



# Genetic Signatures

*3base™*

*The Science Behind EasyScreen™*

*Molecular Diagnostic Assays*

*Nucleic Acid Conversion Technology*



# Topics

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- 3base™ Technology Overview
- Technical Advantages of 3base™
- Validation of 3base™ Specificity
- Validation of *EasyScreen* Assays
  - C. difficile, screening panels
- Validation of GS1 automation
- Future developments





# Developed to Improve Subtype Similarity

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e.g. Non-Converted Influenza Sequences

Influenza A virus H5N1	TGTGTGTGCA	GGGATAATTG
Influenza A virus H7N3	TGTATATGTA	GGGACAATTG
Influenza A virus H5N8	TGTGTTTGTA	GAGACAACTG
Influenza A virus H5N3	TGTATATGTA	GGGACAATTG
Influenza A virus H5N2	TGTGTTTGCA	GAGATAATTG
Influenza A virus H6N6	TGCATTTGCA	GGGACAATTG
Influenza A virus H2N9	TGCACTTGCA	GGGATAATTG
Influenza A virus H6N5	TGCGTTTGCC	GAGATAATTG

Consensus TGYRYDTGYM GRGAYAAYTG  
**768** Possible combinations  
55% Homology



# Conversion to 3base™ Improves Subtype Similarity and Reduces Variation – Enabling Analysis via PCR

## e.g. Non-Converted Influenza Sequences

Influenza A virus H5N1	TGTGTGTC	CA	GGGATAATTG	
Influenza A virus H7N3	TGTATATGTA	GGG	CAATTG	
Influenza A virus H5N8	TGTGTTTGTA	GAG	CAACTG	
Influenza A virus H5N3	TGTATATGTA	GGG	CAATTG	
Influenza A virus H5N2	TGTGTTT	CA	GAGATAATTG	
Influenza A virus H6N6	TC	CATTT	CA GGG	CAATTG
Influenza A virus H2N9	TC	CACTT	CA GGG	AATTG
Influenza A virus H6N5	TC	GTTT	CC	GAGATAATTG

Consensus TGYRYDTGYM GRGAYAAYTG  
768 Possible combinations  
55% Homology

## 3base™ Converted Influenza Sequences

Influenza A virus H5N1	TGTGTGTCTA	GGGATAATTG	
Influenza A virus H7N3	TGTATATGTA	GGGCAATTG	
Influenza A virus H5N8	TGTGTTTGTA	GAGCAATTG	
Influenza A virus H5N3	TGTATATGTA	GGGCAATTG	
Influenza A virus H5N2	TGTGTTTCTA	GAGATAATTG	
Influenza A virus H6N6	TC	CATTTCTA GGG	CAATTG
Influenza A virus H2N9	TC	CATTTCTA GGG	AATTG
Influenza A virus H6N5	TC	GTTTCTT	GAGATAATTG

Consensus TGTRTDTGTW GRGATAATTG  
24 Possible combinations  
80% homology

Cytosines are converted to Thymines – *resulting in non-natural 3-base DNA/RNA*

Sufficient information is retained after conversion for genotyping equivalent to native (4base) genomic assays

No Loss of clinical specificity is observed by this base conversion

*e.g. HPV clinical trial showed superior performance vs. Digene HC2 Assay in reducing False Positives*



# 3base™ Technology

*The cornerstone of EasyScreen™ assays*

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- Universally applicable to all specimen types - converts and detects both DNA and RNA
  - Works with multiple specimen types simultaneously
  - Stool is first commercially available
- Allows for simple multiplexed assays
  - More targets detected per specimen - e.g. 22 gastroenteritis causing targets are simultaneously detected from stool
- Real-Time PCR format
  - No post-PCR handling; virtually eliminates potential for contamination
- Open platform
  - Suits all laboratories – no need to purchase new equipment



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# Technical Advantages of 3base™ Technology



# Technical Advantages of 3base™ Assays

## One primer set detects multiple species

### Universal primer F1

Gi   4566774   G	2007	TA	ACTACTGA	TTG	AAAA	TA	CTGTCTCA	ACTGGAGAT	T	TG	AAAGTACA	Parainfluenza-1
Gi   67906100	10794	TA	ACTACTGA	TCT	TGCTAAA	TA	CTGTCTTC	AATGGAGATA		TC	AGCCATA	Parainfluenza-2
Gi   332716   Gb	5805	TA	ACAACAGA	TCT	CAAAAA	TA	CTGTCTTA	ATTGGAGATA		TG	AAACACA	Parainfluenza-3
Gi   194394351	12057	TA	ACAACAGA	TTT	CAAAAA	TA	CTGTCTTA	ATTGGAGATA		CC	AAACAATA	Parainfluenza-4

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Gi   4566774   G	2057	GC	ATTCTTCG	GT	CAAAGATG	TA	ATGAGATA	TT	CGGCTTTA	AA	ACTTCTTT	Para-1 probe
Gi   67906100	10844	AT	CCATTTTG	CT	GAACATT	AA	AGAATG	TAT	GGACTTC	CA	CATTTATT	Para-2 probe
Gi   332716   Gb	5855	GC	TCTATTG	G	AAACTTG	CA	ACCAATA	TT	TGGATTAA	AT	AAATTGTT	
Gi   194394351	12107	AT	ACCCTTTG	CT	AGAACACT	AA	ATCGAATG	TAC	GGATATC	CT	CATCTCTT	

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### Universal primer-R1

Gi   4566774   G	2107	TA	ACTGGATG	CAC	CTATTC	TAG	AAAAAAG	TAC	GATTTAT	GT	AGGAGATC	
Gi   67906100	10894	TG	AATGGATT	CAT	CTTCGTT	TA	ATAGATC	TAC	ATTAT	GT	TGGTGATC	
Gi   332716   Gb	5905	TA	ATGGTTA	CAC	CTCGTC	TT	GAGGAAG	TA	CAATC	GT	AGGTGATC	Para-3 probe
Gi   194394351	12157	CG	AATGGATT	CAT	TTAAGAT	TA	ATGAAATC	AA	CTTCTAT	GT	AGGTGACC	Para-4 probe

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Gi   4566774   G	2157	CT	TACTGTCC	AG	TACCTGAT	AG	GATGCACA	A	AGA	ACTCCA	AG	ATCATGAT
Gi   67906100	10944	CA	TTCAATCC	TC	CTGCCGCA	AC	TGATGCTT	TC	GATCTAGA	T	AA	AGTATTA
Gi   332716   Gb	5955	CT	TATGTCC	T	CCATCAGAT	A	AGGAACATA	T	ATCAT	TAGA	GG	ATCACCCT
Gi   194394351	12207	CG	TTCAATCC	T	CCATCAGAT	C	ATAATGTGA	CT	GACCTAGA	T	AA	TGCACCA

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# Validation of 3base™ Specificity



# Validation of Specificity

- Single copy genes may be amplified from a 3base™ converted human genome
- High-Risk HPV identified as hardest challenge



Journal of Clinical Virology 42 (2008) 22–26



[www.elsevier.com/locate/jcv](http://www.elsevier.com/locate/jcv)

Comparison of a novel HPV test with the Hybrid Capture II (hcII)  
and a reference PCR method shows high specificity and  
positive predictive value for 13 high-risk  
human papillomavirus infections

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# HPV Validation Study Summary

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- The HGS tests had a statistically significant higher PPV than the HC2 (Digene) test ( $P < 0.001$ )
- The HGS test has a lower rate of false positives at the same level of sensitivity as the HC2 (Digene) test ( $P < 0.001$ )
- The HGS test and the HC2 test showed no statistical difference in NPV for presence of virus ( $p=0.677$ ) or false negatives (sensitivity;  $P=0.398$ )



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# Validation of *EasyScreen*<sup>TM</sup> Clinical Performance



# EasyScreen™ *C. difficile* Detection and Reflex Kits

- Rapid real-time PCR kit for detection of *C.difficile*
- Reflex kit to identify hypervirulent subtypes



Product #	Description	Microorganisms Detected
CDD001	EasyScreen™ <i>C. difficile</i> Detection Kit	Toxicogenic <i>C. difficile</i> (targets both <i>tcdA</i> and <i>tcdB</i> )
CDD002	EasyScreen™ <i>C. difficile</i> Reflex Kit	Hypervirulent <i>C. difficile</i> incl. 027 & 078 (targets (i) <i>tcdC</i> gene deletion at position 117 and (ii) binary toxin gene (iii) <i>gyrA</i> gene mutation (fluroquinolone resistance))

Tom Riley/Kerry Carson to present at ECCMID 2014:  
**sensitivity 93.7% PPVs 96.8%**



# Hypervirulent Strains of *C. difficile*

*Identified in Australia 2010*

## New bacteria found in NSW

From: AAP December 17, 2010 2:53PM **5 comments**

A+ A- S

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**A NEW strain of a bacteria, which causes diarrhoea, has been found for the first time in NSW.**

NSW Health said samples from 21 patients tested positive for the new strain of Clostridium difficile (C difficile) bacteria, also known as 027.

"The 027 strain of C difficile, while common in North America and Europe since 2003, was identified in Australia earlier this year," NSW Health said in a statement.

"C difficile is a bacteria present naturally in the gut of many healthy children under the age of two years and some adults without suffering any ill effects."



# EasyScreen™ Gastrointestinal Screening Panels

Detection Kit	Targets
<i>EasyScreen™</i> Enteric Bacterial Detection Kit (REF: EB003)	<i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Campylobacter</i> spp., <i>Yersinia enterocolitica</i> , <i>Listeria monocytogenes</i> , <i>C. difficile</i> , <i>Aeromonas hydrophila</i> , <i>Vibrio cholera/parahaemolyticus</i> , Shiga toxinogenic <i>E. coli</i> ( <i>stx1/stx2</i> ), Extraction control and Internal Positive Control
<i>EasyScreen™</i> Enteric Protozoan Detection Kit (REF: EP001)	<i>Giardia intestinalis</i> , <i>Cryptosporidium</i> spp, <i>Entamoeba histolytica</i> , <i>Dientamoeba fragilis</i> , <i>Blastocystis hominis</i> , Extraction control and Internal Process Control
<i>EasyScreen™</i> Enteric Viral Detection Kit (REF: EV001)	Norovirus GI, Norovirus GII, Adenovirus hexon, Adenovirus 40/41, Rotavirus A and B, Astrovirus (group 1-7), Sapovirus, and an Extraction control



# EasyScreen™ Protozoan Panel

High performance vs microscopy and RT-PCR

Method	No. Samples	No of Positive samples and sensitivity					Overall Sensitivity <sup>a</sup>	Overall Specificity <sup>b</sup>
		<i>Blastocystis</i>	<i>Cryptosporidium</i>	<i>D. fragilis</i>	<i>E. complex</i>	<i>Giardia</i>		
EasyScreen	358	96% (51/53)	100% (9/9)	95% (41/43)	92% (22/24)	92% (24/26)	92-100%	100%
RT-PCR	358	96% (51/53)	89% (8/9)	95% (41/43)	100% (6/6)*	96% (25/26)	89-100%	100%
Microscopy	358	66% (35/53)	55% (5/9)	74% (32/43)	75% (18/24)	73% (19/26)	55-77%	95-100%

<sup>a</sup> Calculated as follows: (number of true positives/[number of true positives + number of false negative]) x 100

<sup>b</sup> Calculated as follows: (number of true negatives/[number of true negatives + number of false positives]) x 100

- The RT-PCR method used for comparison only targeted *E. histolytica*. A conventional and nested PCR was performed for further confirmation of *E. dispar* and *E. moshkovskii*

**Diagnostic Microbiology & Infectious Disease**  
[Volume 78, Issue 2, Pages 149-152, February 2014](#)





# Clinical Sensitivity and Specificity

As presented by Lee Thomas, Westmead Hospital, Sydney, at ASM 2013

Pathogen detected	EasyScreen™	Sensitivity %	Specificity %	Additional pathogens
Viruses (Noro, Rota, Adeno, Astro)	69	100	97.1%	25
<i>C. difficile</i>	58	84.8	99.4	9
<i>Campylobacter</i> spp.	48	100	100	0
<i>Salmonella</i> spp.	42	97.7	100	1
<i>Shigella</i> spp.	11	100	99.5	0
<i>L. monocytogenes</i>	1	NA	NA	1
<i>Y. enterocolitica</i>	3	100	100	2
<i>D. fragilis</i>	10	100	100	10
<i>B. hominis</i>	17	100	100	16
<i>G. intestinalis</i>	12	92.3	100	7
<i>Cryptosporidium</i> spp.	3	100	100	3
<i>Entamoeba</i> complex	5	NA	NA	5
Totals	279			79

- EasyScreen™ assays identified **79 infections that were missed using traditional methods** and following SOP (28% extra)
- EasyScreen™ results were **generated in ~4 hours, compared to up to 4 days for traditional methods**
- **High sensitivity and specificity** across all targets



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# Validation of GS1 automation

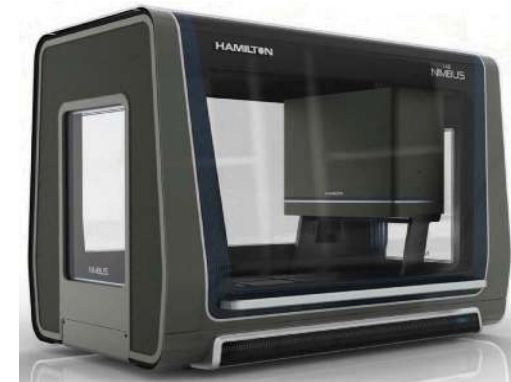


# The New GS1

*Optimised and easy to use for improved workflow*

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- Single platform to perform all sample processing steps (nucleic acid extraction) & set-up of PCR plates (96 or 384 well format)
- Optimised for all *EasyScreen*<sup>™</sup> Assays
- Improves sample throughput
- Eliminates set up errors
- Open system for general lab use
- Developed in partnership with Hamilton Robotics - based on the Nimbus platform





# Clinical Validation of GS1

Ongoing evaluation by Damien Stark, SydPath, Sydney, 221 Specimens

Pathogen	Conventional Methods*	EasyScreen™
<i>Campylobacter</i>	7	9
<i>Salmonella</i>	8	9
<i>Shigella</i>	5	6
<i>C. difficile</i>	3	7
<i>Yersinia</i>	-	1
<i>Cryptosporidium</i>	-	1
<i>Giardia</i>	9	12
<i>Dientamoeba fragalis</i>	4	20
<i>Blastocystis hominis</i>	16	21
<i>Entamoeba histolytica</i>	1	1
Norovirus group II	-	7
Adenovirus	-	1
Adenovirus 40/41	-	1
Sapovirus	-	1
Total	53	97

- EasyScreen™ assays identified **44 infections that were missed using traditional methods** and following SOP (20% extra)
- Extra infections detected are still being validated
- EasyScreen™ results were **generated with <2 mins hands-on time per specimen**

\*Viruses were not tested routinely using conventional methods



# Future Developments

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- Exciting new screening assays in development:
  - MRSA
  - *Mycobacterium tuberculosis/avium*
  - Meningitis
  - STIs
  - Pneumonia
  - Influenza Detection and Typing
  - Respiratory Tract Viral Infections



# Team and Acknowledgements

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  - Dr Douglas Millar
  - Dr Shoo Peng Siah
  - Ms Jiny Nair
  - Dr Kiran Kaur
  - Mr Christopher French
- Tom Olma and Lee Thomas ICPMR
- Thomas Karagiannis and Peter Huntington PaLMS
- Damien Stark and Tamalee Roberts SydPath



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  - Steven Siarakas, Department of Microbiology and Infectious Diseases, Concord Hospital, Hospital Rd, Concord
  - Rogan Lee, Parasitology, CIDMLS, Westmead Hospital, Westmead
  - Ken McPhee, Viral Laboratory, CIDMLS, Westmead Hospital, Westmead
  - Susie Roczo-Farkas, Enteric Virus Group, The Royal Children's Hospital, Victoria



# Genetic Signatures