STANDARD PLATE COUNT, COLIFORM, AND SIMPLIFIED COUNT METHODS

[Unless otherwise stated all tolerances are ±5%]

SAMPLES

1.	Lab	orat	ory	sample requirements (see CP item 33 & 34)	
				STANDARD PLATE AND COLIFORM METHODS	
				DILUTING SAMPLES	
2.	Wor	k Ar	ea		
	a.	Lev	el p	plating bench not in direct sunlight	
	b.	San	itiz	ed immediately before start of plating	
3.	Sel	ecti	ng D	Dilutions	
	a.	Sta	ndar	rd Plate Count	
		1.	Pla	te two decimal dilutions per sample	
		2.		ect dilutions to yield one plate with 250 colonies	
			a.	Raw milk is normally diluted to 1:100 and 1:1000	
			b.	Finished products are normally diluted to 1:10 and 1:100	
			С.	The above are general guidelines and may have to be adjusted on a case by case basis (dilutions below 1:10 not required)	
	b.	Col	ifor	rm Counts	
		1.		milk samples, 1 mL direct and/or decimal utions	
		2.	1:1	all other products, distribute 10 mL of a 0 dilution among three plates, generally high and viscous products	
4.	Ide:	ntif	ying	Plates	
	a.		el e utio	each plate with sample identification and	
	b.	Arr	ange	e plates in order before preparation of dilutions	
5.	Sam	ple	Agit	ation	
	a.			propriate, wipe top of unopened containers cerile, ethyl alcohol-saturated cloth	
(PL	ATE-	1-Re	v. 6	5/05)	

	ν.	of each container	
		 Shake raw and processed sample containers (approx ¾ full) 25 times in 7 sec with 1 ft movement 	
		2. Invert filled retail container 25 times, each inversion a complete down and up motion	
	С.	Remove test portion within 3 min of sample agitation	
5.	Sam	ple Measurement, pipets	
	a.	Use separate sterile pipets for the initial transfers from each container	
		1. Pipets in pipet container adjusted without touching the pipets	
	b.	Pipet tip not dragged over exposed exterior of pipets in container	
	С.	Pipet not dragged across lip or neck of sample container _	
	d.	Pipet not inserted more than 2.5 cm (1") below sample surface (foam avoided if possible)	
	е.	Draw test portion above pipet graduation mark and remove pipet from liquid	
		1. Pipet aid used, mouth pipetting not permitted ()	
	f.	Adjust test volume to mark with lower side of pipet in contact with inside of sample container (above the sample surface)	
	g.	Drainage complete, excess liquid not adhering to pipet	
	h.	Release test portion to petri dish (tip in contact with plate, 45° angle) or dilution blank (with lower side of pipet in contact with neck of dilution blank, or dry area above buffer where appropriate) with column drain of 2-4 sec	
	i.	Blow out last drop of undiluted sample from pipet using pipet aid	
		 Blow out away from main part of sample in plate, do not make bubbles 	
	j.	Pipets discarded into disinfectant, or if disposable into biohazard bags or containers to be sterilized	

⁷ .	Samp	ole Measurement, pipettors ()	
	a.	Each day before use, vigorously depress plunger 10x to redistribute lubrication and assure smooth operation (mechanical pipettors)	
	b.	Before each use examine pipettor to assure that no liquid is expelled from the pipettor nose-cone (contaminated), if fouling is detected do not use until cleaned as per manufacturer recommendation	
	С.	Use separate sterile tip for the initial transfers from each container	
	d.	Depress plunger to first stop (mechanical pipettors)	
	e.	Tip/barrel not dragged across lip or neck of sample container, and pipettor barrel not allowed within sample container	
	f.	Tip not inserted more than 1 cm below sample surface (foam avoided if possible)	
	g.	With pipettor vertical slowly and completely release plunger (for electronic pipettors follow manufacturer instructions)	
	h.	Remove tip from sample and depress plunger completely, re-insert tip into sample and repeat steps f and g, and then remove tip from liquid	
	i.	Touch tip off to inside of sample container above the sample surface, excess liquid not adhering to tip (do not lay pipettor down once sample is drawn up, use vertical rack if necessary)	
	j.	Release test portion to petri dish (tip in contact with plate) by slowly depressing plunger to first stop allowing about 1 or 2 seconds for complete drainage	
	k.	Move tip to a dry spot on plate	
		1. If pipettor only has one (1) stop touch off	
		2. If pipettor has two (2) stops, depress plunger to second stop and touch off	
	1.	Or, dispense test portion to dilution blank (tip in contact neck of dilution blank, or dry area above buffer where appropriate) by slowly depressing plunger to first stop	
	m.	If pipettor has two (2) stops, depress plunger to second stop	
	n.	Tips discarded into disinfectant, biohazard bags or containers to be sterilized	

8.	Dil	ution Agitation	
	a.	Before removal of any portion, shake each dilution bottle 25 times in 7 sec with a 1 ft movement	
	b.	Optionally, use approved mechanical shaker for 15 sec	
	c.	Remove test portion within 3 min of dilution agitation	
9.	Dil	ution Measurement, pipets	
	a.	Use separate sterile pipets for the initial transfers from each container	
		1. Pipets in pipet container adjusted without touching the pipets	
	b.	Pipet tip not dragged over exposed exterior of pipets in container	
	C.	Pipet not dragged across lip or neck of dilution blank	
	d.	Pipet not inserted more than 2.5 cm (1") below dilution surface	
	е.	Draw dilution portion above pipet graduation mark and remove pipet from liquid	
		1. Pipet aid used, mouth pipetting not permitted ()	
	f.	Adjust dilution volume to mark with lower side of pipet in contact with inside of dilution blank neck	
	g.	Drainage complete, excess liquid not adhering to pipet	
	h.	Gently lift cover of petri dish just high enough to insert pipet	
	i.	Hold pipet at 45° angle to dish with tip touching dish (or dilution blank neck)	
	j.	Release dilution portion to dish (or dilution blank) with tip in contact with the bottom of the dish (or dilution blank neck, or dry area above buffer where appropriate) with column drain of 2-4 sec	
	k.	Touch pipet tip once against dry spot on dish bottom (or dilution blank neck)	
	1.	When measuring 0.1 mL, do not re-touch dry area	
	m.	Pipets discarded into disinfectant, or if disposable into biohazard bags or containers, to be sterilized	

10.	Dil	ution Measurement, pipettors ()	
	a.	Use separate sterile tip for the initial transfers from each container	
	b.	Depress plunger to first stop (mechanical pipettors)	
	С.	Tip/barrel not dragged across lip or neck of dilution blank, and pipettor barrel not allowed within dilution blank	
	d.	Tip not inserted more than 1 cm below dilution surface	
	е.	With pipettor vertical slowly and completely release plunger (for electronic pipettors follow manufacturer instructions)	
	f.	Remove tip from dilution and depress plunger completely, re-insert tip into dilution and repeat steps d and e, and then remove tip from liquid	
	g.	Touch tip off to inside of dilution blank neck or dry area above buffer where appropriate, excess liquid not adhering to tip	
	h.	Gently lift cover of petri dish just high enough to insert tip	
	i.	Hold pipettor nearly vertical to dish with tip touching dish	
	j.	Release test portion to petri dish (tip in contact with plate) by slowly depressing plunger to first stop	
	k.	Move tip to a dry spot on plate	
		1. If pipettor only has one (1) stop touch off	
		2. If pipettor has two (2) stops, depress plunger to second stop and touch off	
	1.	Tips discarded into disinfectant, biohazard bags/containers or into spent dilution blanks to be sterilized	
11.	Samp	ples Other than Milk	
	a.	Weigh 11g aseptically into dilution blank	
	b.	Use dilution blanks heated to 40-45C	
12.	Dry	Milk Samples	
	a.	Weigh 11g aseptically into dilution blank heated to $40-45\mathrm{C}$	
		1. Use standard dilution blank	

		2. Or, 2.0% sodium citrate blank (pH<8.0) for relatively insoluble sample (not to be used with Petrifilm)	
	b.	Wet sample completely with gentle agitation (invert)	
	С.	Let soak 2 min, then shake 25 times in 7 sec with 1 ft movement, use within 3 minutes of agitation	
		PLATING	
13.	Pla	ting	
	a.	Melt agar quickly in boiling water, flowing steam not under pressure, or microwave oven (use extreme caution when microwaving)	
	b.	Avoid prolonged exposure to high temperatures during and after melting, establish lab protocol	
	c.	Do not melt more than will be used within 3 hours	
	d.	Do not melt agar more than once	
	e.	Promptly cool melted agar to 45±1C	
		1. Record temperature with other control information	
	f.	Temperature control used for each test medium type	
		1. Contains medium identical to type being used	
		2. In container identical to that being used	
		3. Undergoes <u>same</u> heat treatment and cooling as test medium	
	g.	Select number of samples in any series so that all will be plated within 20 min (pref ≤ 10) after diluting first sample	
	h.	After depositing test portions, promptly pour 10-12 mL medium into each plate of series, or 15-20 mL for > 1 mL portion/plate or where agar weight loss is a problem that can not be corrected by other actions (documentation must be kept to indicate that this is a routine practice)	
	i.	Lift cover of petri dish just high enough to pour medium	
	j.	As each plate is poured thoroughly and evenly mix medium and test portion in petri dish	
		 Multiple plates may be poured and mixed, however, plates may not be stacked prior to mixing 	
	k.	Allow to solidify within 10 min on level surface	
	1.	For dry milk sample, overlay plate with 3-5 mL PCA	
(PL	ATE-	6-Rev. 6/05)	

	m.	For colliorm count, overlay plate with 3-4 mL VRB _	
	n.	Invert and incubate within 10 min of medium solidification	
		CONTROLS	
14.	Con	trols _	
	a.	Check sterility of dilution blanks, medium, petri dishes, and pipets used for each group of samples (AM and PM)	
	b.	Expose a poured plate with cover completely removed or pre-hydrated Petrifilm Aerobic Count (PAC) film (both wet surfaces completely exposed) to air for 15 min during plating, AM and PM	
		1. The air control plate must be the first plate poured immediately before samples are shaken and must be located such that it is in the area of the plating activity (not off to the side)	
		2. If count > 15, take and note corrective actions	
		3. For PAC films see item 45b7	
	c.	Records maintained	
	d.	<pre>Include information on bench, work sheet or report sheet(s)</pre>	
		INCUBATION	
15.	5. Incubation		
	a.	Incubate SPC plates at 32±1C for 48±3 hours (dry milk for 72±3 hours) and incubate coliform plates at 32±1C for 24±2 hours	
	b.	Stack plates no more than 6 high	
	С.	Arrange stacks so each is at least 1" from adjacent stacks and from incubator surfaces	
	d.	Place stacks directly over each other on successive shelves	
		COUNTING COLONIES	
16.	Cou	nting Aids	
	a.	Count colonies with aid of magnification under uniform and properly controlled artificial illumination with a hand tally	

17.	Recording Standard Plate Count				
	a.	After incubating plates, promptly count all colonies on selected plates			
	b.	Where impossible to count at once, store plates at 0-4.4C for not longer than 24 hr (avoid as a routine practice)			
	С.	Record dilutions used and number of colonies on each plate counted			
	d.	Record results of sterility and control tests			
	e.	When possible, select spreader free plates with 25-250 colonies and count all colonies including those of pinpoint size			
		1. Use higher magnification if necessary to distinguish colonies from foreign matter			
		2. Examine edge of petri plates for colonies			
	f.	If consecutive plates yield 25-250 colonies, count all colonies on plate(s) from both dilutions			
g. Spreaders		Spreaders			
		 Count colonies on representative portion only when colonies are well distributed and area covered or repressed does not exceed 25% of plate 			
		2. Do not count if repressed growth area > 25% of plate area			
		3. When spreaders must be counted, count each as a single colony			
		4. Count chains/spreaders from separate sources as separate colonies			
		5. If 5% of plates are more than ¼ covered by spreaders, take immediate steps to eliminate and resolve problem			
	h.	If there is no 25-250 colony plate, use plate having nearest to 250 colonies			
	i.	If plates from all dilutions exceed 250 colonies, estimate counts as follows			
		1. Count colonies in portions representative of distribution and estimate total			

		۷.	colonies in 12 squares, selecting 6 consecutive squares horizontally across the plate and six consecutive squares at right angles	
		3.	When there are 10 or more colonies/sq cm, count 4 representative squares	
		4.	Multiply average number colonies/sq cm by area of plate in sq cm	
	j.		plates yield < 25 colonies each, record actual ber in lowest dilution	
	k.		all plates from a sample show no colonies, record ant as 0	
18.	Col	ifor	rm Count	
	a.	Aft	er incubating plates, promptly count colonies	
	b.	4.4	ere impossible to count at once, store plates at 0- CC for not longer than 24 hr (avoid as a routine actice)	
	С.	on	ck red colonies measuring 0.5 mm or more in diameter agar plates are considered coliforms in plates ataining ≤154 colonies	
	d.		crowded plates, coliform colonies may be atypical; int and confirm presence of lactose fermentors	
	e.	Con	firmation of colonies	
		1.	Pick 10% up to 10 representative colonies per plate with relative percentages of each colony type and inoculate into brilliant green lactose bile broth; incubate 24-48 hr at 32±1C as appropriate	
		2.	Presence of any gas in a BGB tube constitutes a confirmed test	
		3.	Record the number of picked colonies and the number of colonies that produced gas (if necessary calculate % confirmed and multiply by total number of colonies)	
	f.	Ιf	no colonies appear on plate(s), record count as 0	
	g.		there are 1-154 colonies on a plate, record number inted	
	h.		> 154 colonies develop in highest dilution plate, ord number as > 150	
	i.		en multiple plates of a dilution are used, sum counts plates	

	a.		naccurate counting due to carelessness, fatigue, ired vision	
	b.		r cause and correct if unable to duplicate your nts on the same plate	
	С.		monthly counting	
			3 or more analysts use the RpSm method, see rent SMEDP, records maintained	
		agr	less than three analysts, comparative counts ee $\leq 8\%$ for the same analyst and $\leq 10\%$ between analysts, records maintained	
			REPORTS	
20.	Com	puting a	nd Reporting Counts	
	a.		y number of colonies (or estimated number ssary) by the reciprocal of the dilution	
	b.		ecutive dilutions yield 25-250 colonies, compute sing formula below (see current SMEDP)	
		N =	$\Sigma C / [(1 \times n_1) + (0.1 \times n_2)] d$	
		Where,	N = number of colonies per milliliter or gram ΣC = sum of all colonies on all plates counted n_1 = number of plates in lower dilution counted n_2 = number of plates in next highest dilution counted d = dilution from which the first counts were obtained	
		Example	: 1:100 = 244 colonies 1:1,000 = 28 colonies	
		N = = = = = = = =	(244 + 28)/ [(1 x 1) + (0.1 x 1)]0.01 272/[1.1]0.01 272/0.011 24,727 [25,000 (reported)]	
		Note:	In the NCIMS Program the denominator will always be 0.11 for 1:10 dilutions and 0.011 for 1:100 dilutions	
	С.	Report :	SPC and coliform counts only if inhibitors are ected	
	d.		computed count as Standard Plate Count/mL or /mL or SPC/g) when taken from plate(s) in the range	

19. Personal Errors

	e.	Report count as Coliform Count (confirmed)/mL or /g when taken from plate(s) in the 1-154 range	
	f.	If no colonies appear on SPC plates, report as < 25 times the reciprocal of the dilution and report as estimated	
	g.	If no colonies appear on coliform plates, report as < 1 times the reciprocal of the dilution and report as estimated	
	h.	Report SPC plate counts of 0 to 24 as < 25 times the reciprocal of the dilution and report as estimated	
	i.	When colonies on SPC plates exceed $100/\text{sq}$ cm, compute count by multiplying $100 \times \text{dilution}$ factor x area of plate in sq cm and report as > computed count estimated	
	j.	Computed counts from SPC plates outside the 25-250 range are reported as Estimated SPC (ESPC)	
	k.	Counts from coliform plates > 154 are reported as > 150 Estimated Coliform Count (ECC)	
	1.	If for any reason, an entire plate is not counted, the computed count is reported as Estimated	
	m.	Report only first two left-hand digits	
		1. If the third digit is 5 round the second number using the following rules	
		a. When the second digit is odd round up (odd up, 235 to 240)	
		b. When the second digit is even round down (even down, 225 to 220)	
	n.	If all plates from a sample have excessive spreader growth, report as spreaders (SPR), or are known to contain inhibitor(s) report as growth inhibitors (GI)	
	ο.	If a laboratory accident renders a plate uncountable, report as laboratory accident (LA)	
		PLATE LOOP COUNT METHOD	
		APPARATUS	
21.	Loo	p 0.001 mL	
	a.	True circle, welded I.D. 1.45 ± 0.06 mm, calibrated to contain 0.001 mL, made of appropriate wire	
	b.	Loop fits over a No. 54 but not a No. 53 twist drill bits (lab must have set), checked monthly, records maintained	

	С.	Modified by making a 30° bend $3-4$ mm from loop, compare to template before use	
	d.	Opposite end of wire kinked in several places	
22.	Нур	odermic Needle, Luer-Lok	
	a.	13 gauge (sawed off 24-36 mm from the point where the barrel enters the hub)	
	b.	Kinked end of loop wire shank inserted into needle until bend is 12-14 mm from end of barrel, compare to template before use	
23.	Cor	nwall Continuous Pipetting Outfit	
	a.	Consisting of metal holder, Cornwall Luer-Lok syringe and filling outfit	
	b.	Syringe, 2 mL capacity, adjusted to deliver 1.0 mL	
		1. Calibrated by checking ten 1 mL discharges (10 mL) using a 10 mL Class A graduate cylinder each day of use, records maintained	
	С.	With Luer-Lok of needle attached to Luer-Lok fitting of syringe	
		PREPARATION	
24.	Hea	t Treatment of Pipetting Equipment	
	a.	Sterilize assembled outfit wrapped in kraft paper or in a closed container by autoclaving at 120±1C for 15 min	
25.	Ass	embly of Complete Apparatus for Use	
	a.	Place end of rubber supply tube (attached to syringe) in sterile dilution buffer blank and depress syringe plunger several times to pump buffer into syringe	
	b.	Briefly flame loop and allow to cool 15 sec	
	С.	Discharge several 1 mL portions to waste, then discharge 1 mL portion of buffer into instrument control plate	
		PROCEDURE	
26.	Com	parative Test with SPC	
	a.	Comparisons done by each analyst performing test	

		1.	Comparison is valid only if done using similar plate count methods, i.e. SPC agar with pipets (or pipettors) to SPC agar with the PLC device or Petrifilm with pipets (or pipettors) to Petrifilm with the PLC device. Mixing methods is not permissible	
		2.	Results must be shown to be acceptable prior to official use of test in laboratory	
	b.		y of comparison and results in QC record (or easily essible file in laboratory)	
27.	Ider	ntif	ying Plates (as item 4)	
28.	Samp	ole 2	Agitation (as item 5)	
29.	Ino	cula	ting Plates	
	a.	sha	loop into each sample (avoiding foam) to bend in nk and withdraw vertically from surface three times sec with uniform movement of 2.5 cm	
	b.	loom	se cover of petri dish (just high enough to insert p), insert loop and depress plunger causing sterile ution buffer to flow across charged loop washing sured 0.001 mL of sample into dish	
	С.		not depress plunger so rapidly that buffer fails flow across loop	
30.	Plat	ing		
	a.	As o	described in item 13 or 44	
	b.	Pou	r plates with 12-15 mL agar	
			CONTROLS	
31.	Cont	rol	S .	
	a.	See	item 14	
	b.	Ini	tial rinse control, see item 25c and 29c	
	С.		ermine if loop is free rinsing by preparing a rinse trol plate after every 20 samples plated	
	d.		er all samples have been run discharge a final se to a control plate	
			INCUBATION	
32.	Incı	ıbat	ion (see item 15)	
	a.	48±	3 hr at 32±1C	

COUNTING COLONIES

33.	Counti	ing Aids (see item 16 or 47b)	
34.	Record	ding Plate Loop Counts (see item 17 or 48)	
35.	Persor	nal Errors (see item 19 or 49)	
		REPORTS	
36.	Report	ting Counts	
	a. Se	ee item 20 or 50	
	Es	f 0 to 24 colonies on plate report as < 25,000 stimated Plate Loop Count/mL (EPLC/mL) or if etrifilm used, EPPLC/mL	
		f count is between 25 and 250, report count as LC/mL or PPLC/mL	
	d. If	f colony count is > 250, report as EPLC/mL or EPPLC/mL	
	mι	nen colonies exceed 100/sq cm, compute count by altiplying 100 x dilution factor x area of plate a sq cm and report as > computed count estimated	
		PETRIFILM AEROBIC COUNT METHOD	
		APPARATUS	
37.	Petrif	film Aerobic Count (PAC) Films	
38.	Plasti	ic Spreader	
		rovided with Petrifilm films, concave (ridge) side sed	
		PROCEDURE	
39.	Identi	ifying Films (as item 4)	
40.	Sample	e Agitation (as item 5)	
41.	Sample	e Measurement (as item 6&7)	
42.	Diluti	ion Agitation (as item 8)	
43.	Diluti	ion Measurement (as item 9&10)	
44.	Proced	dure	
	a. Pl	lace the film on a level surface	
	di	ift the top film and deposit 1 mL of sample or ilution onto the center of the base film, touching ff the last drop	

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		⊥.	can be used; 10 fold dilution will have to be made)	
		2.	Or, deposit samples with pipettor (capable of making a 1:10 dilution in the tip)	
		3.	Or, deposit sample with PLC apparatus (item 29)	
	С.	Car	efully drop the top film onto the inoculum	
	d.	(ite	ce the plastic spreader with the ridge side down em 38) on the top film over the sample and press n gently on the center of the spreader to distribute culum to the circular ridge of the spreader	
	e.	Lea	ve film undisturbed for 1 min for gel solidification	
	f.	Inc	ubate within 10 min of solidification	
45.	Con	trol	S _	
	a.	See	item 14 above except for air plates	
	b.	Air	plates	
		1.	Inoculate PAC film with dilution buffer (1 mL)	
		2.	Drop film down onto the dilution buffer and spread as described in item 44d above	
		3.	Leave film undisturbed for 1 minute for solidification of gel	
		4.	The film must be the first one prepared immediately before samples are shaken and must be located such that it is in the area of the plating activity (not off to the side)	
		5.	Roll top film back and away from bottom film and expose film for 15 min	
		6.	After the 15 min roll top film back down and incubate with other films as usual	
		7.	<pre>Incubated, exposed films should contain ≤ 10 colonies, if count > 10, take and note corrective actions</pre>	
			INCUBATION	
16.	Inci	ubat	ion _	
	a.	Pla	ce films in horizontal position, clear side-up	
	b.	Sta	ck films no more than 20 high	
	С.	Inc	ubate 48±3 hr at 32±1C	

COUNTING COLONIES

47.	Cour	nting	g PA	C Films	
	a.	See	ite	m 16, or	
	b.	Opt	iona	lly, count using an approved Petrifilm reader	
		1.		er to manufacturer's instructions for set-up operation information	
		2.	3M	Petrifilm Information Management System (PIMS)	
			a.	Store control cards in a clean, dry and enclosed container	
			b.	Scan and record control card result prior to the start of and at the end of each operation period	
			С.	Scan and record control card result hourly with continuous operation	
			d.	Control card result must fall in the 92 to 108 range, if outside of this range an alarm will sound to alert the operator of a failure	
				 If alarm sounds, inspect card for defects, if defect(s) are observed replace control card, scan and report result of new card 	
				2. Do not proceed unless control card gives acceptable result, seek technical assistance	
		3.	3M	Petrifilm Plate Reader	
			a.	Store System Verification Cards in a clean, dry and enclosed container	
			b.	Scan and record System Verification Card result prior to the start of and at the end of each operation period	
				1. Use Log File feature to automatically save electronic pass/fail result	
			С.	Scan and record System Verification Card result hourly with continuous operation	
				1. Use Log File feature to automatically save electronic pass/fail result	
			d.	System Verification Cards used to check the function of the Petrifilm Plate Reader prior to reading test films (red, green and blue top lights, and backlights flash)	

			results in an error re-insert card	message, remove and	
			. If an error occurs a card for visible dir and re-insert card	second time, inspect t or defects, clean —	
			. If card gives a thir scan and report resu	d error replace card, lt of new card	
			. Do not proceed unles Card gives an accept technical assistance		
	4.	Adva	ced Instruments PetriSc	an Reader	
		a.		r spots and if necessary -free cloth with a mild	
		b.	lace templates 1 and 2, rowth in the PetriScan		
		С.	ount and record all res f and at the end of eac	ults prior to the start h operation period	
		d.	can, count and record tilm results hourly with		
		e.	emplate 1 gives count b	etween 27 and 33	
		f.	emplate 2 gives count b	etween 190 and 210	
		g.	o growth films give a c	ount of zero	
		h.	f any results out of ra	nge	
			. Inspect templates an scanner glass for sp	d films for defects and ots	
			. If defect(s) found r and scan, count and	eplace template or film	
				template and no growth e results, seek technical —	
	5.	Main	ain records		
С.		mine read	ach test film visually	prior to placing into —	
	1.	For	ilms with no growth, as	sure reader count is	

		 For atypical films, spreader, confluent growth, excessive growth around edge of film, etc., do not count with reader, record as appropriate using items 17 & 48 	
48.	Pet	rifilm Count	
	a.	Count all colonies stained various shades of red, even those outside the circular indentation left by the spreader	
	b.	See item 17	
	С.	Select spreader free films with 25-250 colonies and count all red colonies	
	d.	If films from all dilutions yield < 25 colonies each, record actual number in lowest dilution	
	е.	If all films from a sample show no colonies, record count as 0	
	f.	If films from all dilutions exceed 250 colonies, estimate (as per manufacturer specification)	
49.	Per	sonal Errors	
	a.	See item 19, or	
	b.	If using an approved film reader (item 47b) analysts must perform monthly visual counts comparing to reader results (reader = one analyst)	
		 If one analyst, count must be ≤ 10% between visual and the reader result 	
		2. If two or more analysts, use RpSm method (see current SMEDP) using the reader result as an analyst count	
		REPORTS	
50.	Rep	orting Counts	
	a.	See item 20	
	b.	If the count is between 25 and 250, report count as Petrifilm Aerobic count/mL (PAC/mL)	
	С.	If count is 0 to 24, report as $<$ 25x reciprocal of the dilution as Estimated PAC/mL (EPAC/mL)	
	d.	If count is > 250, report as EPAC/mL	
	е.	When colonies exceed 100/sq cm, compute count by multiplying 100 x dilution factor x 20 sq cm and report as > computed count estimated	

PETRIFILM COLIFORM COUNT METHOD

APPARATUS

51.	Pet:	rifilm Coliform Count (PCC) Films	
52.	Pla	stic Spreader	
	a.	Provided with Petrifilm films, smooth, flat side used	
		PROCEDURE	
53.	Sel	ecting Dilutions	
	a.	For milk samples, 1 mL direct and/or decimal dilutions	
	b.	For other milk products use 1/10 dilution, must plate 10 mL, i.e., use 10 PCC films or see 64d	
54.	Ide	ntifying Films (as item 4)	
55.	Sam	ple Agitation (as item 5)	
56.	Sam	ple Measurement (as item 6 & 7)	
57.	Pro	cedure	
	a.	Place films on level surface	
	b.	Lift top film and deposit 1 mL of sample above the center of the base film, touching off the last drop	
	С.	Carefully roll the top film into the inoculum, avoid trapping air bubbles	
	d.	Place the plastic spreader with the flat side down (item 52) on the top film and press down gently on the center of the spreader to distribute inoculum over growth area	
	e.	Leave films undisturbed for 1 min for gel solidification	
	f.	Incubate films within 10 min of solidification	
		INCUBATION	
58.	Inc	ubation	
	a.	Place films in horizontal position, clear side up	
	b.	Stack films no more than 20 high	
	С.	Incubate 24±2 hr at 32±1C	

COUNTING COLONIES

59.	Cou	nting Aids (see item 16)	
60.	Pet	rifilm Count	
	a.	Count only red colonies having 1 or more gas bubbles within 1 colony diameter	
	b.	Colonies with gas bubbles are confirmed, no other testing is required	
		REPORTS	
61.	Rep	orting Counts	
	a.	See item 20	
	b.	If the count is between 1 and 154, report count as Petrifilm Coliform count/mL (PCC/mL)	
	c.	If count is 0, report as < 1 Estimated PCC/mL (EPCC/mL)	
	d.	If count is > 154, report as > 150 EPCC/mL	
		PETRIFILM HIGH-SENSITIVITY COLIFORM COUNT METHOD	
		APPARATUS	
62.	Pet	rifilm High-Sensitivity Coliform Count (HSCC) Films	
63.	Pla	stic Spreader for HSCC Films	
		PROCEDURE	
64.	Sel	ecting Dilutions	
	a.	For milk samples, apply 5 mL direct and/or make decimal dilutions	
	b.	1:5 minimum dilution required for: chocolate milk, cottage cheese, dip, evaporated milk, frozen yogurt, heavy and light cream, ice cream, sour cream, sweetened condensed milk and/or decimal dilutions	
	С.	1:10 minimum dilution required for: butter, buttermilk, cheese, dry dairy products, yogurt and/or decimal dilutions	
	d.	1:10 dilutions of milk or milk products test 10 mL (5 mL on two films)	
65.	Ide:	ntifying Films (as item 4)	
66.	Sam	ple Agitation (as item 5)	
67.	Sam	ple Measurement (as item 6 & 7)	
(PL	ATE-	20-Rev. 6/05)	

68.	Pro	cedure	
	a.	Place film on level surface	
	b.	Lift top film and deposit 5 mL of sample or dilution just above the center of the bottom film, touching off the last drop	
	С.	Carefully roll the top film onto the sample gently to prevent pushing the inoculum off the film and to avoid trapping air bubbles	
	d.	Place the plastic spreader (item 63) on the top film over the inoculum	
	е.	Distribute sample with a gentle downward pressure on the handle of the spreader to distribute inoculum to the circular ridge of the spreader	
	f.	Leave film undisturbed for 2-5 min for gel to solidify	
	g.	Incubate within 10 min of solidification	
		INCUBATION	
69.	Inc	ubation (see item 58)	
	a.	Stack films no more than 10 high	
		COUNTING COLONIES	
70.	Cou	COUNTING COLONIES nting Aids (see item 16)	
		nting Aids (see item 16)	
71.	Pet	nting Aids (see item 16) rifilm Count (see items 18 & 60)	
71.	Pet	nting Aids (see item 16) rifilm Count (see items 18 & 60) REPORTS	
71.	Pet:	nting Aids (see item 16) rifilm Count (see items 18 & 60) REPORTS orting Counts	
71.	Pet:	nting Aids (see item 16) rifilm Count (see items 18 & 60) REPORTS orting Counts See items 20 and 61	
71.	Pet:	nting Aids (see item 16) rifilm Count (see items 18 & 60) REPORTS orting Counts See items 20 and 61 On 5 mL direct films report:	
71.	Pet:	rifilm Count (see items 18 & 60) REPORTS orting Counts See items 20 and 61 On 5 mL direct films report: 1. 1 to 4 colonies as < 1 coliform/mL or gm	
71.	Pet:	rifilm Count (see items 18 & 60) REPORTS orting Counts See items 20 and 61 On 5 mL direct films report: 1. 1 to 4 colonies as < 1 coliform/mL or gm 2. 5 colonies as 1 coliform/mL or gm 3. > 5 colonies as 1 coliform for every 5 colonies counted, rounding up to the next number if not even multiples of 5 (ex. 11=3 coliforms/mL or gm)	