A high-throughput qPCR system for flexible and cost-effective large panel assay testing

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that's GOOD science!®

Overview

1

Why molecular methods of detection?

Traditional vs. molecular methods

2

Why large panels?

Need for high-throughput qPCR system

3

Large panel example

Comprehensive UTI combo panel (96 assays)

Spike-in control and its advantages

4

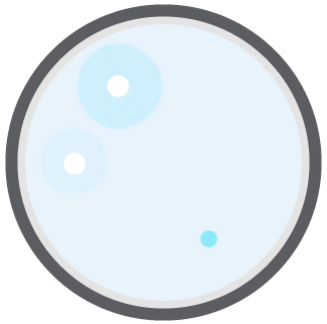
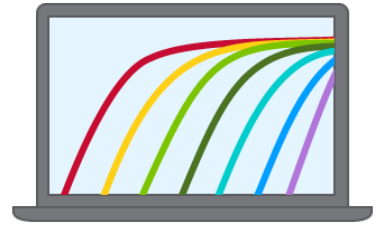
Workflow for SmartChip ND® system

5

Summary

TaKaRa

Molecular methods for pathogen detection preferred over traditional methods

Traditional bacterial culture	Primary challenges	Key benefits	Molecular methods: PCR/qPCR
	Long turnaround time (>24–48 hr)	Fast turnaround time (3 hr)	
	Limited targets	Comprehensive detection	
	Lower sensitivity	Higher sensitivity	
	Limited detection of AMR* genes	Larger coverage of AMR genes	
	Costly in the long term	Cheaper in the long term	

*Antimicrobial Resistance

References

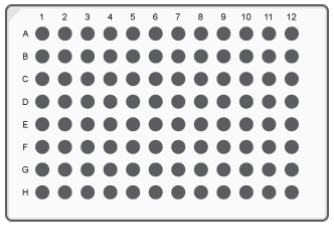
- Gu et al. Molecular diagnostics for infectious diseases: Novel approaches, clinical applications and future challenges. *Frontiers in Microbiology* (2023).
- Pfaller. Molecular approaches to diagnosing and managing infectious diseases: practicality and costs. *Emerging Infectious Diseases* (2001).
- Schmitz et al. Forty years of molecular diagnostics for infectious diseases. *Journal of Clinical Microbiology* (2022).

Additional benefits

- ✓ Detection of novel pathogens
- ✓ Smaller sample volumes
- ✓ Surveillance capability
- ✓ Characterization of infectious agents
- ✓ Epidemiological benefits

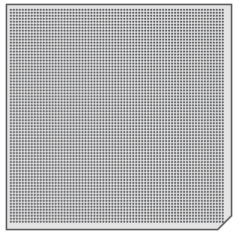
Large panels provide more information

Small panel



Primary challenges	Key benefits
Detects limited number of species	Faster detection
Inadequate for detecting rare species that cause a large portion of UTIs	Results for more species
Insufficient for detecting most AMR genes	Broader scope of detection
High likelihood of incomplete information	More complete information

Large panel



Additional benefits

- ✓ Improved accuracy and sensitivity
- ✓ Increased efficiency and decreased costs

References

- Upadhyay et al. Expanded PCR Panel Testing for Identification of Respiratory Pathogens and Coinfections in Influenza-like Illness. Diagnostics (Basel) (2023).

Why high-throughput qPCR?

Medium-sized lab

- ~100 samples/day
- UTI test with 72 assays

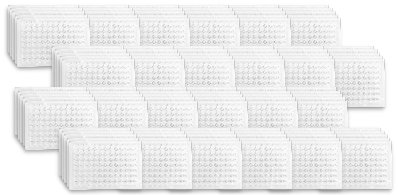
>20,000 reactions (with 3 replicates)

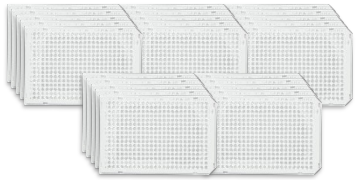
Requires more

- X Time
- X Money
- X Reagents

Plate-based qPCR

- >200 96-well plates
- >50 384-well plates



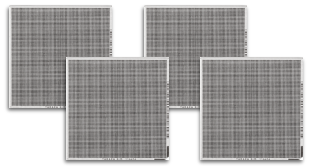


SmartChip ND

Requires less

- ✓ Time
- ✓ Money
- ✓ Reagents

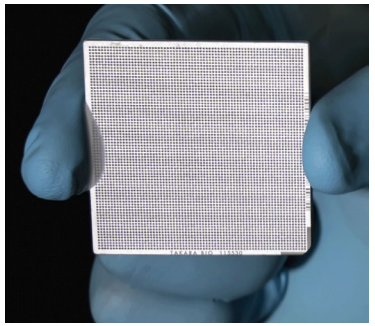
- ~4 chips



SmartChip ND Real-Time PCR System

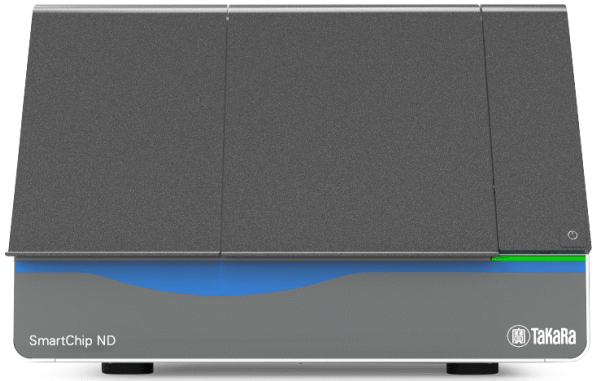
High-throughput pathogen detection made easy

Nanowell chip



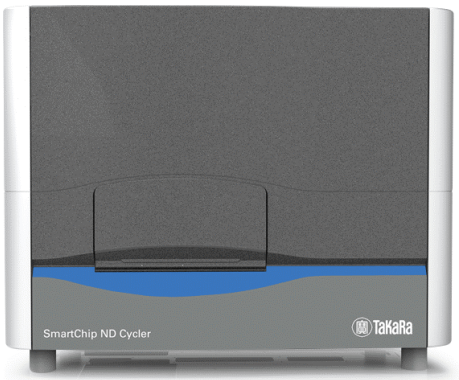
5,184 (100 nl) reactions/chip

SmartChip ND



<1 hr sample and assay dispense

SmartChip ND Cycler



~2 hr qPCR run to data

Case-study: wound, UTI, and STI panel

Wound

1. *Bacteroides fragilis*
2. *Kingella kingae*
3. *Streptococcus pyogenes*
4. AAC (6')-Ib
5. AAC (6')-Ib-cr
6. ANT (3")-IIa/aadA
7. APH (3')-VIa
8. ermA
9. ermB
10. mefA
11. tetM

UTI

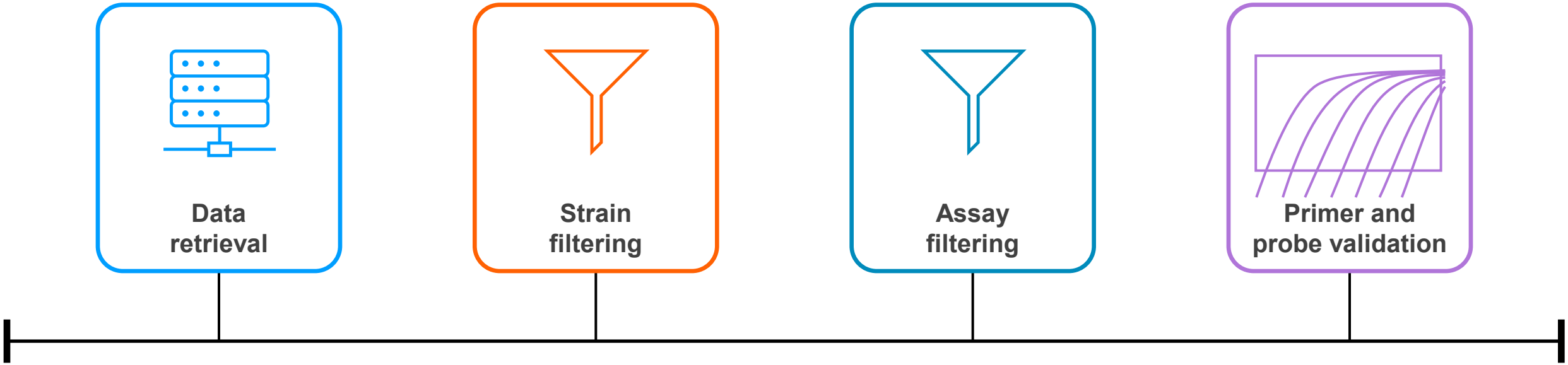
1. *Acinetobacter baumannii*
2. *Actinobaculum schaalii*
3. *Aerococcus urinae*
4. *Bacillus atrophaeus*
5. *Candida albicans*
6. *Candida auris*
7. *Candida glabrata*
8. *Candida parapsilosis*
9. *Candida tropicalis*
10. *Citrobacter freundii*
11. *Citrobacter koseri*
12. *Corynebacterium riegelii*
13. *Enterobacter aerogenes*
14. *Enterobacter cloacae*
15. *Enterococcus faecalis*
16. *Enterococcus faecium*
17. *Escherichia coli*
18. *Klebsiella oxytoca*
19. *Klebsiella pneumoniae*
20. *Morganella morganii*
21. *Mycoplasma hominis*
22. *Proteus mirabilis*
23. *Proteus vulgaris*
24. *Providencia stuartii*
25. *Pseudomonas aeruginosa*
26. *Serratia marcescens*
27. *Staphylococcus aureus*
28. *Staphylococcus epidermidis*
29. *Staphylococcus haemolyticus*
30. *Staphylococcus lugdunensis*
31. *Staphylococcus saprophyticus*
32. *Streptococcus agalactiae*
33. *Streptococcus anginos*
34. *Streptococcus oralis*
35. *Ureaplasma urealyticum*
36. blaACC
37. blaACT/blaMIR
38. blaCMY
39. blaCTX-M 1
40. blaCTX-M 2
41. blaCTX-M 8/25
42. blaCTX-M 9
43. blaDHA
44. blaFOX
45. blaGES
46. blaIMP-1
47. blaIMP-7
48. blaIMP-16
49. blaKPC
50. blaMOX
51. blaOXA-1
52. blaOXA-23
53. blaOXA-40
54. blaOXA-48
55. blaOXA-72
56. blaPER-1
57. blaPER-2
58. blaSHV
59. blaTEM
60. blaVEB
61. blaVIM
62. dfrA1
63. dfrA5
64. mecA
65. nfsA
66. QnrA
67. QnrB
68. QnrS
69. sul1
70. sul2
71. vanA
72. vanB
73. vanC
74. Alien
75. RNaseP
76. 16s

STI

1. *Candida dubliniensis*
2. *Chlamydia trachomatis*
3. *Haemophilus ducreyi*
4. HSVI
5. HSV2
6. *Mycoplasma genitalium*
7. *Neisseria gonorrhoeae*
8. *Treponema pallidum*
9. *Trichomonas vaginalis*

Legend: Fungus Parasite Virus Bacteria Antibiotic resistance gene Control

Integrating in silico design and assay validation



Relevant strain information collected from databases such as GISAID, GenBank, and whole-genome sequencing (WGS) repositories

Based on clade classification and date of collection to ensure up-to-date and relevant coverage

Based on sequence alignment to guarantee strain inclusivity, exclusivity, and adherence to qPCR design criteria, thus preventing non-specific amplification

Final forward and reverse primers, along with FAM-labeled probes, were selected for laboratory testing

Rigorous in-lab testing

1. Selection of assays with the best R^2, efficiency, and S-curve
2. Inclusivity test with proficiency panel
3. Exclusivity test with nearest-neighbor species
4. Validated with human samples

Comprehensive UTI combo panel (96 assays)

Comprehensive detection across multiple targets is a growing need!

Broad target coverage = more accurate information

- Avoid misidentification and retesting delays
- Quickly identify antibiotic resistance

Better controls = higher reliability

- Process validation from extraction to detection
- Assurance of data quality → true negative vs. false negative
- Alien spike-in control → process control with non-homologous sequence
- RNaseP → internal control
- 16S → bacteria load control
- *Bacillus atrophaeus* → extraction control

Growing need for AMR detection worldwide

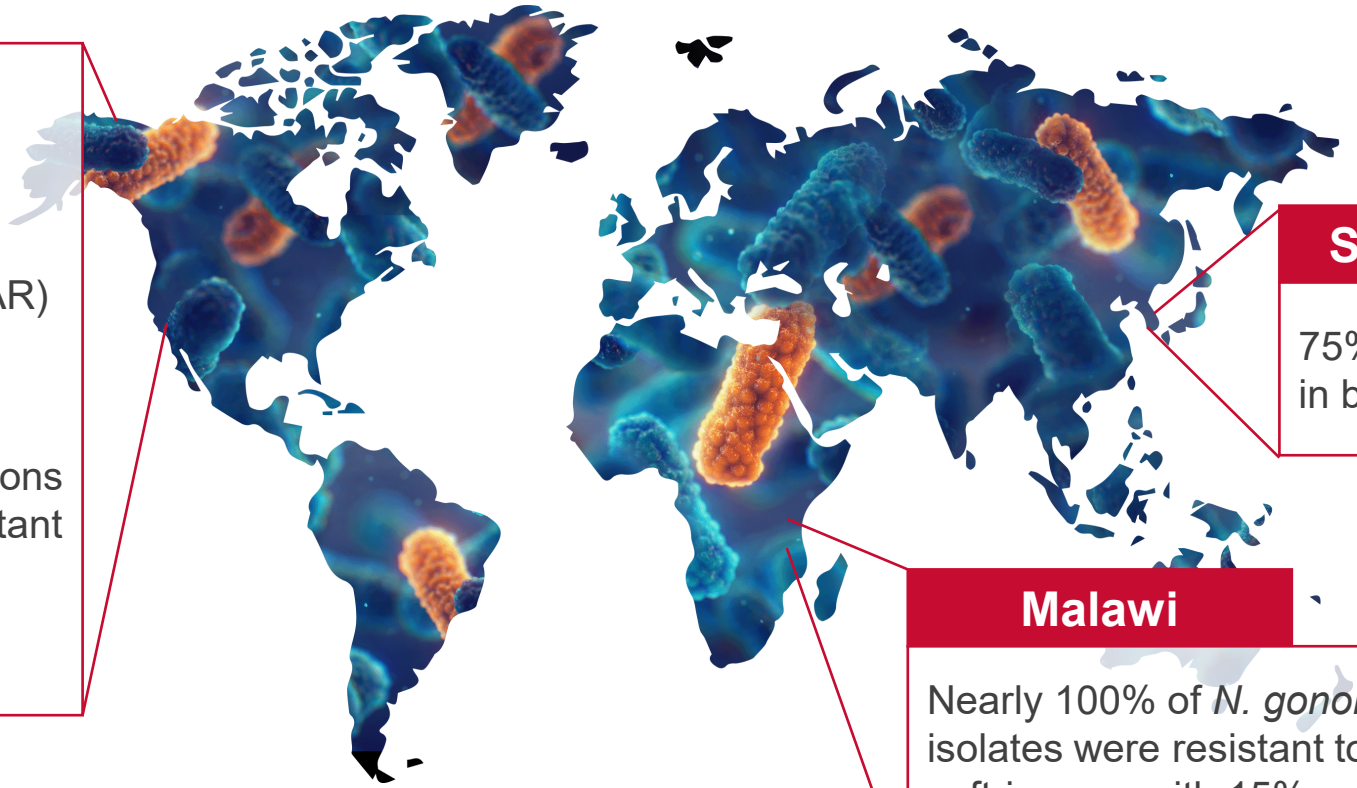
United States

Since 2013

- Over 2 x 10^6 illnesses caused by AR bacteria
- More than 23,000 deaths due to antibiotic resistant (AR) bacteria

California

- >50% of urinary tract infections (UTIs) due to bacteria resistant to ≥1 antibiotic class
- ~13% of UTIs resistant to ≥3 antibiotic classes



South Korea

75% of *Acinetobacter* isolates in blood showed resistance

Malawi

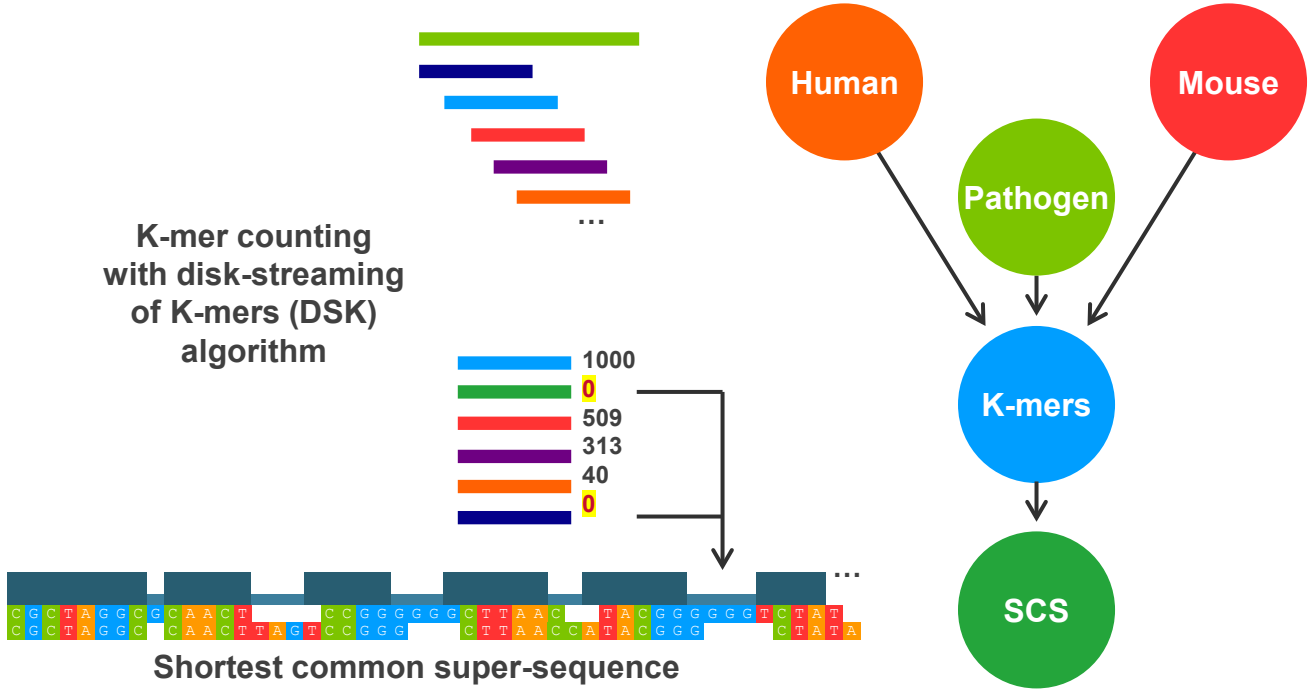
Nearly 100% of *N. gonorrhoeae* isolates were resistant to ceftriaxone, with 15% resistant to azithromycin

Source

- Van Beusekom. Data show rising antibiotic resistance with repeat urinary tract infections. Center for Infectious Disease Research and Policy. 2024.

Alien spike-in control

- To monitor entire molecular detection workflow
- 1 kb exogenous sequence non-homologous to human, mouse, or human-pathogen genome sequences
- Generation of shortest common super-sequence (SCS)
  - Bioinformatics to create an algorithm (avoid repeat sequences)
  - Result: nonrepetitive 1 kb sequence packed with “alien” subsequences

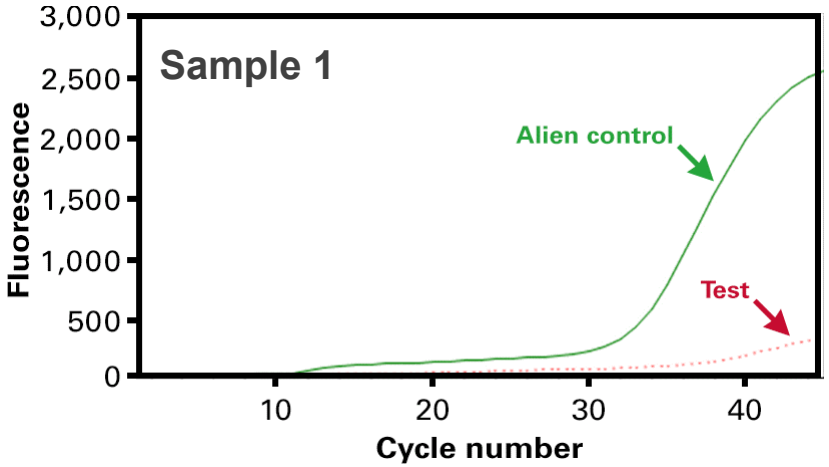


Choose your spike-in control

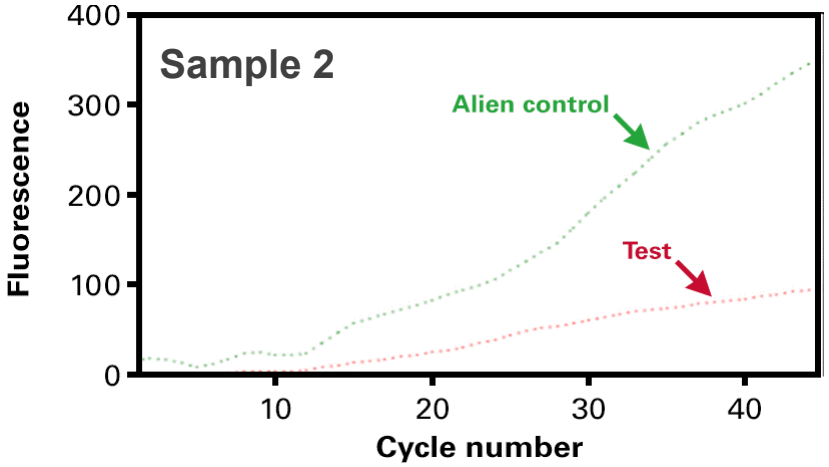
- ✓ DNA version
- ✓ RNA version with virus-like particles (VLPs)

Advantages of alien spike-in control

Process control

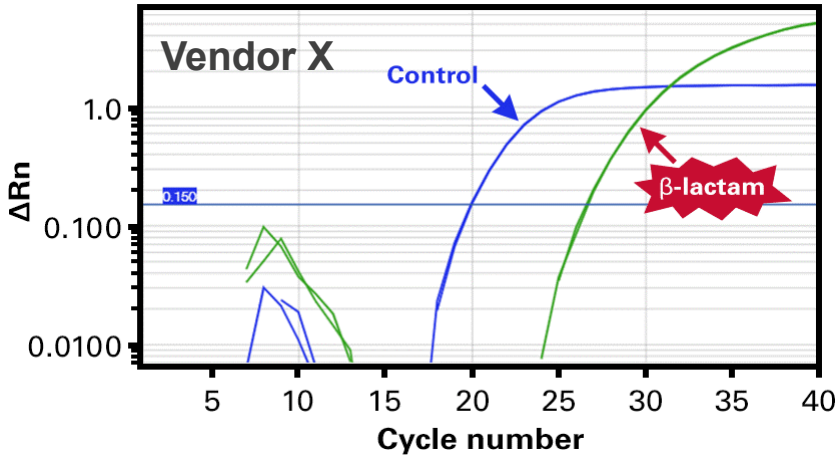


True negative

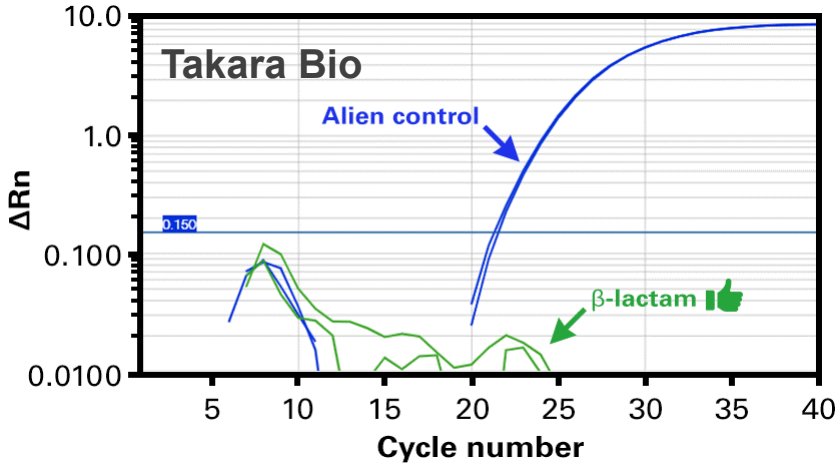


False negative
(process fail)

No cross reactivity



Control from Vendor X
cross-reacts with
β-lactam assay

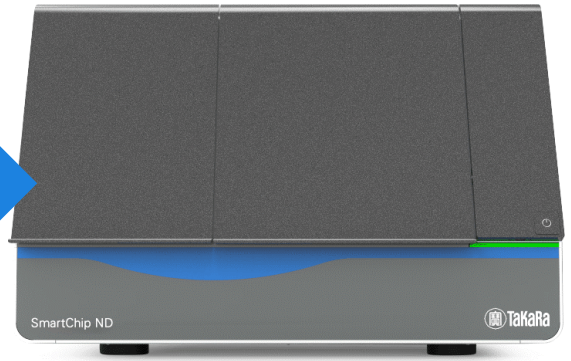


Alien control
does not cross-react
with β-lactam assay

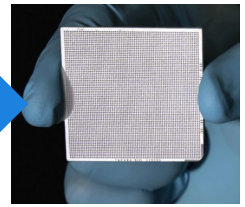
Workflow for UTI, STI, and wound panel

IsoPure Mini or IsoPure 96

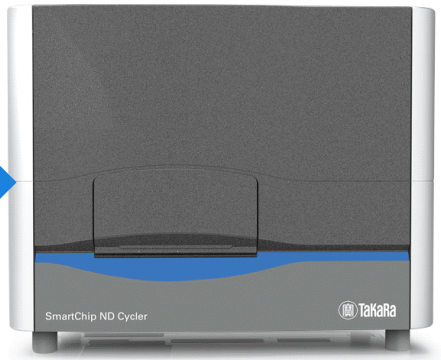
Sample prep

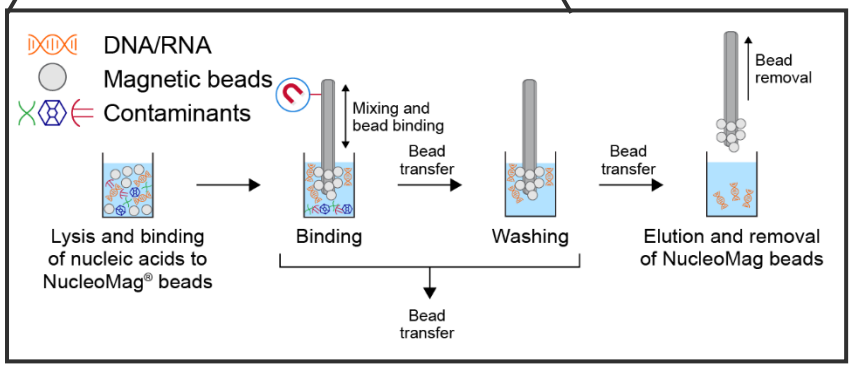


Nanodispensing



PCR



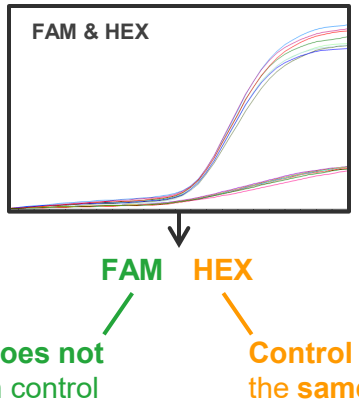


SIGNIFICANT ADVANTAGE!

Confidence in each sample/result

Data analysis

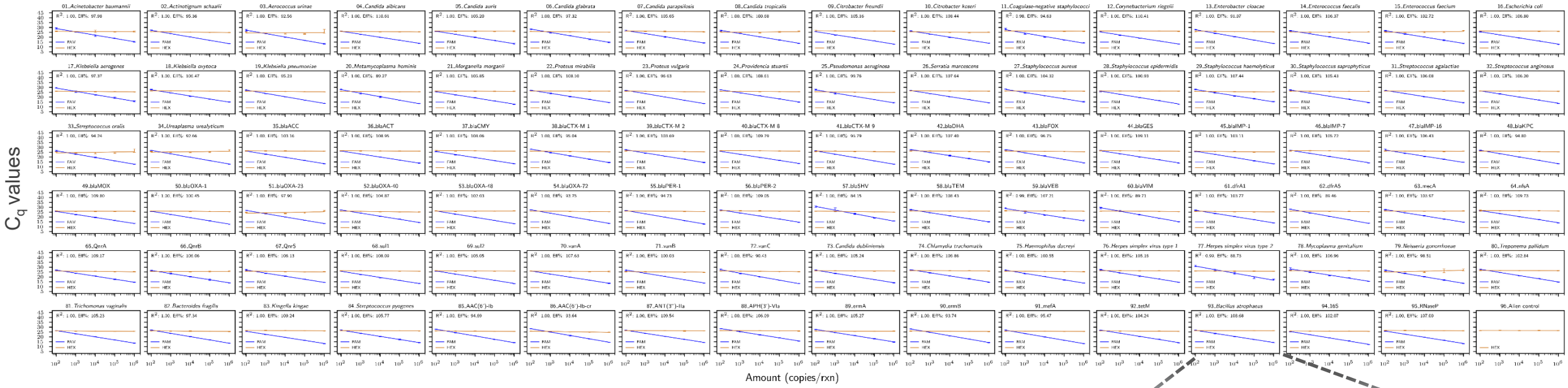
Duplex reaction to monitor full qPCR workflow (including extraction)



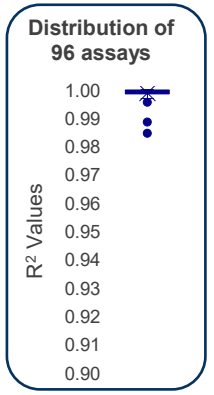
Sample **assay does not cross-react** with control

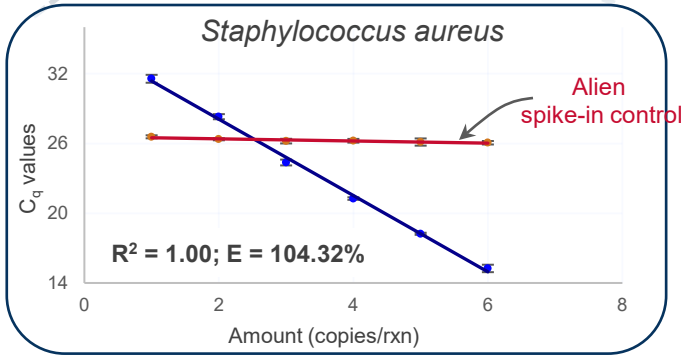
Control in the **same** well

Best performing assays were selected



- Each assay tested in singleplex and duplex formats
- Excellent efficiency (Eff) and R^2 values





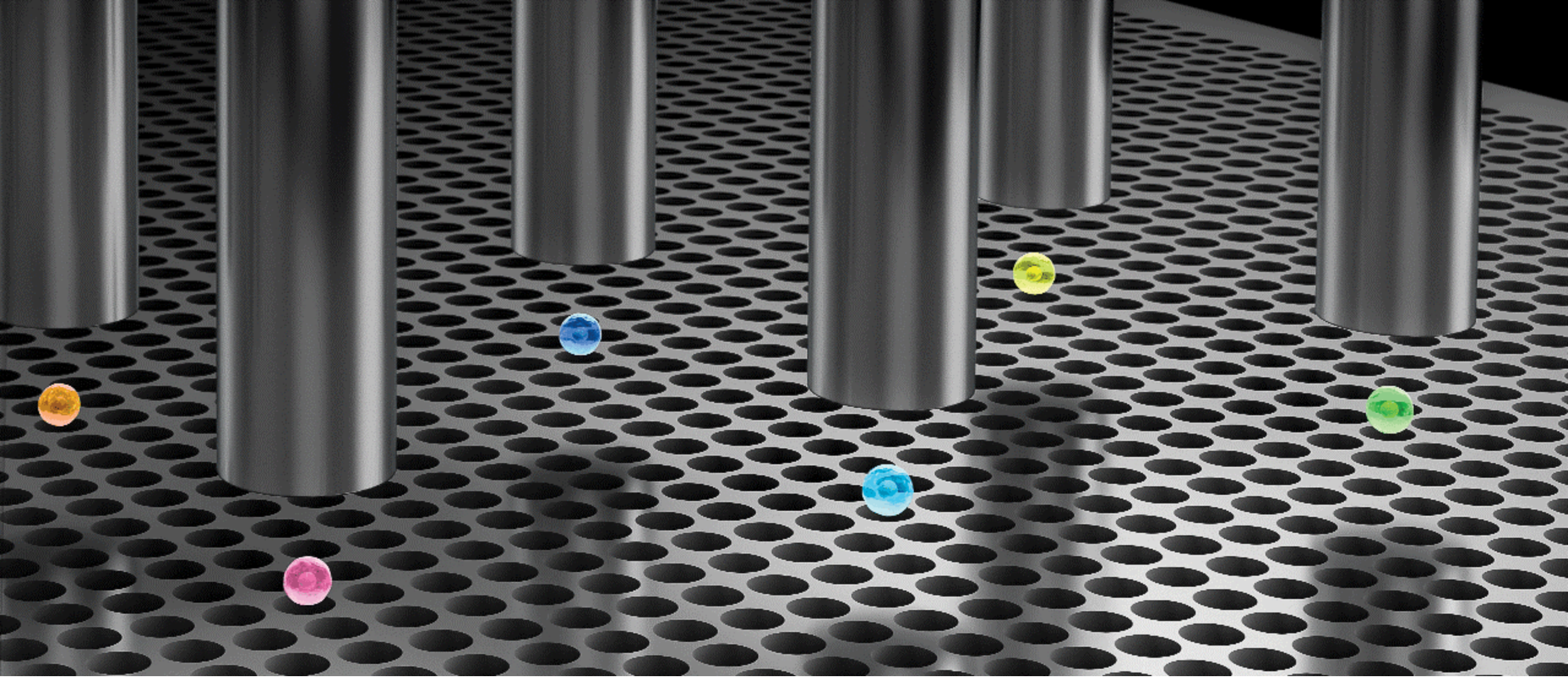
Achieved required sensitivity

All 96 assays were very sensitive and can detect as low as 10 copies per reaction

- 20–40 replicates tested for the calculation
- Limit of Detection (LoD) criteria: target detected for ≥95% of replicates

Acinetobacter baumannii	*Citrobacter freundii*	*Klebsiella aerogenes*	*Pseudomonas aeruginosa*	*Streptococcus oralis*	blaCTX-M 9	blaMOX	blaSHV*	QnrA	*Candida dubliniensis*	*Trichomonas vaginalis*	ermB
Actinotignum schaalii	*Citrobacter koseri*	*Klebsiella oxytoca*	*Serratia marcescens*	*Ureaplasma urealyticum*	blaDHA	blaOXA-1	blaTEM	QnrB	*Chlamydia trachomatis*	*Bacteroides fragilis*	mefA
Aerococcus urinae	*Coagulase-negative staphylococci* (CoNS)	*Klebsiella pneumoniae*	*Staphylococcus aureus*	blaACC	blaFOX	blaOXA-23	blaVEB	QnrS	*Haemophilus ducreyi*	*Kingella kingae*	tetM
Candida albicans	*Corynebacterium riegelii*	*Metamycoplasma hominis*	*Staphylococcus epidermidis*	blaACT	blaGES	blaOXA-40*	blaVIM*	sul1	*Herpes simplex virus type 1*	AAC(6')-Ib	*Bacillus atrophaeus*
Candida auris	*Enterobacter cloacae*	*Morganella morganii*	*Staphylococcus haemolyticus*	blaCMY	blaIMP-1	blaOXA-48	dfrA1	sul2	*Herpes simplex virus type 2*	AAC(6')-Ib-cr	16S*
Candida glabrata	*Enterococcus faecalis*	*Proteus mirabilis*	*Staphylococcus saprophyticus*	blaCTX-M 1	blaIMP-7	blaOXA-72	dfrA5	vanA	*Mycoplasma genitalium*	ANT(3")-IIa	RNaseP
Candida parapsilosis	*Enterococcus faecium*	*Proteus vulgaris*	*Streptococcus agalactiae*	blaCTX-M 2	blaIMP-16	blaPER-1	mecA	vanB	*Neisseria gonorrhoeae*	APH(3')-VIa	
Candida tropicalis	*Escherichia coli*	*Providencia stuartii*	*Streptococcus anginosus*	blaCTX-M 8	blaKPC	blaPER-2	nfsA	vanC	*Treponema pallidum*	ermA	

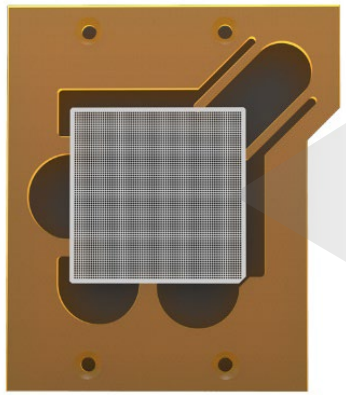
* Four assays (including 16S and β-lactam) were detected at ≤50 copies per reaction.

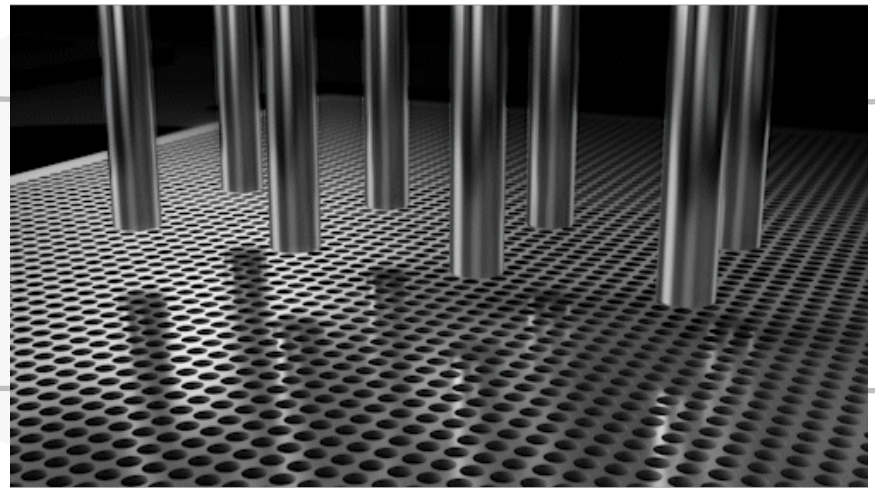


Advantages of the SmartChip ND System

Advantages of the SmartChip ND system

Scalability
- Can handle large panels
- Ideal for small to larger lab facilities





Flexibility
- Customizable assays and sample combinations
- Can be used for any assay panel (not just UTI)

Automated workflow
- Less human error
- Better reproducibility
- Less hands-on time

Lower cost per reaction

Low reagent consumption

Flexible panels to fit your application needs

Examples of molecular assays

- High-throughput SARS-CoV-2 testing
  6 assays/panel = 768 samples/chip
- Nail fungus pathogen panel (21 assays)
- UTI panel (≤72 assays)
- TaKaRa UTI plus panel (96 assays)
- Highly parallel ARG detection panel (384 assays)

	Samples/chip	
Small panel	6	768
	12	384
	24	216
	36	144
	48	108
	54	96
	72	72
	80	64
	96	54
	120	42
	144	36
	216	24
	248	20
	296	16
Large panel	384	12

References

- Stedtfeld et al. Primer set 2.0 for highly parallel qPCR array targeting antibiotic resistance genes and mobile genetic elements. *FEMS Microbiology Ecology* (2018).

Reduced reagent and time consumption

Less reagents + Less time = More savings

5,184 reactions on SmartChip ND vs. plates

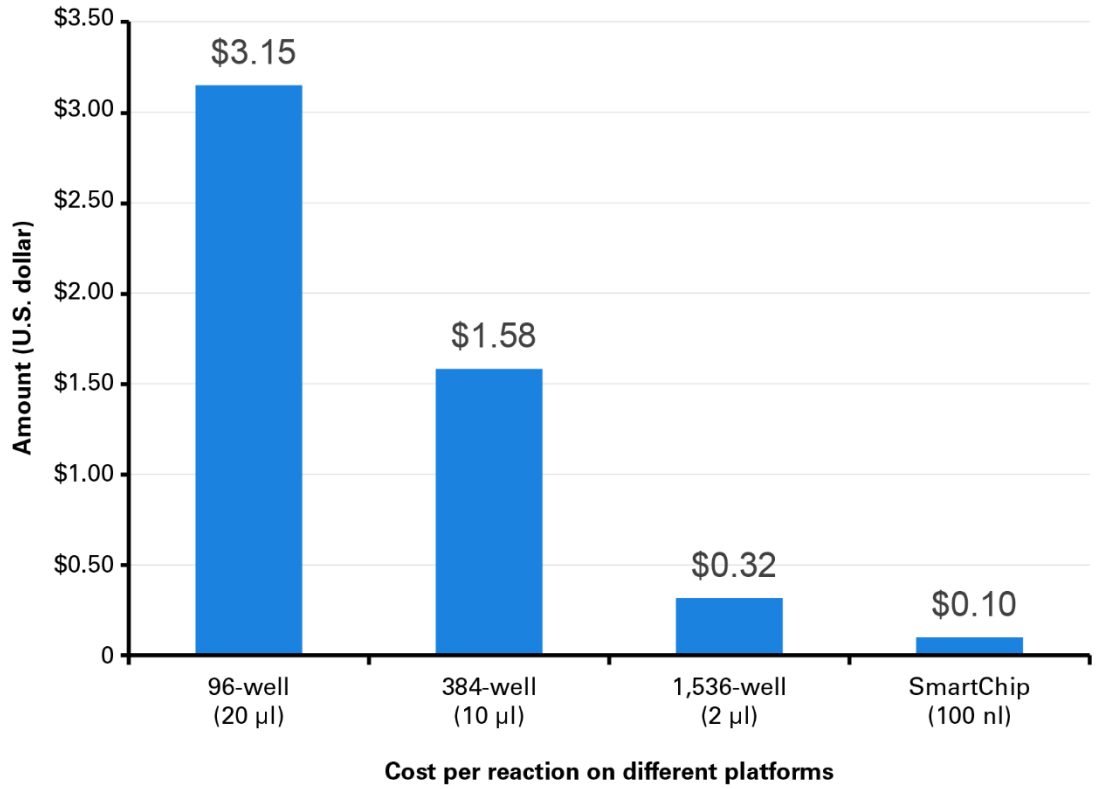
Category	384-well plate (10 µl rxn)	SCND	Fold difference
Master mix	**>30 ml**	**<0.5 ml**	**>60**
Assay mix	>3 ml	~100 µl	>30
Turnaround time	**~25 hr**	**~4 hr**	**>6**

60-fold reagent savings
compared to traditional plate-based qPCR

Turn around time is
6 times faster

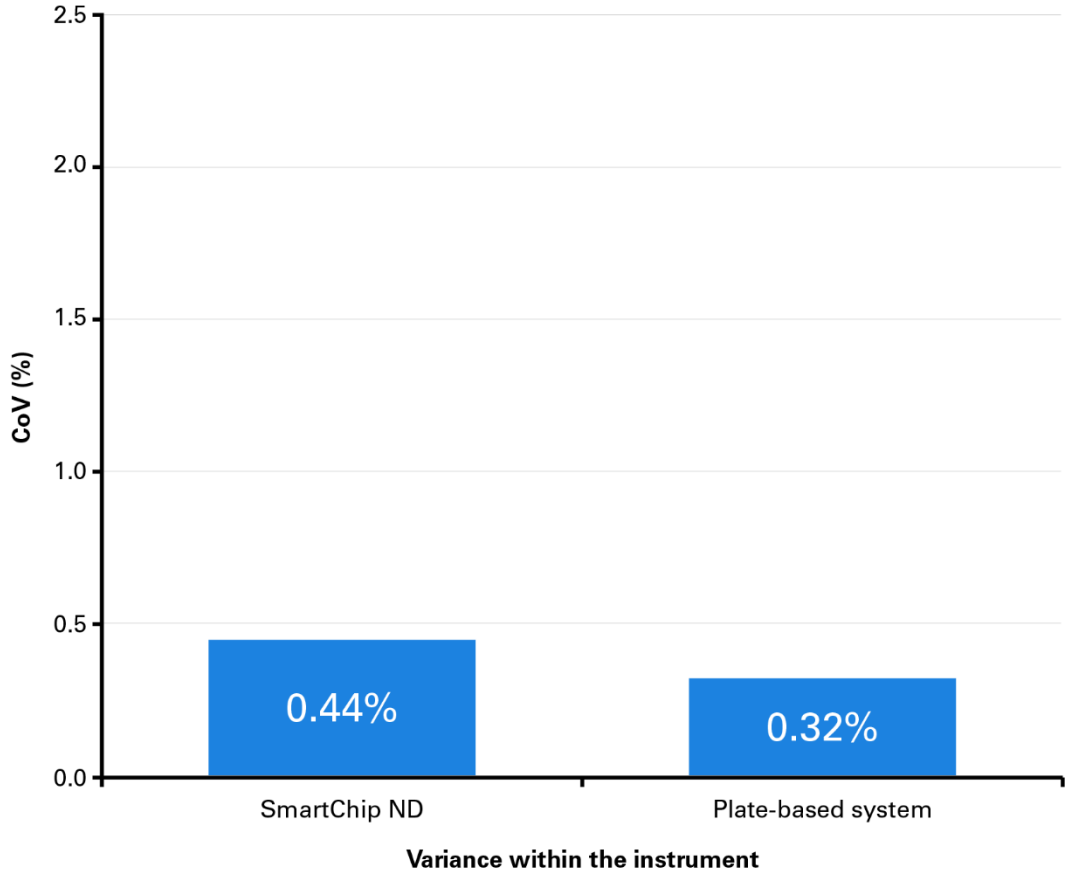
Long term cost savings

Less reagents + Less time = More savings

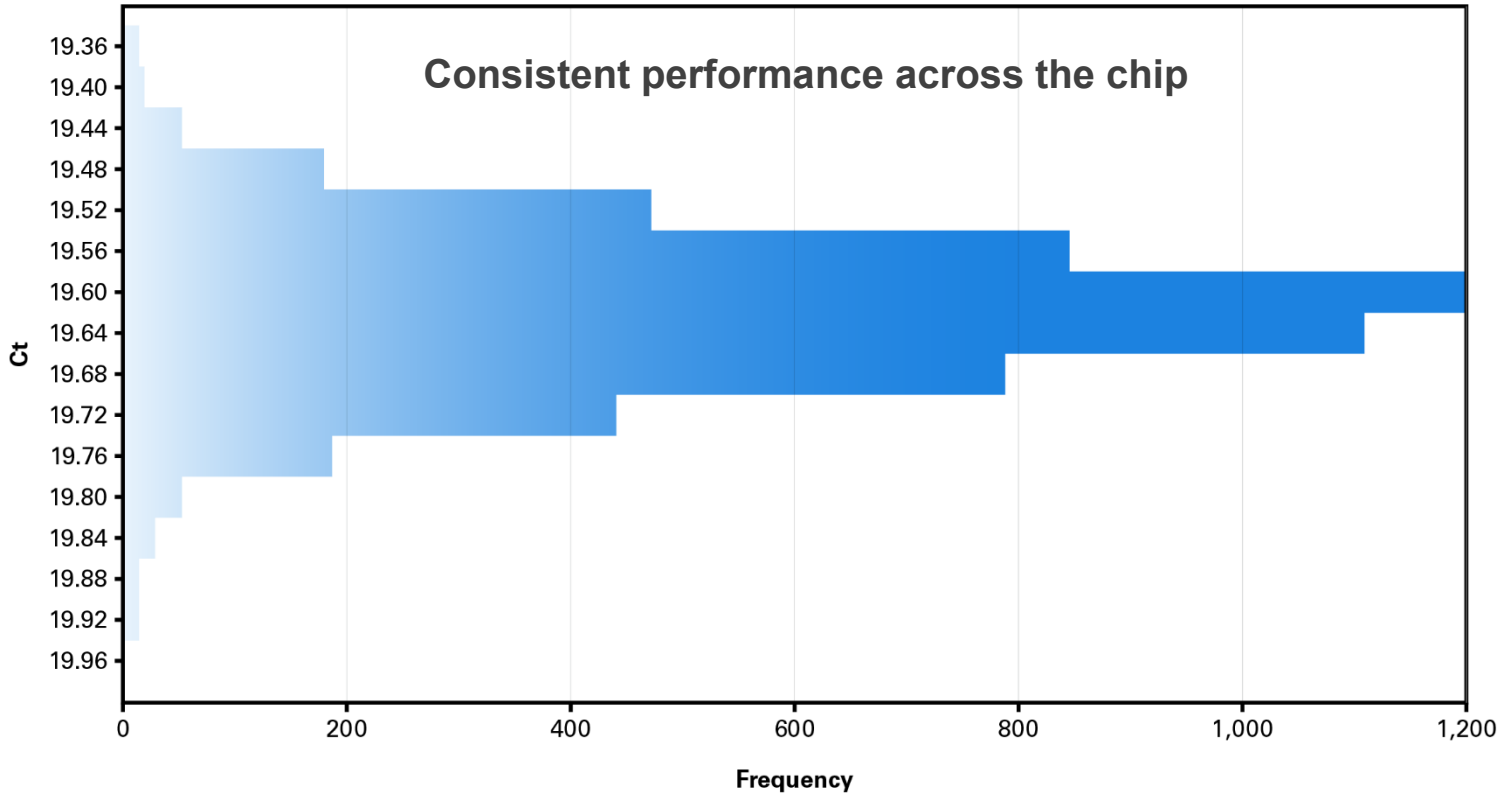


Highly consistent results at a large scale

Highly comparable variability



Reproducible and accurate results within a run

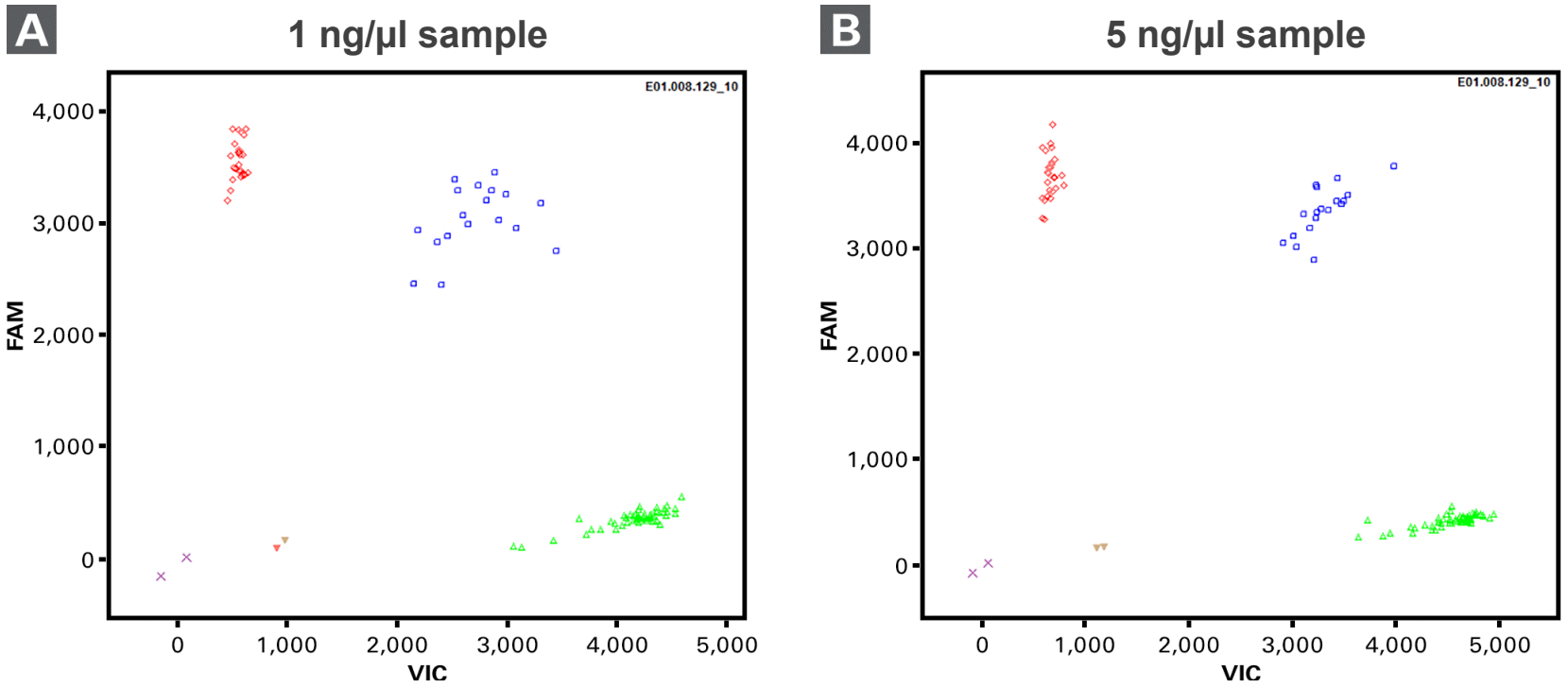


Enclosed, humidified environment and chip cooling station ensure thermal uniformity.

Highly reproducible gene expression data. A single assay run was performed with input from a single sample. The Ct values range from 19.36–19.96, with a low standard deviation (<0.1).

Confidence in genotype clusters

Robust calling for sample concentrations as low as 1 ng/µl



The SmartChip ND system provides highly accurate and sensitive detection, which is critical for making calls when genotyping.
Multiple 1 ng/µl samples **(Panel A)** and 5 ng/µl samples **(Panel B)** were run on the SmartChip ND system using the same genotyping assay. Although the 1 ng/µl samples are more diffuse, the clustering still enables calling.

Summary

- Growing demand for larger pathogen detection panels
  - qPCR a better option than culture-based methods
- SmartChip ND system for high throughput qPCR
  - Significant time and cost savings
  - 5,184 reactions per run
- Comprehensive, 96-assay qPCR panel for UTI, STI, and wound infections
  - Broadens range of detection for pathogens and antibiotic resistance genes
  - Analytical LoD values of 50–10 copies per reaction
- Alien spike-in control ensures qPCR accuracy
  - Detects inhibitors
  - Prevents false negatives

TaKaRa

that's
GOOD
science!®