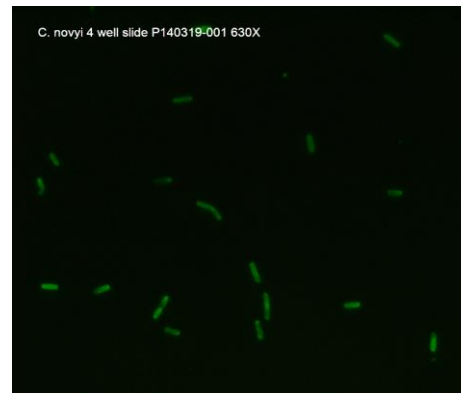
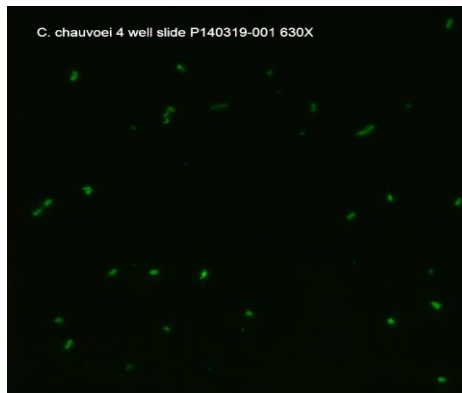


CERTIFICATE OF ANALYSIS

Clostridium spp. 4-way

FA Substrate Slide

Catalog No.:	SLD-IFA-C4
Size:	4 well
Well Capacity:	10 µl
Lot:	P140319-001
Expiration:	07 April 2018

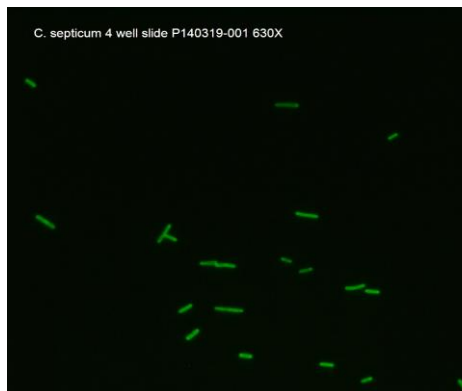


Agents:

Clostridium chauvoei, *C. novyi*, *C. septicum*, *C. sordellii*

Description:

Slides contain *Clostridium chauvoei*, *C. novyi*, *C. septicum* and *C. sordellii* bacteria on the surface of Teflon-masked slides. They are supplied fixed and unstained in moisture-free foil pouches.



Quality Control Method:

Direct FA using conjugates: *C. chauvoei* (catalog no. CJ-F-CCO-1ML or 10ML), *C. novyi* (catalog no. CJ-F-CNO-1ML or

10ML), *C. septicum* (catalog no. CJ-F-CSE-1ML or 10ML), and *C. sordellii* (catalog no. CJ-F-CSO-1ML or 10ML).

Specific Reaction: The *C. chauvoei* well had 3-4+ fluorescence, the *C. novyi* well had 3-4+ fluorescence, the *C. septicum* well had 4+ fluorescence, and the *C. sordellii* well had 3-4+ fluorescence. There are greater than 10 organisms per high-power field on each well.

Other Comments: NA

Pattern Of Fluorescence:

Individual rod-shaped bacteria. Some clumped bacteria. May react with large flagella.

Interpretation of Results:

Staining wells with corresponding Direct FA Conjugate should show positive fluorescence.

Intended Use:

Useful as a control for VMRD's *Clostridium chauvoei*, *C. novyi*, *C. septicum* and *C. sordellii* Direct FA Conjugates. Not intended as a control for *clostridial* conjugates of other manufacturers.

Storage:

Store sealed in foil pouch at -20°C. Avoid self-defrosting freezers.

Recommended Staining Procedure for Indirect FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. FASDB-100ML) however if high background due to anti-bovine IgG activity is present it may be advisable to use SSDB-100ML.
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry around wells by pressing blotter (included in pouch) to front surface. Place labeled anti-IgG or IgM on the wells.
6. Incubate as in step 3.
7. Rinse as in step 4.
8. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
9. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be made at 400X.

Recommended Staining Procedure for Direct FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place direct FA conjugate on the designated wells.
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
6. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

Serum Diluting Buffer (pH 7.2):*

- Na₂HPO₄.....1.19 gm
- NaH₂PO₄.....0.22 gm
- NaCl.....8.55 gm
- BSA.....10.0 gm
- DI/dH₂O.....Q.S. to 1 liter

*This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 2-7°C. Add 0.09% NaN₃ if diluted serum is not going to be used within one week.

4X FA Rinse Buffer (pH 9.0):

- Na₂CO₃.....11.4 gm
- NaHCO₃.....33.6 gm
- NaCl.....8.5 gm
- DI/dH₂O.....Q.S. to 1 liter

Final pH should be 9.0-9.5. This is a 4X concentrate and should be diluted 1/4 with DI/distilled water for use as a working buffer. Keep in a tightly stoppered container at room temperature. MOUNTING FLUID is made by mixing glycerol and FA rinse buffer, pH 9.0, in equal proportions.

FOR *IN VITRO* LABORATORY USE ONLY.