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Molecular Testing at the Point of Care: Past, Present, and Future

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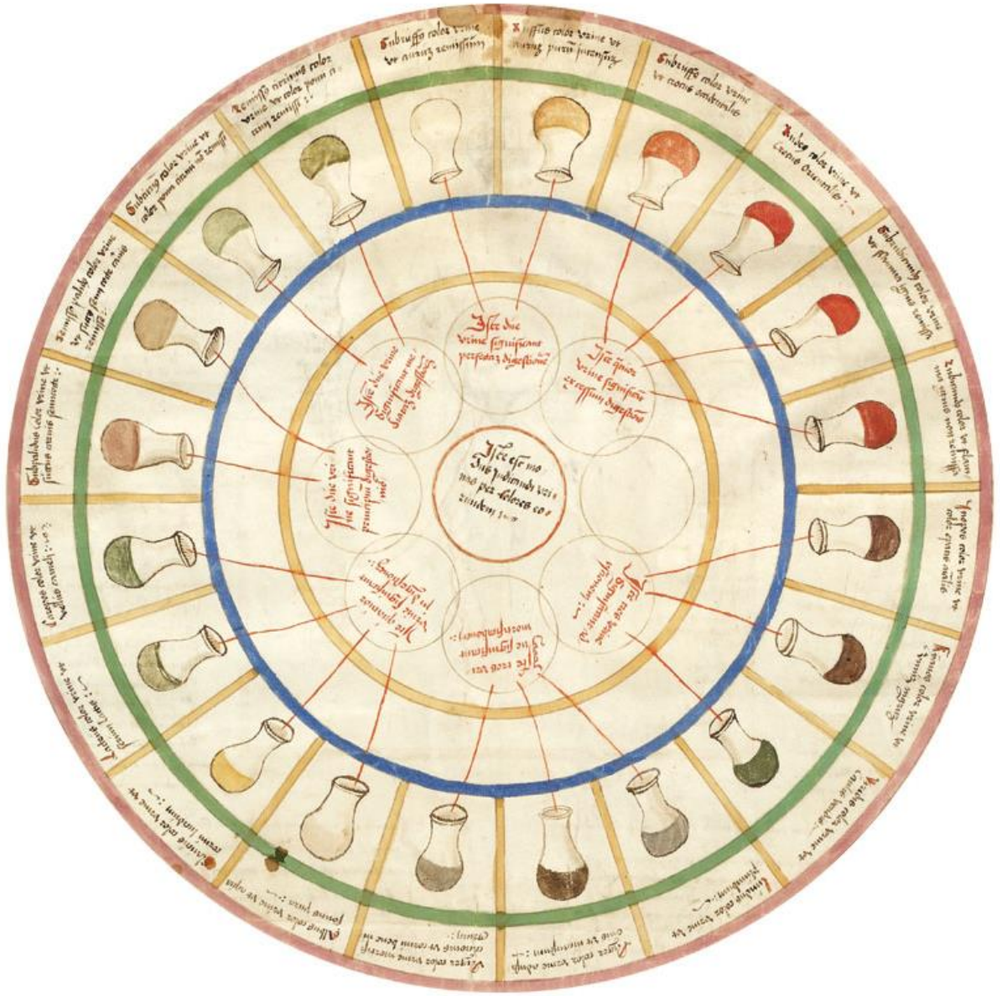
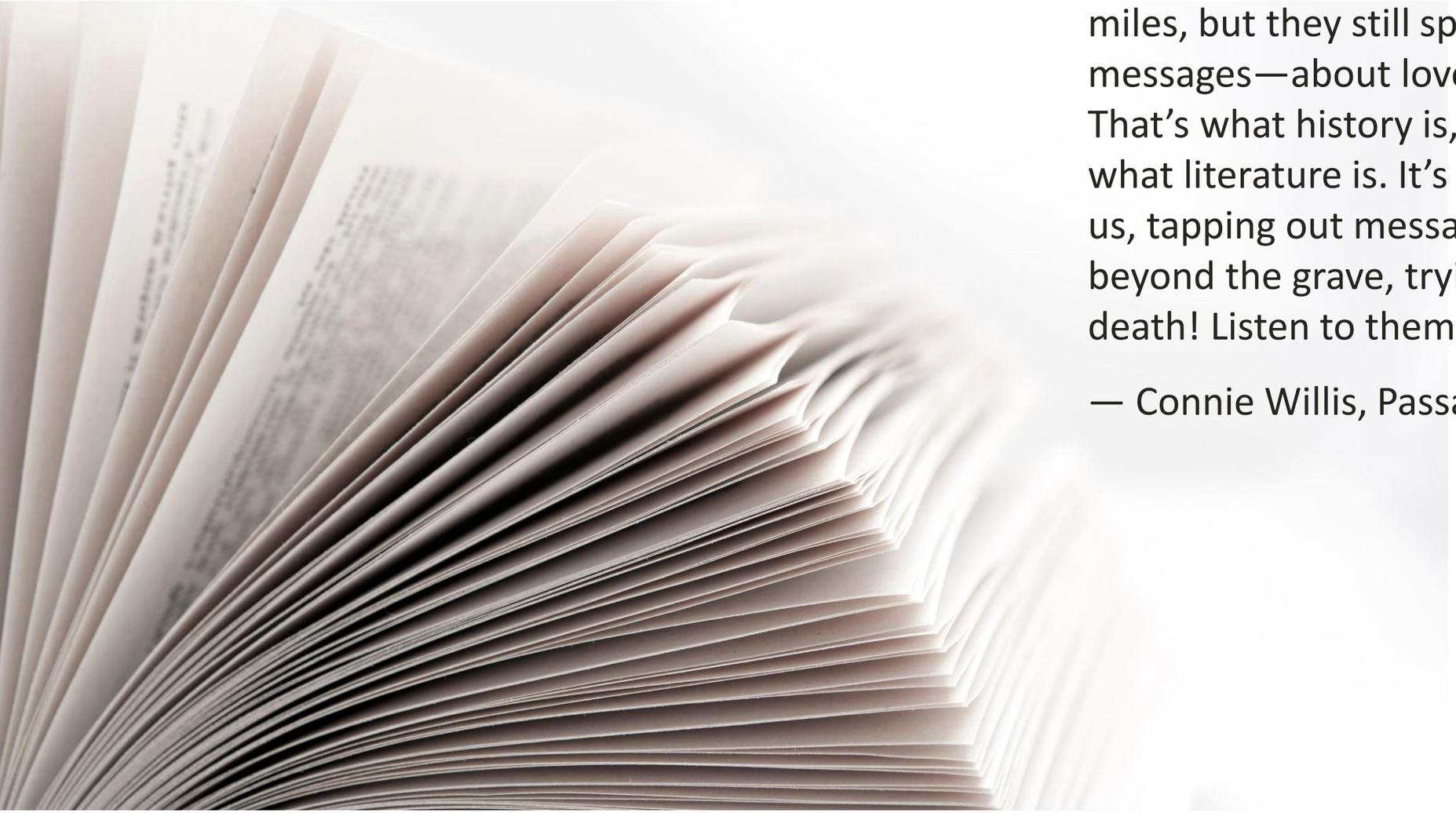
Opinions expressed in this presentation are those of the speaker and do not express the views or opinions of Cardinal Health

Learning Objectives

- At the end of this webinar, participants will be able to:
  - Relate the history of point of care testing to current practice, from ancient uroscopy to current molecular tests
  - Describe the core workflow of point-of-care molecular tests
  - Analyze quality practices for point of care molecular testing
  - Recognize the relationship of molecular and antigen tests for diagnosis of respiratory infections
  - Recognize drivers and non-drivers of molecular POCT in the future



History



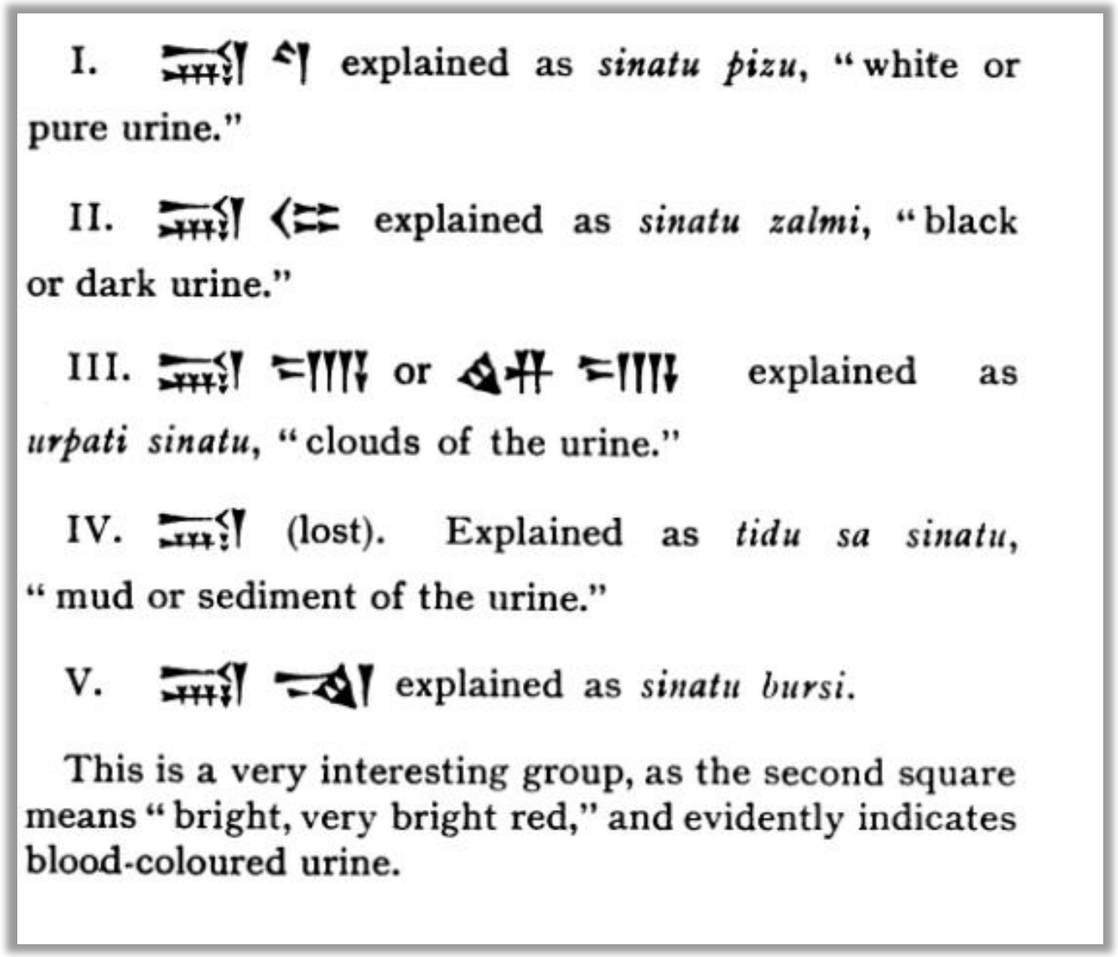
"They say the dead can't speak, but they can! The people in this book died over sixty years ago, in the middle of the ocean, with no one around them for miles, but they still speak to you. They still send us messages—about love and courage and death! That's what history is, and science, and art. That's what literature is. It's the people who went before us, tapping out messages from the past, from beyond the grave, trying to tell us about life and death! Listen to them!"

— Connie Willis, Passage

Uroscopy as POC in the Ancient World

A Sumerian Syllibarium (dictionary) c. 4000 BCE lists body parts and alludes to changes in color and constitution of urine observed by physicians.

The beginnings of lab testing, but (of course) performed at the point of care.



I. [illegible] explained as *sinatu pizu*, "white or pure urine."

II. [illegible] explained as *sinatu zalmi*, "black or dark urine."

III. [illegible] or [illegible] explained as *urpati sinatu*, "clouds of the urine."

IV. [illegible] (lost). Explained as *tidu sa sinatu*, "mud or sediment of the urine."

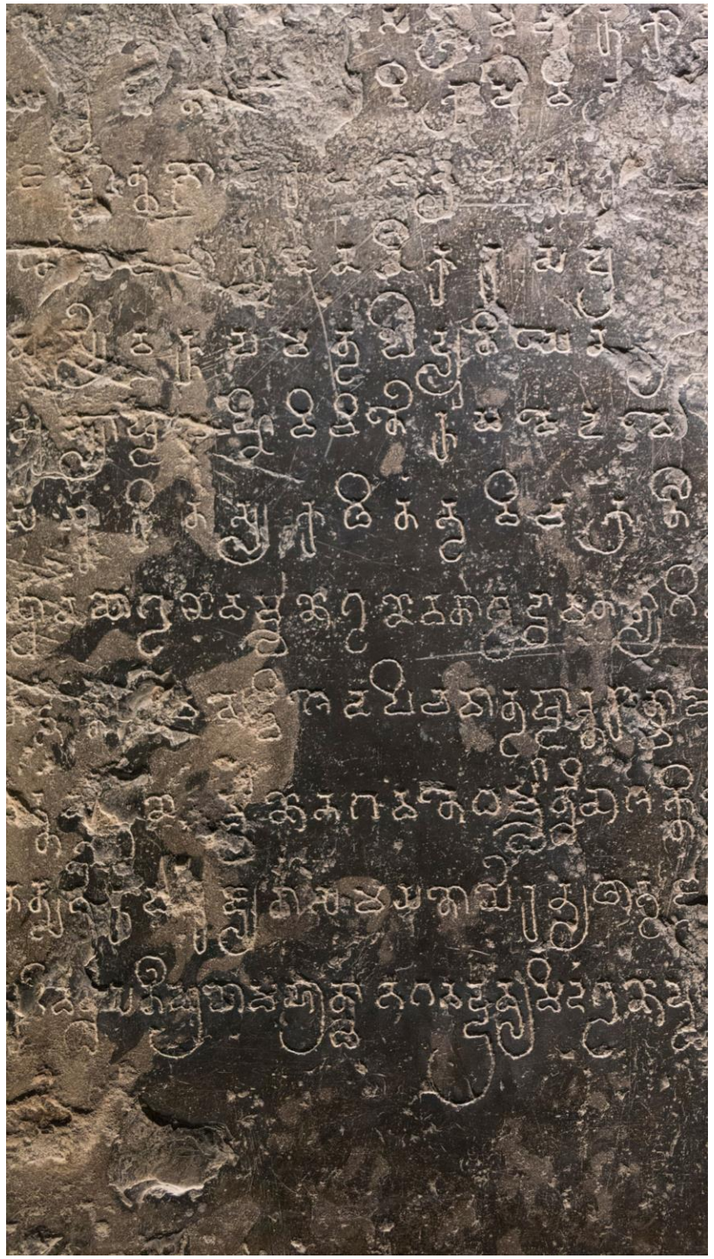
V. [illegible] explained as *sinatu bursi*.

This is a very interesting group, as the second square means "bright, very bright red," and evidently indicates blood-coloured urine.

No, I was *not* personally around for this.

Some Sanskrit Diagnoses:

- *IKSUMEHA*, CANE-SUGAR JUICE URINE.
- *KSUERMEHA*, POTASH URINE.
- *SONITAMEHA*, URINE CONTAINING BLOOD.
- PISTAMEHA, FLOURY-WHITE URINE.
  - WHEN THE PATIENT PASSES THIS TYPE OF URINE THE HAIR ON THE BODY BECOMES ERECT, AND THE URINE LOOKS AS THOUGH MIXED WITH FLOUR. URINATION IS PAINFUL.
- *HASTIMEHA*, ELEPHANT URINE.
  - “THE PATIENT CONTINUOUSLY PASSES TURBID URINE LIKE A MAD ELEPHANT.”
- *MADHUMEHA*, HONEY URINE.
  - TRAINS OF LONG BLACK ANTS ARE ATTRACTED BY THE URINE.



Advances in Urine Analysis

Theophilus (610-641 AD) employed heat to further the analysis of urine; arguably the first analytic technique in medicine.

Alsahavarius (c. 1085) noted the effect of certain foods on the color of the urine and cautioned physicians against being fooled by intentional ingestions.

Actuarius (d. 1283) recommended the use of a graduated glass for measuring sediments.

A Greek Physician examining the Urine
and
a Diagram showing the Various
Colours of Morbid Urine
From an illuminated MS. of the XIV century

Specimen Guidelines

Ismail of Jurjani (c. end of 11th century), a Persian physician

- Includes container specifications, time of collection, storage conditions, and patient instructions.
- Goes on to provide detailed recommendations for examination of urine.

Uroscopy as practised by the early Persians

"The urine which is for the physician to examine," he states, "must be collected in a bottle, which must be large, transparent and clean, and if possible should be in the shape of a bladder. It should be of a large size, so as to contain the whole of the urine (24 hours), for the reason, if there be something (sediment) in it, it should be detected at once. The shape of the bottle is devised like a bladder for the reason that the urine should be in natural position as in that viscus. Urine should be well guarded against heat, cold and the sun, because extremes of temperature change its natural state, and heat makes it burn, and its thin sediments are consumed thereby. Cold makes urine congealed.

"Urine sent for examination should be that of the early morning after a good sleep. It should be passed before eating or drinking anything, because partaking of certain foods changes the colour of the urine. One should not rely upon urine that has been passed during starvation, sorrow, weakness or sleeplessness, or after coition, because above conditions change its colour. After food and wine the natural heat of the body increases for the purpose of digestion, the urine becomes colourless. Often in hot diseases it becomes white and puts the physician off his guard. After hunger, sleeplessness, sorrow and trouble, urine changes its colour, because heat (bodily) in such conditions moves about (in the body) and makes the urine appear coloured. Often one passes colourless urine after sleeplessness, because heat (bodily) is dissipated through insomnia, the urine passed is rather turbid and not clear and light, because food cannot be well digested in sleeplessness; food remains kham (uncooked, unasssimilated); that is also the reason why one gets darkish and muddy water from uncooked food.

Comprehensive QA for Uroscopy

PHYSICIAN EXAMINING A SAMPLE OF URINE BROUGHT BY A PATIENT

From a woodcut of the XVI century

Gilles de Corbeil, early 12th Century

Poem written in dactylic hexameter, which I dare anyone here to write a scientific publication in today.

Gilles de Corbeil, who graduated at the School of Salerno at the beginning of the twelfth century, and was first physician to Phillipe Auguste, wrote an elaborate poem on the urine, entitled "Liber de urinis," which gives a good idea of the state of medical knowledge at the period in which he lived. He begins by studying the etymology of the word urine, and then, referring to the composition of this excretion, remarks that "urine is composed of the residue left in the blood and other humours in the kidneys." Next, he proceeds to lay down in detail, rules for its examination, placing, for the guidance of the uroscopist, special emphasis on the aspects, the consistence, the quantity, the nature, and the things contained therein. He enjoins the physician to take into consideration, also, the circumstances of place, the number, the time, the age, the sex, the exercises indulged in, as well as the temperament and diet of his patient.

Gilles de Corbeil and his poetical treatise on urine

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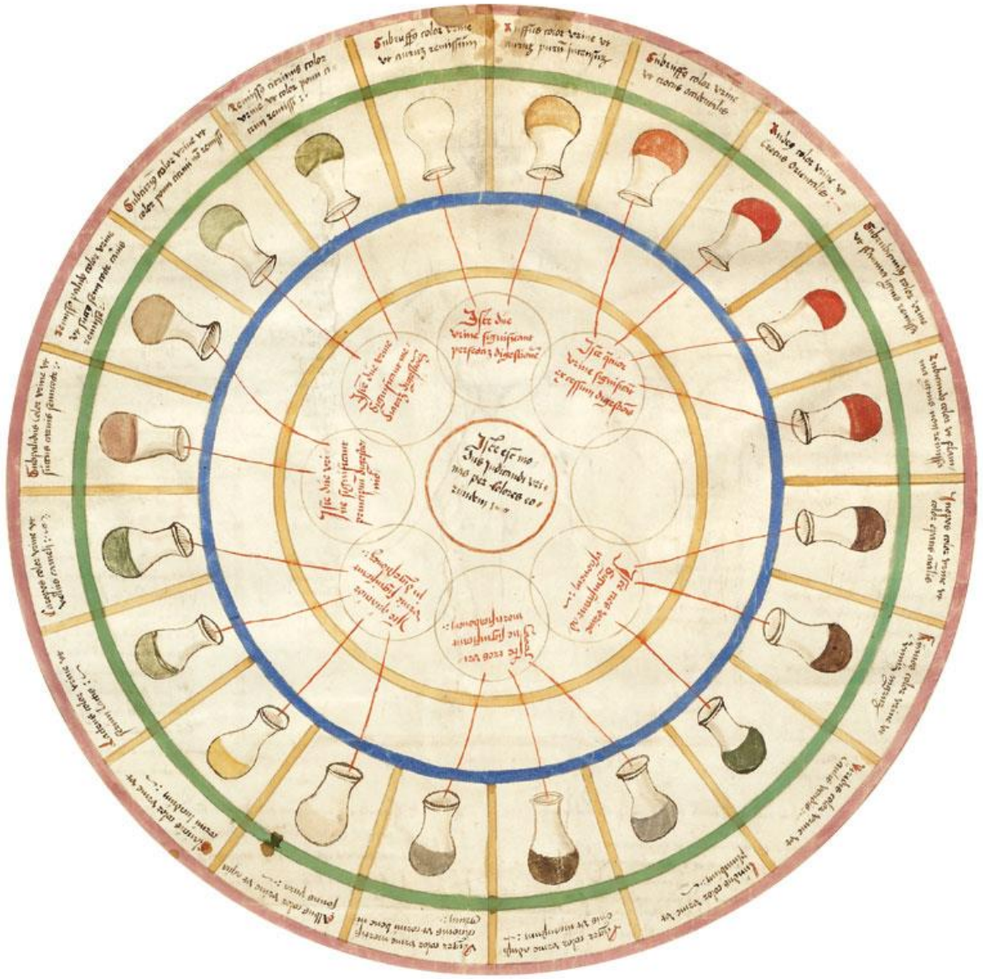
Historical Attempts to Comply with CLIA

The urine-glass disc was used as a colorimetric standard (the first ones known date from 1400 or before) in urine diagnosis.



History of Uroscopy – Lessons

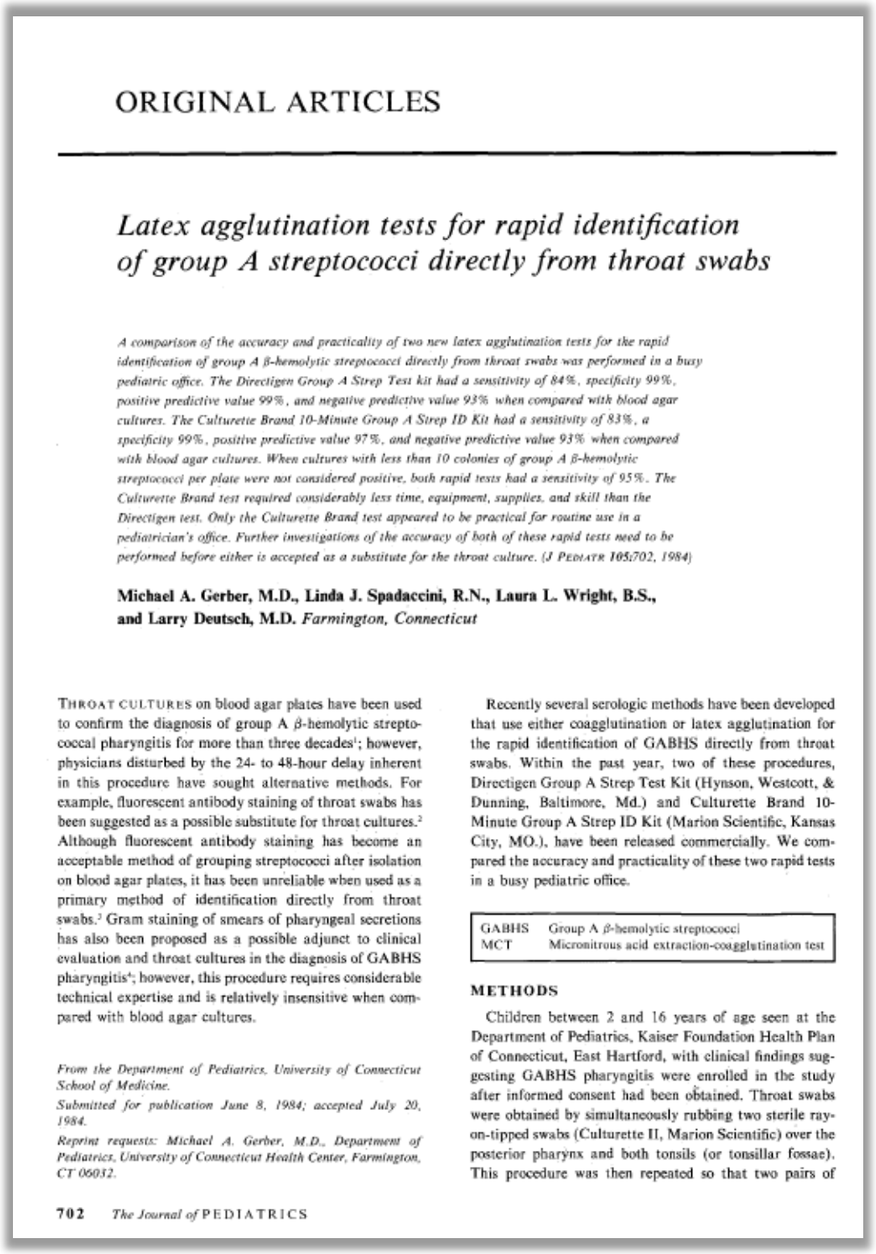
Like us, the ancient uroscopists:

- Paid attention to pre-analytical, analytical, and post-analytical components of testing
- Attempted to standardize procedures and practices
- Attempted to train, and assess and ensure competency
- Attempted to improve the practice of their craft

Title Page of "The Judycyall of Uryns"
Printed about 1512

The Modern Era of POCT: Rapid Antigen Tests

- For infectious disease, the first antigen tests for POC use were rapid strep latex tests
- Required a simple extraction followed by latex agglutination on a glass slide
- **WHY Group A Strep!!?**
  - *A single test allows for treatment*
  - *Limited differential*
  - *No need for imaging or other tests to* ***complete the encounter***



ORIGINAL ARTICLES

Latex agglutination tests for rapid identification of group A streptococci directly from throat swabs

A comparison of the accuracy and practicality of two new latex agglutination tests for the rapid identification of group A β-hemolytic streptococci directly from throat swabs was performed in a busy pediatric office. The Directigen Group A Strep Test kit had a sensitivity of 84%, specificity 99%, positive predictive value 99%, and negative predictive value 93% when compared with blood agar cultures. The Culturette Brand 10-Minute Group A Strep ID Kit had a sensitivity of 83%, a specificity 99%, positive predictive value 97%, and negative predictive value 93% when compared with blood agar cultures. When cultures with less than 10 colonies of group A β-hemolytic streptococci per plate were not considered positive, both rapid tests had a sensitivity of 95%. The Culturette Brand test required considerably less time, equipment, supplies, and skill than the Directigen test. Only the Culturette Brand test appeared to be practical for routine use in a pediatrician's office. Further investigations of the accuracy of both of these rapid tests need to be performed before either is accepted as a substitute for the throat culture. (J PEDIATR 105:702, 1984)

Michael A. Gerber, M.D., Linda J. Spadaccini, R.N., Laura L. Wright, B.S., and Larry Deutsch, M.D. *Farmington, Connecticut*

THROAT CULTURES on blood agar plates have been used to confirm the diagnosis of group A β-hemolytic streptococcal pharyngitis for more than three decades[1]; however, physicians disturbed by the 24- to 48-hour delay inherent in this procedure have sought alternative methods. For example, fluorescent antibody staining of throat swabs has been suggested as a possible substitute for throat cultures.[2] Although fluorescent antibody staining has become an acceptable method of grouping streptococci after isolation on blood agar plates, it has been unreliable when used as a primary method of identification directly from throat swabs.[3] Gram staining of smears of pharyngeal secretions has also been proposed as a possible adjunct to clinical evaluation and throat cultures in the diagnosis of GABHS pharyngitis[4]; however, this procedure requires considerable technical expertise and is relatively insensitive when compared with blood agar cultures.

From the Department of Pediatrics, University of Connecticut School of Medicine.

Submitted for publication June 8, 1984; accepted July 20, 1984.

Reprint requests: Michael A. Gerber, M.D., Department of Pediatrics, University of Connecticut Health Center, Farmington, CT 06032.

Recently several serologic methods have been developed that use either coagglutination or latex agglutination for the rapid identification of GABHS directly from throat swabs. Within the past year, two of these procedures, Directigen Group A Strep Test Kit (Hynson, Westcott, & Dunning, Baltimore, Md.) and Culturette Brand 10-Minute Group A Strep ID Kit (Marion Scientific, Kansas City, MO.), have been released commercially. We compared the accuracy and practicality of these two rapid tests in a busy pediatric office.

GABHS	Group A β-hemolytic streptococci
MCT	Micronitrous acid extraction-coagglutination test

METHODS

Children between 2 and 16 years of age seen at the Department of Pediatrics, Kaiser Foundation Health Plan of Connecticut, East Hartford, with clinical findings suggesting GABHS pharyngitis were enrolled in the study after informed consent had been obtained. Throat swabs were obtained by simultaneously rubbing two sterile rayon-tipped swabs (Culturette II, Marion Scientific) over the posterior pharynx and both tonsils (or tonsillar fossae). This procedure was then repeated so that two pairs of

702 *The Journal of* PEDIATRICS

Gerber MA, Spadaccini LJ, Wright LL, Deutsch L. Latex agglutination tests for rapid identification of group A streptococci directly from throat swabs. *J Pediatr*. 1984;105(5):702-705. doi:10.1016/s0022-3476(84)80286-3

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Molecular Testing

“We've been merging with tools since the beginning of human evolution, and arguably, that's one of the things that makes us human beings.”

-Franklin Foer

A Breakthrough in Testing!

A physician examining a urine specimen in which a faint figure of a baby is visible, a female patient is crying and being shouted at by her angry mother, indicating that she is pregnant.

What is Molecular Diagnostics?

- Molecular diagnostics have found widespread application with the advent of ***amplification methods*** (PCR and related approaches).
- Huge scope
  - From single-target molecular detection of pathogens…
  - To pharmacogenomic analysis of metabolism genes for drug dosing…
  - To whole genome sequencing for disease susceptibility and everything else.

Molecular Diagnostic Testing

Specimen

- Specimen type important, specimen integrity is crucial.

DNA/RNA Extraction

- Extraction steps simple for easy specimen types; more elaborate for stool, etc.

Amplification of Target

- Many amplification technologies available: thermal cycling vs. isothermal.

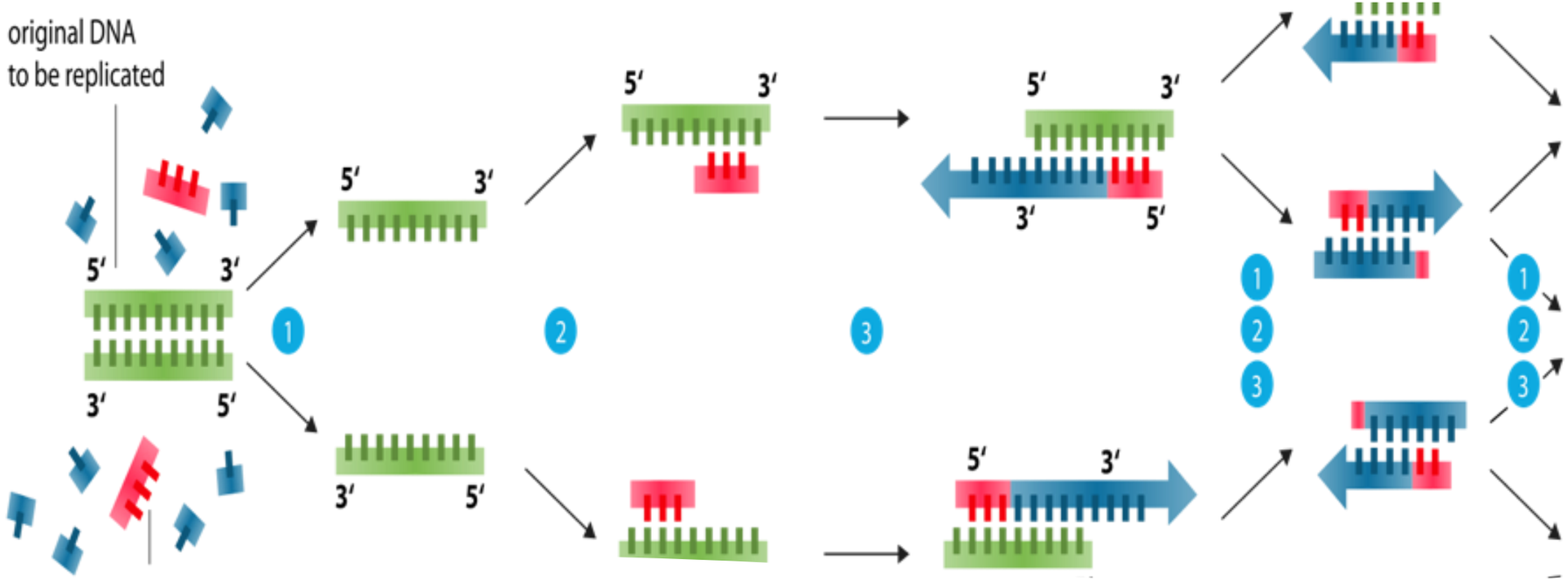
Detection of Amplified Material

Interpretation and Clinical Use

Polymerase Chain Reaction (PCR)

IT ALL STARTED WITH PCR…

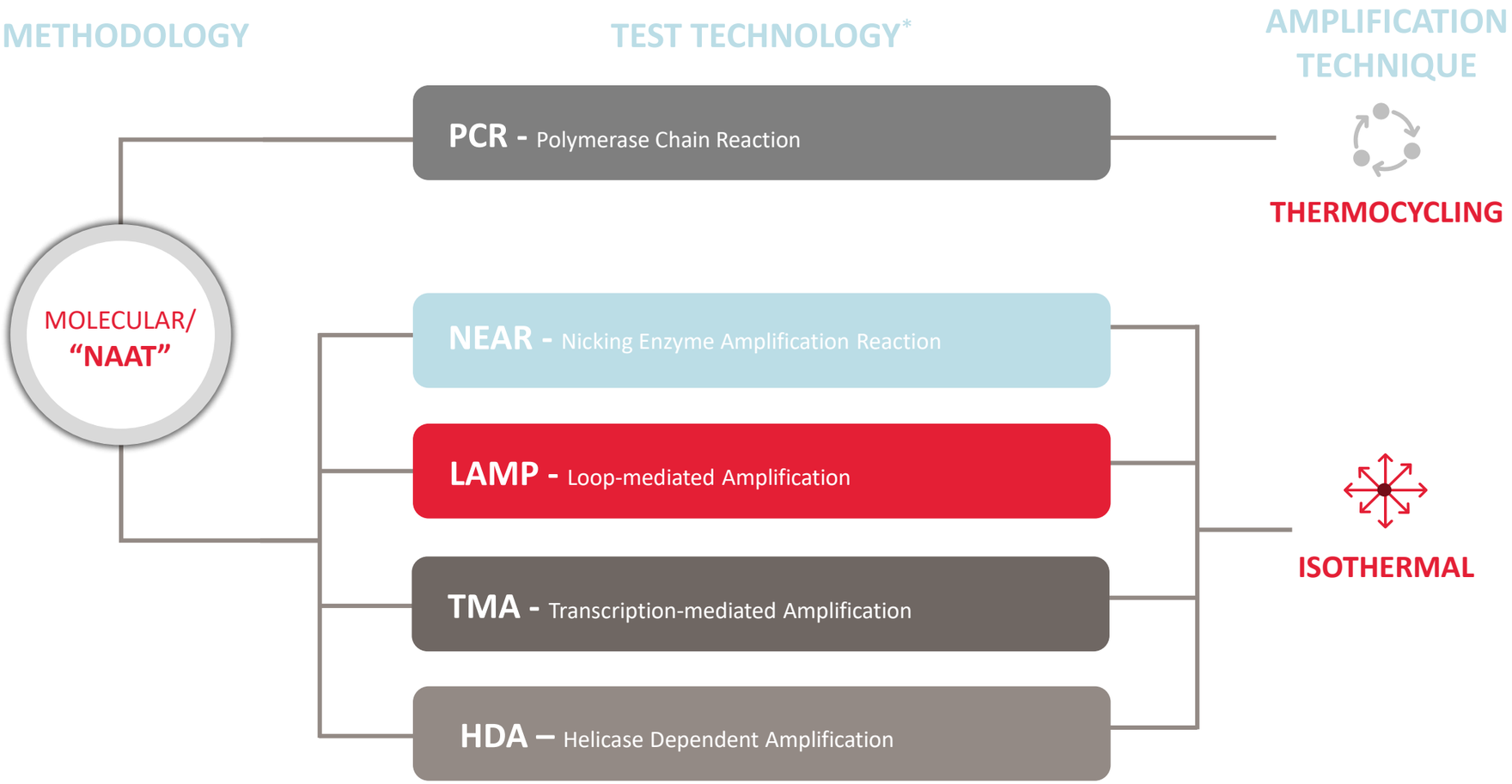
Basically, you pick a target sequence out of a bunch of other DNA and make a jillion copies of it, then detect those copies.



BUT THERE ARE MANY OTHER AMPLIFICATION TECHNOLOGIES.

Image By Enzoklop - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=32003643

Molecular (NAAT) Tests



NAAT, nucleic acid amplification test.
*Multiple NAATs amplify nucleic acids, not a comprehensive list.
CDC, Nucleic Acid Amplification Tests (NAATs), updated June 16, 2021. Accessed July 21, 2021.

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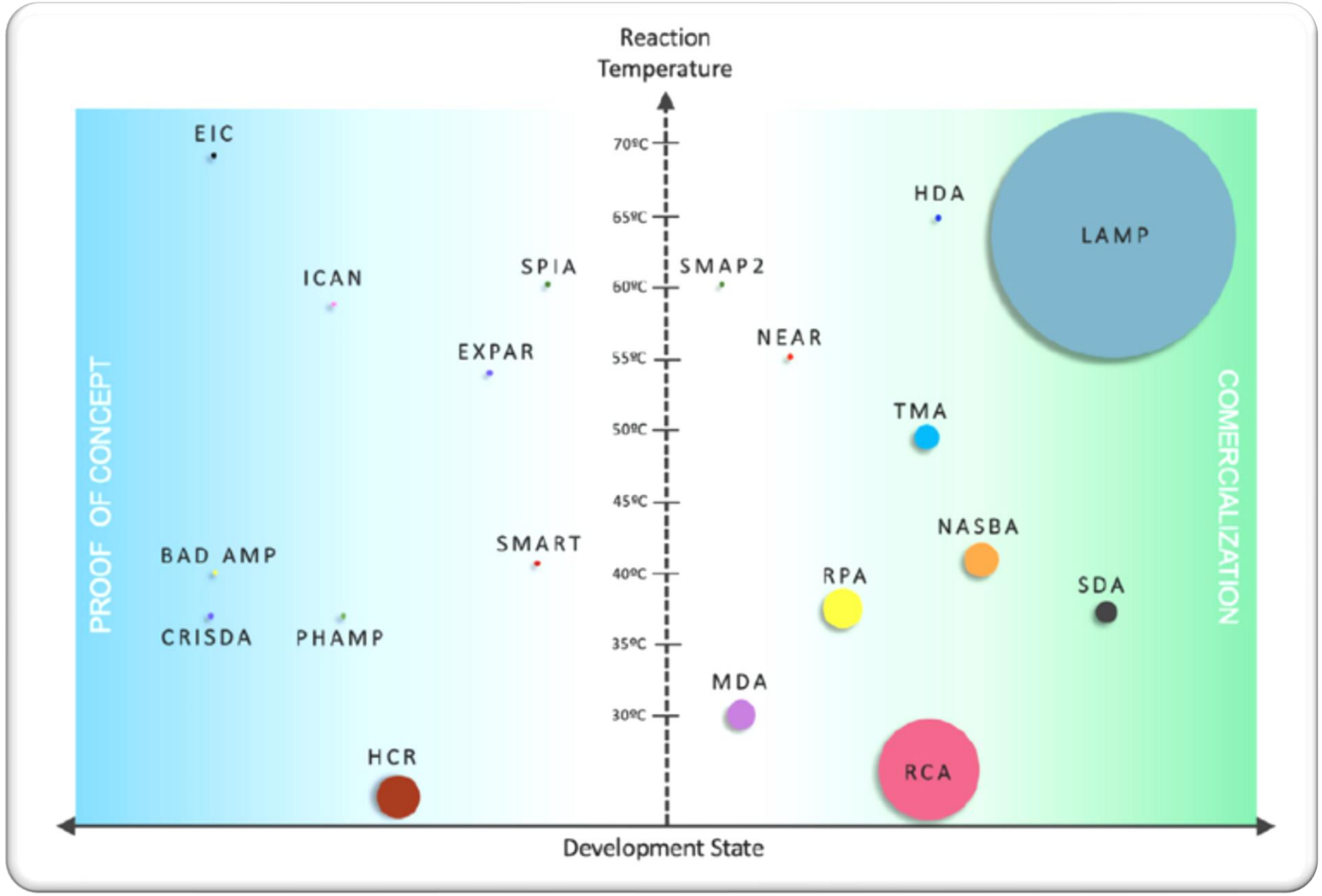
Isothermal Amplification



Comparison between isothermal amplification mechanisms.
Circle size is proportional to the number of scientific items in literature.

- Multiple isothermal amplification technologies are available and in the pipeline.
- Isothermal amplification allows for simpler instrument design but tends to make for chemically-complex assays; more enzymes/primers/probes.

From: Oliveira BB, Veigas B and Baptista PV (2021) Isothermal Amplification of Nucleic Acids: The Race for the Next “Gold Standard”. Front. Sens. 2:752600

Managing POC Molecular

All the usual QC and QA, plus:

Interferences

- Extraction efficiency
- **Inhibition** by
  - Blood
  - DNA
- Internal amplification / extraction controls
- Interferences in other testing, maybe more in molecular

Contamination

- **Extraordinarily sensitive methods**
- **Specimen** cross-contamination
  - Native material transferred from a positive to a negative specimen
  - Collection devices
  - Ports, racks, hands
- **Amplicon** contamination
  - From amplified material
  - How well is the product contained?
  - Waste disposal
- Molecular people are very aware of this, lab people are pretty aware of this, **clinical/POC people are entirely unaware of this.**

Quality Practices Particular to Molecular POCT

“Unfortunately, it's also true to say that good management is a bit like oxygen - it's invisible and you don't notice its presence until it's gone, and then you're sorry.”

— Charles Stross, The Fuller Memorandum

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Suggested POC Molecular Practices

Problem	Approach
Contamination!	Monitor positivity rates
Specimen quality and preservation	Have procedures to assure clinically-relevant specimens and maintain specimen integrity and identification
You're testing for dangerous things	Address staff safety in testing procedures and practices
Different methods perform differently	Include method and relevant information in final report

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Monitoring for False-Positives

Problem	Approach
Contamination!	Monitor positivity rates

How do you monitor for the presence of false positive results (eg, due to nucleic acid contamination)?

- What do you do if:
  - Your rate of influenza positives jumps to 10% in the middle of the summer?
  - You have three positives in a single run with a test that normally generates one positive every week?
  - In the middle of a covid spike, you have no positives for three days in a row?
- Think about what to monitor and what actions to take in response.

Specimen Integrity

Problem	Approach
Specimen quality and preservation	Have procedures to assure clinically-relevant specimens and maintain specimen integrity and identification

How might you prevent specimen loss, alteration, or contamination during collection, transport, processing and storage?

- Specimen loss: Is that relevant to POC? When?
- Specimen alteration: Can it get hot or cold? Could the transport media deteriorate?
- Contamination: How might this happen between specimens?
- Transport: When is it relevant to POC?
- Processing: Could specimens be lost or cross-contaminate?
- Storage: Where do you keep specimens if testing doesn't happen immediately so they're not lost, harmed, or mixed up?

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Safety

Problem	Approach
You're testing for dangerous things	Address staff safety in testing procedures and practices

How do you safely handle and process specimens, including those suspected to contain highly infectious pathogens?

- You need a plan!!
- OK, so maybe the policy says, 'run in circles screaming'. At least you know what to do, right?
  - (No, that's not a recommendation.)
  - Think about it ahead of time!
  - How could collection/testing personnel be exposed?
  - What PPE should be used?
  - What environmental/engineering controls do you need?
  - What are safe work practices for the hazards you anticipate?

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Report the Method

Problem	Approach
Different methods perform differently	Include method and relevant information in final report

How do you ensure that providers know what test they're getting and how it performs?

- The final report should include a summary of the test method and information regarding clinical interpretation, if appropriate.
- Different methods for POC testing – especially antigen vs molecular, but even different molecular tests – can have markedly different sensitivity/specificity/interferences.

Molecular POCT in The Broader Diagnostics Context

Never make predictions, especially about the future.
- Casey Stengel

Molecular Testing for Respiratory Pathogens in 2019…

- Real-time molecular methods can provide result in <1h
- Molecular methods as a class exceed culture in sensitivity (probably due to viral loss in transport)
- Detection properties vary from system to system
- Moderately to very expensive equipment
- **Clearly the 'gold standard' (cue ominous music…)**

Where We Stood in Late 2019

- Molecular testing for respiratory viruses was standard-of-care.
- Automated readers for antigen tests improved performance, but not to the level of molecular tests.
  - Antigen tests were on the way out?

File:Albert Bierstadt - The coming storm.jpg. Wikimedia.org.. https://commons.wikimedia.org/wiki/File:Albert_Bierstadt_-_The_coming_storm.jpg

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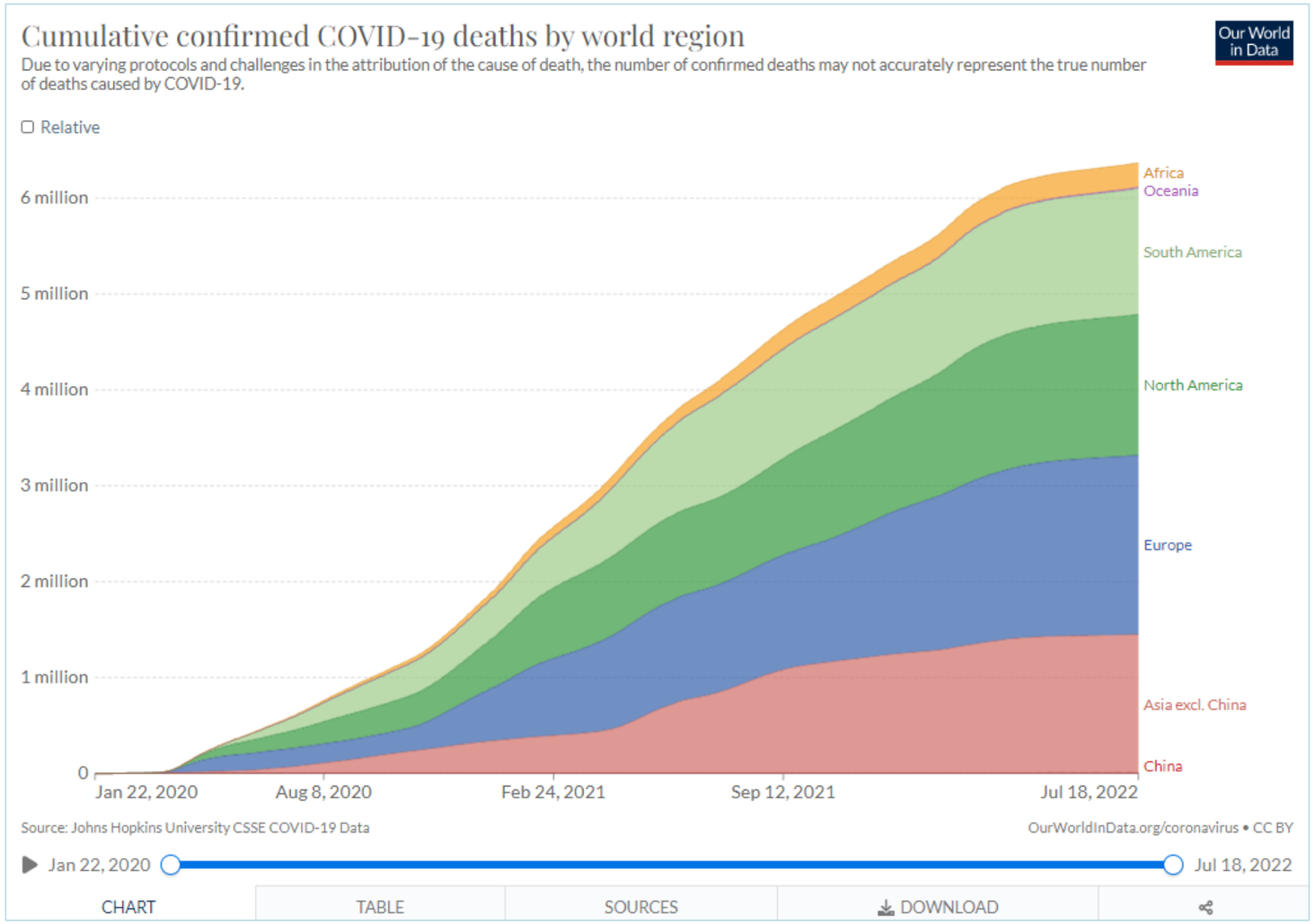
COVID-19

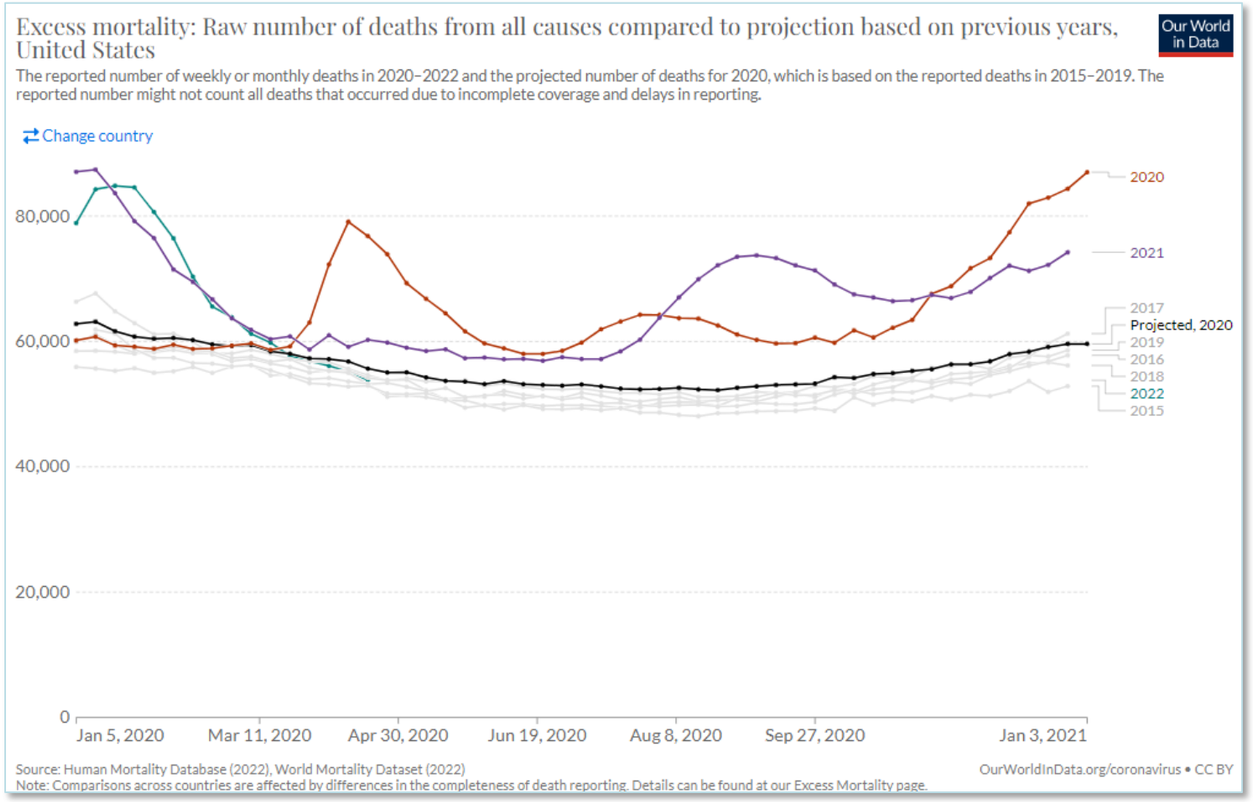
- Global pandemic; began in Wuhan, Hubei Province, China, in late 2019.
- Caused by SARS-CoV-2 coronavirus.
- Has since spread worldwide, with in excess of 6 million deaths so far.



Cumulative confirmed COVID-19 deaths by world region. Our World in Data. https://ourworldindata.org/grapher/cumulative-covid-deaths-region

Impact of COVID-19 Pandemic

- Globally
  - Global stock markets worst crash since 1987.
  - In the first three months of 2020 the G20 economies fell 3.4% year-on-year.
  - Between April and June 2020, an equivalent of 400 million full-time jobs were lost across the world.
  - Income earned by workers globally fell 10 percent in the first nine months of 2020, equivalent to a loss of over US $3.5 trillion.
- In 2020, the U.S. GDP contracted at a 3.5% annualized rate. It was the biggest contraction since 1946 and the first contraction since 2009.



Excess mortality: Raw number of deaths from all causes compared to projection based on previous years. Our World in Data. Accessed October 20, 2022. https://ourworldindata.org/grapher/excess-mortality-raw-death-count

POC In the COVID Pandemic (Controversial, like everything else)

- Molecular
  - Sensitive, maybe too sensitive.
  - Expensive when lots of tests needed.
  - Labs are connected to LIS and report to public health.
- Antigen
  - Insensitive; except maybe not.
  - Cheap, except not really.
  - Home-based testing is widely and rapidly available.

My daughter's (+) COVID test; did not get reported to public health. Did get loaded to Instagram.

SCIENCE ADVANCES | RESEARCH ARTICLE

CORONAVIRUS

Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening

Daniel B. Larremore[1,2]*, Bryan Wilder[3], Evan Lester[4,5], Soraya Shehata[5,6], James M. Burke[4], James A. Hay[7,8], Milind Tambe[3], Michael J. Mina[7,8,9]*†, Roy Parker[2,4,6,10]*†

The COVID-19 pandemic has created a public health crisis. Because SARS-CoV-2 can spread from individuals with presymptomatic, symptomatic, and asymptomatic infections, the reopening of societies and the control of virus spread will be facilitated by robust population screening, for which virus testing will often be central. After infection, individuals undergo a period of incubation during which viral titers are too low to detect, followed by exponential viral growth, leading to peak viral load and infectiousness and ending with declining titers and clearance. Given the pattern of viral load kinetics, we model the effectiveness of repeated population screening considering test sensitivities, frequency, and sample-to-answer reporting time. These results demonstrate that effective screening depends largely on frequency of testing and speed of reporting and is only marginally improved by high test sensitivity. We therefore conclude that screening should prioritize accessibility, frequency, and sample-to-answer time; analytical limits of detection should be secondary.

SCIENCE ADVANCES | RESEARCH ARTICLE

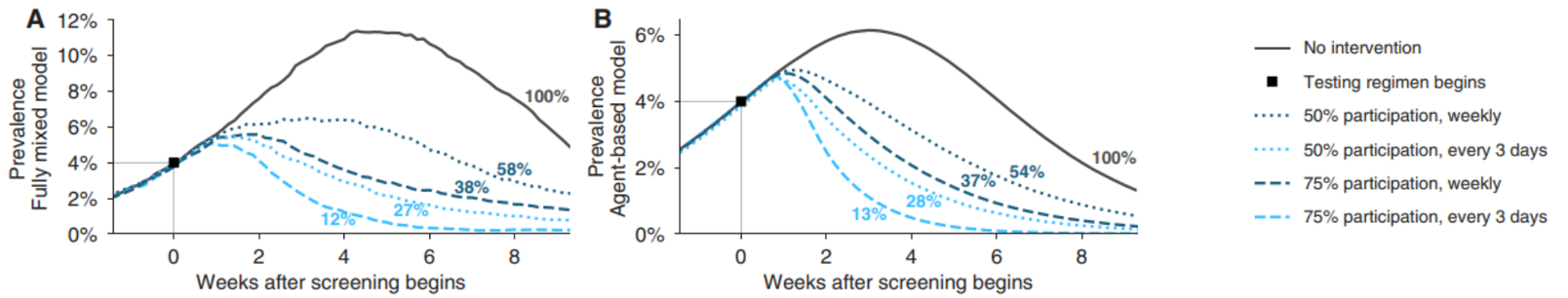


Fig. 6. Repeated population screening suppresses an ongoing epidemic. Widespread testing and isolation of infected individuals drive prevalence downward for both (**A**) the fully mixed compartmental model and (**B**) the agent-based model. Time series of prevalence, measured as the total number of infectious individuals, are shown for no intervention (solid) and population screening scenarios (various dashed lines; see legend) for individual stochastic simulations. Screening began only when prevalence reached 4% (box), and time series are shifted such that testing begins at $t = 0$. Scenarios show the impact of a test with LOD 10^5, no delay in results, and with 10% of samples assumed to be incorrectly collected (and therefore negative) to reflect decreased sensitivity incurred at sample collection in a mass testing scenario. Annotations show total number of post-intervention infections, as a percentage of the no-intervention scenario, labeled as 100% (see fig. S8 for identical simulations using a test with LOD 10^6).

Dilemmas

- Causes of late Ct (low-level) positives:
  - Timing of specimen collection
  - Antiviral therapy
  - Specimen type / quality / stability
  - PCR inhibitors

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COMMENTARY

Considerations regarding Interpretation of Positive SARS-CoV-2 Molecular Results with Late Cycle Threshold Values

Stephanie L. Mitchell,[a] Michael J. Loeffelholz[b]

[a]Medical Affairs, Cepheid, Sunnyvale, California, USA
[b]Scientific Affairs, Cepheid, Sunnyvale, California, USA

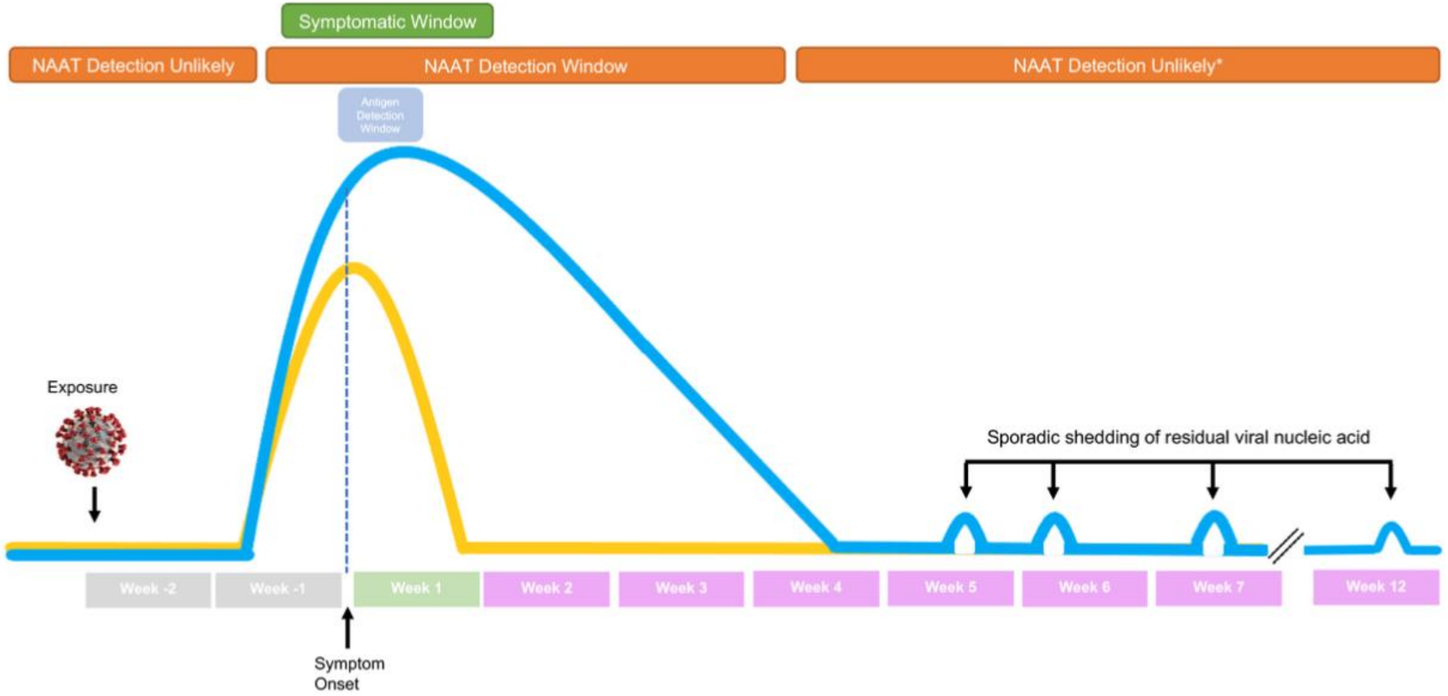


FIG 1 SARC-CoV-2 viral load kinetics and nucleic acid detection.

More Considerations…

- Symptomatic persons at any Ct value considered infected; viral shedding varies
- Immunosuppressed persons often shed longer
- If low prevalence, false-positives are relatively more common
- Retesting can be problematic; around and below the test LoD positives are not necessarily reproducible
- Clinical vs. analytical specificity

Characteristics of Direct Tests

- Published April 29, 2022
- 225 patients
- All infections confirmed by RT-PCR

JAMA Internal Medicine | Original Investigation

Comparison of Home Antigen Testing With RT-PCR and Viral Culture During the Course of SARS-CoV-2 Infection

Victoria T. Chu, MD, MPH; Noah G. Schwartz, MD; Marisa A. P. Donnelly, PhD; Meagan R. Chuey, PhD, RN; Raymond Soto, PhD; Anna R. Yousaf, MD; Emily N. Schmitt-Matzen, DVM, MPH; Sadia Sleweon, MPH; Jasmine Ruffin, MPH; Natalie Thornburg, PhD; Jennifer L. Harcourt, PhD; Azaibi Tamin, PhD; Gimin Kim, BS; Jennifer M. Folster, PhD; Laura J. Hughes, PhD; Suxiang Tong, PhD; Ginger Stringer, PhD, MPH; Bernadette A. Albanese, MD, MPH; Sarah E. Totten, DrPH; Meghan M. Hudziec, BS; Shannon R. Matzinger, PhD; Elizabeth A. Dietrich, PhD; Sarah W. Sheldon, MS; Sarah Stous, MPH; Eric C. McDonald, MD, MPH; Brett Austin, MA; Mark E. Beatty, MD, MPH; J. Erin Staples, MD, PhD; Marie E. Killerby, VetMB, MPH; Christopher H. Hsu, MD, PhD; Jacqueline E. Tate, PhD; Hannah L. Kirking, MD; Almea Matanock, MD, MS; for the COVID-19 Household Transmission Team

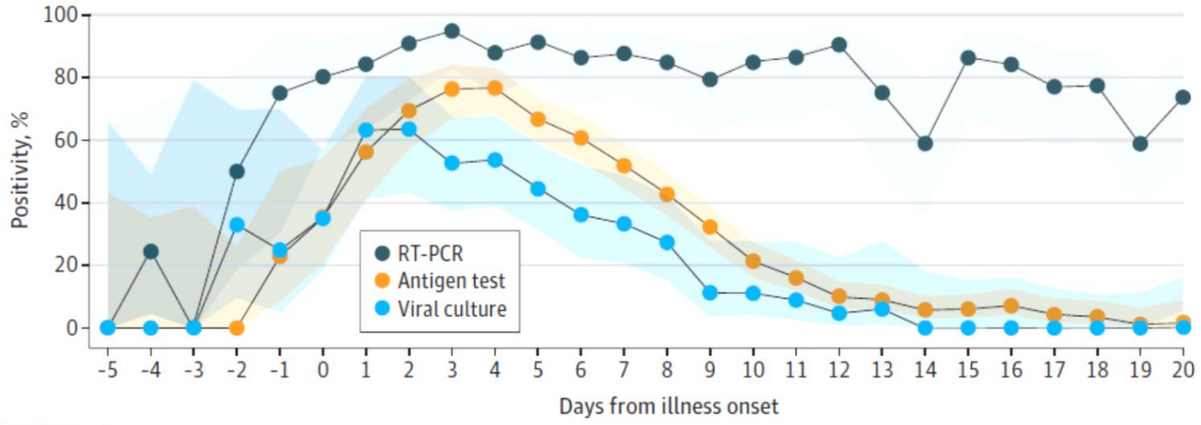
IMPORTANCE As self-collected home antigen tests become widely available, a better understanding of their performance during the course of SARS-CoV-2 infection is needed.

OBJECTIVE To evaluate the diagnostic performance of home antigen tests compared with reverse transcription–polymerase chain reaction (RT-PCR) and viral culture by days from illness onset, as well as user acceptability.

RT-PCR vs. Antigen vs. Culture

- RT-PCR more sensitive early and late in infection
  - Stays positive a long time in a lot of patients
- Antigen and culture track closely – maybe antigen correlates with infectivity
  - We're not likely to get a better measure of this

Figure 1. Daily Percentage of Positive SARS-CoV-2 Tests in Participants With Reverse Transcription–Polymerase Chain Reaction (RT-PCR)–Confirmed Infection



No. of participants

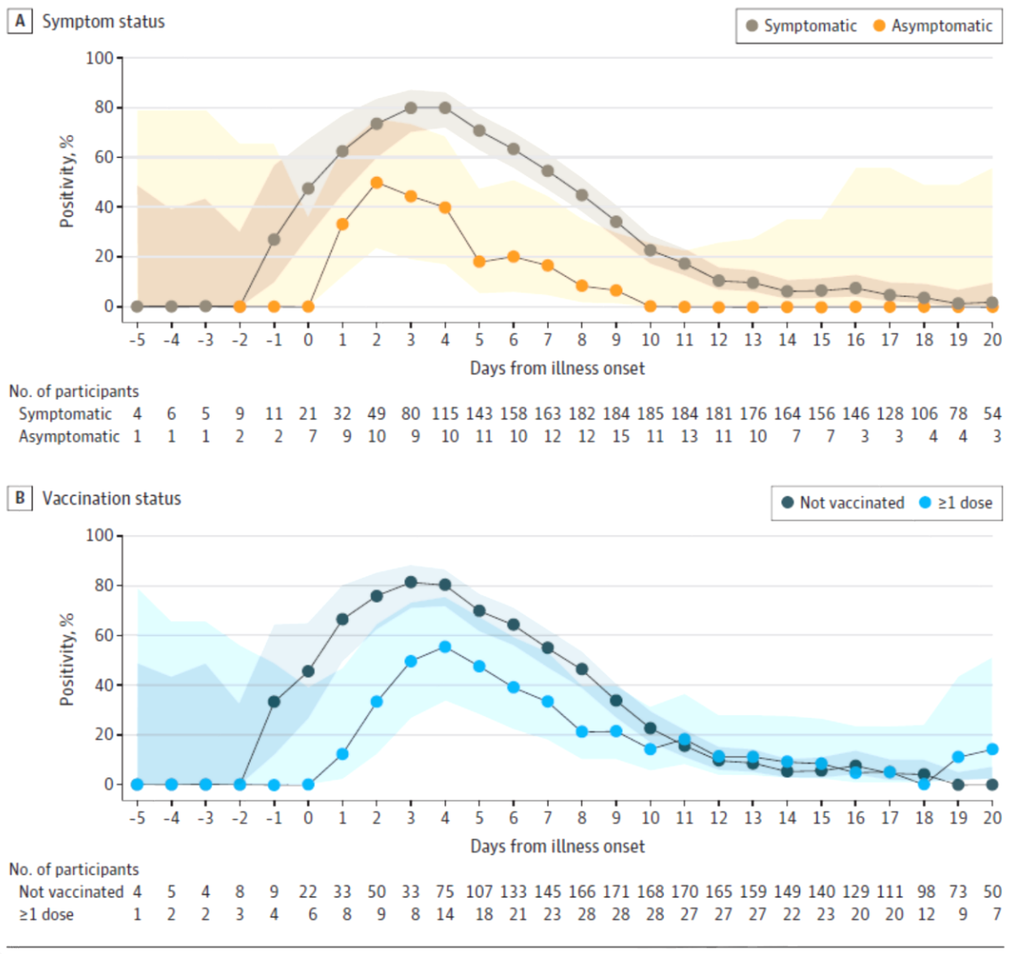
	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RT-PCR	2	4	1	6	4	20	19	22	38	41	47	36	40	33	29	27	22	21	16	17	22	19	26	31	29	19
Antigen test	5	7	6	11	13	28	41	59	89	125	154	168	175	194	199	196	197	192	186	171	163	149	131	110	82	57
Viral culture	2	4	1	6	4	20	19	22	38	41	47	36	39	33	27	27	22	21	16	17	21	19	26	31	29	19

Daily percentage of positive SARS-CoV-2 tests (lines) and 95% CIs (shaded areas) of RT-PCR tests, home antigen tests, and viral culture among 225 participants with RT-PCR–confirmed SARS-CoV-2 infection. If the participant was symptomatic, illness onset was defined as the symptom onset date; if asymptomatic, illness onset was the collection date of the first positive RT-PCR test result. Confidence intervals were calculated by the Wilson score interval method.

Limitations of Antigen Testing

- Antigen is better in symptomatic patients than in asymptomatic, and better in the unvaccinated than in the vaccinated

Figure 2. Daily Percentage of Positive Home Antigen Tests by Symptom Status and Vaccination Status



Daily percentage of positive SARS-CoV-2 tests (lines) and 95% CIs (shaded areas) of home antigen tests among 225 participants with reverse transcription–polymerase chain reaction (RT-PCR)–confirmed SARS-CoV-2 infection by symptom status (A) and vaccination status (B). Participants were considered symptomatic if they reported symptoms that fulfilled the clinical criteria for COVID-19 adopted by the Council of State and Territorial Epidemiologists on August 5, 2020 (https://ndc.services.cdc.gov/case-definitions/coronavirus-disease-2019-2020-08-05/). Symptoms were captured via the enrollment questionnaire and daily symptom questionnaires during the 15-day enrollment period. Confidence intervals were calculated using the Wilson score interval method.

The Future

“Although now that I'm in middle management I'm supposed to call it "refactoring the strategic value proposition in real time with agile implementation,” or, if I’m being honest, “making it up as I go along.”

— Charles Stross, The Apocalypse Codex

Drivers, Not Drivers

- Technological Drivers
- Clinical Drivers / Not Drivers
  - What questions might POCT help answer?
  - What questions is POCT misdirected for?

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Technological Drivers

Trends in Biotechnology CellPress

Review

Emerging Technologies for Next-Generation Point-of-Care Testing

Sandeep Kumar Vashist,[1,*] Peter B. Luppa,[1] Leslie Y. Yeo,[2] Aydogan Ozcan,[3,4,5] and John H.T. Luong[6,*]

Considerable advances in point-of-care testing (POCT) devices stem from innovations in cellphone (CP)-based technologies, paper-based assays (PBAs), lab-on-a-chip (LOC) platforms, novel assay formats, and strategies for long-term reagent storage. Various commercial CP platforms have emerged to provide cost-effective mobile health care and personalized medicine. Such assay formats, as well as low-cost PBAs and LOC-based assays, are paving the way to robust, automated, simplified, and cost-effective POCT. Strategies have also been devised to stabilize reagent storage and usage at ambient temperature. Nevertheless, successful commercialization and widespread implementation of such clinically viable technologies remain subject to several challenges and pending issues.

Vashist SK, Luppa PB, Yeo LY, Ozcan A, Luong JHT. Emerging Technologies for Next-Generation Point-of-Care Testing. Trends Biotechnol. 2015 Nov;33(11):692-705

Key Table

Table 1. Conceptual Potential of Emerging Technologies for Next-Generation POCT

Parameter	CP (1)	PBA (2)	LOC (3)	Next-Generation POCT[a] (1 + 2 + 3)
Performance				
Suitability for POCT	🟢	🟢	🟢	🟢
Technology penetration	🟢	🔴	🔴	NA[b]
Utility in epidemics and emergencies	🟢	🟢	🟢	🟢
Prerequisite of prolonged storage of reagents	✓	✓	✓	✓
Prerequisite of rapid assay	✓	✓	✓	✓
Portability	🟢	🟢	🟢	🟢
Cost-effectiveness of consumables	🟢	🟢	🟢	🟢
Overall cost-effectiveness	🟢	🟢	🟢	🟢
Quantitative	✓	✗	✓	✓
Sensitivity	🟢	🔴	🟢	🟢
Specificity	🟢	🟢	🟢	🟢
Throughput	🟢	🟢	🟢	🟢
Precision	🟢	🔴	🟢	🟢
Reproducibility	🟢	🔴	🟢	🟢
Capable of mass production	✓	✓	✓	✓
Compliance with regulatory guidelines	✓	✗	✓	✓
Ease of Operation				
Ease of operation	🟢	🟢	🟢	🟢
Labor intensiveness	🟢	🔴	🔴	🔴
Need for power supply	✗	✓	✓	✗
Need for readout instruments	✗[c]	✗	✓	✗[c]
Standalone analysis	✓	✓	✓	✓
Personalized	✓	✗	✗	✓
Accessibility of POCT results anytime, anywhere	✓	✗	✗	✓
Basic skill set required for operation	✓	✓	✗	✓
Connectivity				
Connectivity to cloud	✓	✗	✗	✓
Smart applications and portal services	✓	✗	✗	✓
Test history and data patterns	✓	✗	✓ (limited)	✓
Spatiotemporal mapping	✓	✗	✗	✓
Demographic data and statistics	✓	✗	✗	✓
Telemedicine support	✓	✗	✗	✓
Text alerts	✓	✗	✗	✓
Preventive health-care tools	✓	✗	✗	✓

🟢, high; 🟢, medium; 🔴, low.

[a]This column has been computed conceptually taking into account the characteristics of the component technologies.

[b]NA, not applicable.

[c]A smartphone attachment or interfaced instrument would be required for nonoptical signal detection such as in the case of electrochemical readout.

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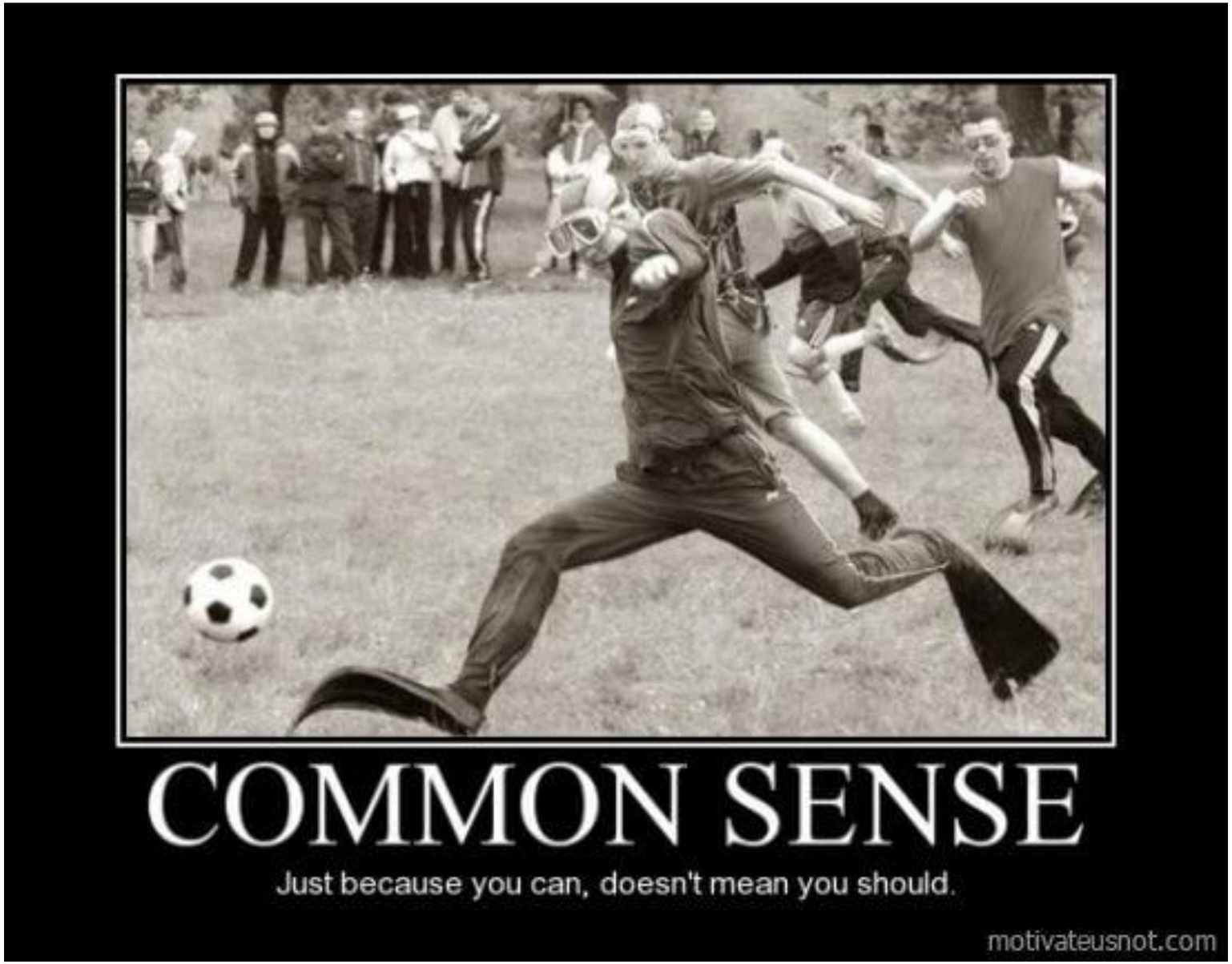

I have this fabulous new <test><biomarker> for <your favorite disease> and I want to make it a point-of-care-test!!!

(Non) Drivers of POCT: Campbell's laws of POCT, and Corollaries

The Laws

1. Almost nobody goes into medicine or nursing to do diagnostic testing
2. No POCT, however simple, is easier than filling in one more box on a laboratory order

The Inpatient Corollary

An Inpatient POC test is useful only if:

- The time for transport to the laboratory for THAT SINGLE ANALYTE significantly and negatively impacts care, OR
- The test is performed on an easily obtained sample (eg, fingerstick blood) MORE FREQUENTLY than routine blood draws are obtained

The Outpatient Corollary

An outpatient POC test is useful only if:

- The test result is available during the patient visit AND a decision can be made or action taken on the basis of it without waiting for other laboratory results, OR if you can make money doing it

Strengths	Weaknesses
• Everything everyone loves about POC • Not novel to MDs and PTs; accustomed to GAS and Flu Ag tests • Current assays (e.g. NAAT, more sensitive Ag assays) have improved performance • Some POC NAAT comparably sensitive to culture and lab-based methods • Many specimens readily available: urine, mucosal swabs, whole blood	• Instrumentation costs • Assay / Reagent costs • Specimen type restrictions (e.g. eSwab v. conventional swab) • Serum or plasma beyond POC scope • Limited ID conditions where AST is not relevant • Quality of testing performance by non-laboratory staff. • Arbitrary / limited menus limit clinical impact • Small number of analytes per platform limit scalability
Opportunities	**Threats**
• Continuing advances in testing: NAAT workflow, TAT, “Lab on a Chip” • Antimicrobial stewardship (AMS) increased importance nationally with regulatory bodies • Development of biomarkers for AMS → Negative Predictive Value • Development of new antivirals to broaden clinical actions (e.g. RSV) • Implementing tests at specific sites (e.g. public health / STI clinics) • Ability to facilitate new models of care • Microbiology laboratory consolidation may necessitate more local infectious disease testing	• Changes in reimbursement models • Inertia in physician offices • Theranos-effect → Disproportionally increased scrutiny of assays / methods and/or disproportionate fear of regulatory oversight for novel tests / methods • Turf wars between pharmacies, urgent cares, offices, EDs and potential regulation

Environment of care…

Table 1
Microbiological POC in various environments

Care Setting	Clinical Environment	Types of Infections and Problems Seen	Turnaround Time for Impact	Other
Inpatient	Clinical laboratory on-site; often clinically complex patients.	Sepsis; HAI.	Transport time to laboratory has to be long enough to make it worth doing the test at the POC.	Wide range of potential pathogens in many cases.
Emergency	Clinical laboratory on-site	Acute infectious syndromes; some screening.	Test turnaround time strongly impacts throughput.	Tests that can speed discharge strongly favored.
Urgent care	No dedicated laboratory; test availability impacts scope of care available. Space and personnel limited. Volume of testing must justify capital expenses.	Acute infectious syndromes.	Test turnaround time strongly impacts throughput.	Availability of some tests may allow expansion of scope of care available on-site.
Ambulatory	POL on site, or only CLIA-waived tests. Space and personnel limited. Volume of testing must justify capital expenses.	Common health maintenance, screening, and acute ambulatory illnesses.	Test results must be available during the encounter to streamline care.	
Telemedicine	Laboratory may or may not be on-site, depending on the telemedicine model.	Common health maintenance, screening, and acute ambulatory illnesses.	Depends on care model.	Evolving models for telemedicine. In some cases will be linked to other services—pharmacy, imaging. Extent of laboratory tests available at POC may impact scope of care.
Outreach	Specific programs, targeting particular diseases or vulnerable populations. No on-site laboratory; limited, often temporary space.	STI; HIV, HCV.	Rapid—30 min or less for success.	
Home	Patient centered; clinical and interpretive support limited.	STI; acute infectious syndromes; chronic disease screening.	Somewhat flexible; some mail-in testing has been successful.	An evolving area; will expert systems increase the possibilities for home testing?

Abbreviations: HAI, healthcare-associated infection; HCV, hepatitis C virus; HIV, human immunodeficiency virus; POC, point-of-care; POL, physician's office laboratory; STI, sexually transmitted infection.

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Information Technology and the Future of POCT

Opportunities

- Outreach to underserved populations via widely available devices, e.g. smart phones
- Run complex analytics, computer vision, interpretation, NGS data analysis, remotely
- Rapid reaction to emerging infections

Challenges

- How can the variety of POCT plug into the EMR and the public health system?
- Development of heterogeneous data universe would be bad
- Validation of complex multisite testing at POC
- Security. Also, security and security.

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Future of POCT – Theranos?*

- Decentralizing testing will be essential for decentralized models of health care
- But will require more than just technology
  - POC Testing
  - Imaging and vital signs
  - IT Support
  - Changes in training and organization
  - Reimbursement
  - And more…

European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1015–1022
https://doi.org/10.1007/s10096-019-03492-4

REVIEW

CrossMark

The successful uptake and sustainability of rapid infectious disease and antimicrobial resistance point-of-care testing requires a complex 'mix-and-match' implementation package

John P. Hays[1] · Konstantinos Mitsakakis[2] · Saturnino Luz[3] · Alex van Belkum[4] · Karsten Becker[5] · Ann van den Bruel[6] · Stephan Harbarth[7] · John H. Rex[8] · Gunnar Skov Simonsen[9] · Guido Werner[10] · Valentina Di Gregori[11,12] · Gerd Lüdke[13] · Tjeerd van Staa[14] · Jacob Moran-Gilad[15,16] · Till T. Bachmann[17] · on behalf of the JPIAMR AMR-RDT consortium

Clinical Chemistry 64:8
1136-1142 (2018)

Q&A

There's No Place Like Home: Exploring Home-Based, Acute-Level Healthcare

Moderators: Michelle L. Parker[1] and Paul M. Yip[1,2*]
Experts: Linda V. DeCherrie,[3] Christian Escobar,[4] Anna K. Füzéry,[5] Christopher P. Price,[6] and Andrew St John[7]

*Only without the hype, fraud, lies and crazy.

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The Distant Future

- POCT and changes in care models. Note that POL testing exists in large practices now; how different is this?
- Decentralized testing, along with decentralized imaging and other diagnostic support services, may drive decentralization of care.
- Highly-complex analyses will be laboratory-performed for the foreseeable future, but new models of laboratory practice will evolve as decentralized testing becomes more prevalent.
  - How do you manage QC for analyzers in fifty decentralized telemedicine / pharmacy sites?
  - In ten thousand homes?
- POC will still need to close the clinical encounter to have impact; but perhaps the clinical encounter will change, too.

Sources and Acknowledgements

- Much of the discussion and tables are from:
  - Peaper DR, Durant T, Campbell S. Distributed Microbiology Testing: Bringing Infectious Disease Diagnostics to Point of Care. Clin Lab Med. 2019 Sep;39(3):419-431.
- For information on uroscopy:
  - Melissa Grafe, Ph.D.
    John R. Bumstead Librarian for Medical History
    Cushing/Whitney Medical Library, Yale University
  - The evolution of urine analysis; an historical sketch of the clinical examination of urine. Wellcome, Henry S. Sir, 1853-1936. London, Burroughs Wellcome [1911].
    - *Of this 305-page monograph, only the first 92 pages pertain to uroscopy; the rest consists of advertisements for Wellcome products.*