

A NEW IMMUNOCHROMATOGRAPHIC (IC) RAPID TEST FOR THE DETECTION OF SUSPECTED HYPER-VIRULENT STRAINS (027 OR 017) OF *CLOSTRIDIUM DIFFICILE*

von Eichel-Streiber C.¹, Murillo R.² and Villacampa M.²

tgcBIOMICS
The gene carrier company

¹ tgcBIOMICS GmbH, Carl-Zeiss Str. 51, D-55129 Mainz, Germany

² OPERON S.A. Immunodiagnostics, Cuarte de Huerva, Zaragoza, Spain



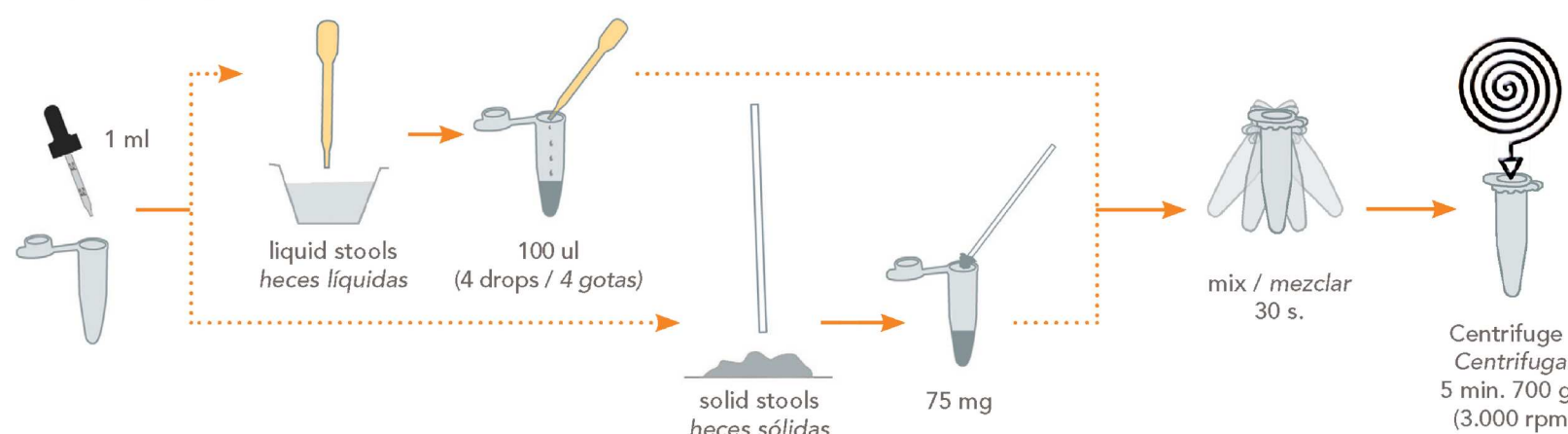
INTRODUCTION

In recent years endemic outbreaks have been reported with ribotype **017** and **027** *Clostridium difficile* strains. CDI due to such strains eventually occurs even at younger age, also in outpatients and is connected with a higher mortality rate. That is why especially ribotype 027 strains have been called hypervirulent. Such strains require extensive hygiene measures so that an early diagnosis is highly beneficial for the patient and also for the clinical setting, restriction of spread will save thousand € per patient.

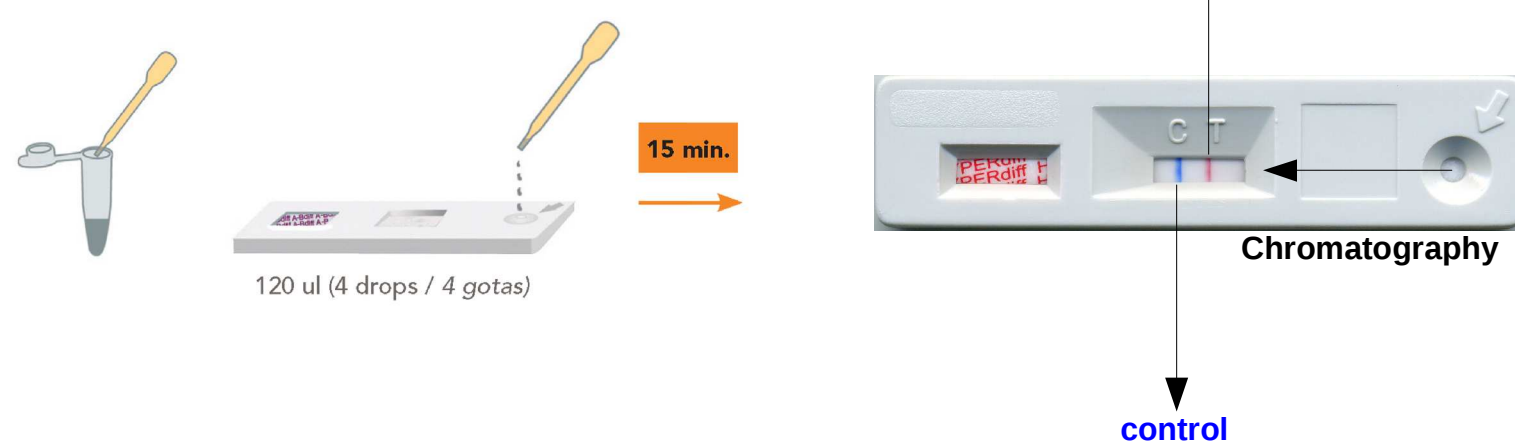
OPERON & tgcBIOMICS here introduce an innovative one step rapid test (designated **HYPERdiff** assay): a negative **HYPERdiff** result indicates the absence of such hypervirulent strains, positive results are highly indicative for CDI due to **027** or **017** strains. Approval is achieved using Operon's **2A-Bdiff** test and finally by PCR-ribotyping. Test results are obtained within ~20 minutes following sample collection, no expensive equipment is needed, and the tests can be done at the point of care. Since a single faecal extraction buffer is used for both **HYPERdiff** and **2A-Bdiff** the tests can be run at any order. The two tests will facilitate and accelerate diagnosis of hypervirulent *Clostridium difficile* Infection.

TEST PROCEDURE

Sample preparation /



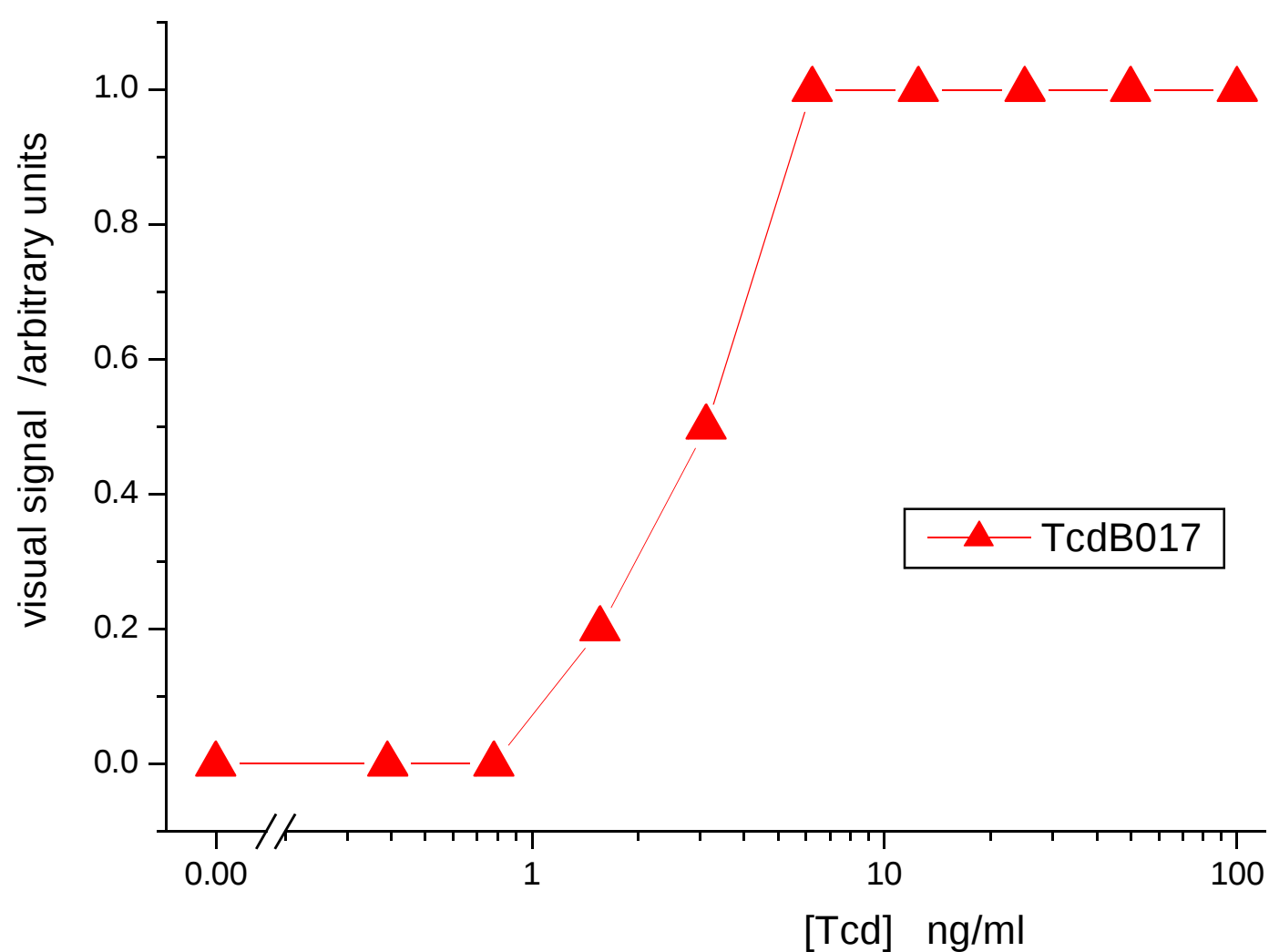
Simple HYPERdiff



ANALYTICAL SENSITIVITY

The test analytical sensitivity was measured using TgcBiomics toxins B (TcdB017 and TcdB027). The detection limits obtained were in both cases 2.5 ng/ml.

At the same time, the TcdB-10463 –the standard strain in many laboratories - is not detected with **HYPERdiff** (even not at 100 ng/ml).



Panel of Common *C. difficile* REA Types (according to Hines VA-Dale Gerding):

SAMPLE	HYPERdiff
Type Y-1	neg
Type BK-6	neg
Type K-12	neg
Type G-1	neg
Type B-1	neg
Type BI-17	pos
Type J-7	neg
Type CF-1	neg
VPI 11186	neg
Media Control	neg

Panel of Common *C. difficile* PFGE Types

PFGE type	Toxinotype	HYPERdiff	PFGE type	Toxinotype	HYPERdiff
NAP 1	III	pos	NAP 5	0	neg
NAP 1	N.A.	pos	NAP 6	0	neg
NAP 1	III	pos	NAP 6	0	neg
NAP 1	III	pos	NAP 7	V	neg
NAP 1	N.A.	pos	NAP 7	V	neg
NAP 2	N.A.	neg	NAP 7	V	neg
NAP 2	0	neg	NAP 7	V	neg
NAP 2	0	neg	NAP 8	V	neg
NAP 2	0	neg	NAP 8	VIII	neg
NAP 3	0	neg	NAP 10	0	neg
NAP 4	0	neg	NAP 11	0	neg
NAP 4	N.A.	neg	NAP 12	0	neg
NAP 5	0	neg	N.D	IX/XXIII	pos

Ribotype strains discovered in the European survey published by Barbut et al. 2007 most abundant types in Europe (322 ribotypes samples):

Ribotype	%	Toxinotype	HYPERdiff	Ribotype	%	Toxinotype	HYPERdiff
001	12,7%	N.A.	neg	023	2,2%	IV	neg
014	9,6%	N.A.	neg	005	2,2%	N.A.	neg
027	6,2%	III	++++	087	1,9%	N.A.	+/-
020	5,9%	N.A.	neg	081	1,9%	N.A.	neg
017	5,9%	VIII	++++	015	1,6%	N.A.	+/-
002	5,9%	N.A.	neg	169	1,2%	N.A.	n.t
012	4,0%	I	neg	156	1,2%	N.A.	neg
168	3,4%	N.A.	n.t	094	1,2%	N.A.	n.t
077	3,4%	N.A.	++	049	1,2%	N.A.	neg
126	2,8%	N.A.	neg	018	1,2%	N.A.	+/-
078	2,8%	V	neg	095	1,0%	N.A.	+/-
048	2,8%	N.A.	neg	050	1,0%	N.A.	neg

CONCLUSIONS

We here introduce our **HYPERdiff** test and show that it is: simple to perform in the laboratory and at the point of care, the fastest assay to diagnose ribotype **027** and **017** *Clostridium difficile* Infection (when combined with Operon's **2A-Bdiff**), it is highly sensitive, and reliable to exclude or diagnose a putative **027** and **017** CDI within ~20 min! The test will simplify and accelerate diagnosis of hypervirulent CDI at the point of care and in laboratories.

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Contact:

Operon S.A.
Camino del Plano 19
50410-Cuarte de Huerva (Spain)

<http://www.operon.es>
info@operon.es