D-197 Development of a New Rapid Latex Agglutination Test for the Detection of all Established and Emerging *Campylobacter* species from Colonies Isolated from Clinical, Veterinary, Food and Environmental Samples

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ABSTRACT

Thermophilic *Campylobacter* species produce the highest numbers of cases of bacteria associated human and animal enteritis annually. At the University of Cape Town an alternative isolation procedure has been used routinely for a number of years which has demonstrated the isolation of a large number of *Campylobacter* species which cannot be isolated using conventional high temperature incubation at 42ºC and antibiotic selection. A number of these new species have been identified as potential emerging pathogens e.g. *C. concisus*. The alternative isolation procedure utilizes a membrane filtration step, modified hydrogen enriched atmosphere, no antibiotics and incubation at 37ºC to isolate these emerging species. To enable large scale surveys to be performed rapidly and to generate a rapid confirmation that an emerging *Campylobacter* species colony has been isolated a new latex reagent has been developed, based on rabbit antisera raised against one of the emerging species. This new latex reagent, when used in combination with the established latex agglutination kit has been shown to detect all of the *Campylobacter* species, both emerging and established species. This new dual latex combination can therefore be used to facilitate more research into the clinical significance and environmental reservoirs of these emerging *Campylobacter* species. Initial studies using an extensive panel of 56 *Campylobacter* species isolates, comprising 21 different species and sub-species, have confirmed the wide specificity of this dual latex test. This dual latex test is currently being used to detect colonies being isolated from human clinical specimens, veterinary sources, foods and environmental samples to determine the prevalence of these emerging species from a range of clinical, veterinary, food and environmental sources.

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PROCEDURE

Quality Control:
The following check with the Positive Control should be performed each time the kit is used to confirm that the reagents are functioning correctly:
1.A single 50µL drop of Positive Control should be dispensed on to three adjacent areas on the test slide.
2.These should be tested with both of the Test and Control Latex Reagents as described in "Test Procedure" below.
3.Deterioration of a reagent should be suspected if:
•There is no reaction between the Test Latex Reagents and the Positive Control OR the reaction shows a significant loss of strength with time.
•The Control Latex Reagent reacts with the Positive Control
•A latex reagent becomes discoloured or forms lumps which do not disperse with gentle shaking.

Test Procedure:
1.Bring all reagents to room temperature. Gently shake the latex reagents to ensure a homogeneous suspension.
2.Dispense 50µL of isotonic saline on to each of three ovals of the agglutination slide.
3.Use an inoculating loop to remove several colonies with Campylobacter-like morphology. If evidence of microbial growth is sparse, take a broad sweep of the agar surface. Mix the bacteria into each of the three drops of isotonic saline on the slide to form even suspensions.
4.Add 1 drop (50µL) of Control Latex reagent to one of the bacterial suspensions on the slide. Similarly dispense 1 drop (50µL) of each of the two Test Latex reagents to the other two bacterial suspension wells.
5.Mix the bacterial suspensions with the latex reagents using a mixing stick, **starting with the Control Latex reagent.** Spread the mixtures to the edges of the oval areas.
6.Gently rock the slide, to keep the fluid suspensions in constant movement, for 2 minutes. Observe for agglutination.
7.Read the test results (see INTERPRETATION below)
8.Discard the used mixing sticks and slides into a suitable disinfectant.

INTERPRETATION
An agglutination reaction is indicated by visible aggregation of the latex particles. The strength of the reaction may vary, and can be assessed according to the following guidelines.
\+ reaction - fine, but readily discernible granularity against a milky background.
++ reaction - coarse granularity against a milky background.
+++ reaction - heavy clumping of particles around the periphery of the test oval, against a clear background.

Campycheck Campylobacter results should be interpreted as follows:

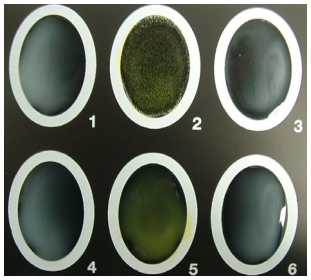
Thermophilic Latex	Species Latex	Control Latex	Interpretation
+	-	-	Thermophilic Campylobacter
+	+	-	Thermophilic Campylobacter
-	+	-	Non-Thermophilic Campylobacter
+ or -	+ or -	-	Auto-agglutination*

***An isolate that causes the Control Latex reagent to agglutinate cannot be tested by Campycheck Campylobacter.**

SUMMARY OF RESULTS ON CAMPYLOBACTER ISOLATES (ARS, USDA, Albany, CA.)

Bacterial Species	Thermophilic Latex		Campylobacter spp. Latex		Control Latex		Detection Rate
	Positive	Negative	Positive	Negative	Positive	Negative	
Campylobacter jejuni	19	0	19	0	0	19	100%
	1	0	0	1	0	1	100%
Campylobacter coli	27	0	27	0	0	27	100%
	22	0	0	22	0	22	100%
Campylobacter upsaliensis	8	0	8	0	0	8	100%
Campylobacter hyointestinalis	0	12	12	0	0	12	100%
Campylobacter lari	0	2	2	0	0	2	100%
Salmonella Thompson	0	1	0	1	0	1	0%
Escherichia coli 0113	0	1	0	1	0	1	0%
Escherichia coli 0128	0	1	0	1	0	1	0%

LATEX AGGLUTINATION



PRODUCT PRESENTATION

THE CAMPYCHECK STRAIN SET

CAMPY CHECK	TAXON	STRAIN NUMBER	NOTES	SOURCE (clinical/geographic)
CC 1 CC 2 CC 3	*C. coli*	RM 2228 Lab 33 300.97	genome sequenced hyoilei genotype	Chicken/ USA Pig/DK Human, diarrhoea/SA
CC 4 *CC 5* *CC 6*	*C. concisus*	CCUG 13144 CCUG 19995 Lastovica 396/96	type strain genospecies 2 large genome size, ca. 2.186 mB	Human, oral/USA Human, diarrhoea/Belgium Human,diarrhoea& vomiting/SA
CC 7 *CC 8* *CC 9*	*C. curvus*	SSI 19296 Lastovica 525.92 Lastovica 13A		Human, diarrhoea/DK Human, oral/SA Human, oral /SA
CC 10 *CC 11* *CC 12*	*C. fetus subsp. fetus*	CCUG 32114 Abdn 1076 241.99		Human abscess, Sweden Sheep abortion, Scotland Human, loose stools/SA
CC 13 *CC 14* *CC 15*	*C. fetus subsp. venerealis*	CCUG 11287 Armi 4402 Abdn SM5		Human/France Bovine/Australia Aborted bovine fetus/N. Ireland, UK
CC 16 *CC 17*	*C. gracilis*	CCUG 27720 CCUG 13143		Human, periodontitis (USA) Human, periodontitis (USA)
CC 18 *CC 19*	*C. helveticus*	CCUG 30566 CCUG 34016		Cat diarrhoea/Switzerland Cat faeces/Sweden
CC 20 *CC 21*	*C. hominis*	NCTC CH001 NCTC CH003	type strain	Human faeces/UK Human faeces/UK
CC 22 *CC 23* *CC 24* *CC 25*	*C. hyointestinalis subsp. hyointestinalis*	LMG 7538 LMG 9260 176.96 234.95		Human diarrhoea, Sweden Human diarrhoea, Belgium Human neonate, diarrhoea/SA Human infant, loose stools/SA
CC 26 *CC 27*	*C. hyointestinalis subsp. lawsonii*	CHY 5 CCUG 27631	type strain	Pig stomach/UK Pig stomach/Sweden
CC 28 *CC 29* *CC 30*	*C. jejuni subsp. doylei*	SSI 5384 CCUG 18266 269.97	serotype 0:17	Human/DK Human gastric biopsy/Germany Human blood, Kwashiorkor/SA
CC 31 *CC 32* *CC 33* *CC 34* *CC 35*	*C. jejuni subsp. jejuni*	NCTC 11168 RM 1221 SVS 4039 RM 1864 (=81-176) 47.97	genome sequenced genome sequenced	Human diarrhoea/UK Chicken/USA Cattle/DK Human diarrhoea/USA Infant, blood/SA
CC 36 *CC 37*	*C. lanienae*	NCTC 13004 DARDNI G718D		Human faeces/UK Pig faeces/N. Ireland, UK
CC 38 *CC 39*	*C. lari*	RM 2100 (NARTC), CCUG 22395 (UPTC)	genome sequenced	Human faeces/USA Human diarrhoea/France
CC 40 *CC 41*	*C. mucosalis*	CCUG 21559 CCUG 23201	serotype A serotype B	Pig necrotic colitis/Scotland, UK Pig intestine/Scotland, UK
CC 42 *CC 43*	*C. rectus*	CCUG 20446 CCUG 11645	type strain	Human, oral/USA Human periodontitis/Sweden
CC 44 *CC 45*	*C. showae*	CCUG 30254 CCUG 11641	type strain	Human, oral/Japan Human periodontitis/Sweden
CC 46 *CC 47* *Cc 48*	*C. sputorum*	Lastovica 86.92 LMG 11764 CCUG 20703	bv. sputorum bv. paraureolyticus bv. fecalis	Infant diarrhoea/SA Human faeces/Canada Ovine faeces/UK
CC 49 *CC 50* *CC 51*	*C. upsaliensis*	RM 3195 CCUG 19559 CCUG 19607	genome sequenced	Human diarrhoea/SA Human diarrhoea/UK Canine faeces/Sweden
CC 52 *CC 53*	*B. ureolyticus*	CCUG 18470 Rigshospitalet 9880		Human urine/Sweden Human faeces/DK
CC 54 *CC 55* *CC 56*	*A. butzleri*	SSI 71032 29.97 167.97		Human faeces/DK Infant, loose stools /SA SA
CC 57 *CC 58*	*A. cryaerophilus*	CCUG 17801 SSI 70952	type strain: subgroup 1	Bovine abortion/N. Ireland, UK Human faeces/DK
CC 59	*A. skirrowii*	BU 30CC 8B1		Poultry/UK

RESULTS ON CAMPYCHECK STRAIN SET (TEAGASC, EIRE)

Campycheck Code	Species	Original Microgen Latex	New Microgen Latex	Comments
Cc1	*C. coli*	+	+	
Cc2	*C. coli*	+	+	
Cc3	*C. coli*	+	+	
Cc4	*C. concisus*	+	+	
Cc5	*C. concisus*	+	+	
Cc6	*C. concisus*	+	+	
Cc7	*C. curvus*	+	+	
Cc8	*C. curvus*	+	+	
Cc9	*C. curvus*	+	+	
Cc10	*C. fetus*	+	+	
Cc11	*C. fetus*	+	+	
Cc12	*C. fetus*	+	+	
Cc13	*C. venerealis*	+	+	
Cc14	*C. venerealis*	+	+	
Cc15	*C. venerealis*	+	+	
Cc16	*C. gracilis*	+	+	
Cc17	*C. gracilis*	+	+	
Cc18	*C. helveticus*	+	+	
Cc19	*C. helveticus*	+	+	
Cc20	*C. hominis*	+	+	
Cc21	*C. hominis*	-	+	Missed by original Latex
Cc22	*C. hyointestinalis*	-	+	Missed by original Latex
Cc23	*C. hyointestinalis*	+	+	
Cc24	*C. hyointestinalis*	+	+	
Cc25	*C. hyointestinalis*	+	+	
Cc26	*C. lawsonii*	+	+	
Cc27	*C. lawsonii*	+	+	
Cc28	*C. doylei*	+	+	
Cc29	*C. doylei*	+	+	
Cc30	*C. doylei*	+	+	
Cc31	*C. jejuni*	+	+	
Cc32	*C. jejuni*	+	+	
Cc33	*C. jejuni*	+	+	
Cc34	*C. jejuni*	+	+	
Cc35	*C. jejuni*	+	+	
Cc36	*C. lanienae*	+	+	
Cc37	*C. lanienae*	+	+	
Cc38	*C. lari*	+	+	
Cc39	*C. lari*	+	+	
Cc40	*C. mucosalis*	+	+	
Cc41	*C. mucosalis*	+	+	
Cc42	*C. rectus*	+	+	
Cc43	*C. rectus*	-	+	Missed by original Latex
Cc44	*C. showae*	-	+	Missed by original Latex
Cc45	*C. showae*	-	+	Missed by original Latex
Cc46	*C. sputorum*	+	+	
Cc47	*C. sputorum*	+	+	
Cc48	*C. sputorum*	-	+	Missed by original Latex
Cc49	*C. upsaliensis*	+	+	
Cc50	*C.upsaliensis*	+	+	
Cc51	*C. upsaliensis*	+	+	
Cc52	*B. ureolyticus*	+	+	
Cc53	*B. ureolyticus*	-	-	Missed by both Latexes
Cc54	*A. butzleri*	+	+	
Cc55	*A. butzleri*	+	+	
Cc56	*A. butzleri*	-	+	
Cc57	*A. cryaerophilus*	-	-	Missed by both Latexes
Cc58	*A. cryaerophilus*	+	+	
Cc59	*A. skirrowii*	+	+	

CONCLUSIONS:

- This latex kit provides the first rapid method to detect the emerging *Campylobacter* species
- The kit will be a critical tool for the monitoring of the emergence of *Campylobacter* species
- The reaction pattern with the dual latex test allows the operator to make a presumptive assessment of the type of *Campylobacter* species which has been isolated i.e. Thermophilic or emerging species
- It is clear from the CAMPYCHECK strain set that specific atmospheric control, limited antibiotic use and incubation temperature (35-37ºC) will be critical for laboratories wishing to routinely isolate the wide range of emerging *Campylobacter* species

SYNOPSIS OF THE CAMPYCHECK PROJECT

CAMPYCHECK is an EU project which aims to assess the prevalence of emerging *Campylobacter* species in environment, food, veterinary & clinical samples.

The project aims to develop culture methods and detection / identification tools to enable laboratories to monitor these emerging *Campylobacter* species.

The project consortium comprises a group of leading European laboratories, the Agricultural Research Service, USA and The University of Cape Town, South Africa.

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