# Mycobacteria sputum culture with Ogawa-Kudoh

### *Purpose*

Different methods for culturing mycobacteria are used worldwide. Some were developed aiming at simplicity, low cost and feasibility in settings with little infrastructure. A simple and inexpensive method for the culture of sputum samples was reported by Kudoh in 1974.

Laboratories sometimes do not have all of the biosafety equipment recommended for performing the Petroff method, particularly centrifuges with protection against the production of aerosols containing bacilli. In addition to being inexpensive, fast, and easy to perform, the Kudoh modified Ogawa method is advantageous for microbiologists because of the low risk of contamination once the sample is inoculated using swabs, and no centrifugation process is necessary. It can even be performed in the field. Another advantage of the Ogawa-Kudoh method is a reduction of the time of onset of visible colonies in culture.

### *Principle*

Briefly, each of two sterile swabs impregnated with a good amount of the sputum sample is submerged into a tube containing 2 ml of 4% NaOH during two minutes for decontamination and immediately inoculated onto a slant of Kudoh modified Ogawa medium (egg-based medium with a slightly acidic pH to neutralize the NaOH).

# 1. Equipment, materials and reagents

## 1. Equipments

- Class II Biosafety Cabinet (BSC), with exhaust air ducted or vented to the outside
- Autoclave, Autoclave tape, Spore test for autoclave
- Incubator
- Refrigerator

## 2. Materials

- N95, FFP2, or equivalent respirators
- Lab gowns
- Disposable gloves
- Biohazard bags
- Safety Bunsen burner, with device to light on demand, or micro-incinerator
- Rack for tubes
- Pipettes for 1.0 ml, preferably sterile, single-use, plastic, Pasteur pipettes (with graduation)
- Pipetting aids
- Mortar or blender (for biopsy)
- Sterile forceps (for swabs)

- Tuberculocidal disinfectants
- Separate autoclavable waste containers for pipettes and disposables
- Waste receptacles
- Vortex mixer
- Timer

## 3. Reagents and solutions

Avoid use of previously opened reagent bottles.

Use aliquot stock solutions, one vial per specimen

- Sodium hydroxide, 4%
- Ogawa modified medium pH 6.4 (for Kudoh method)

Article-code : O041.34.0007 (7 mL) OGAWA MOD, Packing Unit (PU) : 40 pcs

TRITIUM MICROBIOLOGY http://www.tritium-microbiologie.nl/en/products

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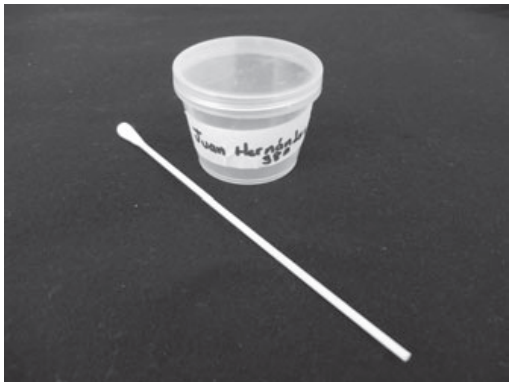
## 4. Biosafety and infection control

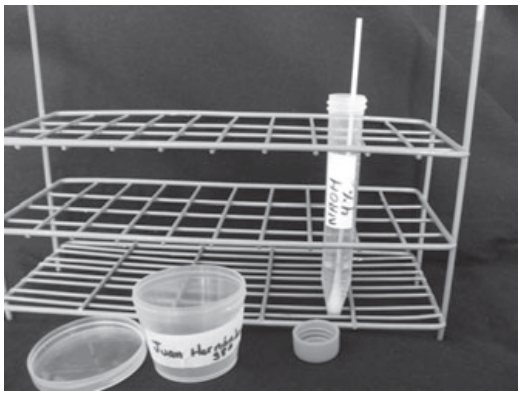
See http://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf, p.8-10

# 2. Procedure

1) Sputum is picked up onto a sterile cotton swab.



2) The swab is immersed in a sterile 4% sodium hydroxide solution in a test tube.



3) After 2 min the swab is removed from the test tube and the specimen is directly inoculated into two tubes containing modified Ogawa culture medium (pH 6.4) by smearing and squeezing the swab over the surface of the media

The Ogawa-Kudoh method (or the substitution of Ogawa for the acidified IUATLD medium) is an easy to perform tool for diagnosing pulmonary TB. It requires less biosafety equipment than the Petroff method, has a low cost, and has good sensitivity for detecting Mycobacteria, although it has been reported to be 10% less sensitive than the Petroff method.

# 3. References

- KUDOH, S.; KUDOH, T. A simple technique for culturing tubercle bacilli. Bull Word Health Org 1974; 51: 71-82.

- Mycobacteriology Laboratory Manual, First Edition, April 2014

A publication of the Global Laboratory Initiative a Working Group of the Stop TB Partnership http://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf

- Evaluation of the Kudoh method for mycobacterial culture: Gambia experience. Jobarteh T, Otu J, Gitteh E, Mendy F, Faal-Jawara TI, Ofori-Anyinam B, Ayorinde A, Secka O, Antonio M, Gehre F. Int J Mycobacteriol. 2016 Dec;5 Suppl 1:S166. doi: 10.1016/j.ijmyco.2016.09.049. Epub 2016 Nov 11.