Building COVID-19 Testing Capacity from Scratch

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The situation in Denmark before Covid-19

- Most testing for infectious agents had been decentralized to regional public hospitals
- The central government laboratory (SSI) is only testing for some rare infectious diseases
- SSI still propose new testing methods to the regional public hospitals

Close-down of Denmark in Marts 2020

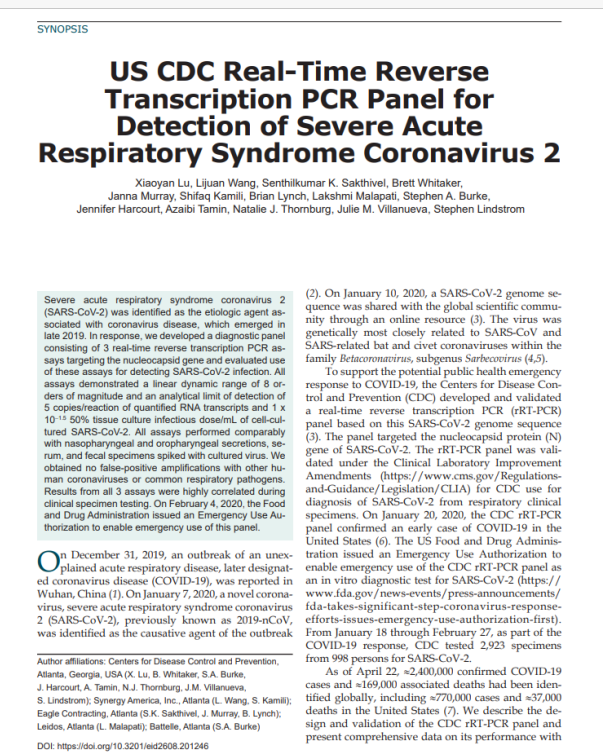
- Marts 11:
  - The prime minister close-down the country
  - Novo Nordisk close-down the research laboratories
  - Change of testing strategy

- Marts 21, Novo Nordisk is asked by the PM to help the hospitals with RT-PCR testing for Covid-19

- Marts 29, Novo Nordisk foundation invites for meeting to start an alternative testing track (Test Center Denmark)

The task given by Rigshospitalet

- Use the same RT-PCR package as Rigshospitalet
  - Primer-probe mixes for:
    - 2019-nCoV_N1: targets viral nucleoprotein gene
    - 2019-nCoV_N2: targets viral nucleoprotein gene
    - RP: targets human RNase P gene



SYNOPSIS

US CDC Real-Time Reverse Transcription PCR Panel for Detection of Severe Acute Respiratory Syndrome Coronavirus 2

Xiaoyan Lu, Lijuan Wang, Senthilkumar K. Sakthivel, Brett Whitaker, Janna Murray, Shifaq Kamili, Brian Lynch, Lakshmi Malapati, Stephen A. Burke, Jennifer Harcourt, Azaibi Tamin, Natalie J. Thornburg, Julie M. Villanueva, Stephen Lindstrom

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the etiologic agent associated with coronavirus disease, which emerged in late 2019. In response, we developed a diagnostic panel consisting of 3 real-time reverse transcription PCR assays targeting the nucleocapsid gene and evaluated use of these assays for detecting SARS-CoV-2 infection. All assays demonstrated a linear dynamic range of 8 orders of magnitude and an analytical limit of detection of 5 copies/reaction of quantified RNA transcripts and 1 x $10^{-1.5}$ 50% tissue culture infectious dose/mL of cell-cultured SARS-CoV-2. All assays performed comparably with nasopharyngeal and oropharyngeal secretions, serum, and fecal specimens spiked with cultured virus. We obtained no false-positive amplifications with other human coronaviruses or common respiratory pathogens. Results from all 3 assays were highly correlated during clinical specimen testing. On February 4, 2020, the Food and Drug Administration issued an Emergency Use Authorization to enable emergency use of this panel.

On December 31, 2019, an outbreak of an unexplained acute respiratory disease, later designated coronavirus disease (COVID-19), was reported in Wuhan, China (*1*). On January 7, 2020, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously known as 2019-nCoV, was identified as the causative agent of the outbreak (*2*). On January 10, 2020, a SARS-CoV-2 genome sequence was shared with the global scientific community through an online resource (*3*). The virus was genetically most closely related to SARS-CoV and SARS-related bat and civet coronaviruses within the family *Betacoronavirus*, subgenus *Sarbecovirus* (*4*,*5*).

To support the potential public health emergency response to COVID-19, the Centers for Disease Control and Prevention (CDC) developed and validated a real-time reverse transcription PCR (rRT-PCR) panel based on this SARS-CoV-2 genome sequence (*3*). The panel targeted the nucleocapsid protein (N) gene of SARS-CoV-2. The rRT-PCR panel was validated under the Clinical Laboratory Improvement Amendments (https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA) for CDC use for diagnosis of SARS-CoV-2 from respiratory clinical specimens. On January 20, 2020, the CDC rRT-PCR panel confirmed an early case of COVID-19 in the United States (*6*). The US Food and Drug Administration issued an Emergency Use Authorization to enable emergency use of the CDC rRT-PCR panel as an in vitro diagnostic test for SARS-CoV-2 (https://www.fda.gov/news-events/press-announcements/fda-takes-significant-step-coronavirus-response-efforts-issues-emergency-use-authorization-first). From January 18 through February 27, as part of the COVID-19 response, CDC tested 2,923 specimens from 998 persons for SARS-CoV-2.

As of April 22, ≈2,400,000 confirmed COVID-19 cases and ≈169,000 associated deaths had been identified globally, including ≈770,000 cases and ≈37,000 deaths in the United States (*7*). We describe the design and validation of the CDC rRT-PCR panel and present comprehensive data on its performance with

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- Avoid competing with any of the Danish hospitals for reagents, kits or plasticware
  - ABI 9600 for RT-PCR
  - Biomek i5 and i7 robots for liquid handling including nucleic acid purification

- Validate assay by testing difficult sample sets from Rigshospitalet to demonstrate comparability before first diagnostic use and after any subsequent change of method

The strategy we followed from day 1

1. Start testing real patient samples from the hospital as fast as possible

2. Change to multiplex RT-PCR both for simplicity and for increased capacity

3. Move towards sourcing reagents and plasticware in Denmark. Scandinavia or EU as possible alternatives

4. Could we possibly push a method back to the hospitals where we could guarantee the supply of reagents?

Criticality of simultaneous diagnostic testing and method development

- One week after being contacted by the PM, we were running 400 diagnostic samples/day
- Copy number controls included on all plates for:
  - Viral RNA
  - Viral DNA
  - Cell copy number
- Two partially overlapping teams established for method development and for diagnostic testing
- Transfer to Biomek robots i5 and i7 increased the testing capacity to 2000 diagnostic samples/day two weeks later

Change to multiplex RT-PCR both for simplicity and for increased capacity

- 2.000 diagnostic samples per day corresponds to approximately 7.000 RT-PCR reactions including controls
- The number of ABI 9600s had become the bottleneck
- An opportunity to reduce reagents and plasticware
- April 24, we could run 6.000 diagnostic samples/day using multiplex RT-PCR without compromising sensitivity or robustness

Berlingske

FREDAG 24.04.2020 2. SEKTION

Business

CORONA KRISEN

Ulf Bech Christensen ejer PentaBase, der har udviklet en ny dansk metode til at teste for coronavirus. Men han kunne ikke trænge igennem til myndighederne trods den store efterspørgsel på test. Gennembruddet kom efter en henvendelse fra Novo Nordisk, og nu satser han på et eksporteventyr – og tiltrængt kapital.

Ulfs opfindelse gav pote efter opkald fra Novo Nordisk

HJÆLPEPAKKE
Rejsebureauer vildledt af ministerium

OVERDOMMERNE
Pas på, Anders Holch. Sailing Group klar til kamp

C25 CAP
1,7%

ANALYSE
Svensk økonomi hårdt ramt trods åben strategi

How to avoid stock-out?

- Internally targeting 10.000 samples/day and a total of 2 million tests
- Ordered 1200 kg GuSCN in China (lysis buffer and RNA purification)
- Started own production of silica-coated magnetic beads but ended up sourcing at The Technical University in Oslo, Norway
- Initiated production of DNA polymerase and Reverse Transciptase
- The flexibility of the Biomek robots allowed the use of different plates
- Developed method for reuse of pipette tips
- Constantly developing and sourcing for 1-2 backup methods

Could we possibly push a method back to the hospitals where we could guarantee the supply of reagents?

- July 1, 2020, we transferred the method, and by that a testing capacity of 10.000 samples/day for the Danish hospitals to the Technical University of Denmark

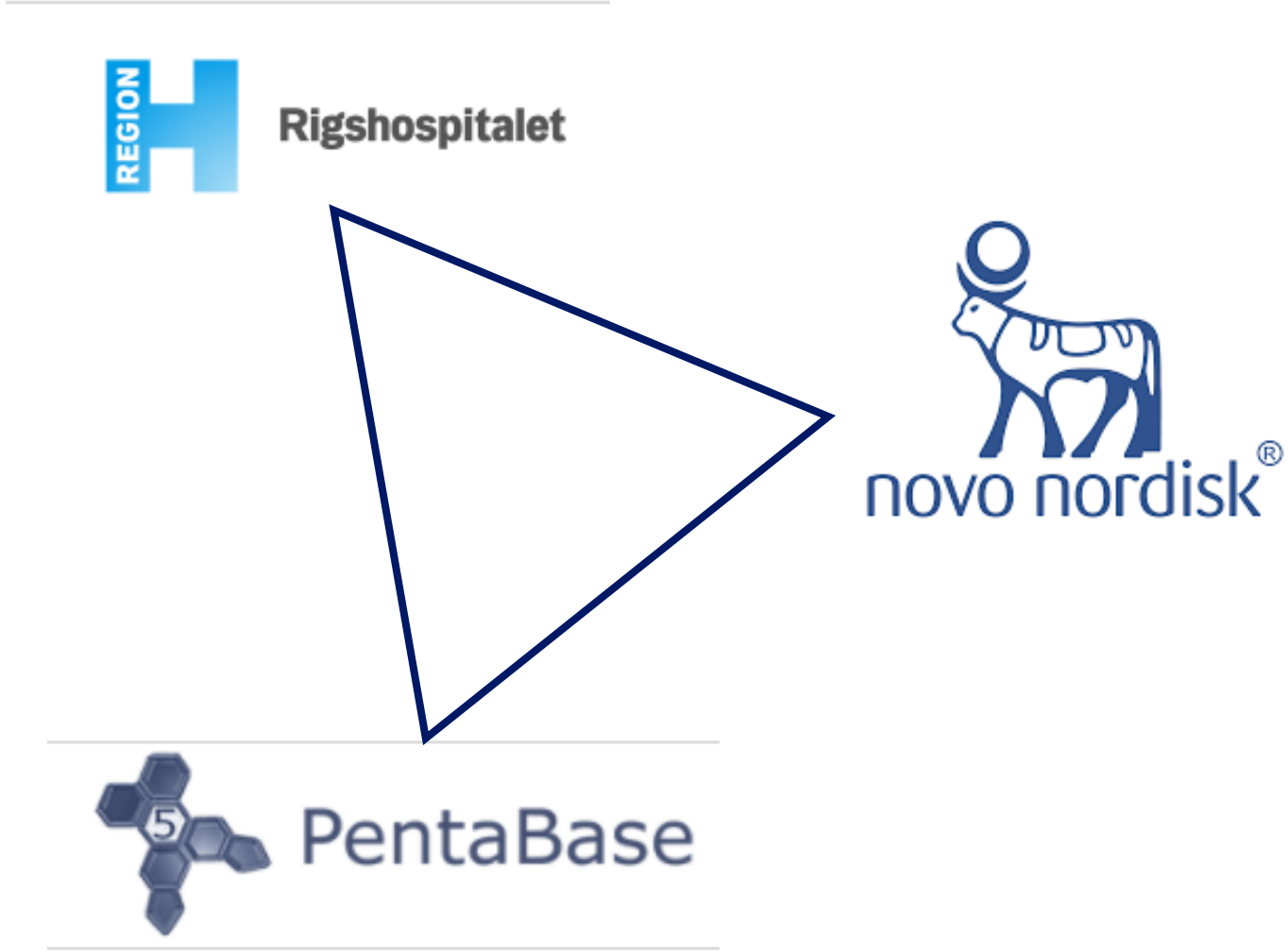
- PentaBase is on a commercial basis offering testing in Denmark, and have made a business out of exporting kits with the method developed

- Only 3 hospitals in Denmark have implemented the method, and only for a subset of their testing capacity

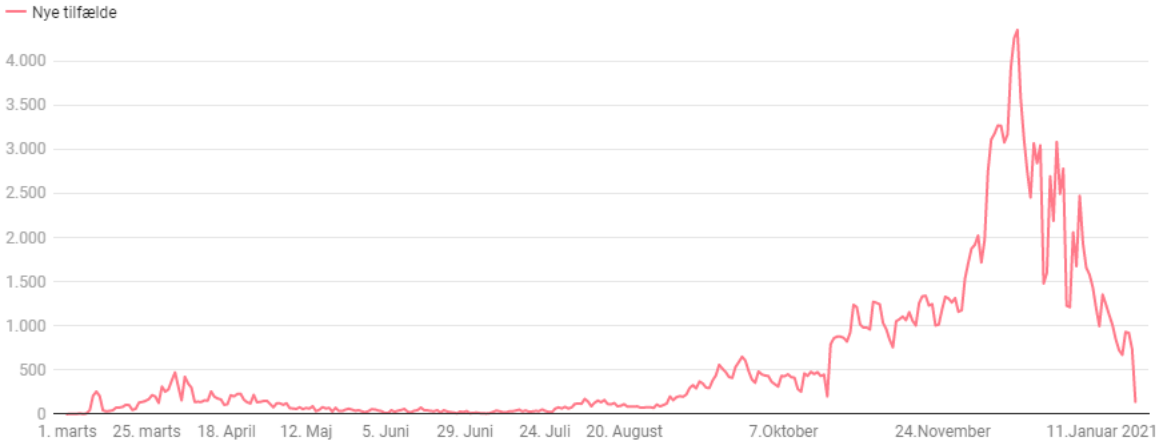
Three key parameters that I forgot to control

- Standardized tubes with lysis buffer and barcodes at the hospitals
- Supply of swab sticks to the hospitals
- Physical location of equipment

Thank you!



RT-PCR positive/day



Grafik: TV 2 Lorry. Tallene for de seneste dage vil stige, som tests bliver registreret. De nye smittede er placeret på grafen efter tidspunktet for deres test. • Kilde: Statens Serum Institut • Hent data • Lavet med Datawrapper

Dead with covid-19/day

Dødstallene er vist for datoen, hvor de blev erklæret døde, ikke for datoen hvor tallet blev offentliggjort. Tallene for de seneste dage bliver løbende opjusteret efterhånden, som myndighederne får meldinger ind, og vil derfor stige.

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