TITLE: Anaerobic Identification	Revision Date: 20-April- 2018	Issue Date: 20-April-2016
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PURPOSE:

Anaerobic bacteria are found as normal components of most body surfaces and mucous membranes. They exist in large numbers throughout the entire GI tract from the mouth to the colon with the exception of the stomach and esophagus, as well as the female genito-urinary tract. They can cause a variety of infections varying from wound infections, abscesses, appendicitis, peritonitis, chronic otitis media and sinusitis, bacteremia, endocarditis and gas gangrene. Sterile body fluids and deep wounds or abscesses will be cultured for anaerobic micro-organisms.

Special potency antimicrobial disks can be used for the presumptive identification of certain anaerobic bacteria. All identifications are considered PRESUMPTIVE and should be confirmed at Dynalife.

SAMPLE INFORMATION:

Source	Proven anaerobic culture ~18-24 hrs old
Stability	Allow to come to room temperature prior to opening
Storage Requirements	Store at -20°C

REAGENTS and/or MEDIA:

- Oxoid An-ident Discs (Cat#DD0006A): Erythromycin(60ug), Rifampicin(15ug), Colistin(10ug), Penicillin(2 units), Kanamycin(1000ug), and Vancomycin (5ug)
    - Store at -20C, allow to come to room temperature before opening (~1hr)
- Blood Agar Plate(BAP)

SUPPLIES:

- Forceps
- Anaerobic jar
- Anaerogen Pack
- Anaerobic indicator
- Ruler

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing An-Ident Testing	
1	Make a suspension using several colonies of a pure culture that has been proven to be anaerobic via aerotolerance testing in approximately 1 mL of Thioglycolate broth.
2	Using a sterile swab inoculate a BAP and streak in three directions
3	Apply the 6 discs to the plate using sterile forceps
4	Incubate the plate for 24-48hrs anaerobically at 35°C

5	Read and record zone sizes
6	In the Plate log - order media **"ANID"** Using the keypad provided, choose the appropriate combination of results
7	See following table for interpretation

INTERPRETATION:

IF	THEN
Zone size is less than 10mm	RESISTANT
Zone size is equal to or greater than 10mm	SENSITIVE

Interpretation Table:

Bacteria	Erythromycin 60 µg	Rifampicin 15 µg	Colistin 10 µg	Penicillin 2 units	Kanamycin 1000 µg	Vancomycin 5 µg
Bacteroides fragilis	**S^r**	**S**	**R^s**	**R**	**R**	**R**
Prevotella melaninogenica	**S**	**S**	**V**	**S**	**R^s**	**R**
Prevotella oralis	**S**	**S**	**S**	**S**	**R**	**R**
Bacteroides urealyticus	**S**	**S**	**S**	**S**	**S**	**R**
Fusobacterium species	**R^s**	**R^s**	**S**	**S**	**S**	**R**
Gram-positive cocci	**S**	**S**	**R**	**S**	**S**	**S**
Gram-negative cocci	**S**	**S**	**S**	**S**	**S**	**R**

S= sensitive, S^r = occasionally strains resistant, V=variable, R=resistant, R^s=occasionally strains sensitive

All identifications are considered PRESUMPTIVE and are to be sent to Dynalife for confirmation

REFERENCES:

- Garcia, L. S. (2007). Identification by Using Special-Potency Disks. In *Clinical Microbiology Procedures Handbook, volume 3* (p. 4.6.5).
- Oxoid. (n.d.). An-Ident Discs.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Dec13	Initial Release	A. Darrach
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell