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# **Validation of a novel test for hypervirulent strains of *Clostridium difficile* including BI/NAP1/027**

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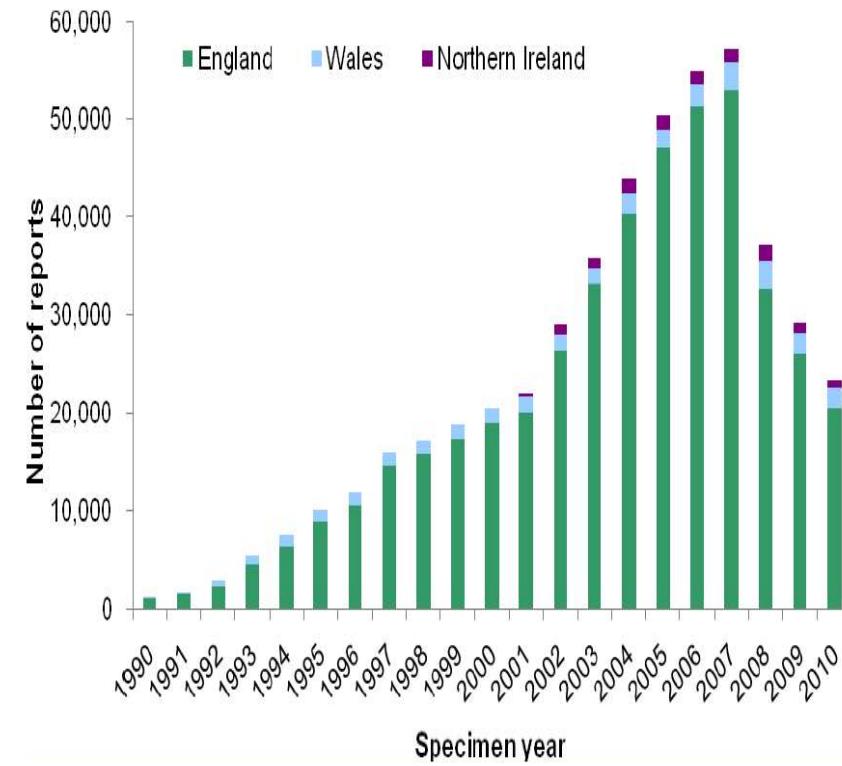
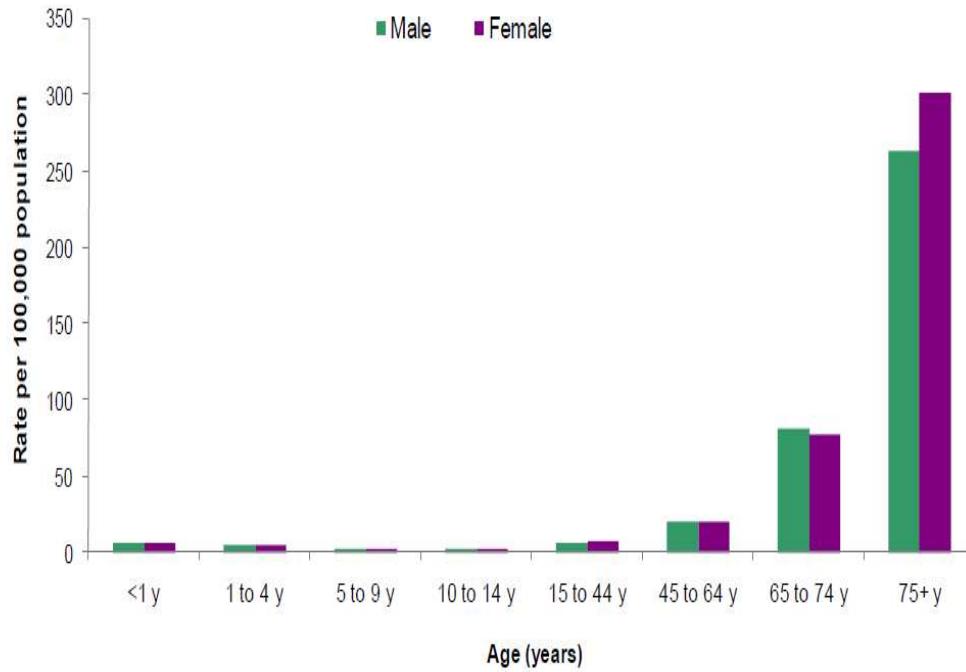
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Prof Thomas Riley (UWA)

Dr Douglas Millar (HGS)



# Increasing incidence of *Clostridium difficile* infection (CDI) reported

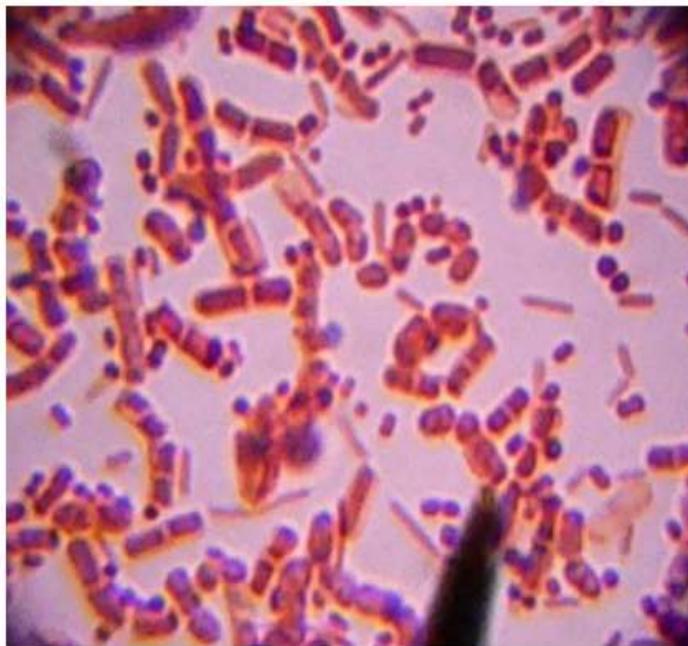




# *Clostridium difficile* infection: The most common nosocomial infection

## Superbug Clostridium Difficile About to Overtake MRSA in Hospitals

by Robert Rister / Healthy Living 



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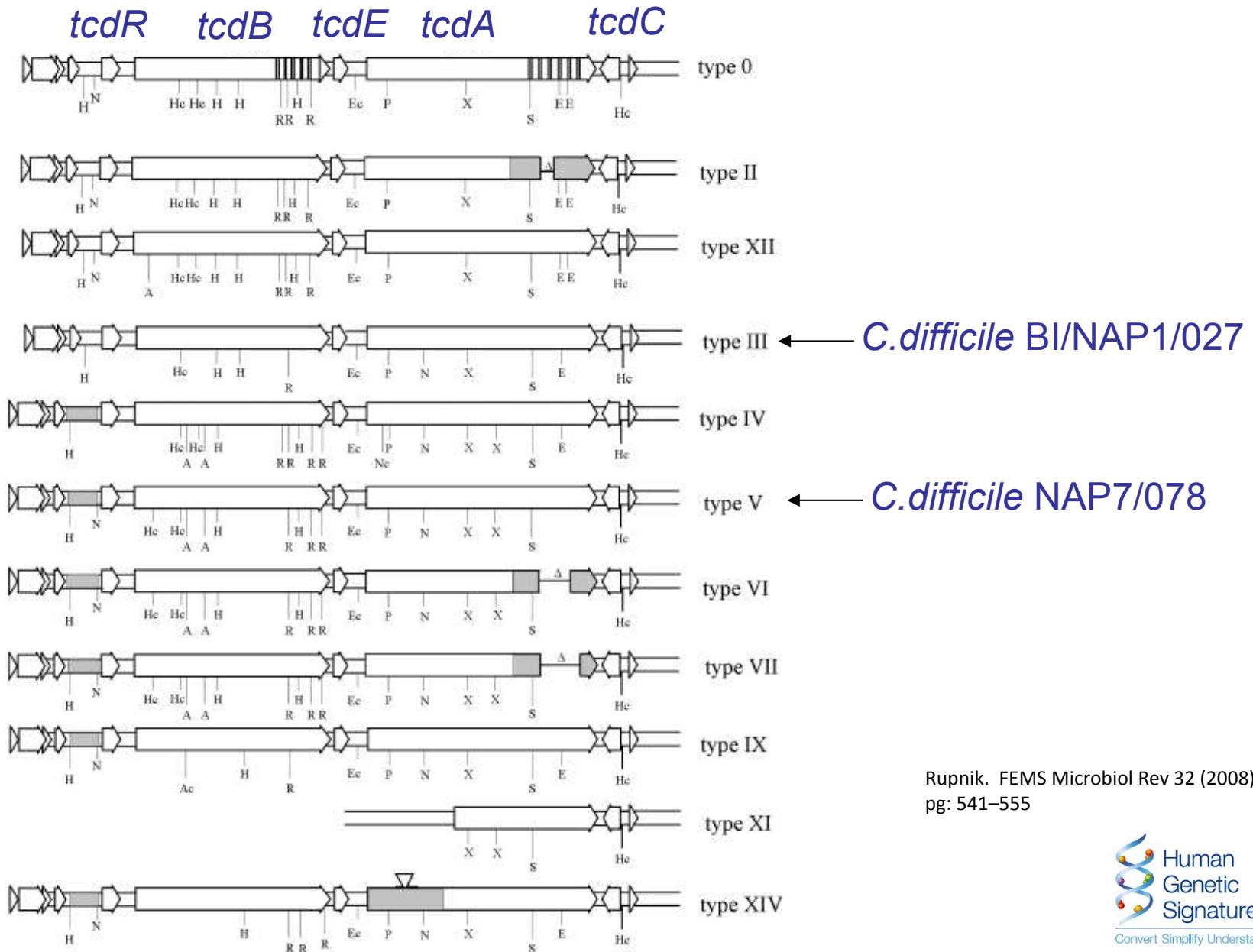
### "Super-bug" - MRSA or methycillin-resistant *Staphylococcus aureus*

For over seven years the news has been filled with scary reports about the "super-bug" MRSA, methycillin-resistant *Staphylococcus aureus*. This flesh-eating bacterium has been known to haunt

[http://www.steadyhealth.com/articles/Superbug\\_Clostridium\\_Difficile\\_About\\_to\\_Overtake\\_MRSA\\_in\\_Hospitals\\_a1317.html](http://www.steadyhealth.com/articles/Superbug_Clostridium_Difficile_About_to_Overtake_MRSA_in_Hospitals_a1317.html)



# Variability in *C.difficile* toxinotypes = difficulties in detection?





# Markers of hypervirulent *C.difficile*

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- *tcdC* single base pair deletion at nt 117 ( $\Delta 117tcdC$ )
  - found in 027 and 078 ribotypes
- repeat unit deletion downstream of  $\Delta 117tcdC$ 
  - 18bp in 027 ribotype
  - 39bp in 078 ribotype
- Threonine to Isoleucine mutation (codon 82) at *DNA gyrase A gene (gyrA)*
  - confers resistance to fluoroquinolone in newer hypervirulent strains (027, 078)
- Presence of binary toxin genes (*cdtA* and *cdtB*)



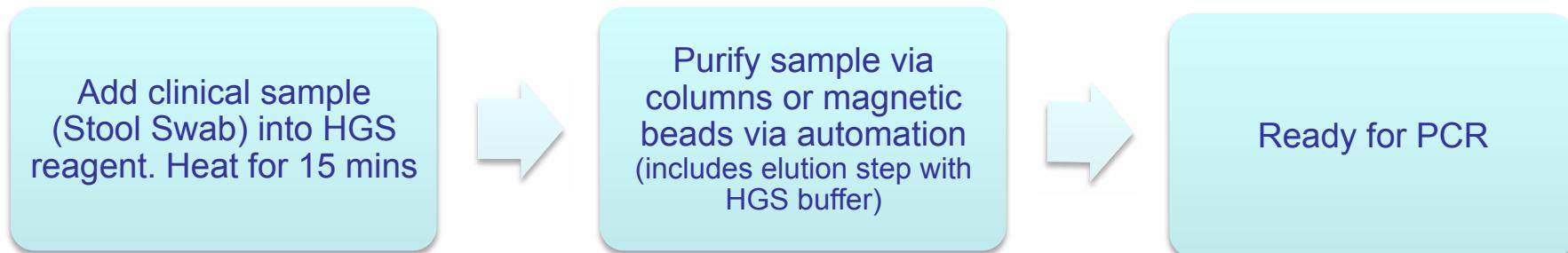
# Introducing the EasyScreen *C. difficile* assays

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- Detection assay
  - *tcdA* and *tcdB*
  - PCR amplification control
  - Extraction control
- Reflex assay
  - Identifies hypervirulent strains
    - “Quebec” strain (BI/NAP1/027)
  - *binary toxin* gene (*cdtA*)
  - *tcdC* single base pair deletion at nucleotide 117 ( $\Delta 117\text{tcdC}$ )
  - fluoroquinolone resistance marker (*gyrA*)
  - PCR amplification control



# Rapid and automatable work flow



Total Hands-on time ~ 10 mins/ Incubation times ~ 15 mins/ Sample to result time ~ <3 hrs



# Instrument compatibility

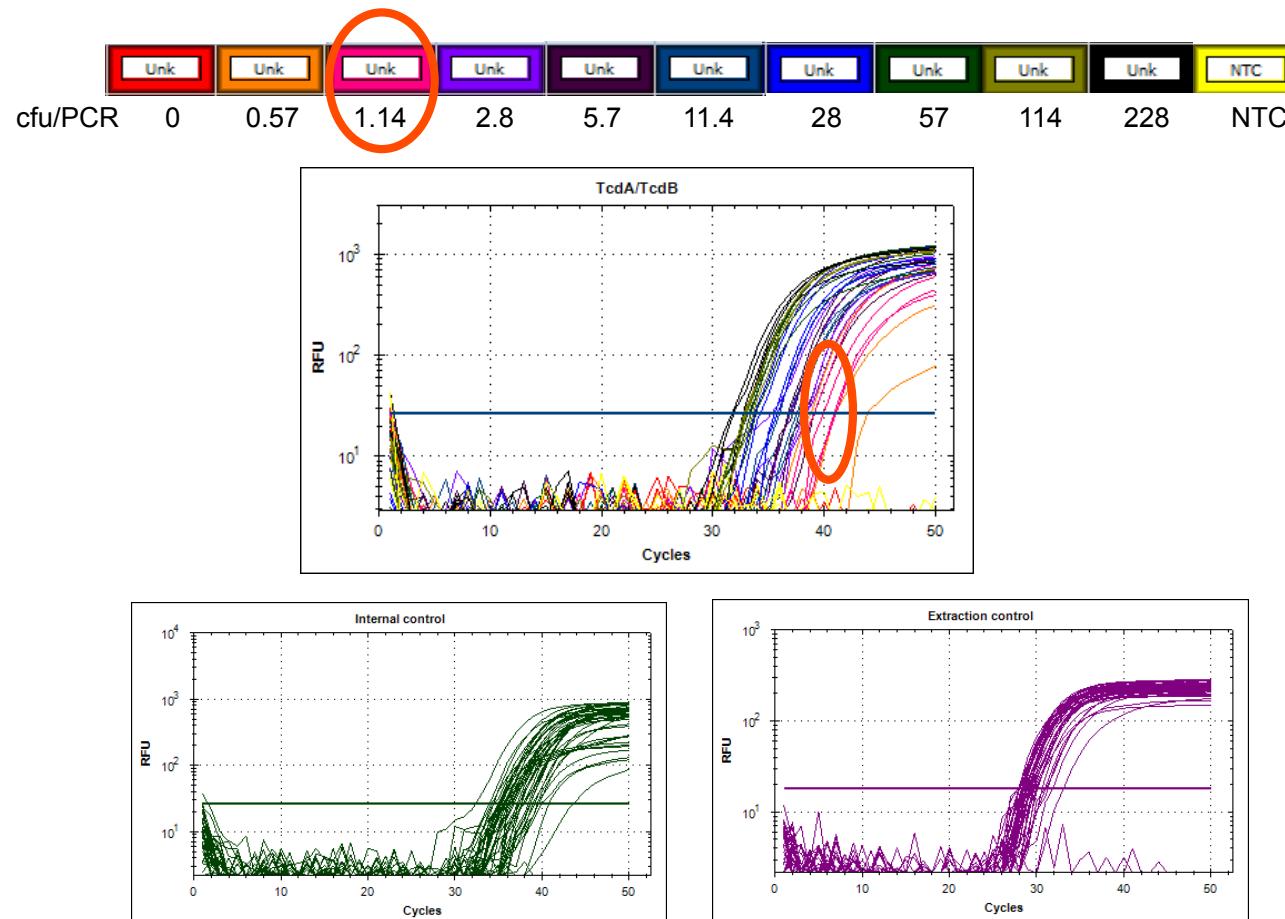
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- Compatible with multiple extraction platforms.
  - QiaSymphony SP
  - Qiagen Biorobot M48
  - Qiagen Biorobot EZ1
  - Roche MagNApure
  - Thermo Fisher Scientific's Kingfisher
- Compatible with various real-time PCR systems
  - Roche LightCycler 480 instruments
  - Biorad CFX96
  - Agilent (Stratagene) MX3000
  - Qiagen Rotorgene-Q (or Corbett Rotorgene 6000)
  - Cepheid Smartcycler



# Sensitive detection of *tcdA* and/or *tcdB*

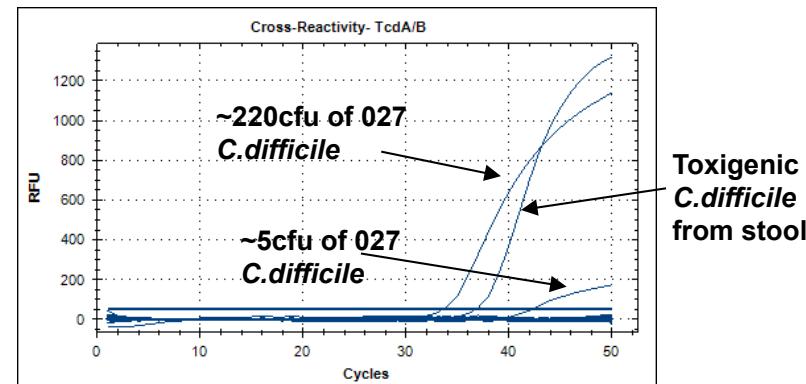
- ~20cfu of *tcdA* or *tcdB* positive *C.difficile* in stool
  - Equivalent ~1 cfu/PCR





# Specific detection of *tcdA* and/or *tcdB*

- Does not cross react with
  - Closely related *Clostridium* sp.
    - *C.perfringens*
    - *C.sordelli*
  - Common enteric pathogens
    - *Camplyobacter jejuni*
    - *Salmonella enterica*
    - *Shigella flexneri*
    - *Escherichia coli*
    - *Listeria monocytogenes*
    - *Bacillus cereus*
  - *Helicobacter pylori*
  - Parasites (data not shown)
    - *Giardia*
    - *Cryptosporidium*
    - *Entameoba*
    - *Dientameoba*
  - *Candida* sp.
  - *Streptococcus* sp.
  - *Staphylococcus* sp.





# EasyScreen kit results

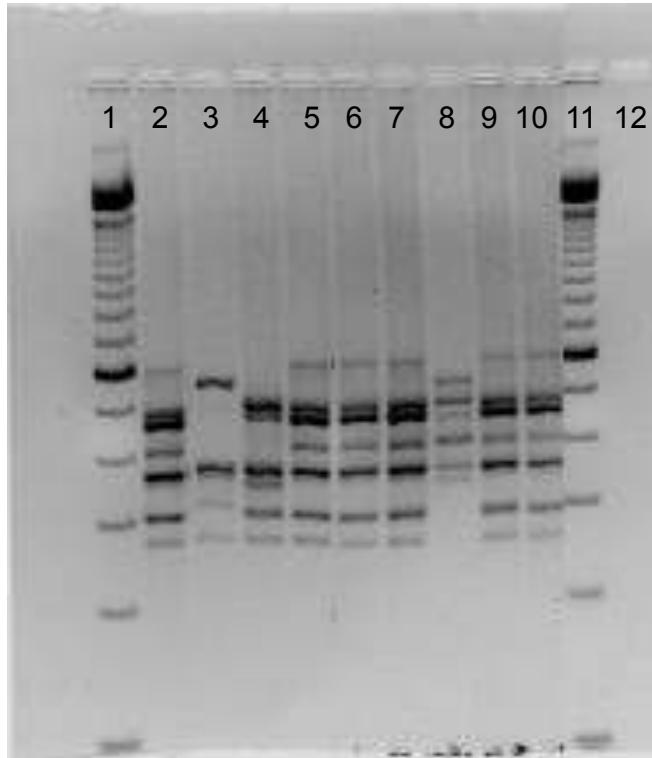
	Set 1 (n=70)		Set 2 (n=73)	
	Positive	%	Positive	%
Culture	67/70		63/72	
EIA <sup>1</sup>	43/70	61.4	31/71	43.7
Illumigene <sup>2</sup>	N/A	N/A	56/73	76.7
QuikChek <sup>3</sup>	N/A	N/A	35/52	67.3
<i>EasyScreen</i> Detection <sup>4</sup>	53/70	75.7	56/73	76.7
<i>EasyScreen</i> Reflex <sup>4</sup>	12/70	17	11/73	15.1

Screening results for the 143 samples comparing

1. Techlab ® Wampole™ *C.difficile* Tox A/B II™ EIA,
2. Meridian Bioscience illumigene™ *C.difficile* DNA amplification assay,
3. TechLab® Quik Chek Complete™ and
4. Human Genetic Signatures EasyScreen™ *C.difficile* methods.



# Ribotyping results



Lane	Sample ID	Binary toxin	$\Delta 117\ tcdC$	<i>gyrA</i>	<i>tcdC</i> repeat deletion	027 Ribotype
1	DNA ladder					
2	Reference 027					
3	n17	+	+	-	38bp	No
4	R9	+	+	-	18bp	No
5	H25	+	+	+	18bp	Yes
6	H7	+	+	+	18bp	Yes
7	R22	+	+	+	18bp	Yes
8	R27	+	+	+	38bp	No, 078
9	H23	+	+	+	18bp	Yes
10	H20	+	+	+	18bp	Yes
11	DNA ladder					
12	Water control					



# Interpretation of results

TcdA/B	$\Delta 117\ tcdC$	Binary toxin	gyrA	IPC	Status
-	-	- / +	- / +	+	Negative
+	-	+	-	+	Toxigenic <i>C.difficile</i>
+	+	-	-	+	Toxigenic <i>C.difficile</i>
+	+	+	-	+	Toxigenic <i>C.difficile</i>
+	+	+	+	+	Hypervirulent <i>C.difficile</i>
-	-	-	-	-	Inhibited



# Summary

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- The HGS EasyScreen *C.difficile* assays sensitively and specifically detects toxigenic and/or hypervirulent *C.difficile*
  - <3 hours
  - Validated on multiple DNA extraction/purification platforms
  - Compatible with multiple real-time PCR machines
  - Quick, 10mins hands-on time if using automated purification platforms.
- Only commercially available test that incorporates gyrA as marker for hypervirulence
  - Reduce false positive identification of potentially hypervirulent strain



# Team and Acknowledgements

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- HGS
  - Dr Douglas Millar\*
  - Dr John Melki\*
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  - Dr Kiran Kaur
  - Dr Nicola Boulter
- Mr Peter Huntington\* (Abstract 624) from PaLMS
- Mr Thomas Karagiannis\* from PaLMS, RNSH
- Prof. Tom Riley\*, UWA
- Prof. William Rawlinson\* from SEALS, PoWH