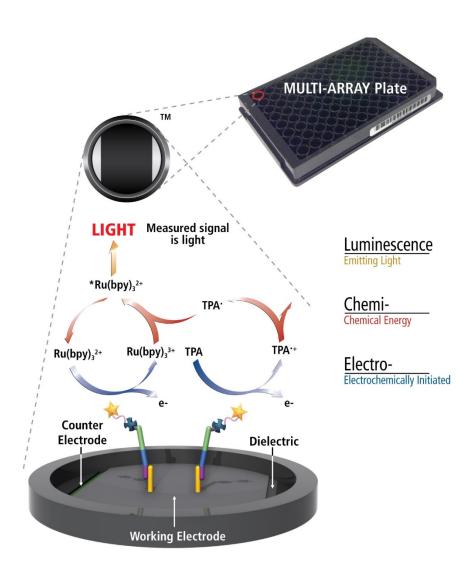


Slide 4



Methodology for SNP assessment

- The N-PLEX platform (Meso Scale Diagnostics) was used for viral SNP determination
- The method relies on electrochemiluminescence detection from SULFO-TAG[™] labels that are attached near the surface of the plate
- SULFO-TAG labels only emit light upon electrochemical stimulation at the electrode surfaces in MULTI-ARRAY[®] plates
- This leads to reduced non-specific background and strong specific signal, giving high signal-to-background ratios
- N-PLEX plates have 10-spots per well in a 96-well plate format
- See next slide for SNP detection methodology





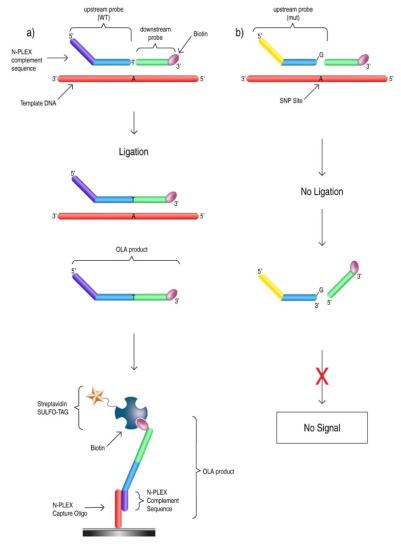
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Oligonucleotide Ligation Assay (OLA) for SNP discrimination

- Following RNA extraction and reverse transcription/amplification, 3 probes and Taq DNA ligase are added and used to join probes that are aligned exactly against the target DNA
 - Multiple rounds of ligation increase signal
 - The ligation temperature helps give specificity
 - All targets are multiplexed in the same amplification and OLA reactions
- OLA products are hybridized to the N-PLEX plate with the N-PLEX complement sequence in the probe
- Streptavidin labeled with SULFO-TAG binds to biotin in the ligated probe
- An MSD[®] instrument is used to detect signal generated from SULFO-TAG
- Two spots are needed for each SNP discrimination, one for wild type and one for the mutated base



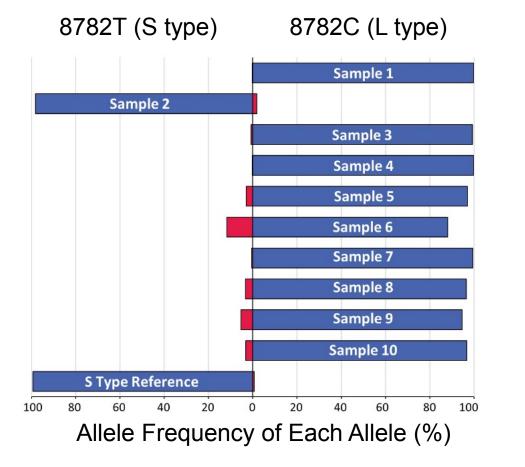


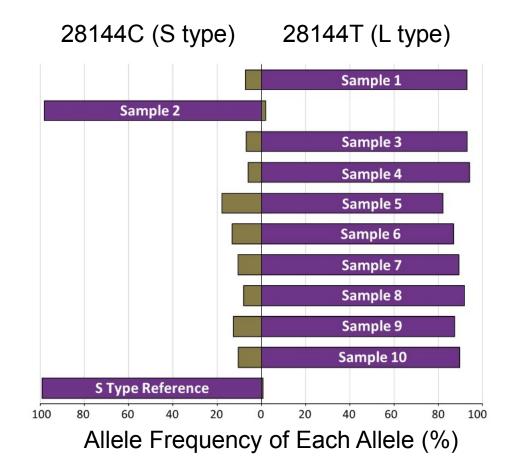
Slide 6

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Multiplex assays for differentiating L and S strains in samples and a reference strain

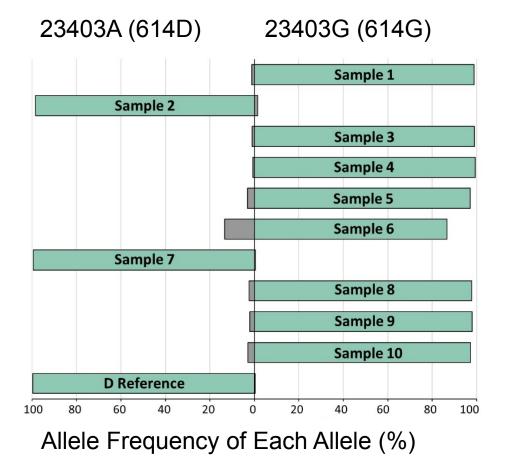








The pathogenic 614G strain is highly represented in the sample cohort



 80% of patients from this sample set were positive for the more pathogenic 614G strain

 The 614D reference strain is also confirmed as such with the N-PLEX assay

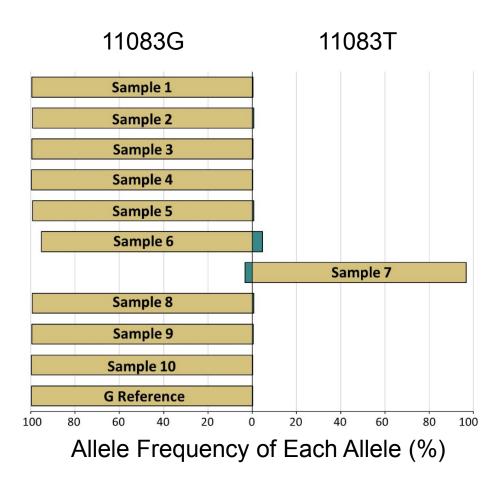


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An N-PLEX SNP assay can be used to identify the SNP associated with asymptomatic infections



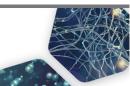
- Only one of the patients in the sample cohort was positive for the mutation associated with asymptomatic infection (11083T)
- The low prevalence of the 11083T mutation could be due to individuals not showing symptoms or having mild symptoms and, thus, not being present in the sample set



Conclusions

- All 4 SARS-CoV-2 SNP assays developed for assessment on the N-PLEX platform show good specificity and match known references
- The ability to multiplex SNP assays highly increases throughput and does not impact the ability to identify the polymorphic base
- Assay time after RNA extraction is ~6 hours for a full 96-well plate, with the time-limiting step being the RT-PCR, as reading a plate on an MSD instrument takes about 2-3 minutes
- Development of new SARS-CoV-2 SNP assays is very quick, with a turnaround time of ~3 weeks
- See poster: Development of a High-Throughput SARS-CoV-2 Strain-Typing Assay





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Meso Scale Discovery

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