C.DIFFICILE RAPID LATEX TEST KIT

For professional *in Vitro* diagnostic use only

Latex slide agglutination test for the confirmatory identification of *C. difficile* colonies

INTENDED USE
C.difficile Rapid Latex Test Kit is a rapid latex agglutination test intended for confirmatory identification of *C.difficile* cultured on selective solid media from faecal samples from patients with suspected pseudomembranous colitis, antibiotic-associated diarrhoea and post-operative diarrhoea. The kit is intended for professional laboratory use only.

PRINCIPLE OF THE TEST
Latex particles are coated with rabbit IgG antibodies specific for C. difficile cell wall antigens. When the sensitised latex particles are mixed with a suspension of C. difficile colonies, a sensitive and specific immunochemical reaction takes place causing the finely dispersed latex particles to agglutinate rapidly into aggregates that are easily visible to the unaided eye.

REAGENTS AND MATERIALS PROVIDED
REAG TEST CD1: C. difficile Latex Reagent: 2.5 mL - Latex particles coated with rabbit antibodies to C. difficile antigens. Preserved with 0.099% sodium azide. (White cap)
CONTROL +: Positive Control: 0.5mL – Suspension of inactivated *C. difficile* antigens reactive with Test Latex Reagent. Preserved with 0.099% sodium azide. (Red cap)
SAMPLE DILUENT: 0.90% Saline: 5.0mL. Preserved with 0.099% sodium azide. (Black cap)
DISPOSABLE AGGLUTINATION CARDS (SLIDE) : 9 cards, each with 6 black agglutination areas
MIXING STICKS (2x25) : 50 disposable mixing sticks
DISPOSABLE PIPETTE: 1 disposable transfer pipette
INSTRUCTIONS FOR USE

MATERIALS REQUIRED BUT NOT SUPPLIED
Bacteriological loops
C. difficile selective agar plates
Plastic tubes 1 mL
Timer

WARNINGS AND PRECAUTIONS
Safety:
- The reagents supplied in this kit are for in vitro diagnostic use only
- Sodium azide, which is used as a preservative in the kit reagents can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.
- Appropriate precautions should be taken when handling or disposing of potential pathogens. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30minutes. Liquid waste containing acid must be neutralised before treatment.
- The positive control has been inactivated during the manufacturing process. However, it should be handled as through potentially infectious.

Procedural:
- *C.difficile Rapid Latex Test Kit* should be used according to the kit instructions.
- Allow all reagents to reach room temperature before use.
- Do not dilute any of the kit reagents
- Do not intermix reagents from different batches of kits.
- Do not freeze any of the kit reagents
- Do not allow the latex reagent dropper to touch the bacterial samples.
- Ensure the agglutination slide is clean and dry prior to use.
- The kit should not be used if the latex reagent fails to agglutinate with the positive control, or if the latex reagent agglutinates in isotonic saline only. Replace with a new kit.

STORAGE AND SHELF LIFE
C.difficile Rapid Latex Test Kit should be stored at 2-8°C when not in use. The kit should not be used after the expiry date printed on the label.

SPECIMENS
Isolates derived from faecal samples should be cultured on selective medium (example C.difficile selective medium Biolife Ref. 541308) anaerobically at 37°C for 48 hours. Colonies with morphology resembling C. difficile are removed for testing with *C.difficile Rapid Latex Test Kit* (see below). The use of alcohol shock prior to plating is useful to remove other non-spore forming faecal organisms.

PROCEDURE
Quality Control:
The following controls should be performed each time the kit is used to confirm that the reagents are functioning correctly:.
1-Reagent Control: Add one drop of **REAG TEST CD1** to 1 drop of isotonic saline in the same circle on an agglutination slide. Mix with a mixing stick and observe for agglutination. No agglutination should be seen. If this control shows agglutination, at least one of the reagents is contaminated and they should be discarded.
2-Positive Control: Gently mix the Positive Control by inverting several times. Place 1 drop on a circle of an agglutination slide. Add 1 drop of C. difficile Latex Reagent to the same circle and mix. Agglutination should be visible within 2 minutes. If no agglutination is seen the reagents should be discarded.
Test Procedure:
1. Dispense 1 drop (50µL) of **SAMPLE DILUENT** on to 1 circle of a clean, dry agglutination slide.
2. Using an inoculating loop, remove a suspected *C. difficile* colony from the selective agar plate. Only select colonies whose morphology resembles that of *C. difficile*.
3. Emulsify the colony in the drop of **SAMPLE DILUENT** on the test slide to produce a heavy, smooth suspension.
4. Observe the suspension for any agglutination or clumping which would indicate auto-agglutination. If the suspension remains smooth, proceed to Step 5. If auto-agglutination is seen, the organism cannot be tested using *C.difficile Rapid Latex Test Kit*. Alternative test methods should be used.
5. Gently mix the Test Latex Reagent by inverting the vial several times. Add 1 drop of the colony suspension on the slide. Do not allow the dropper to touch the organism suspension.

6. Mix the latex reagent and organism suspension together with a clean mixing stick for 30 seconds. Continue mixing by rocking the slide.
7. Examine for agglutination after 2 minutes from initial mixing of latex and sample.
8. After reading, discard the used mixing sticks and slides into suitable disinfectant.

INTERPRETATION
Agglutination within 2 minutes is a positive result and indicates the presence of *C. difficile*. No agglutination within 2 minutes is a negative result.

LMITATIONS OF USE
1. Results should be interpreted by the clinician in the context of all available clinical and laboratory information. The isolation of *C. difficile* does not constitute a diagnosis of pseudomembranous colitis or antibiotic-associated diarrhoea.
2. Identification of *C. difficile* using *C. difficile Rapid Latex Test Kit* should be performed on cultures grown on selective media as this increases the isolation rate.
3. Culture-derived suspensions which auto-agglutinate cannot be tested by *C. difficile Rapid Latex Test Kit*. Alternative methods should be used.

PERFORMANCE CHARACTERISTICS
C. difficile Rapid Latex Test Kit has been evaluated as a culture confirmation test at both an independent microbiology laboratory and in-house. In total, 137 bacterial isolates were cultured on selective agar plates and colonies tested by *C. difficile Rapid Latex Test Kit* and a well-established commercially available test.

		C. difficile Rapid Latex Test Kit		
		+ve	-ve	Total
Commercial test	+ve	85*	0	85
	-ve	0	52**	52
	Total	85	52	137

Sensitivity: 85/85 = 100% Specificity: 52/52 = 100% Diagnostic Efficiency: 137/137 = 100%

*Of the 85 isolates in this group, 18 were cross-reactants in both tests. However, 16 of these either will not grow on C. difficile selective medium or the colonies do not resemble C. difficile. The remaining two isolates (both C. glycolicum) will grow slightly but do not exhibit colony fluorescence which is a characteristic of the majority of C. difficile strains.
** 2 of these isolates were classified as C. difficile (serogroups A9, A10). One of these (serogroup A10) exhibited slightly irregular colony morphology. The remaining 50 isolates comprised a wide variety of bacterial species including 5 Clostridium spp. Most of these isolates either do not grow on C. difficile selective agar or exhibit atypical colony morphology. Overall, the results obtained with *C. difficile Rapid Latex Test Kit* correlate closely with those obtained using the established commercial product.
Although a number of organisms have the potential to cause false positive reactions in both tests, they either do not grow in C. difficile selective culture media or their colony morphologies are not typical of *C. difficile*.

REPRODUCIBILITY
Intra-batch reproducibility was established by testing one batch of product on three separate occasions using a different operator for each occasion. Sensitivity was tested using serial dilutions of reference and kit control antigens and specificity was confirmed using a QC organism panel. No substantial differences were seen between the results obtained by the three operators. **Inter-batch reproducibility** was examined by testing the sensitivity and specificity of three batches of product using reference and kit control antigens and the QC organism panel. No differences in sensitivity or specificity were seen between the three batches of product.

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (50 tests) **REF 271085**

REAG TEST CD1:	2.5 mL (dropper white cap)
CONTROL +:	0.5 mL (dropper red cap)
SAMPLE DILUENT	5.0 mL (black cap)
DISPOSABLE AGGL. CARDS (SLIDE)	9 cards with 6 wells each
MIXING STICKS:	2x25 disposable mixing sticks
DISPOSABLE PIPETTE:	1 disposable transfer pipette
INSTRUCTIONS FOR USE	1 item

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