

I have seen the future and it works:

Molecular Microbiology Dx

Willem JG Melchers



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Molecular Microbiology: L'inizio



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Molecular revolution in Microbiology

1985 PCR

1986 Taq polymerase

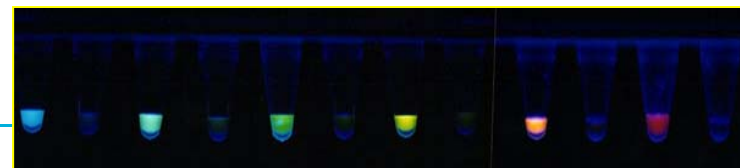
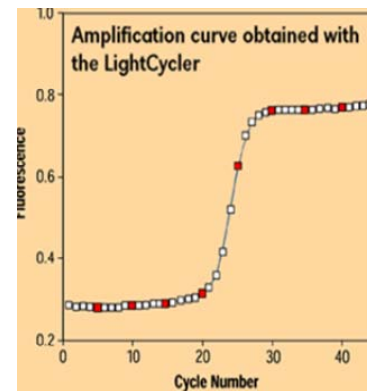
1988 First diagnostic applications

1993 Monitoring Fluorescence during PCR

1997 Real-time PCR (LC/ABI)

1999 Multiplex real-time PCR

Diagnostic Applications



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Diagnosics Strategy – Increase Testing Efficiency

Superior platforms and technologies connected via IT

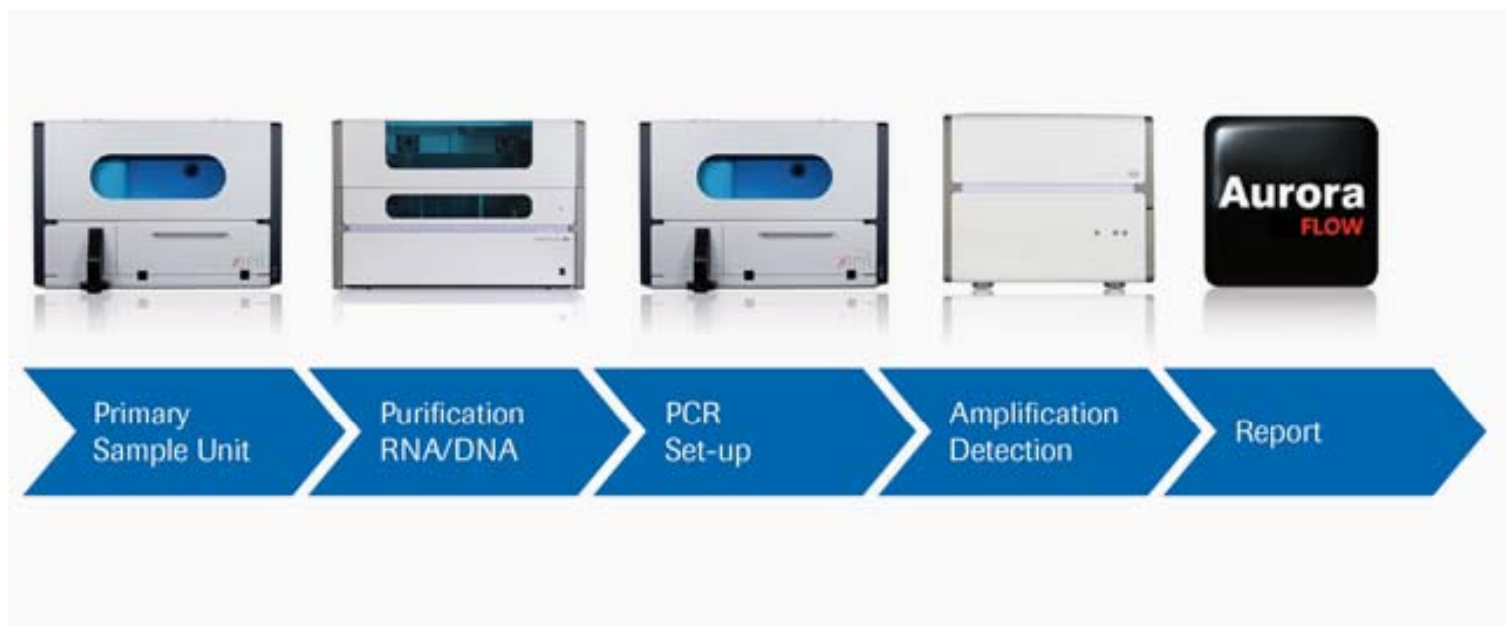


Molecular Microbiology: The Overture



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Automated Workflow Molecular Diagnostics



Other options



Qiagen



Siemens



EliTech

Fully automated platforms



Beckman Coulter



Abbott



Roche



Roche

Hologic



BD



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cobas[®] 6800 & 8800 Systems and cobas p 680 Instrument

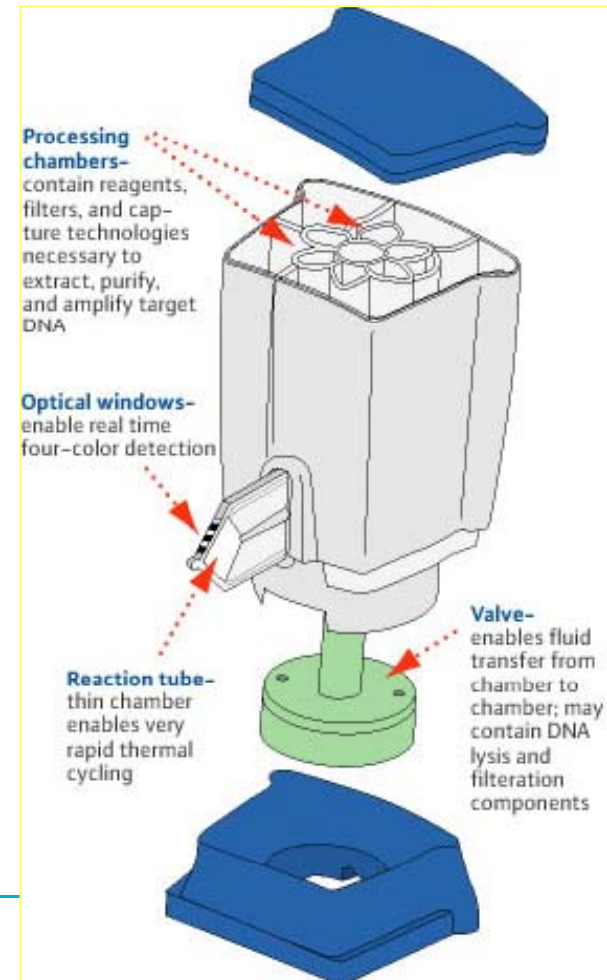
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Molecular diagnostics today and tomorrow

Some Sample-to-Result // Point-of Care MDx systems

- Atlas Genetics - Velox
- Becton Dickinson - BD Max
- BioCartis - Ydilla
- bioMerieux - Biofire
- Cepheid - GeneXpert
- Curetis - Unyvero
- DxNA - Gene STAT
- Enigma - FL, ML
- Eppendorf - RAP
- GenePoc - diagnostics
- Genewave - Genewave systems
- GenMark Dx - eSensor
- Great Basin Corporation - Great Basin System
- Icube - Icube IC
- Instant Labs - Hunter
- Luminex (GenturaDx) - Aries
- Nanosphere - Verigene, NextGen
- Quidel - Savanna Dx
- Rheonix - CARD/Encompass MDx
- Roche - LIAT
- Samsung - GenSpector
- Serigene - RT Reader
- Smith Detection - Bioseq

Cepheid, GeneXpert®: First Cartridge based assay



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Portfolio GeneXpert

Most extensive portfolio: still expanding

CE-IVD Tests

Healthcare Associated Infections

- Xpert MRSA
- Xpert SA Nasal Complete
- Xpert MRSA/SA SSTI
- Xpert MRSA/SA BC
- Xpert C. difficile
- Xpert vanA/vanB
- Xpert Norovirus
- Xpert Carba-R

Critical Infectious Diseases

- Xpert Flu/RSV XC
- Xpert MTB/RIF
- Xpert Flu
- Xpert EV
- Xpert Ebola
- Smart Norovirus

Virology

- Xpert HIV-1 Viral Load
- Xpert HIV-1 Qual
- Xpert HCV Viral Load

Sexual Health

- Xpert TV
- Xpert HPV
- Xpert CT/NG
- Xpert CT
- Xpert GBS

Oncology/Genetics

- Xpert BCR-ABL Monitor
- Xpert FII & FV

Available platforms



I



II



IV

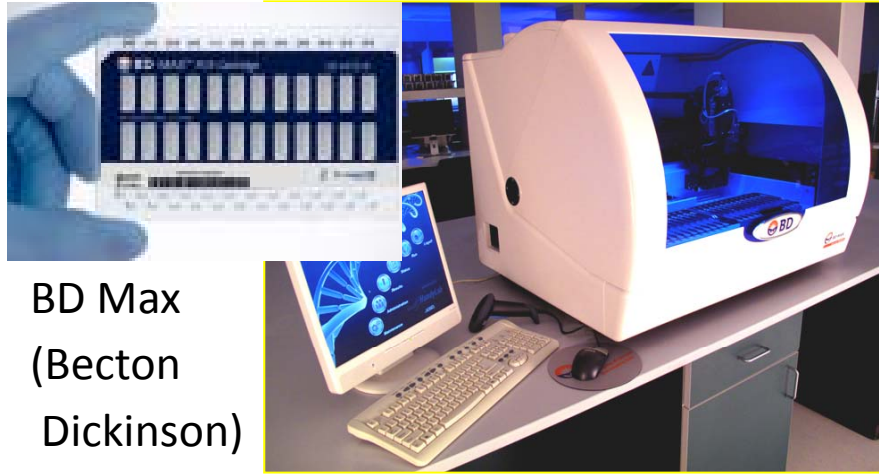


XVI



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Other STA devices: TAT 1-2.5h



BD Max
(Becton
Dickinson)

Focus 3M cyclor
(Focus Diagnostics/
Diasorin)

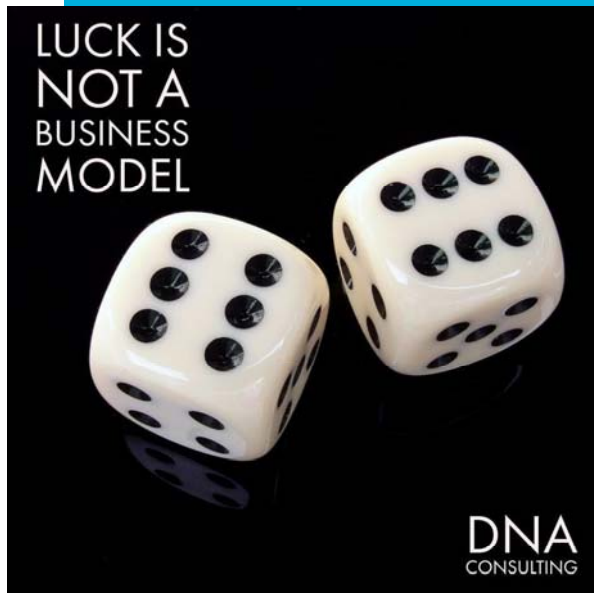


LMX Aries
(Luminex)



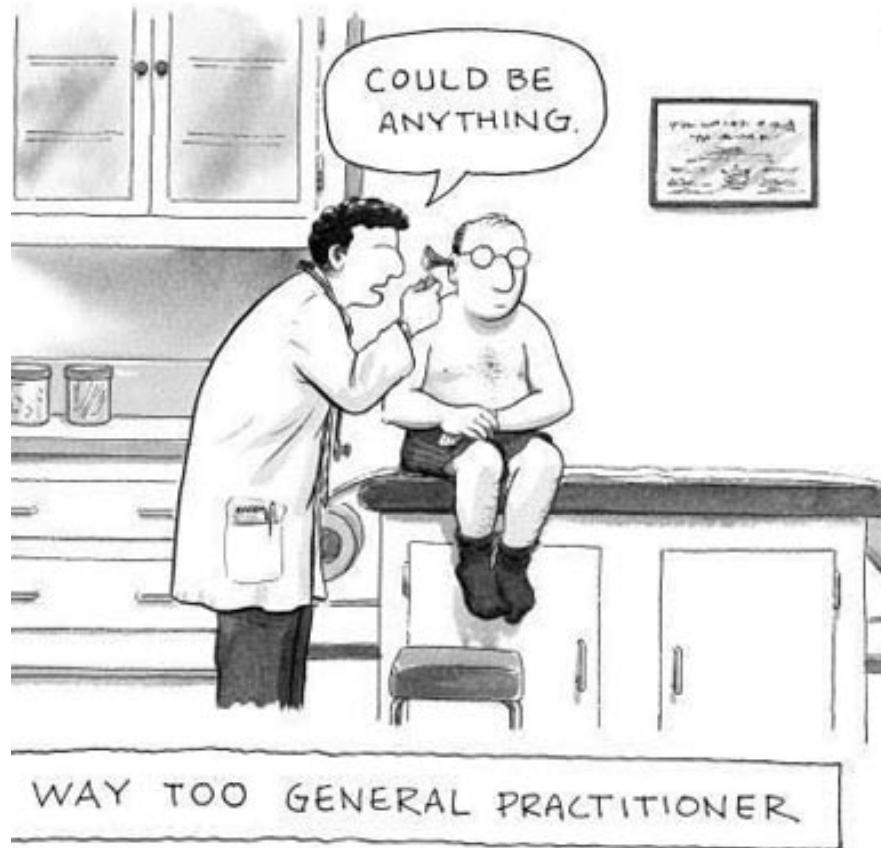
**Limited number of
targets!**

Molecular Microbiology: Crescendo



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Syndromic testing indicated



**In many infections,
presentation of
disease is similar**

Respiratory infection

Gastroenteritis

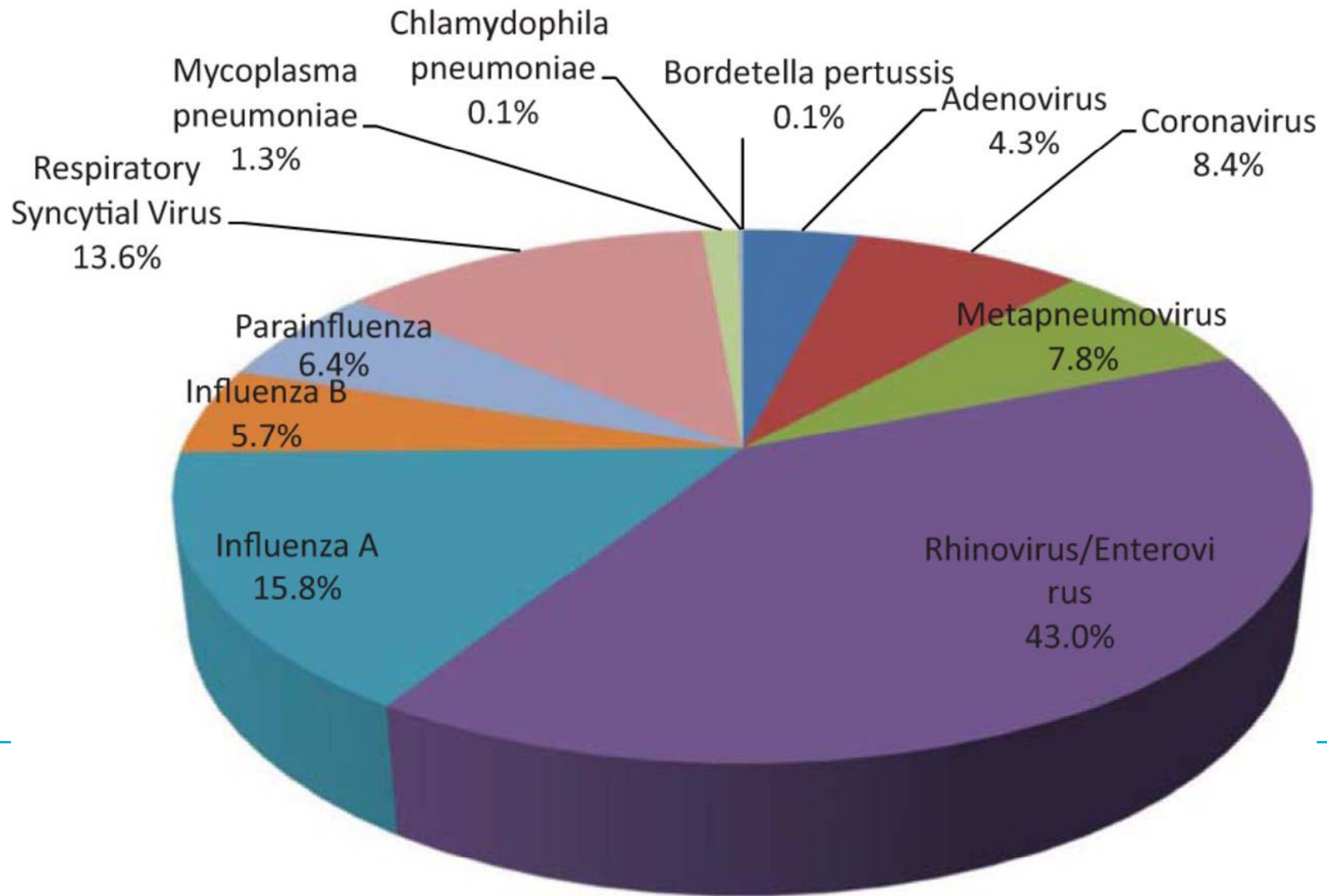
Meningitis

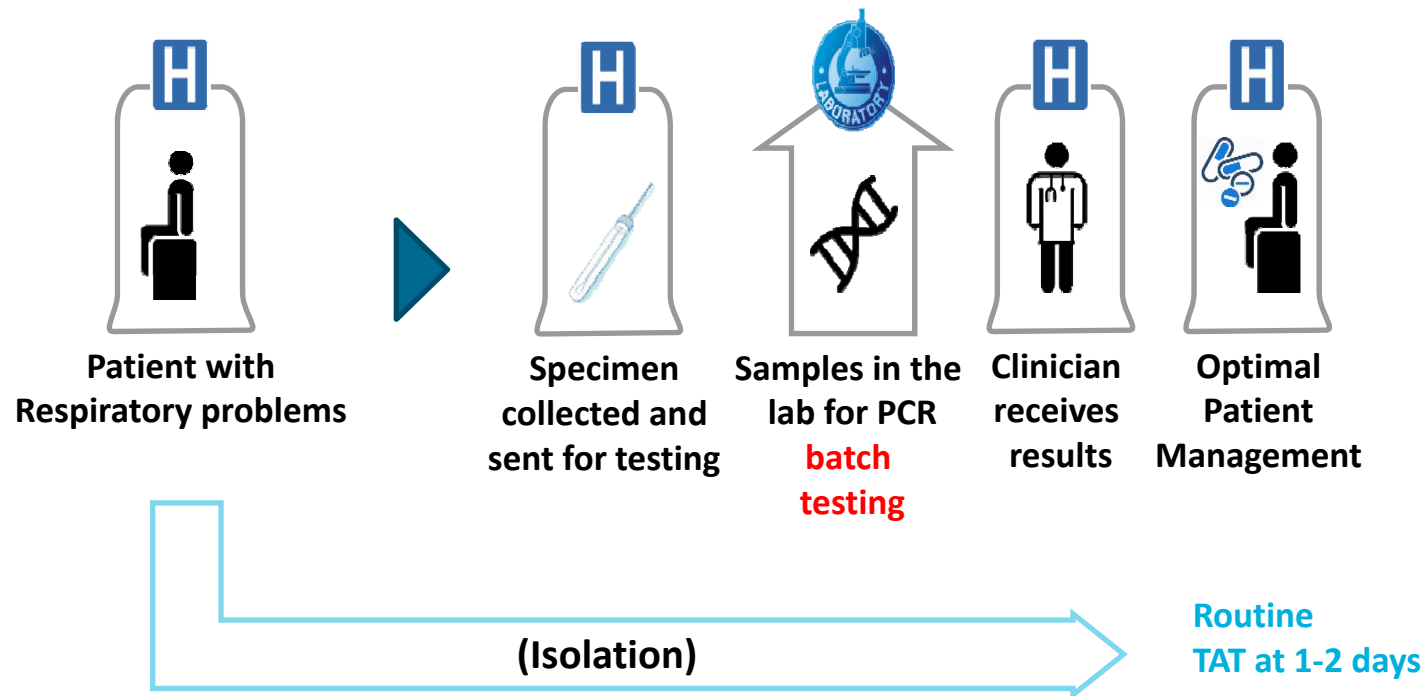
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Diagnostics required

Even during the influenza season, in a large proportion of patients with respiratory tract infections, other viruses and non-cultivable bacteria have been found to cause substantial morbidity and mortality

**Incidence of viruses present in respiratory specimens (n=1,528) at Loyola UMC,
1 October 2013 to 27 September 2014.**



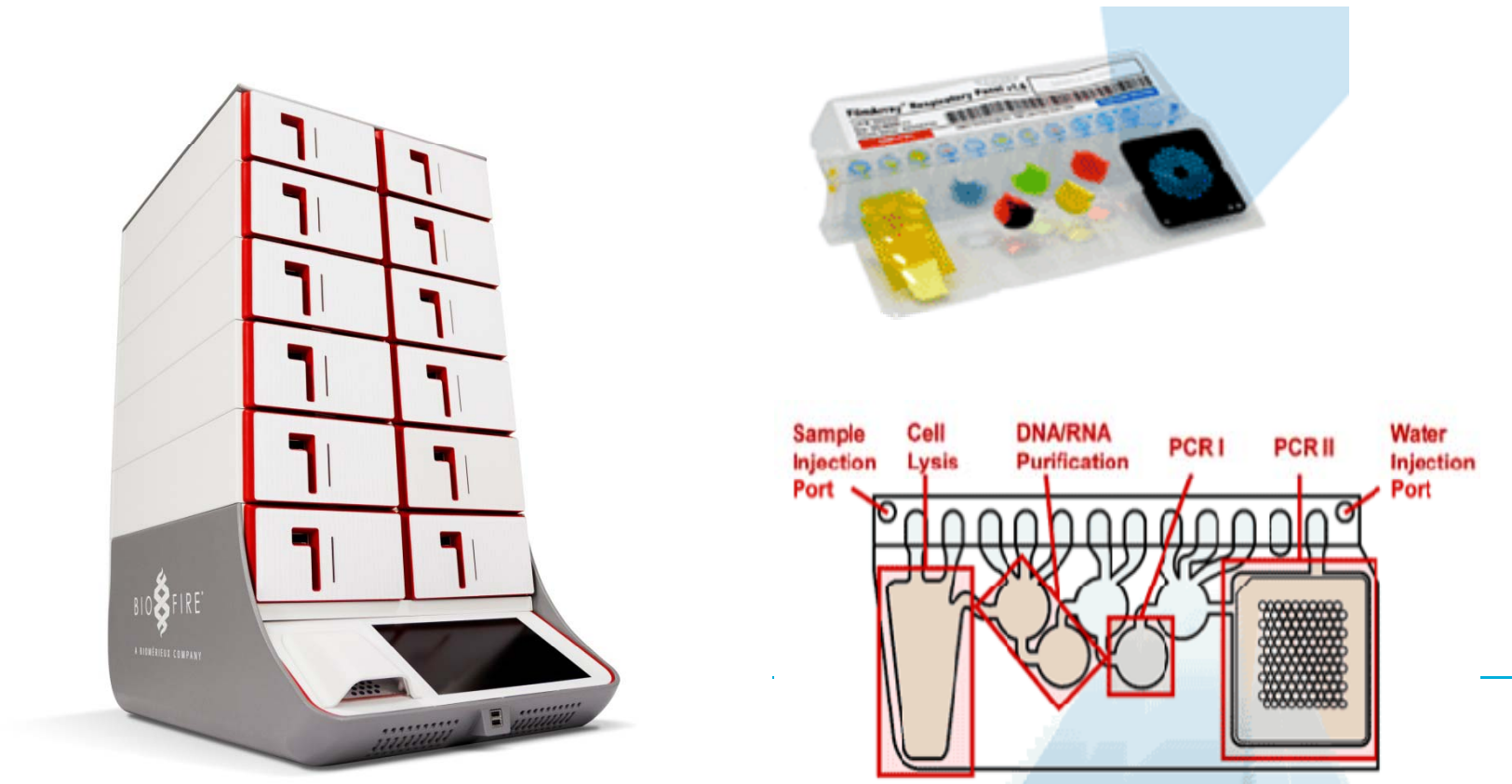


Challenges/unmet needs:

- Turn around times are long
- No 24/7 diagnostics
- Patients may remain in isolation until diagnostics
- Correct patient management (treatment) is delayed
- Need for rapid diagnosis

Multiplex Rapid STA devices

BioMerieux Film Array: result in ± 1 hour

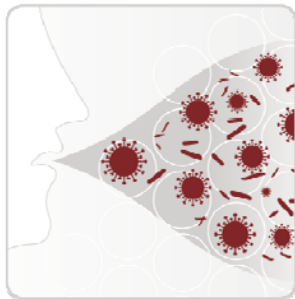


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FilmArray

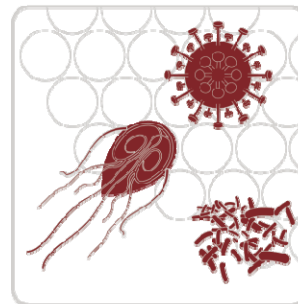
Panels cover a wide range of targets involved in causing respiratory, gastrointestinal, and bloodstream infections

**Respiratory
Panel**



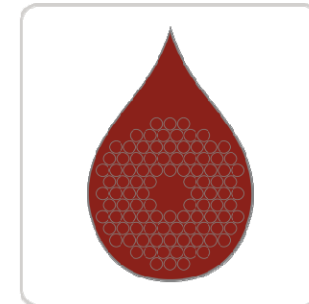
20
targets

**Gastrointestinal
Panel**



22
targets

**Blood Culture
Identification Panel**



27
targets

GenMark's ePlex System



Improves Testing Efficiency

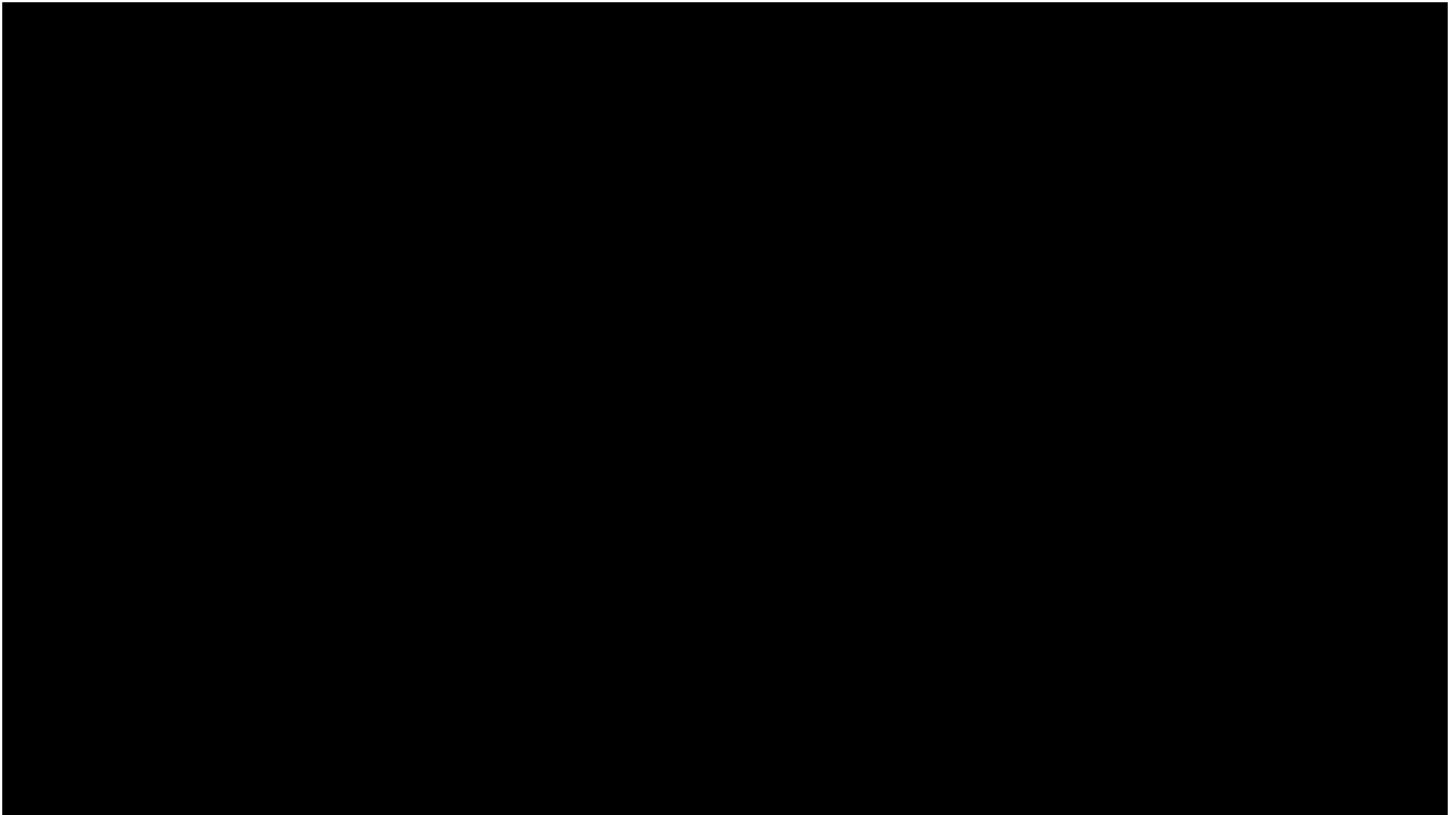
- Fully integrated, sample-to-answer testing
- True random & continuous access
- Modular & scalable

Drives Informed Clinical Decisions

- Comprehensive multiplex panels
- Rapid time to result
- Designed for high sensitivity & specificity

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GenMark Dx ePlex System



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ePlex Respiratory Pathogen (RP) Panel

20 Viral Targets

Adenovirus
Coronavirus 229E
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Middle East Respiratory Syndrome
 Coronavirus (MERS-CoV)
Human Bocavirus
Human Metapneumovirus
Human Rhinovirus/Enterovirus
Influenza A
Influenza A H1
Influenza A H1-2009
Influenza A H3
Influenza B
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Respiratory Syncytial Virus A
Respiratory Syncytial Virus B

4 Bacterial Targets

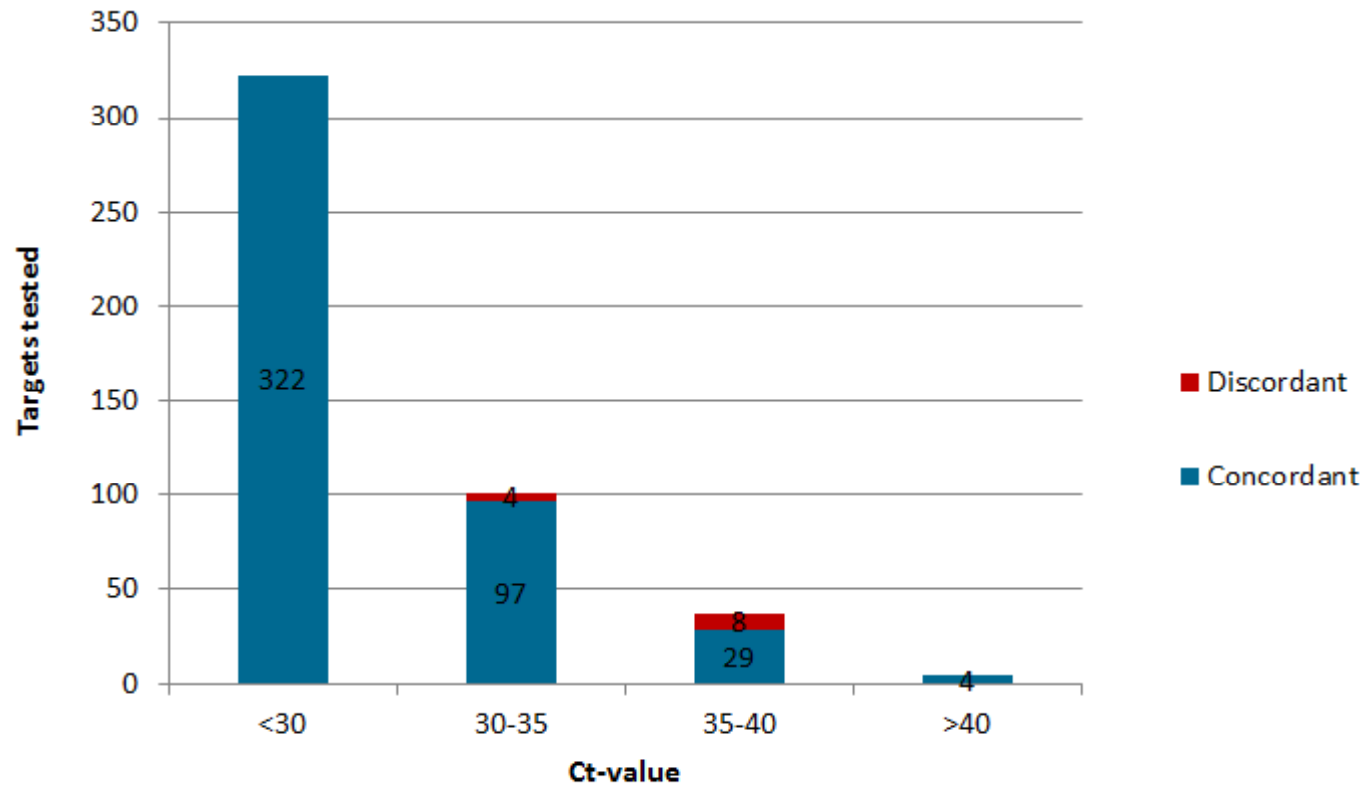
Bordetella pertussis
Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae



GenMark eplex: respiratory tract infections – clinical validation

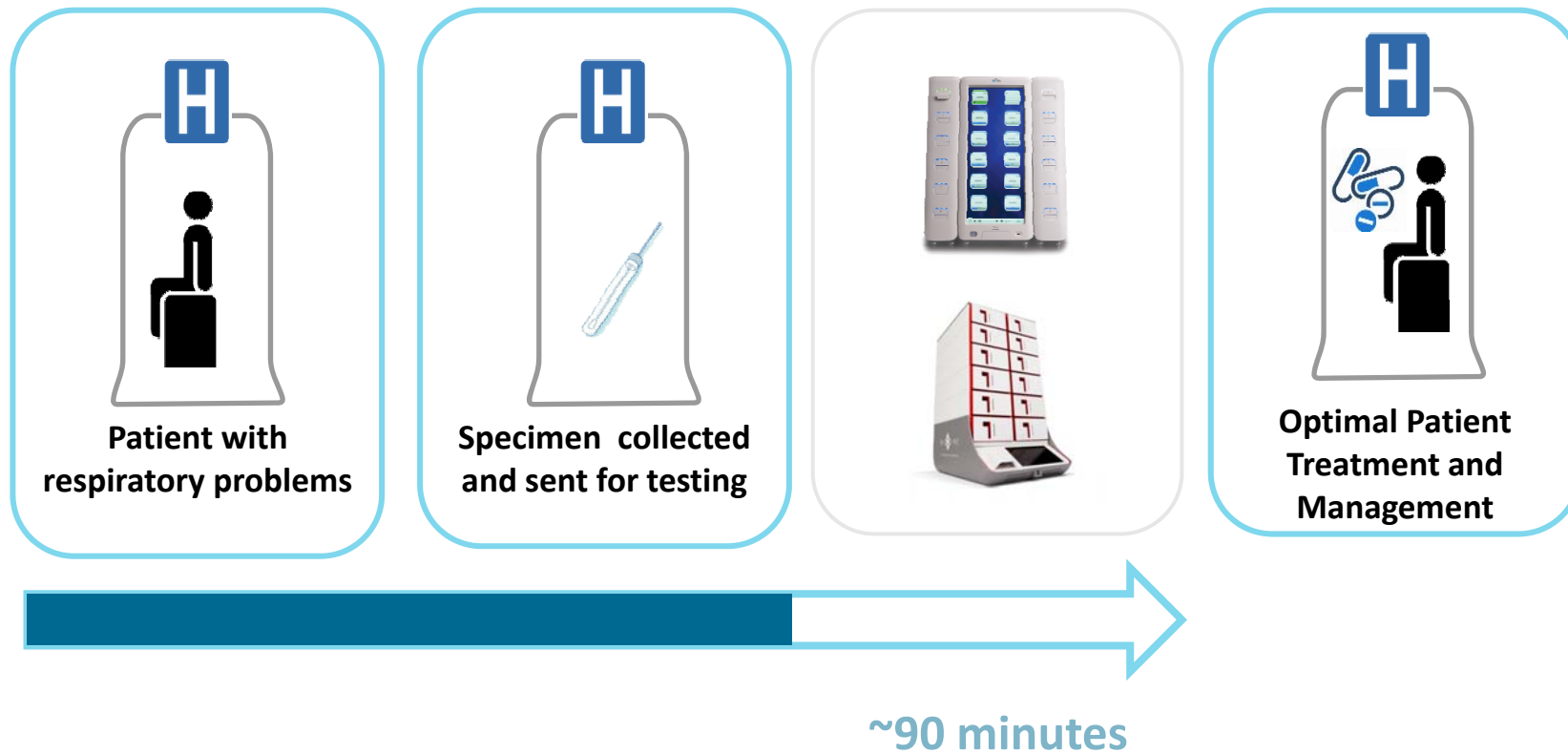
Clinical samples positive for	Tested in Radboudumc	PCR+/RP+	PCR-/RP+	Tested in Leiden	PCR+/RP+	PCR-/RP+	Total amount tested	
adeno	5	4		39	39	4	44	8,4%
corona 229E	2	2		7	6	1	9	1,7%
corona HKU1	0	0		12	11		12	2,3%
corona NL63	2	1	1	7	7	1	9	1,7%
corona OC43	1	1		9	8	1	10	1,9%
Hboca	3	3		27	24	1	30	5,7%
Hmpv	7	6		28	25		35	6,7%
Hrhino	10	10		107	105		117	22,2%
entero	1	1	2	27	27	6	28	5,3%
influenza A	1	1		1	1		2	0,4%
influenza H1	0	0		5	5		5	1,0%
influenza A 2009 H1N1	2	2		18	18	1	20	3,8%
influenza A H3	8	8		17	17		25	4,8%
influenza B	3	3		20	20		23	4,4%
parainfluenza 1	1	1		11	11		12	2,3%
parainfluenza 2	2	1		12	12		14	2,7%
parainfluenza 3	4	4		15	15		19	3,6%
parainfluenza 4	1	1		2	2	2	3	0,6%
RSVA								
RSVB	5	5		80	80		85	16,2%
Bordetella pertussis	2	1		6	6		8	1,5%
Chlamydomphila pneumoniae	0	0		0	0		0	0,0%
Legionella pneumophila	1	1		6	6		7	1,3%
Mycoplasma pneumoniae	1	1		8	8		9	1,7%
total	62	57		464	453		526	100,0%
concordance		92%			97,6%		97,0%	

GenMark Dx ePlex: Respiratory tract infections – Pathogen concordance by Ct-value



Nijhuis et al. JCM 2017;55:1938-45.

Implementation and workflow



Implementation: Point-of-Impact within hospital (MML, Front-office, Back-office)

ePlex cartridges



Respiratory Pathogen Panel (RP)

Viral and bacterial targets from nasopharyngeal swab



Gastrointestinal Pathogen Panel (GI)

Bacterial, viral, and parasitic targets from stool samples



Blood Culture Identification Gram-Positive Panel (BCID-GP)

Bacterial & resistance targets from positive blood culture



HCV Genotyping Panel (HCVg)

Typing and subtyping of HCV 1a, 1b, 2a/c, 2b, 3, 4, 5, and 6 from plasma or serum



Central Nervous System Panel (CNS)

Bacterial, viral, and fungal targets from cerebrospinal fluid samples



Blood Culture Identification Gram-Negative Panel (BCID-GN)

Bacterial & resistance targets from positive blood culture

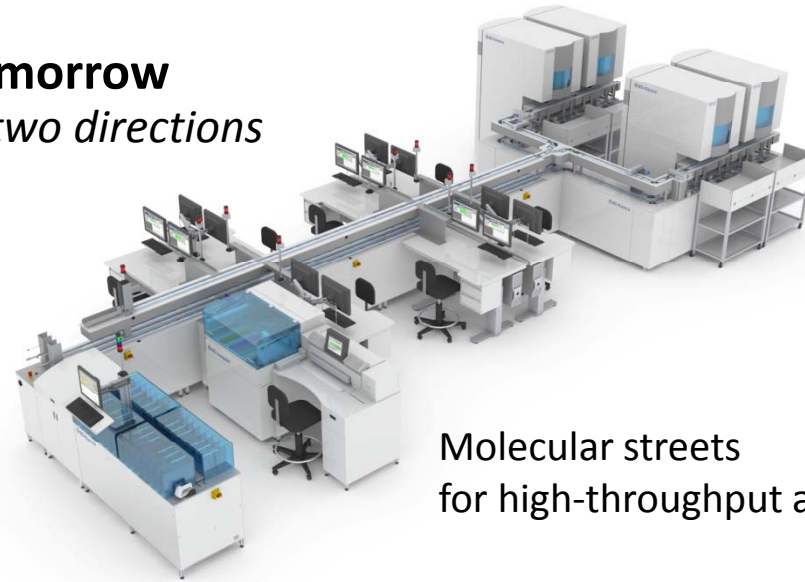


Fungal Pathogen Panel (FP)

Fungal targets associated with bloodstream infections from positive blood culture

Molecular diagnostics today and tomorrow

Innovations in work flow will move into two directions



Molecular streets
for high-throughput analysis



Sample-to-Result MDx

Molecular Microbiology: The Intermezzo



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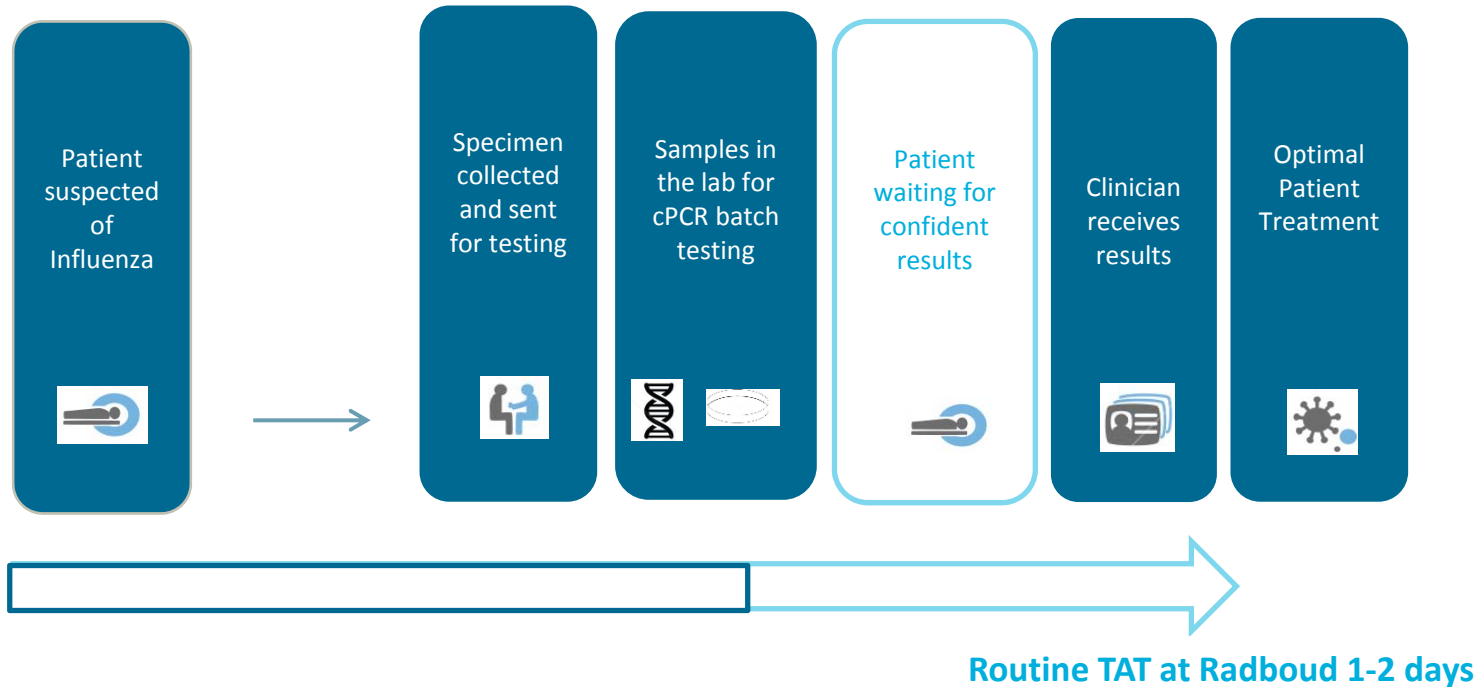
Molecular diagnostics today and tomorrow

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- Enigma - FL, ML
- Eppendorf - RAP
- GenePoc - diagnostics
- Genewave - Genewave systems
- GenMark Dx - eSensor
- Great Basin Corporation - Great Basin System
- Icube - Icube IC
- Instant Labs - Hunter
- Luminex (GenturaDx) - Aries
- Nanosphere - Verigene, NextGen
- Quidel - Savanna Dx
- Rheonix - CARD/Encompass MDx
- **Roche - LIAT**
- Samsung - GenSpector
- Serigene - RT Reader
- Smith Detection - Bioseq

Flu Testing Today

Radboud university medical centre Nijmegen, the Netherlands



Challenges:

- Turn around times are long
- Patients remain in isolation until diagnostics
- No 24/7 diagnostics
- Rapid treatment
- Need for rapid diagnosis, preferable point-of-care

cobas® Liat® System: *The system*

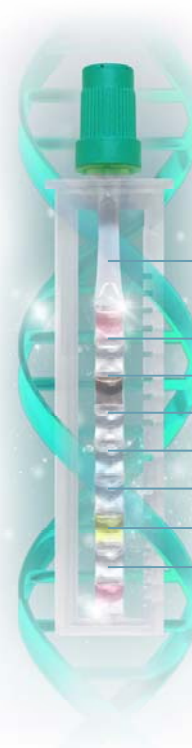
The **cobas® Liat® System**, comprised of the **cobas® Liat® Analyzer** and the **cobas® Liat® assay tube**, is a compact, innovative real-time PCR platform designed for on-demand STAT testing, enabling confidence in rapid patient management, at the point of care or in the laboratory.

cobas® Liat® Analyzer



19.0 cm

11.4 cm



Sample

Internal process control

Proteinase K

Magnetic beads

Lysis buffer

Wash buffer

Elution buffer

PCR reagents

Pre-packed reagents in a flexible assay tube

Automated sample preparation & silica bead-based nucleic acid extraction

Completely closed system

Cobas[®] Liat[®] System: *Influenza A*

Pre Discrepant Analysis

Liat Diagenode	+	-	Total
+	51	5	56
-	0	65	65

Sensitivity: 96%
Specificity: 100%

*: H3N2, H3, H1N1

**:
Of 5 Diagenode positive and Liat negative samples,
2 samples positive by Cepheid GeneXpert; CT>38.5
3 samples negative by Cepheid GeneXpert

Cobas[®] Liat[®] System: *Influenza B*

Liat Diagenode	+	-	Total
+	30	0	30
-	0	90	90

Sensitivity: 100%
Specificity: 100%

*: Wisconsin, Yamagata

Cobas[®] Liat System: *POC Implementation*



- **Implementation: Point-of-care within hospital**

Coming up:
GeneXpert-Omni
Portable
Small
Use everywhere
Integrated battery



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PanNAT System: Molecular Testing for the Non-Molecular Lab





micronics
Real Patient Molecular Testing

The PanNAT System

Molecular Testing for the Non-Molecular Lab

The PanNAT System is a revolutionary solution for molecular diagnostic testing. Combining a compact instrument and test cartridges with complete reagent integration, the PCR technology can deliver sample to result for multiple pathogens/targets simultaneously in approximately 1 hour.

- **Integrated closed test cartridge**: all reagents and controls integrated into the test cartridge; room temperature storage with minimal waste
- **Ease of use**: no sample pre-conditioning or treatment with minimal sample prep steps and unique sample transfer accessory
- **Portable**: lightweight for use across a range of settings; battery backup, Wi-Fi, no external computer required
- **Flexible**: low to high multiplexing using end point PCR with capability for real time PCR and melt curves
- **Multiple Markets**: Designed to meet needs in CLIA highly and moderately complex labs as well as CLIA waived environments



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Technical advancements

Nanotechnology results in downsizing of MDx tools

Handheld PCR systems

Faster NA extraction, amplification and detection protocols



True Point of Care testing enabled

Faster decisions on treatment options

Faster outbreak response and isolation procedures

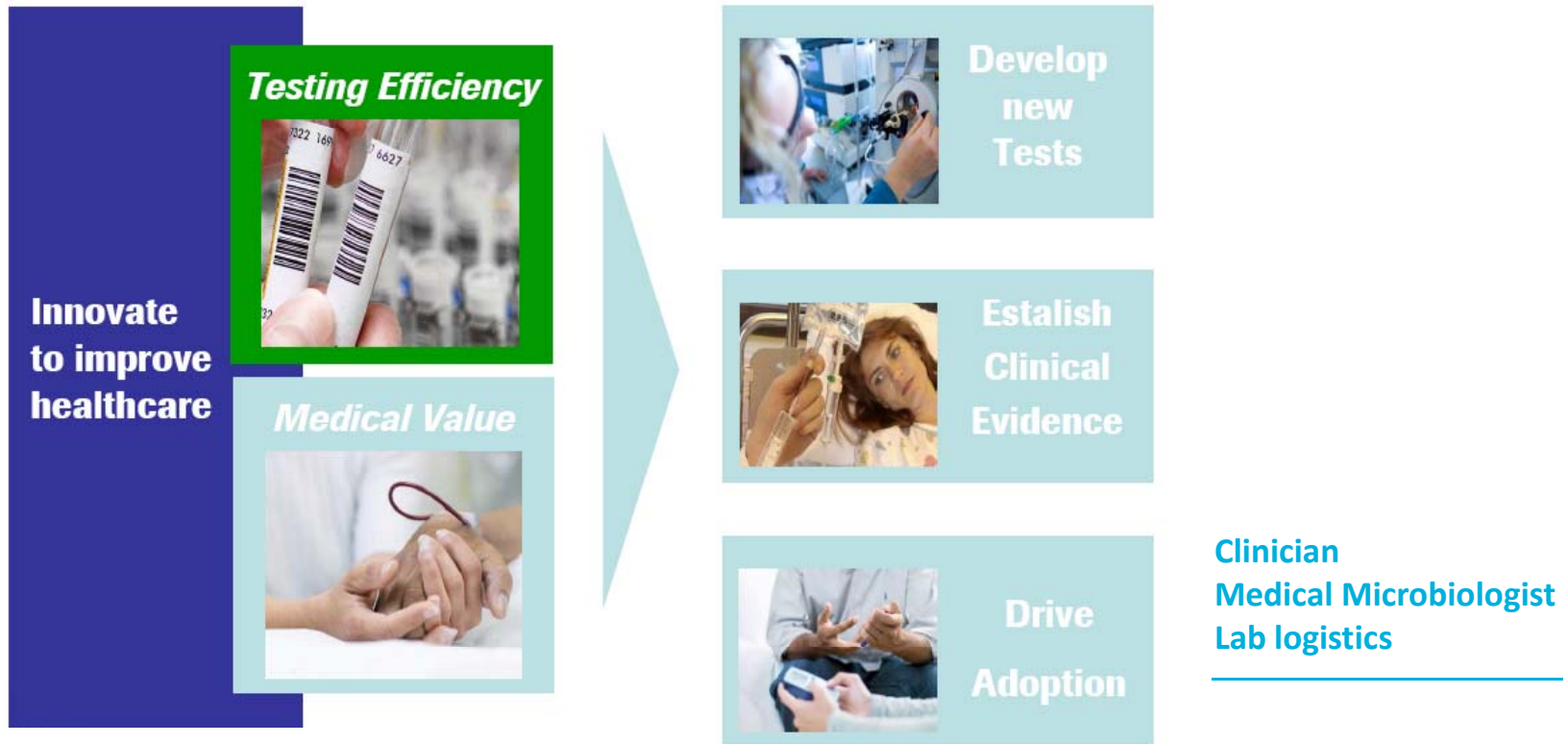
Closer to the patient (Africa, India)



QuantuMDX: Q-Poc

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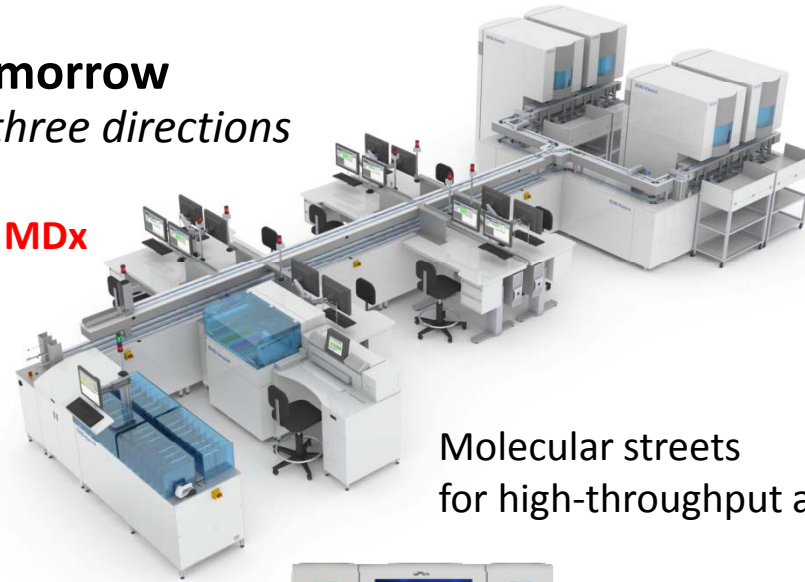
Diagnostics strategy – Improve Medical Value of Molecular Diagnostics



Molecular diagnostics today and tomorrow

Innovations in work flow will move into three directions

Hospital street MDx



Molecular streets
for high-throughput analysis

Backoffice MDx



Sample-to-Result MDx

Point-of-Care MDx



Intermezzo Conclusions

- **Molecular Point-of-care/Impact diagnostics is a reality**
- **Portfolio in MDx-POC/I is increasing**
- **Demand for POC/I will simultaneously increase**
- **MDx POC/I will have a significant effect on patient healthcare**
- **MDx POC/I is a major step in individualized diagnostics**
- **24/7 diagnostics becomes available**
- **New MDx developments will influence microbiological logistics**
- **A mind shift in microbiological diagnostics is required**

When looking at patient outcomes in relationship to use molecular diagnostics/syndromic panels, several outcomes should be measured:

1. Quicker access to treatment
2. Shorter duration of symptoms
3. Less time out of work or school
4. Shorter emergency room times
5. Shorter hospital stay if admitted
6. Implementation of infection control measures, including cohorting of patients, the use or nonuse of isolation precautions based on the known etiologic agent
7. Reduction in pharmacy cost due to less antibiotic usage
8. Reduction in laboratory costs due to less need for additional follow-up tests, including additional diagnostic assays and determination of antibiotic peak and trough levels
9. Reduction in collateral side effects from antibiotics such as adverse drug reaction
10. Reduction in total medical cost for the particular medical encounter



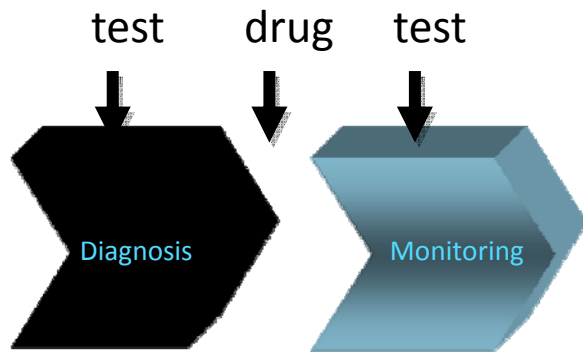
Paul C. Schreckenberger, and Alexander J. McAdam. *J. Clin. Microbiol.* 2015;53:3110-3115

Molecular diagnostics today and tomorrow

Diagnostics will significantly expand its role in clinical practice

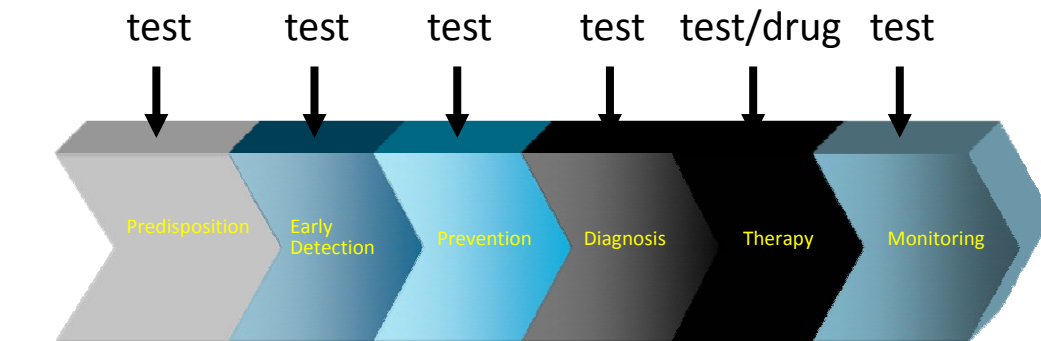
Yesterday

Traditional Diagnostics



Today & Tomorrow

Expanded Testing Market



Providing Health Information:

-
- Disease Risk
 - Health Status
 - Drug Choice
 - Therapy Efficacy

What's next in Medical Molecular "Microbiology" ?

Integrated Diagnostics



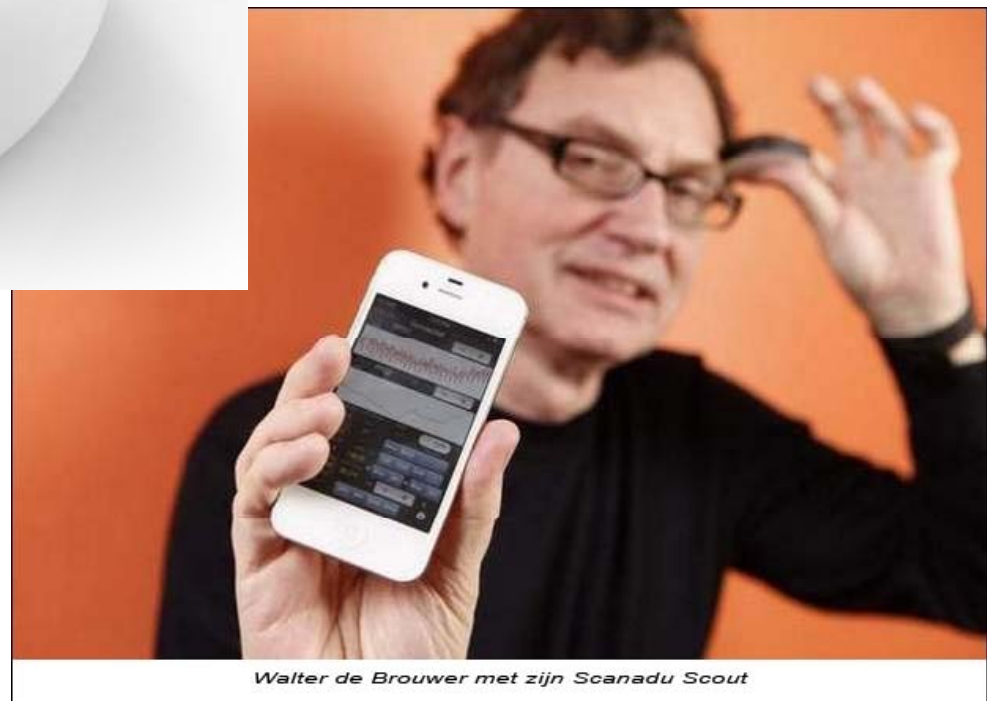
Molecular Microbiology: The accelerazione



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Scanadu Scout is a sensor that people hold to their temple. In less than 10 seconds, it will collect data on vital signs, including pulse, heart rate, respiratory rate, blood pressure, temperature, and even emotional stress.



Rapid infectious diseases diagnostics using Smartphones

Matthew Bates^{1,2}, Alimuddin Zumla^{1,2,3}

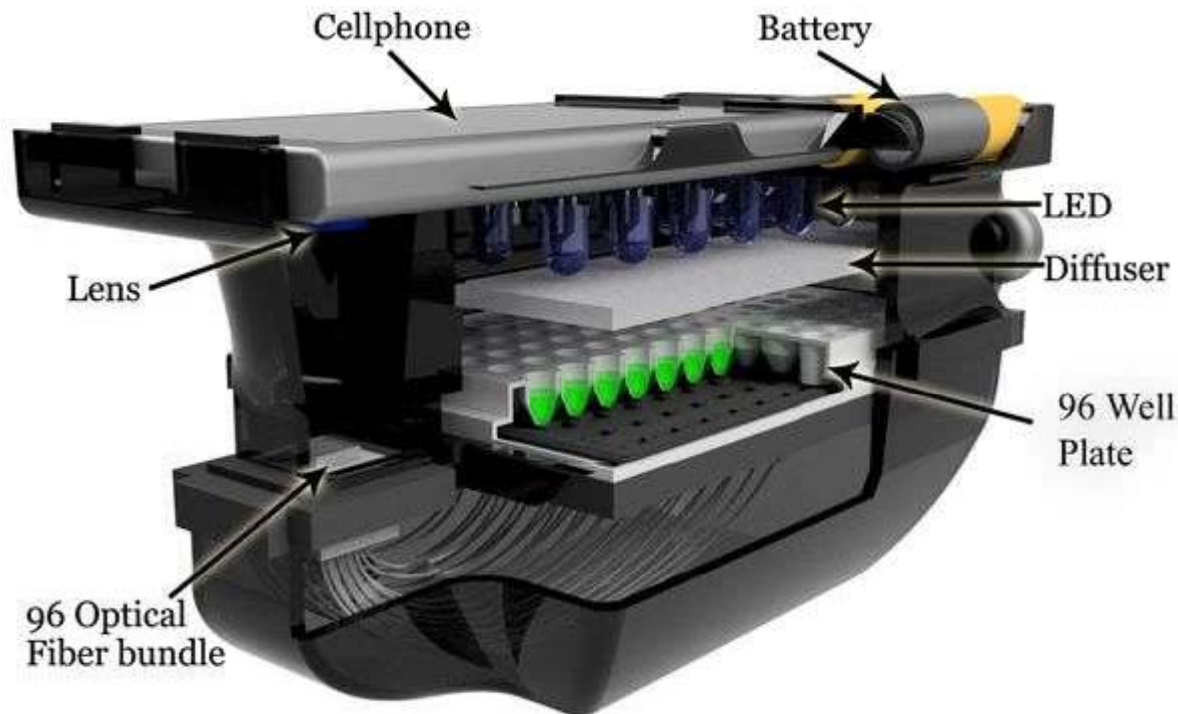
¹Department of Paediatrics, UNZA-UCLMS Research and Training Programme, University Teaching Hospital, Lusaka, Zambia; ²Division of Infection and Immunity, Centre for Clinical Microbiology, University College London, London, UK; ³NIHR Biomedical Research Centre, UCL Hospitals, London, UK

Correspondence to: Alimuddin Zumla, FRCP, Department of Paediatrics, UNZA-UCLMS Research and Training Programme, University Teaching Hospital, Lusaka, Zambia; Division of Infection and Immunity, Centre for Clinical Microbiology, University College London, London, UK; NIHR Biomedical Research Centre, UCL Hospitals, London, UK. Email: a.zumla@ucl.ac.uk

This year, NSF funded 10 Partnerships for Innovation: Building Innovation Capacity (PFI:BIC) projects for smart, human-centered service systems:

1. Pathtracker: A smartphone-based system for mobile infectious disease detection and epidemiology: Brian Cunningham of the University of Illinois at Urbana-Champaign, principal investigator.

Molecular revolution in Microbiology: Antimicrobial resistance using smartphones



A special plates prepared with 17 different antibiotics targeting *Klebsiella pneumoniae*, a bacteria containing highly resistant antimicrobial profiles. During the clinical tests, they used 78 samples from patients. Their results showed that the mobile-phone-based reader meets the FDA-defined criteria for laboratory testing, with a detection accuracy of 98.2 percent

Steve Feng et al. High-throughput and automated diagnosis of antimicrobial resistance using a cost-effective cellphone-based micro-plate reader, *Scientific Reports* (2016). DOI: [10.1038/srep39203](https://doi.org/10.1038/srep39203)

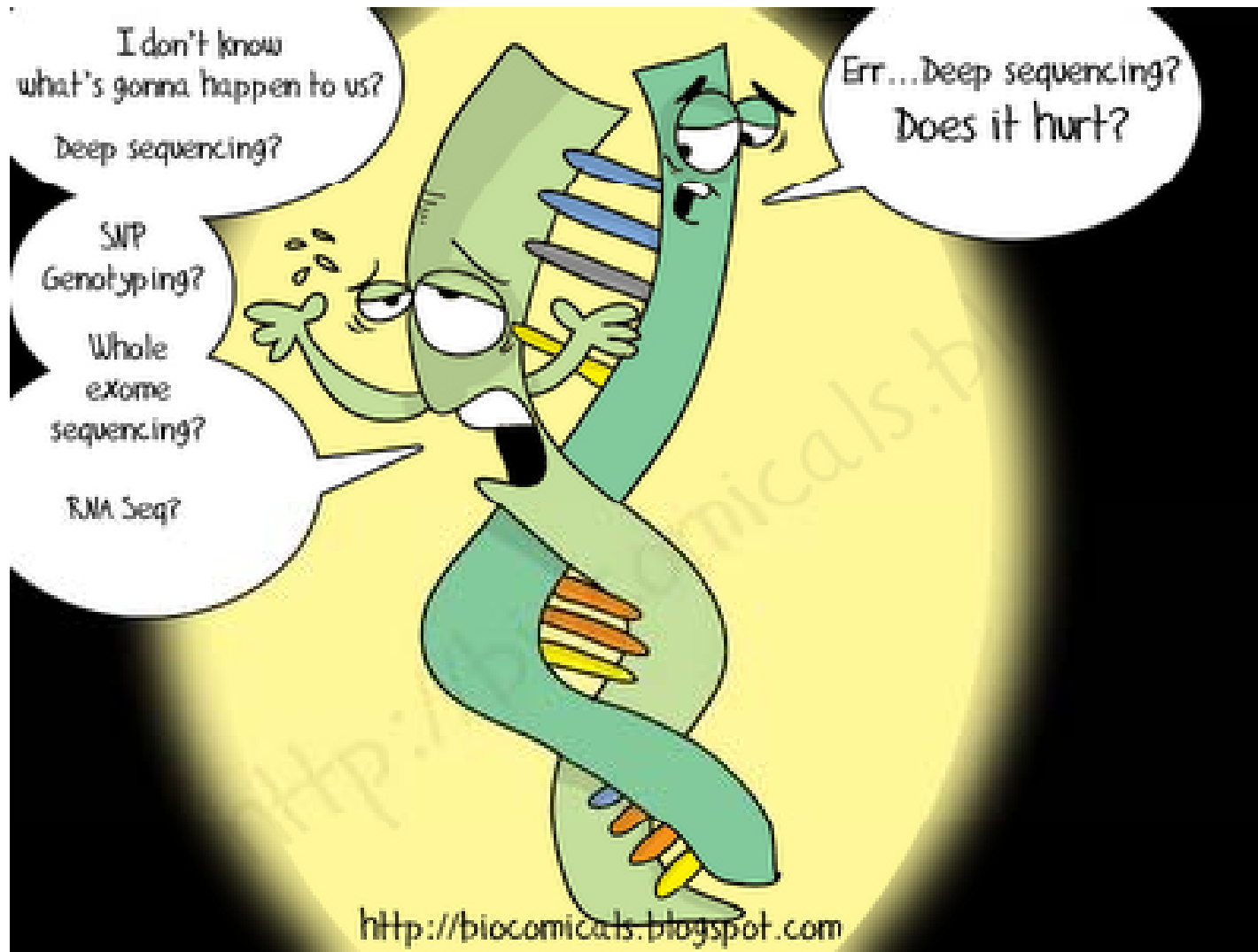


Enzyme-linked immunosorbant assay, or ELISA, is a diagnostic tool that identifies antigens such as viruses and bacteria in blood samples. ELISA can detect a number of diseases, including HIV, West Nile virus and hepatitis B, and it is widely used in hospitals. It can also be used to identify potential allergens in food, among other applications.

This mobile platform can be used for point-of-care testing, screening populations for particular diseases, or tracking vaccination campaigns in most resource-poor settings.

The new device, which is created with a 3D printer and attaches to a smartphone, illuminates the ELISA plate with an array of light-emitting diodes.

"Cellphone-Based Hand-Held Microplate Reader for Point-of-Care Testing of Enzyme-Linked Immunosorbent Assays." *ACS Nano*, Article ASAP DOI: [10.1021/acsnano.5b03203](https://doi.org/10.1021/acsnano.5b03203)



2011

The MinION device is a miniaturised single-molecule analysis system, designed for single use and to work through the USB port of a laptop or desktop computer



2012

The MinION device is adaptable for DNA sequencing, protein sensing and other nanopore sensing techniques



2014

Nature doi:10.1038/ SEPTEMBER 2014

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Molecular Microbiology: The Apotheose



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1 The accelerating pace of change ...



2 ... and exponential growth in computing power ...

Computer technology, shown here climbing dramatically by powers of 10, is now progressing more each hour than it did in its entire first 90 years

COMPUTER RANKINGS

By calculations per second per \$1,000



Analytical engine
Never fully built, Charles Babbage's invention was designed to solve computational and logical problems



Colossus
The electronic computer, with 1,500 vacuum tubes, helped the British crack German codes during WW II



UNIVAC I
The first commercially marketed computer, used to tabulate the U.S. Census, occupied 943 cu. ft.

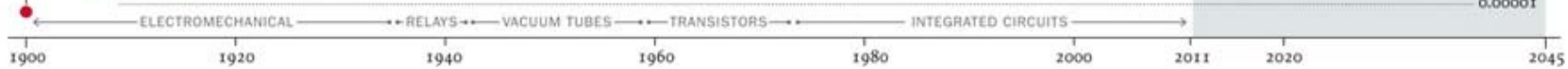
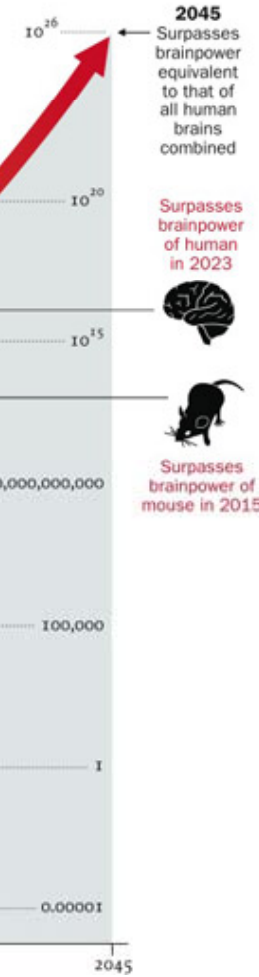


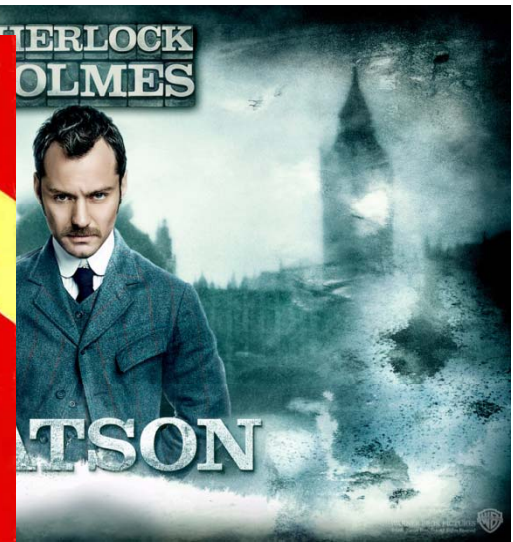
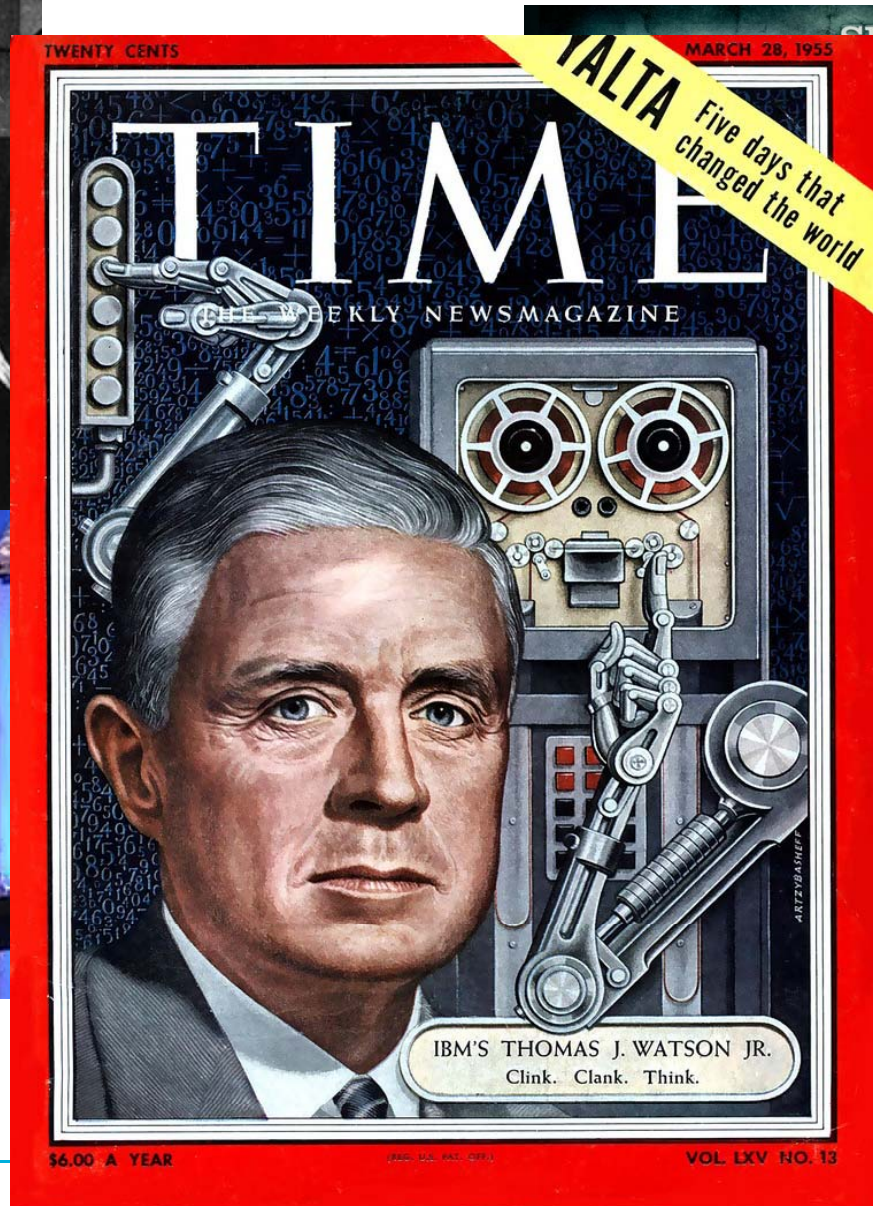
Apple II
At a price of \$1,298, the compact machine was one of the first massively popular personal computers



Power Mac G4
The first personal computer to deliver more than 1 billion floating-point operations per second

3 ... will lead to the Singularity





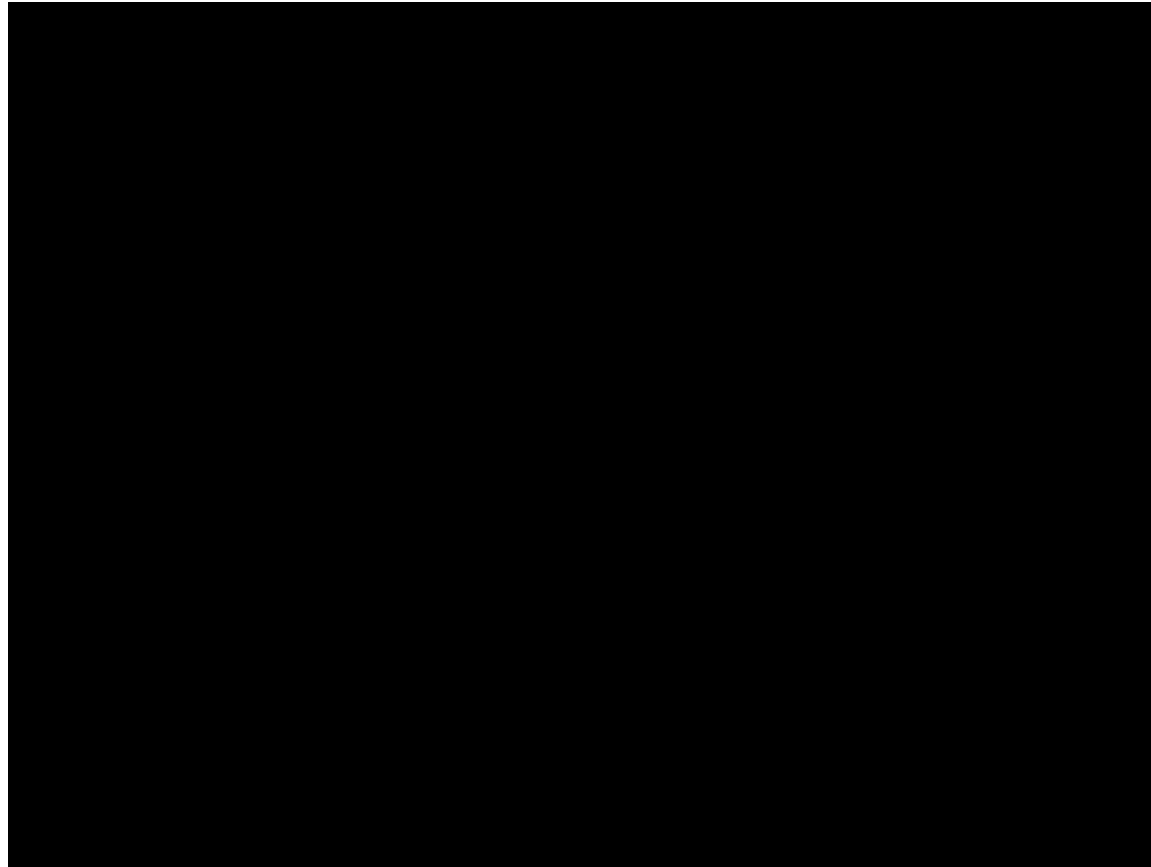
Meet: WATSON







Erica is in charge



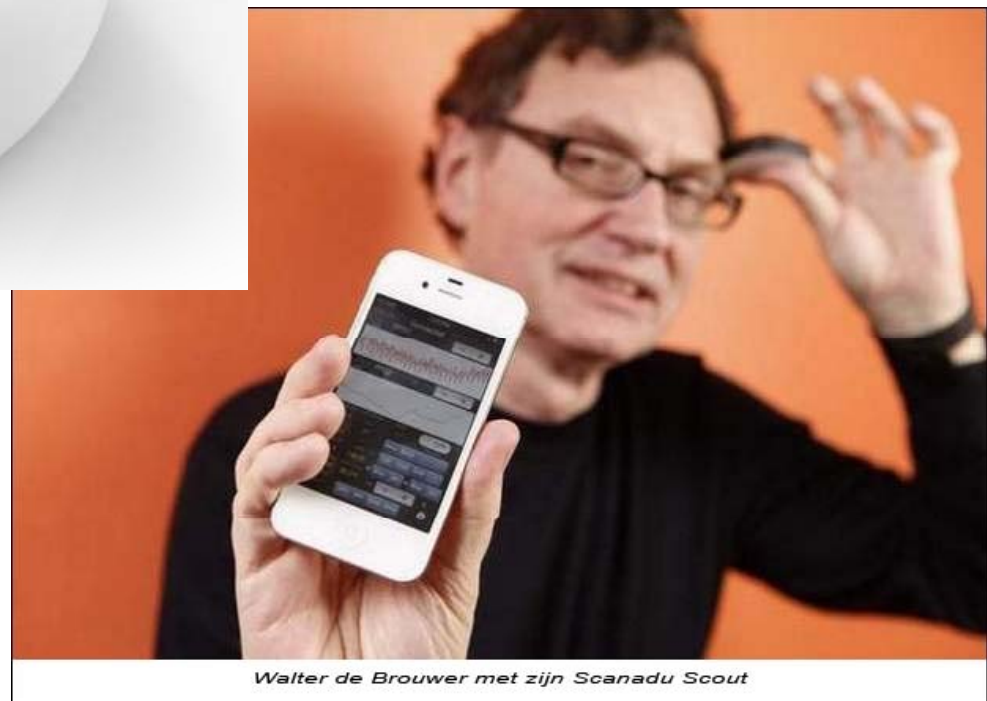
<https://www.theguardian.com/technology/ng-interactive/2017/apr/07/meet-erica-the-worlds-most-autonomous-android-video>







Scanadu Scout is a sensor that people hold to their temple. In less than 10 seconds, it will collect data on vital signs, including pulse, heart rate, respiratory rate, blood pressure, temperature, and even emotional stress.







The Apotheose: the final countdown

- Centralization of hospital labs in the Netherlands is a no-go
- Centralization of hospital labs within the hospital is the future **2020**
- Turn-around-Time will be the basis of diagnostics **2017**
- Integrated diagnostics (all specialism's) will be the core Dx **2025**
- New platform technologies should be integrated **onwards**
- Smart phone Dx will becomes important (patient in the lead) **2025**
- Hand-held Dx devices is the new direction **2030**
- Software Dx becomes central **2030**

- I will go with retirement **2028**

***Lincoln Steffens' famous quote about the
future (1919):***

I have seen the future and it works



(But not in the future)

