



Genetic Signatures

3base™

The Science Behind EasyScreen™

Molecular Diagnostic Assays

Nucleic Acid Conversion Technology



Topics

- 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



Overview of 3base™

Basic technical functionality: Moving from 4base to 3base™

Regular Cell Lysis and Preparation Steps

Native
Sequence

3base™
proprietary Sodium
Bisulfite Treatment

CGTAGCCTCACTTCCAGGACTGGC
↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓
TGTAGTTTATATTTTAGGATTGGT

Original (Native) 4-base
Microbial genome

Converted 3base™ Microbial
Genome

3base™
Sequence

Standard Real-Time PCR Instrument



Developed to Improve Subtype Similarity

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**CA**ATTG
 Influenza A virus H5N8 TGTGTTTGTA GAG**CA**ACTG
 Influenza A virus H5N3 TGTATATGTA GGG**CA**ATTG
 Influenza A virus H5N2 TGTGTTT**CA** GAGATAATTG
 Influenza A virus H6N6 T**CA**TTT**CA** GGG**CA**ATTG
 Influenza A virus H2N9 T**CA**CTT**CA** GGGATAATTG
 Influenza A virus H6N5 T**CA**GTTT**CA** GAGATAATTG

Consensus TGYRYDTGYM GRGAYAAYTG
 768 Possible combinations
55% Homology

3base™ Converted Influenza Sequences

Influenza A virus H5N1 TGTGTGT**CTA** GGGATAATTG
 Influenza A virus H7N3 TGTATATGTA GGG**TA**ATTG
 Influenza A virus H5N8 TGTGTTTGTA GAG**TA**ATTG
 Influenza A virus H5N3 TGTATATGTA GGG**TA**ATTG
 Influenza A virus H5N2 TGTGTTT**CTA** GAGATAATTG
 Influenza A virus H6N6 T**CTA**TTT**CTA** GGG**TA**ATTG
 Influenza A virus H2N9 T**CTA**TTT**CTA** GGGATAATTG
 Influenza A virus H6N5 T**CTA**GTTT**CTA** 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

- 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



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 | TTGAAA | AAA | TACTGTCT | CA | ACTGGAGAT | T | TGAAA | CTACA | Parainfluenza-1 |
| Gi 67906100 | 10794 | TA | ACTACTGA | TCTTGCT | AAA | TACTGTCT | TC | AATGGAGATA | | TCAG | ACCATA | Parainfluenza-2 |
| Gi 332716 Gb | 5805 | TA | ACAACAGA | TCTCAA | AAA | TACTGTCT | TA | ATTGGAGATA | | TGAA | CAACA | Parainfluenza-3 |
| Gi 194394351 | 12057 | TA | ACAACAGA | TTTACA | AAA | TATTGTCT | TA | ATTGGAGATA | | CCAA | CAATA | Parainfluenza-4 |

| | | | | | | | | | | | | |
|------------------|-------|------|---------|-------|---------|-------|--------|-------|-------|-------|--------|--------------|
| Gi 4566774 G | 2057 | GCA | TTCTTCG | GTCAA | AGATG | TAATG | GAGATA | TTCGG | CTTTA | AAAC | TTTCTT | Para-1 probe |
| Gi 67906100 | 10844 | ATCC | ATTTTG | CTCGA | ACATT | AAAC | AGAATG | TATGG | ACTTC | CACAT | TTTATT | Para-2 probe |
| Gi 332716 Gb | 5855 | GCT | CTATTG | GAGAA | ACTTG | CAACC | AAATA | TTTGG | ATTAA | ATAA | ATTGTT | |
| Gi 194394351 | 12107 | ATAC | CCTTTG | CTA | GAACACT | AAAT | CGAATG | TACGG | ATC | CTCAT | CTCTT | |

Universal primer-R1

| | | | | | | | | | | | | |
|------------------|-------|-----|----------|--------|-------|--------|--------|-------|-------|-------|--------|--------------|
| Gi 4566774 G | 2107 | TA | ACTGGATG | CACCCT | ATTC | TAGAAA | AAAG | TACGA | TTTAT | GTAGG | GAGATC | |
| Gi 67906100 | 10894 | TGA | ATGGATT | CATCTT | CGTT | TAAT | TAGATC | TACAT | TAT | GTTGG | TGATC | |
| Gi 332716 Gb | 5905 | TA | ATGGTTA | CACCCT | CGTC | TTGA | AGGAA | TACA | ATC | GTAGG | TGATC | Para-3 probe |
| Gi 194394351 | 12157 | CGA | ATGGATT | CATTT | AAGAT | TAAT | GAAATC | AAC | TTT | GTAGG | TGACC | Para-4 probe |

| | | | | | | | | | | | | |
|------------------|-------|-----|----------|-------|-------|-------|---------|------|----------|------|---------|--|
| Gi 4566774 G | 2157 | CT | TACTGTCC | AGTAC | CTGAT | AGGAT | GCACA | AAGA | ACTCCA | AGAT | CATGAT | |
| Gi 67906100 | 10944 | CAT | TCAATCC | TCCTG | CCGCA | ACTG | ATGCTT | TCG | ATCTAGA | TAA | AGTATTA | |
| Gi 332716 Gb | 5955 | CT | TATTGTCC | TCCAT | CAGAT | AAG | GAACATA | TAT | CATTAGA | GGAT | CACCCT | |
| Gi 194394351 | 12207 | CG | TTCATCC | TCCAT | CAGAT | CATA | ATGTGA | CTG | ACCCTAGA | TAAT | GCACCA | |



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

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

- 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; $P=0.398$)



Validation of *EasyScreen*TM 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 |
|--|--|
| EasyScreen™ 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 |
| EasyScreen™ 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 |
| EasyScreen™ 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 ^a | Overall Specificity ^b |
|------------|-------------|--|------------------------|--------------------|-------------------|----------------|----------------------------------|----------------------------------|
| | | <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% |

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

^b 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



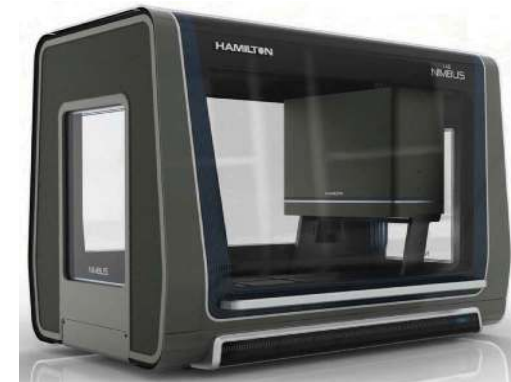
Validation of GS1 automation



The New GS1

Optimised and easy to use for improved workflow

- 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*[™] 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

- 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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Genetic Signatures