Genetic Signatures

Transforming Molecular Diagnostics

EasyScreen™ Detection Kits for the Rapid Screening of Infectious Diseases

For the Screening of Respiratory and Enteric Pathogens

All Genetic Signatures' ***EasyScreen*™ Detection Kits** offer the following advantages

✔ Novel **3base™** Technology

- Simultaneous, specific detection of multiple pathogen targets
- Compatible with both RNA and DNA
- Chemistry prevents amplification of environmental contaminants
- In-built controls in every assay, including independent Inhibition and Extraction controls

✔ Standardised Workflow

- Single efficient workflow for all specimen types and all ***EasyScreen*™ Detection Kits**
- Multiple ***EasyScreen*™ Detection Kits** can be run simultaneously
- Results in under 5 hours (sample to reportable result)
- Reduced staffing requirements and hands-on-time

✔ Adaptable and Automatable

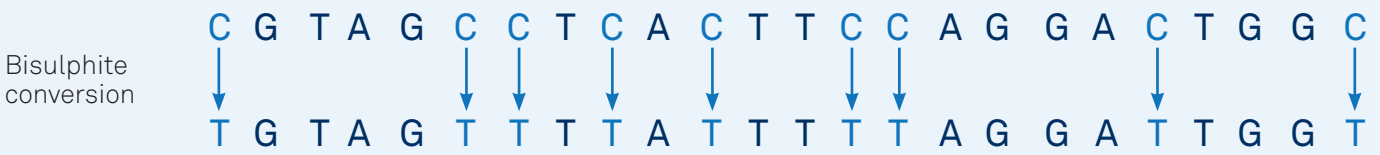
- Compatible with many common nucleic acid extraction and real-time PCR platforms
- GS1 platform available for nucleic acid extraction and PCR setup in molecular and non-molecular grade laboratories
- High and low throughput options available – compatible with 96 and 384 well PCR plates

Highly Sensitive & Specific Pathogen Detection on a Range of Platforms

3base™ Nucleic Acid Modification Technology

- Improves the efficiency of multiplex PCR amplification by enabling conditions in which primers and probes work optimally at similar temperatures
- No need for laboratories to purchase new equipment as **3base™** does not require specialist hardware and is compatible with most automated sample preparation systems and real-time PCR instruments
- Significantly improves consensus primer homology across species variants, and enables the use of single or minimal primers/probes to accurately screen for all/any variants present in a sample

3base™ Mechanism



3base™ Works by Improving Sequence Similarity Across Subtypes

Figure 1a. Example of the **3base™** mechanism. The example sequences below show the increase in homology from 75% ("Before") to 95% ("After") via the **3base™** conversion where all C bases are detected as T bases.

	Before	After
Seq 1	G A T G G **C** G A **T** A T G G T **T** G A **C** A **C**	G A T G G T G A T A T G G T **T** G A T A T
Seq 2	G A T G G **T** G A **C** A T G G T **A** G A **T** A **C**	G A T G G T G A T A T G G T **A** G A T A T
Seq 3	G A T G G **T** G A **T** A T G G T **G** G A **C** A **C**	G A T G G T G A T A T G G T **G** G A T A T
Seq 4	G A T G G **T** G A **T** A T G G T **A** G A **T** A **T**	G A T G G T G A T A T G G T **A** G A T A T
Seq 5	G A T G G **T** G A **T** A T G G T **G** G A **C** A **C**	G A T G G T G A T A T G G T **G** G A T A T
Seq 6	G A T G G **C** G A **C** A T G G T **T** G A **T** A **T**	G A T G G T G A T A T G G T **T** G A T A T
Seq 7	G A T G G **T** G A **T** A T G G T **G** G A **C** A **C**	G A T G G T G A T A T G G T **G** G A T A T
Seq 8	G A T G G **T** G A **C** A T G G T **A** G A **T** A **C**	G A T G G T G A T A T G G T **A** G A T A T
Seq 9	G A T G G **T** G A **T** A T G G T **A** G A **T** A **C**	G A T G G T G A T A T G G T **A** G A T A T
Seq 10	G A T G G **T** G A **T** A T G G T **G** G A **T** A **C**	G A T G G T G A T A T G G T **G** G A T A T
Consensus	G A T G G **Y** G A **Y** A T G G T **D** G A **Y** A **Y**	G A T G G T G A T A T G G T **D** G A T A T
	75% homology over 20 bases	95% homology over 20 bases
	48 possible primer combinations	**3** possible primer combinations

Figure 1b. The regular and **3base™** DNA sequence for 2 primers and 2 probes is shown. The primers and probes for the **3base™** have a more similar melting temperature (Tm) improving the efficiency of multiplex real-time PCR.

Conventional Sequence		Tm
Primer 1	G T A C A C A C C G C C C G T C G C T C C T A C C	77°C
Primer 2	G A A G G A G A A G T C G T A A C A A G	56°C
Probe 1	T G A A T A A A G A G G T G A A A T T C T A G G	59°C
Probe 2	G A A G G G C C G C G A G C C C C C G C G C	87°C

3base™ Sequence		Tm
Primer 1	G T A T A T A T T G T T T G T T G T T T T T A T T	52°C
Primer 2	G A A G G A G A A G T T G T A A T A A G	50°C
Probe 1	T G A A T A A A G A G G T G A A A T T T T A G G	59°C
Probe 2	G A A G G G T T G T G A G T T T T T G T G T	62°C

- The **3base™** converted genome is different from 4-base native genomes; however genomic aspects that identify the presence of disease or microorganisms remain intact
- The converted genome delivers an increase in target homology (similarity) across sub-species
- No loss of specificity, genotyping can be effectively carried out using **3base™** genomes

For further information on 3base™ Technology and *EasyScreen*™ Products, visit our website at www.geneticsignatures.com or email info@geneticsignatures.com

The EasyScreen™ Workflow

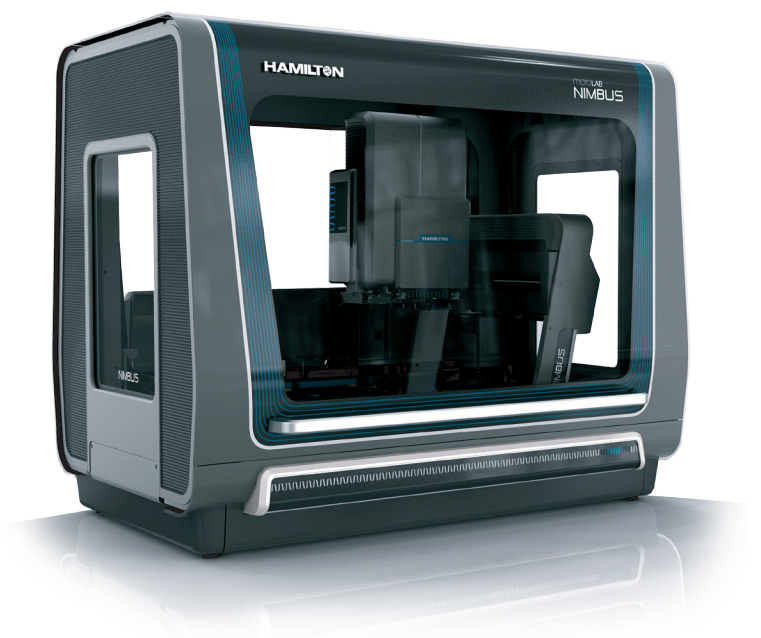
Specimens are inoculated, barcoded and kept at room temperature in the microbiology, molecular or specimen reception laboratory until ready for use.

1. Samples are incubated and processed on one of the validated extraction platforms
2. PCR Setup by scientist or liquid handling platform
3. Amplification on validated real-time PCR platform and results are interpreted

The GS1 Workflow Solution

The GS1 Platform simplifies the *EasyScreen™* Workflow by automating Sample Processing and high-throughput PCR Setup in a contained environment

The GS1 is based on the Nimbus platform (manufactured by Hamilton Robotics, USA). The deck and programs have been customised to be compatible with ***EasyScreen™* Detection Kits** and validated for optimal performance and results.



- Extraction and PCR processes contained within the instrument, allowing for placement in non-molecular laboratories
- Rapid set up of 384 well PCR plates
- UV irradiation for decontamination, combined with **3base™** converted samples, reduces the potential for amplicon and environmental contamination
- Open platform for use with other laboratory work
- Minimises hands-on-time

Validated Nucleic Acid Extraction and Real-Time PCR Platforms

Alternative platforms for use with ***EasyScreen™* Detection Kits**:

Automated Nucleic Acid Extraction Platform Compatibility
Genetic Signatures GS1
bioMérieux NucliSENS easyMAG
Qiagen EZ1
Qiagen QIAsymphony
Roche MagNA Pure 96
Thermo KingFisher Flex

Real-Time PCR Platform Compatibility
Roche LC480 (96 and 384 well formats)
Bio-Rad CFX (96 and 384 well formats)
Qiagen RotorGene
Applied Biosystems 7900HT / 7500
Applied Biosystems QuantStudio™ 5
Stratagene/Agilent Mx

Genetic Signatures
EasyScreen™ Product Range

EasyScreen™ Detection Kits offer the following advantages

- Compatible with most nucleic acid extraction and real-time PCR instruments commonly found in pathology laboratories
- Multiple ***EasyScreen*™ Detection Kits** can be run from a single specimen preparation, allowing the laboratory to test as many targets as desired, either simultaneously or retrospectively
- In-built controls offer a high level of confidence in results. Extraction and Inhibition controls reduces the likelihood of false negative results, providing clarity as to why amplification may not have occurred
- No post-PCR handling, virtually eliminating potential for environmental contamination
- Rapid detection of multiple pathogen targets in under 5 hours, from sample to result

EasyScreen™ Enteric Product List

Product#	***EasyScreen*™** Detection Kit	Microorganisms Detected
EB001	**Enteric Bacteria Detection Kit**	(i) *Salmonella spp.* (ii) *Campylobacter spp.* (iii) *Shigella spp.*/Enteroinvasive *E.coli* (EIEC) (iv) *Yersinia enterocolitica* (v) *toxigenic C. difficile* (vi) *Listeria monocytogenes*
EP001	**Enteric Protozoan Detection Kit**	(i) *Cryptosporidium spp.* (ii) *Giardia intestinalis* (iii) *Dientamoeba fragilis* (iv) *Entamoeba histolytica* (v) *Blastocystis spp.*
EV002	**Enteric Viral Detection Kit**	(i) Norovirus GI (ii) Norovirus GII (iii) Rotavirus (iv) Enterovirus (v) Astrovirus (vi) Sapovirus (vii) Adenovirus universal (viii) Adenovirus 40/41 (ix) Bocavirus
CDD001	***C. difficile* Detection Kit**	(i) Toxigenic *C. difficile* (targets both *tcd*A and *tcd*B)
CDD002	***C. difficile* Reflex Kit**	Hypervirulent *C. difficile* incl. ribotype 027 & 078 targeting: (i) *tcd*C gene deletion at position 117 (ii) binary toxin gene (*cdt*A) (iii) *gyr*A gene mutation (fluoroquinolone resistance)

EasyScreen™ Respiratory Product List

Product#	*EasyScreen*™ Detection Kit	Microorganisms Detected
RP005	**Respiratory Pathogen Detection Kit**	(i) Influenza A (ii) Influenza B (iii) RSV - A/B (iv) Human Metapneumovirus (v) Parainfluenza 1/3 (vi) Parainfluenza 2 (vii) Parainfluenza 4 (viii) Rhinovirus (ix) Enterovirus (x) Adenovirus (xi) *B. pertussis/B. parapertussis* (xii) *M. pneumoniae*
RP003	**Coronavirus Detection Kit**	(i) Coronavirus HKU-1 (ii) Coronavirus OC43 (iii) Coronavirus NL63/229E

The *EasyScreen*™ Sample Processing Kit Range

Product#	Sample Processing Kit Description	Compatible Nucleic Acid Purification Platforms
SP001	Includes reagents for sampling patient specimens and performing **3base™** conversion. To be used on compatible Nucleic Acid Extraction Platforms, with associated extraction reagents.	Qiagen QIAsymphony Qiagen EZ1 Roche MagNAPure 96 bioMérieux NucliSENS easyMAG MagNA Pure 96
SP003	Includes reagents for sampling patient specimens, performing **3base™** conversion, extraction and purification of nucleic acid via magnetic beads.	**Genetic Signatures GS1** Thermo KingFisher Flex
SP004-NS	Includes all reagents in SP003 except for swabs used to sample faecal specimens. Ideal for Respiratory testing.	**Genetic Signatures GS1** Thermo KingFisher Flex

Genetic Signatures ***EasyScreen*™ Sample Processing Kits** are required for the **3base™** conversion of samples, and offer the following flexibilities:

- The SP001 format can be used in conjunction with other manufacturers' extraction platforms and reagents, to adapt to the laboratories current equipment and workflow
- The SP003 format can be used to perform **3base™** conversion, extraction and purification of nucleic acid using the GS1 or Thermo KingFisher Flex

EasyScreen™ Products in Development

- Atypical Pneumonia
- Sexually Transmitted Infections
- Extended Spectrum beta-lactamase (ESBL) and Carbapenemase Producing Organisms (CPO)
- Flavivirus
- Meningitis

Independent Clinical Studies

Clinical Evaluation of the *EasyScreen*™ Enteric Detection Kits in a Multi-Hospital Study

This study examined the clinical performance of the ***EasyScreen*™ Enteric Detection Kits** when screening primary stool specimens for an infectious agent. No cross-reactivity was observed with a wide range of non-target bacterial and viral species.

> ***EasyScreen*™ Detection Kits identified 79 infections that were missed using traditional methods (279 vs 200 detected)**

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
C. difficile	58	84.8	99.4	9
Campylobacter spp.	48	100	100	0
Salmonella spp.	42	97.7	100	1
Shigella spp.	11	100	99.5	0
L. monocytogenes	1	NA	NA	1
Y. enterocolitica	3	100	100	2
D. fragilis	10	100	100	10
Blastocystis spp.	17	100	100	16
G. intestinalis	12	92.3	100	7
Cryptosporidium spp.	3	100	100	3
Entamoeba complex	5	NA	NA	5
Total	**279**			**79**

Independent confirmatory PCR/OCP/Tissue Culture
- Diane Grote, Virology Department, The Children's Hospital, Westmead
- 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
- Damien Stark, SydPath, Sydney

Clinical Evaluation of the *EasyScreen*™ Enteric Protozoan Detection Kit at St. Vincent's Hospital, Sydney

Stark et. al. (2014) used, the ***EasyScreen*™ Enteric Protozoan Detection Kit** to indentify 39 more test-positive samples (17 Blastocystis, 9 *D. fragilis*, 5 Giardia, 4 Entamoeba complex, and 4 Cryptosporidium) than traditional microscopy. The assay also was found not to cross react with various other viral, bacterial, and protozoan faecal pathogens that were tested.

Method	No. Samples	No of Positive samples and sensitivity					Overall Sensitivity	Overall Specificity
		Blastocystis spp.	*Cryptosporidium*	*D. fragilis*	*E. complex*	*Giardia*		
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%

*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*

Clinical Validation of *EasyScreen*™ Enteric Detection Kits on the GS1 Platform

EasyScreen™ Detection Kits identified 44 infections that were missed using In-house methods (97 vs 53 detected)

In this validation, the ***EasyScreen*™ Detection Kits** were compared against multiple methodologies, such as culture, microscopy, EIA and in-house PCR. The ***EasyScreen*™ Detection Kits**, used on the GS1, provided a more sensitive detection of targets in a single, easy-to-use workflow. An additional 44 targets were detected by the ***EasyScreen*™ Detection Kits** when compared to methods used by the laboratory at the time. Viruses were not tested routinely using conventional methods.

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

EasyScreen™ Respiratory Virus Performance Against 2014 QCMD

Performance of the GS Respiratory Viral Panels on 2014 QCMD Panels				
QCMD Panel	Core Samples	GS RVP Result	Educational Samples	GS RVP Result
Influenza A	5	5	2	1
Influenza B	5	5	1	1
RSV	6	6	2	2
Rhinovirus	7	7	3	3
Parainfluenza*	6	6	3	3
Coronavirus	6	6	4	4
Metapneumovirus	8	8	0	0
Adenovirus	9	9	1	1

*Parainfluenza 4 is not detected by this version of the Respiratory Virus Detection Kit (RV001).

Other Publications:

1) D. Stark, T. Roberts, D. Marriott, J. Harkness (2014). **Evaluation of the *EasyScreen*™ Enteric Parasite Detection Kit for the detection of *Blastocystis spp.*, *Cryptosporidium spp.*, *Dientamoeba fragilis*, *Entamoeba complex* and *Giardia intestinalis* from clinical stool samples.** Diagnostic Microbiology and Infectious Disease" 78(2): 149-152.
2) S.P Siah, K. Kaur, J. Nair, P. G. Huntington, T. Karagiannis, D. Stark, J. Merif, W. Rawlinson, T. Olma, L. Thomas, J. R. Melki and D. S. Millar (2014). **Improved detection of gastrointestinal pathogens using generalised sample processing and amplification panels.** Pathology 46(1): 53-59.
3) K. C. Carson, S. P. Siah, D. Millar, B. MacKenzie, J. Melki and T. V. Riley (2014). **Evaluation of the *EasyScreen*™ *C. difficile* Detection Kit for *tcd*A and *tcd*B.** Poster presentation, ECCMID, 2014.
4) L. C. Thomas, T. Olma, S. Chen. ***EasyScreen*™ multiplexed real-time PCR assays for rapid and cost effective routine detection of faecal pathogens.** Proffered paper/Oral presentation, The Australian Society for Microbiology, Annual scientific meeting, 2013.

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Transforming Molecular Diagnostics

For more information on the ***EasyScreen™*** product range, or the underlying **3base™** technology please contact us:

Genetic Signatures Ltd
Level 9, Lowy Packer Building
405 Liverpool Street
Darlinghurst NSW 2010, Australia
Phone: +61 2 9870 7580
Fax: +61 2 9889 4034
Email: info@geneticsignatures.com
Technical support: techsupport@geneticsignatures.com
Web: www.geneticsignatures.com

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