PURPOSE: To determine the presence or absence of *Actinomyces* spp. in intra-uterine devices (IUD).

SAMPLE INFORMATION:

Type	IUD in a dry, sterile container
Storage Requirements	1. Refrigerated
Criteria for rejection	2. Unlabeled/mislabeled specimen. 3. Specimen container label does not match patient identification on requisition.

REAGENTS and/or MEDIA:

- Brucella agar (BRU) and Thioglycollate broth (THIO)

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Biosafety cabinet
- 35° ambient air and 37° CO_2 incubators
- Wooden sticks
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential 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 when there is a known or potential risk of exposure of 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.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

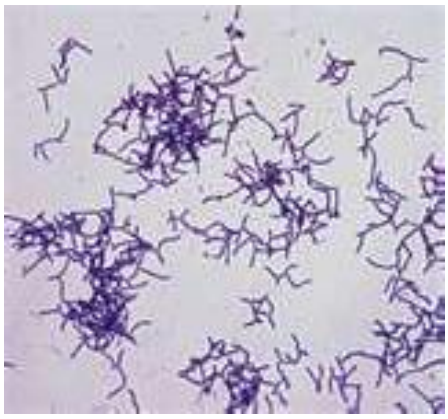
QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures.

PROCEDURE INSTRUCTIONS:

Step	Action
Processing intra-uterine device specimens for culture	
1	In the biosafety cabinet, add Thioglycollate broth to the specimen container containing the IUD and vortex for 30 seconds.
2	Using a sterile pipette, transfer the THIO broth to sterile centrifuge tube (located in TB lab) and centrifuge at 3500 rpm for 10 minutes.
3	After centrifugation is complete, remove the supernatant and use the sediment to: • Inoculate BRU • Add 2 to 5 drops to new THIO broth tube Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
4	Label THIO with Day 2 date, Day 5 date and Day 10 date. Place in THIO rack in O_2 incubator in "Day 2" row.
5	Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation.
6	Examine BRU plate after 48 hours incubation and record your observations in the LIS. Re-incubate for an additional 3 days and examine as above and record your observations in the LIS. If no growth suggestive of *Actinomyces* spp. is observed at 5 days on anaerobic media, re-incubate for an additional 5 days. After 5 days, examine plate record observations in the LIS.
7	Examine THIO at 48 hours, 5 days and 10 days for signs of growth. Record all observations in the LIS. If present, perform gram stain and culture for send out, if organisms resembling *Actinomyces* spp. are seen.

INTERPRETATION OF RESULTS:

Step	Action
1	Examine BRU for growth of colonies typical of *Actinomyces*: white, "molar tooth", pitting the agar. If colonies present, perform gram stain. If gram stain is branching, Gram- positive bacilli suggestive for Actinomyces, report as below. 
2	Although some species of *Actinomyces* can grow well aerobically, anaerobic culture is recommended for optimal recovery.

REPORTING RESULTS:

IF	REPORT
No growth of colonies suggestive of *Actinomyces*	• Report: **"No Actinomyces isolated"**
Growth and gram-stain of colonies suggestive of *Actinomyces*	• Add organism**: "Gram-positive bacilli suggestive of Actinomyces"** • List quantitation as **"Present"** • Add isolate comment **&REF2** to state: **"This organism has been referred for further identification"** • Refer organism to DynaLIFE for identification and as per MIC10510 – Referral of Category B Specimens to DynaLIFE. • Use anaerobic transport media.

	• Freeze isolate and log into stored isolates binder.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven