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10 Aug92, 26 July '94, 23 July '95, 21 July '96, 22 July '97, 1 July 98, 15 July 01, 7 Aug 02, 11 Aug 03, 23July09, 22July10
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Coliform_assays/Single_Colony_Isolation.htm

Robert Koch, in spelling out the criteria for demonstrating the etiologic agent for a disease, emphasized as two of his four postulates, that one must isolate the putative agent in pure culture, and then use the pure culture to experimentally cause the disease in a healthy animal.

He developed a technique to produce a pure culture based on his observation of colonies growing on the spoiling half of a baked potato. Called "single colony isolation" the procedure is to touch a source colony with a sterile loop to pick up a small sample and to "streak it out" on a sterile plate (the primary streak). The loop is sterilized, cooled, and streaked across the primary streak. This sterilization, cooling and streaking is repeated a third time, causing the initial streak to be spread out across the plate in successive cycles of sterilization and cross streaking. The goal is to sufficiently spread out the inoculum so that individual colonies are formed. Rigorous application repeats this process on a second plate.

First: Draw a careful **full-sized plate illustration** in your lab book in which you will practice the fluid movements of streaking (6, 7 & 8). Divide the plate into quadrants, and trace out with a pen the streaks with the proper "elbow" motion (four times, rotating the plate 90° for each streak). Carefully label the streaks on one quadrant to show the primary, secondary and tertiary streaks as shown by the instructor.

Second: Fill in the provided table listing the following for the isolates you streak out:

- a) isolate number, *lac* + or *lac* -, and origin, b) detailed colony description on source plate,
- c) colony description after streak is grown, d) TSI reactions, e) possible identity of the isolate.

1. **PREPARE SOURCE PLATE:** You should have already spread a suitably diluted mixed culture on a plate to give 50 to 500 colonies per plate, and incubated until well-formed colonies appear (24-48 hours). [For example, from coliform plate count on EMB *lac* plates] This is your **source plate**.
2. **DESCRIBE SOURCE COLONIES:** Examine the source plate. How many classes can you identify? For the isolate table: Pick, circle with a wax pencil, and number four highly distinct colonies 1 to 4: colonies which have significantly different traits: size, morphology, color, wrinkled, mucoid, spreading, clear, etc. Three should be unambiguously *lac*-. Record the detailed traits in table provided: next protocol: *Isolation and Traits of Ambient Water Bacteria*.
3. **PREPARE THE STREAK PLATE:** Divide a fresh EMB plate into quadrants with a wax pencil. In small print, enter your initials, seat number and the date at one edge and label the four areas with the numbers of the selected colonies.
4. **ADJUST, STERILIZE LOOP:** Flame a 26 gauge platinum wire bacteriological loop so that the entire wire glows. When cool, adjust the wire to the proper shape: 4 cm straight shank, the last 6 mm bent at a slight angle, tipped with a 1-2 mm loop, at a right angle to axis of handle.
5. **PICK THE COLONY:** Reflame the loop. Open the source plate, touch the hot loop to a sterile part of the agar to insure that it is cool. Then lightly touch the *edge* of the first selected colony so that you pick up a *tiny* sample. You should see the imprint on the colony edge. (Too much, it will not streak out properly, too little, the transfer may not successful.) Close the source plate. For liquid cultures, pick a loopful of the liquid (check the loop to see) and use that for the primary streak, as below.
6. **APPLY PRIMARY STREAK:** On the new plate, with reflected light showing your tracks, apply the primary streak by *lightly* zig-zagging the inoculating loop across the agar at one side of the quadrant with a small wiggling/dragging motion. Do not dig into the agar, nor bend the loop.
7. **PERFORM SECONDARY STREAK:** Reflame and cool the loop as before. With a light sweeping motion (wrist stiff, pivot from elbow, little finger resting on table surface), perform the secondary streak: start at the terminal end of the primary streak, and sweep across the top of the quadrant, cross streaking several times back and forth through the primary streak.
8. **MAKE TERTIARY STREAK:** Reflame and cool the loop as before. Rotate the plate 90°, and cross streak through the secondary streak, starting at the far end, working towards the primary streak, and filling the rest of the quadrant. *Do not again go through the primary streak*.
9. Repeat the streaking procedure (steps 5 through 8) for the other colonies to be purified.
10. **Incubate 24 hours at 37° C** (until colonies are well formed). (48 hours may "overgrow" the streaks.)
11. **Score your streaking abilities:** do you see numerous individual colonies well separated? Great! The primary streak is probably confluent? Score the morphologies of the isolated strains. Are they identical with the original colonies from step 2? Record your results in your two page table.

Cut here..... **Single Colony Isolation Gradesheet** page 53b

You are to streak two *lac*+ and two *lac*- colonies from EMB Levine plates. This is the grade sheet which will be used to evaluate your streaking technique.

(Worth a total of 15 points)

	excel	avg	poor
	+2	+1	0
layout of streaks (3 pts)			
single colonies, #1			
single colonies, #2			
single colonies, #3			
single colonies, #4			
labeling technique			
incubator technique			

GRADING OF SINGLE COLONY ISOLATION STREAKING ABILITY

18 August 1994, 22 July 2010

This grade sheet will be used to evaluate your lab practical assignment of single colony isolation.

A mixed culture of *E coli* and *S typhimurium* has been prepared, possibly from TSI slants with these bacteria on their surfaces (or colonies of each). Student divide and mark their plate appropriately with Ec and St across from each other, the mixed culture streaked in two quadrants across from each other as well. Plates are labeled with the usual information, and the data is entered into their notebooks.

Grade the plates as follows:

Proper technique: initials _____ date _____
streaks: not touching or cross contaminated
single colonies in each streak get 2 points, including both St and Ec in the mixed culture streaks.

Total score: 15 points (3 for technique, 2 for Ec, 2 for St, 2+2 for each of the two mixed cultures.

streak	culture	quantity/quality of single colonies:		
		both strains (2 pts)	one strain or few (1 pt)	none (0 pts)
1	<i>Escherichia coli</i>			
2	mixed culture			
3	<i>Salmonella typhimurium</i>			
4	mixed culture			

FOR CONDUCT OF SINGLE COLONY TECHNIQUE TEST:

Set up four stations at the sides of the room:

Fresh cultures of:

E. Coli B

Salmonella

Prepare mixed culture, equal parts each, four aliquots, one for each station.

Four previously inoculated EMB plates (one per station), each with two species of bacteria:

E coli streaked on one half,
Salmonella on the other half

one EMB Levine plate for each student, room temp.

