行政院及所屬各機關出國報告

（出國類別：實習）

匹茲堡大學榮民聯合醫學中心特殊病菌實驗室退伍軍人症臨床檢體培養實習報告

服務機關：高雄榮民總醫院

出國人職稱：主治醫師、醫檢師

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出國地區：匹茲堡

出國期間：八十九年十一月二十七日至八十九年十二月十六日

報告日期：八十九年三月二十六日

摘要：

退伍軍人桿菌（*Legionella pneumophila*）引起退伍軍人症（Legionnaires' disease）爲肺炎之重要病原菌，其臨床表現不典型，用藥與一般肺炎致病菌不同，而併發症及致命性不亞於其他致病菌，因此早期診斷並快速使用有效之抗微生物劑對於臨床治療非常重要。目前國內對退伍軍人症之診斷多依賴痰液或尿液抗原之檢測或血清學之抗體效價變化。痰液或尿液抗原檢測雖可快速診斷但只限於少數血清型，而血清學之診斷需急性期及恢復期二次效價之比較方能確立診斷，對於病人之臨床治療緩不濟急。國內迄今無本土大規模之退伍軍人桿菌血清型檢測，亦未建立流行病學之本土血清學基本效價參考，因此臨床檢體培養之陽性率對於患者之診斷及治療極爲重要。國內環境檢體之培養較少問題，但臨床檢體培養之靈敏度甚低；美國匹茲堡大學榮民聯合醫學中心對於退伍軍人症之臨床治療及研究極具盛名，而其特殊病菌實驗室之退伍軍人桿菌培養靈敏度高達 80%。此次赴美實習

爲期二週，觀摩臨床患者之治療，並實習環境採檢、處理及培養，痰液及尿液之抗原檢測，血清學效價之酵素免疫法檢測以及最重要的痰液培養：培養基之選擇、配製及品管，和菌落的辨識、菌種之鑑別。

正文

目的：

於匹茲堡大學榮民聯合醫學中心特殊病菌實驗室實地學習退伍軍人桿菌之環境採檢、處理及培養，痰液及尿液之抗原檢測，血清學檢測以及臨床檢體包括痰液之培養，培養基之選擇、配製及品管和菌落的辨識、菌株之鑑別。

過程：

第一週：

第一天先拜訪匹茲堡大學榮民聯合醫學中心感染科主任余博士，臨床微生物科實驗室以及特殊病菌實驗室及其他同仁，並參觀實驗室。第二天觀摩特殊病菌實驗室環境檢體之處理及接種，以及培養基之配置。第三天加護病房恰有退伍軍人症社區肺炎患者，與余博士至加護病房巡視患者，其後兩天觀摩患者痰液培養，痰液、尿液及肋膜液之抗原檢測和血清抗體檢測。

第二週：

與實驗室主任 Dr. Janet Stout 實地採集環境檢體並觀摩其消毒設備，並實習檢體處理及接種，實地操作抗體及抗原檢測，配製培養基並進行品管試驗，比較本院原有培養基和實驗室培養基之差別。

心得：

前言：

退伍軍人症（Legionnaires' disease）佔社區肺炎之第三、四位，次於肺炎雙球菌及流感嗜血桿菌，亦是院內肺炎之重要致病菌，由於一般肺炎致病菌的治療用藥對退伍軍人桿菌無效，死亡率高達 15-30%，因此早期診斷並使用有效之抗微生物劑對於臨床治療非常重要。

一、 退伍軍人桿菌之培養（Legionella culture）

前言：

培養爲診斷退伍軍人症最確定之方法。匹茲堡大學榮民聯合醫學中心特殊病菌實驗室之培養靈敏度高達 80%，醫師在開立相關之檢驗項目時必定包含退伍軍人桿菌培養。

退伍軍人桿菌屬挑剔性格蘭氏陰性桿菌，培養須使用含 yeast extract、charcoal、iron 及 amino acid cysteine 之特殊培養基，培養環境爲 35℃，不添加二氧化碳之

高溼度環境。臨床檢體分爲二類，一類包括肺組織、肋膜液、支氣管沖洗液、氣管吸取液等不易取得且不受口腔及上呼吸道常在菌污染之檢體，不論醫師是否開立檢驗單，一律做退伍軍人菌培養，可提昇分離律；另一類爲一般痰液或氣管抽痰檢體，僅當醫師開立檢驗單才做培養。培養基選用 BCYE（Buffered Charcoal Yeast Extract），PAC（BCYE+Polymyxin B+Anisomycin+cefamandol)和 PAV(BCYE+Polymyxin B+Anisomycin+Vancomycin)。臨床微生物科使用現購培養基；特殊病菌實驗室則自行配製，原因是品質較佳。

操作流程：如附件一

所有檢體均先由臨床微生物科直接接接種（不經任何處理）至 BCYE，PAC，PAV，於 35-37℃溫箱培養，三天後觀察有無競爭性常在菌過度生長，若無則繼續培養 7-10 天，並在解剖顯微鏡下觀察；如果有競爭性

常在菌，則將保存於 4℃冰箱之檢體轉至特殊病菌實驗室做酸-緩衝液前處理 4 分鐘（0.2M HCl-KCl, pH2.2），再接種至 BCYE,PAC,PAV 培養 7-10 天。

檢體的保存與運送：

由於退伍軍人桿菌在 4℃可存活至少一週，在冷凍下更久，因此爲了避免污染菌過度滋生，檢體應於 4℃以下運送與保存。

培養基：

匹茲堡大學榮民聯合醫學中心特殊病菌實驗室使用的培養基有現購與自製的。不論哪一種，使用前均需做培養基品管（medium QC），以確保退伍軍人菌可以生長良好，且含抗生素之選擇性培養基可有效抑制其他菌種。方法爲將標準測試菌種(*Legionella pneumophila, Legionella micdadei, Escherichia coli, Staphylococcus aureus, Candida albicans*)以無菌水調成 0.5 McFarlamd 的濃度，用 0.001mL 定量接種環接種至欲測試的培養基。3 天後觀察並記錄生長情形，結果應爲 *Legionella*

pneumophila 與 *Legionella micdadei* 菌落大小至少 1mm，Escherichia coli 菌落數爲 0-50 個，Staphylococcus aureus 與 Candida albicans 沒有生長。（如附件二）

自製培養基非使用已配好成分之 medium base，而採用個別加入各種基本成分，包括 yeast extract（DIFCO），agar（DIFCO），charcoal（Fisher），ACES buffer（N-(2-acetamido)-2-aminoethanesulfonic acid；Research Organics），α-ketoglutaric acid（Sigma），L-cysteine monohydrochloride（Fisher），ferric pyrophosphate（Sigma），以及 1.0N KOH 用來調整 pH 值。pH 值須控制在 6.85-6.95（6.9±0.05）的範圍內，須測試兩次，autoclave 前以及 autoclave 後加入其它不宜 autoclave 之成分後；pH meter 的測試電極，應爲可測試培養基表面 pH 值之平面電極。而其 charcoal 濃度較市售配方略低，以避免過度吸附抗生素影響抑制雜菌效果。

PAV 及 DGVP 中皆含兩種染料：bromocresol purple（BCP；Fisher），bromothymol blue（BTB；Fisher），目的可使 *Legionella pneumophila* 呈淺綠色菌落，*Legionella micdadei* 呈藍色菌落，便於肉眼觀察。DGVP 培養基含有 Dye，Glycine，Vancomycin 及 Polymixin；Glycine 抑制環境雜菌效果極佳，但對呼吸道常在菌無抑制效果，因此僅做爲環境評估用培養基。

菌落觀察：

退伍軍人桿菌生長緩慢，約需 3-5 天才可觀察到，菌落微突、呈圓形，具毛玻璃狀外觀。年輕菌落邊緣尚可看見綠色、粉紅色或紫色的虹光（idridescence）。隨著菌落老化，呈中央微白邊緣淡紫色虹光，若以接種環挑取，呈黏稠狀（sticky）。在波長 365nm 的紫外燈下觀察，部分退伍軍人菌株會產生藍白色自發性螢光（blue-white autofluorescence），如 *Legionella bozemannii, Legionella dumoffi, Legionella cherrii, Legionella gormanii, Legionella tucsconesis, Legionella*

anisa；某些菌株則產生紅色自發性螢光，如 *Legionella rubrilucens*, *Legionella erythra*。

鑑定方法：如附件三

以解剖顯微鏡（dissecting microscope）和紫外燈觀察疑似菌落，先操作 L-cysteine 需求試驗，即挑取菌落分別接種至 BAP(blood agar plate)和 BCYE。若 BCYE 生長而 BAP 不長，則近一步做免疫螢光染色，以確認是否爲退伍軍人桿菌並鑑定菌株或血清型。匹茲堡大學榮民聯合醫學中心特殊病菌實驗室採用的免疫螢光染色試劑（如附件四）可分爲單株直接螢光分析結合劑套組（廠牌爲 Genetics Systems，含 *Legionella pneumophila* 所有血清型），多株直接螢光分析結合劑套組（廠牌爲 Scimedx 和 m-Tech Monoclonal Technologies，含 *Legionella pneumophila* 單獨血清型及 1-6 血清型）及多價多株間接螢光分析結合劑套組（廠牌爲 Remel，含 33 種退伍軍人菌菌株型）。

免疫螢光分析結果	鑑定
Serogroup 1-6: 陰性 Genetics: 陽性 Remel: 陽性	*Legionella pneumophila*，非血清型 1-6 型
Serogroup 1-6: 陰性 Genetics: 陰性 Remel: 陽性	*Legionella* species
所有免疫螢光分析陰性	非退伍軍人菌

二、 血清學診斷：

匹茲堡大學榮民聯合醫學中心特殊病菌實驗室利用兩種不同酵素免疫分析法（ELISA）來偵測患者血清抗體。一種爲購買的套組試劑：Wampole Laboratories *Legionella pneumophila* IgG/IgM ELISA。另一種爲特殊病菌實驗室自行製作之抗原檢測，MicroELISA。原理相同，將退伍軍人桿菌抗原吸附在 microwell plate，加入患者血清，使患者血中抗體與抗原結合，洗掉多餘抗體，加上結合劑（conjugate，過氧化酶結合抗人類血清），洗掉多餘結合劑，再加上呈色劑，終止呈色劑，最後經由比色測定效價。

兩種方法不同處在於 Wampole 試劑抗原含 *Legionella*

pneumophila 血清型 1-6 型，同時測得 IgG 及 IgM 抗體，操作時間約一小時，靈敏度及專一性不若其自配抗原，作爲初步篩檢用。自配抗原分爲三種：

I—*Legionella pneumophila* 血清型第一型（Philadelphia），II—*Legionella pneumophila* 血清型第二、五型（Togus, Dallas），III—*Legionella pneumophila* 血清型第三、四、六型（Bloomington, Chicago, Los Angeles）。結合劑分爲兩種可分別檢測 IgG 及 IgM。唯自製抗原費時，約需 15 天，檢測過程費時二天；但可提供較多訊息，包括血清型、急性期或恢復期（IgM 或 IgG），因此特殊病菌實驗室用於 Wampole 測試之陽性確認及研究個案。

三、 尿液抗原測定：

退伍軍人症患者尿中會出現專一可溶性抗原，可在感染早期或較晚時期測得，且不受抗生素治療影響，因此爲方便理想的檢測方法。缺點爲目前試劑僅能偵測

Legionella pneumophila 血清型第一型。試劑：Binax, Legionella Urinary Antigen, EIA。

四、 環境檢測：

匹茲堡大學榮民聯合醫學中心每兩個月針對冷熱水儲水槽（storage tank）以及特定之遠端出水口做定期檢測，以監控醫院環境中之退伍軍人桿菌菌落數；同時亦對銅銀離子消毒系統（copper-silver ionization unit）進行溫度、電壓以及水中離子濃度之測試，以確保該系統保養及運作之正常。採檢過程除實驗室人員外亦會同管路負責人員一同前往。

1、 採集方法：

飲用水系統（potable water）直接採集 100ml 以上的水檢體；遠端出水口（水龍頭或蓮蓬頭）利用特殊棉棒拭子（雙棉棒，內含特殊運送培養基）採集表層生物膜（biofilm）；冷熱水儲水槽則經由底部的引流閥（drain valve）收集開閥後立即流出

的水以及流放 15 至 30 秒後的水各 10-15ml。

2、 接種方法：

接種培養基爲 BCYE、DGVP 及 CCVC（BCYE 添加 cephalothin, cylohexamide, vancomycin, colistin）。飲用水檢體直接取 0.1ml 接種至 BCYE、DGVP、CCVC，再以 L 形玻棒推勻；同時利用 0.2μm 濾紙過濾濃縮後，經酸緩衝液前處理 3 分鐘後接種。棉棒拭子則須經酸緩衝液前處理 3 分鐘後接種。冷熱水儲水槽則直接取 0.1ml 接種即可。

當環境監測結果之陽性分離率大於 30%，院方則需對供水系統進行消毒措施。（如附件五）

建議：

一、 退伍軍人症之診斷：臨床醫師懷疑患者爲非典型肺炎，除做血清學檢查及考慮用藥外，應送檢體培養並做痰及尿液之抗原檢驗，已提高確定診斷率。

二、 培養基可採用匹茲堡大學榮民聯合醫學中心配方，並注意 pH 值之調整。

三、 退伍軍人桿菌之培養使用特殊培養基，應建立品質管制，以提高培養靈敏度。

四、 國內可考慮做流行病學，調查一般人之血清抗體效價，建立本土血清學診斷之參考值。

五、 分析醫院檢驗室培養之問題，與臨床醫師配合，解決可能造成培養陰性的情況，提高診斷率。

附件二

PROCEDURE FOR PROCESSING CLINICAL SAMPLES FOR LEGIONELLA CULTURE

CULTURE AUTOMATICALLY	CULTURE ON REQUEST
Lung Tissue Pleural Fluid* Transtracheal Aspirates Bronchial washings*	Sputum Transtracheal Suction

DIRECT CULTURE ONTO BCYE, PAV, PAC INCUBATE @ 35-75°C FOR 3 DAYS**

HEAVY GROWTH OF COMPETING MICROFLORA

NO	YES
CONTINUE INCUBATION FOR TOTAL OF 7-10 DAYS EXAMINE PLATES UNDER STEROSCOPE	ACID-BUFFER PRE-TREAT SAVED SPECIMEN, FOLLOWED BY CULTURE ONTO BCYE, PAV, PAC INCUBATE TOTAL OF 7-10 DAYS LEGIONELLA DFA STAIN OF SPECIMEN PERFORMED AT THIS POINT

*Concentrate by centrifugation prior to inoculating media
**Save specimen at 4°C for 5-7 days

附件三

QUALITY CONTROL

Media Testing

Inoculating Methods

The following methods are used to test media for proper reactivity or growth characteristics. These methods are exclusive for media used for isolation and identification of the *Legionellceae* family. The chart following this section lists media, methods of inoculation, stock isolates, and expected results when the procedures listed below are utilized.

Method 1. Suspend stock isolate from a 1 to 2 day agar culture (prepared from the working stock) into 2.0 ml sterile D.I. water at a 0.5 McFarland turbidity. Streak a .001 ml loopful for colony isolation onto the plate media for testing.

NOTE: *Candida albicans* suspension is a McFarland #3 (25-50 yeast cells per 400x field).

STOCK ISOLATE LIST	VAMC #	ATCC#	STORAGE MEDIUM
Legionella pneumophila	#20	43111	BCYE slant
Legionella micdadei	#594	VAMC	BCYE slant
Candida albicans	#212	14053	TSA slant
Escherichia coli	#210	25922	TSA slant
Staphylococcus aureus	#209	25923	TSA slant

Recording Results

(1) Media information such as date tested, date prepared, and medium type are logged onto a log sheet in Quality Control log book.

(2) Results are logged onto the log sheet, as indicated, dated, and initialed. (See Sample Log Sheet.)

(3) All media not meeting expected results are discarded and remade. Failures are recorded in the Quality Control Log book.

File: Media Testing
G drive: Documents/Stout/Special Pathogens

7.Hepatic disease	1 yes 2 no 9nil	**10.Other surgery**	1.cardiac 3.lung 4.brain 5.skeletomuscular 6.skin 7. BPH s/p TURB **2. No. 9. Nil** 8. Sinusitis s/p op 9. Thyroid 10. Bteast 11. Hemorrhoid 12 Laryngeal Ca
1. Etiology *0721=9221*	1.acute hepatitis A 3.Chronic hepatitis B 4. Chronic hepatitis C 5. Hepatitis D superimposed 6. Chronic nonB nonC hepatitis 7.alcoholic hepatitis 8. Hepatic failure **9.nil** 10.liver cirrhosis B 11. Liver cirrhosis C 12 alcoholic liver cirrhosis 13 ascites 14 SBP 15 hepatic encephalopathy 16. Hepatorenal syndrome 17 lepatopul syndrome 18.EV bleeding 19 GV bleeding **2 No** 20 polycystic liver dz 21 fatty liver 22 spleenomegaly 23 hepatomegaly 25 carrier B 26 carrier C 27 Shock liver 28 liver function impairment ,cause 29 LC,cause? 30 spleen abscess 31 LC, non B non C 32 chronic liver dz	1.Years before entry	Years 2.No 9.Nil
		11.CV Dz	1.Y 2.N 9.nil
		1. Etiology	1.AMI 3. Old MI 4.CAD 5.unstalbe angina 6. HTN 7. ISM 8.DCM **9.nil** 10 CHF 11. Pul edema 12 pericarditis **2. No** 13.myocarditis 14.pericardial effusion 15.vavular heart dz 16. Cardiac aarrhythmia 17. AAA 18 Aortic dissection 19PAOD 20.DVT 21dyslipidemia 22. Aortic aenurysm type A 23. Aortic aneurysm type B 24.Mycotic aneurysm 25 IE 26 RHD 27. HCVD
		12.CNS	1.Y 2.N 9.nil
8.GI tract dz	1.yes 2.no 9. nil	**1. Etiology**	1. CVA 3. Meningitis 4.encephalitis 5.brain abscess 6 ICH 7. IICP 8. EDH **9 nil** 10 SDH 11 SAH 12.brain edema 13 hydrocephalus **2.no** 14 senile dementia 15. SDH 16 TIA
1. Etiology	1. peptic ulcer **2 no** 3. GU 4. DU 5 gastritis 6 diverticulitis 7 colitis 5. IBD **9 nil** 10 UGI bleeding 11 EU 12 LGI bleeding 13. Angildysplasia 14 ischemia bowel dz 15 abd internal bleeding 16 malabsoption 17 irritable bowel syndrom 18.short bowel syndrome 19.gastrostomy 20 jejunostomy 21 colonostomy 22. GERD 23 Barrett's esophagitis 24 entrtitis	**13. renal dz**	1.Y 2.N 9.nil
		1. Etiology	1.ARF **2.no** 3. CRI 4. CRF 5.Uremia +H/D 6. Nephrotic syndrom 7. Nephitis 8. GM **9. Nil** 10 renal cysts 11 polycystic kidney 12. Renal abscess 13 perinral abscess 14 APN 15 UTI 16 renal stone 17 uretral stone 18 U-GB stone 19. Staghrone stone 20 hydronephosis 21 BPH 22 Rhadomyolysis 23 acute epididimyitis 24 prostitis
9.Neoplasm	1.Y 2.N 9 nil	**14.Pul dz**	1. yes 2. no 9. nil
1.Hematology	1.AML 3.ALL 4.CML 5 CLL 6.Hodgkin's lymphoma 7. Non-Hodgkin's lymphoma 8.MM 10. MDS **99.nil** 11..Preleukemia 12. Others **2.No.**	**1. etiology**	1. COPD **2.no** 3. Asthma 4. Community pneumona 5.nosocomial pnemonia 6. Aspiration pneumonia 7. Necrotizing pneumonia 8. Lung abscess **9 nil** 10 viral pnemonitis 11 pcp 12 fungal pneumonia 13 active pul TB 14 old pul TB s/p treatment 15. Empyema 16.pleural effusion 17pneumothroax 18 hemothroax 19acure tonsilitis 20.URI 21 ILD 22.resp failure 23.ARDS 24 acute pul edema 25 embolic lung 26 URI
2.Solid tumor .	1.Brain 3.esophageal 4. Gastric 5. Intestine 6 colon 7 HCC 8. GB 10. CBD **99 nil** 11. holangiocarcinoma 12. Ut Ca 13. Ovarian Ca 14 Cx Ca 15. Skin 16 Lung Ca 17 Cardiac tumor 18 parotid tumor. **2.No. 19. Du Ca 20 carcinomatosis, abd 20 lung metastasis 21 liver metastasis 22 renal tumor 23 tumor of unknown 24 pancrate Ca 25. Prostate Ca 26. NPC 27. Laryngeal Ca**		

MEDIA TESTING CHART

MEDIUM	ISOLATE(S)	RESULTS (3D)
BCYE	*L. pneumophila*	1 mm colony
	L. micdadei	1 mm colony
Selective #2 (PAV)	*L. pneumophila*	1 mm colony/green
	L. micdadei	1 mm colony/blue
	C. albicans	No growth
	E. coli	0-50 colonies
	S. aureus	No growth
Selective #1 (PAC)	*L. pneumophila*	1 mm colony
	C. albicans	No growth
	E. coli	0-50 colonies
	S. aureus	No growth
DGVP	*L. pneumophila*	1 mm colony/green
	L. micdadei	1 mm colony/blue
	E. coli	0-50 colonies
	S. aureus	No growth

7.Hepatic disease		1 yes 2 no 9nil	**10.Other surgery**		1.cardiac 3.lung 4.brain 5.skeletomuscular 6.skin 7. BPH s/p TURB **2. No. 9. Nil** 8. Sinusitis s/p op 9. Thyroid 10. Bteast 11. Hemorrhoid 12 Laryngeal Ca
1. Etiology *0721=9221*		1.acute hepatitis A 3.Chronic hepatitis B 4. Chronic hepatitis C 5. Hepatitis D superimposed 6. Chronic nonB nonC hepatitis 7.alcoholic hepatitis 8. Hepatic failure **9.nil** 10.liver cirrhosis B 11. Liver cirrhosis C 12 alcoholic liver cirrhosis 13 ascites 14 SBP 15 hepatic encephalopathy 16. Hepatorenal syndrome 17 lepatopul syndrome 18.EV bleeding 19 GV bleeding **2** No 20 polycystic liver dz 21 fatty liver 22 spleenomegaly 23 hepatomegaly 25 carrier B 26 carrier C 27 Shock liver 28 liver function impairment ,cause 29 LC,cause? 30 spleen abscess 31 LC, non B non C 32 chronic liver dz	1.Years before entry		Years 2.No 9.Nil
			11.CV Dz		1.Y 2.N 9.nil
			1. Etiology		1.AMI 3. Old MI 4.CAD 5.unstalbe angina 6. HTN 7. ISM 8.DCM **9.nil** 10 CHF 11. Pul edema 12 pericarditis **2. No** 13.myocarditis 14.pericardial effusion 15.vavular heart dz 16. Cardiac aarrhythmia 17. AAA 18 Aortic dissection 19PAOD 20.DVT 21dyslipidemia 22. Aortic aenurysm type A 23. Aortic aneurysm type B 24.Mycotic aneurysm 25 IE 26 RHD 27. HCVD
			12.CNS		1.Y 2.N 9.nil
8.GI tract dz		1.yes 2.no 9. nil	**1. Etiology**		1. CVA 3. Meningitis 4.encephalitis 5.brain abscess 6 ICH 7. IICP 8. EDH **9 nil** 10 SDH 11 SAH 12.brain edema 13 hydrocephalus **2.no** 14 senile dementia 15. SDH 16 TIA
1. Etiology		1. peptic ulcer **2 no** 3. GU 4. DU 5 gastritis 6 diverticulitis 7 colitis 5. IBD **9 nil** 10 UGI bleeding 11 EU 12 LGI bleeding 13. Angildysplasia 14 ischemia bowel dz 15 abd internal bleeding 16 malabsoption 17 irritable bowel syndrom 18.short bowel syndrome 19.gastrostomy 20 jejunostomy 21 colonostomy 22. GERD 23 Barrett's esophagitis 24 entrtitis	**13. renal dz**		1.Y 2.N 9.nil
			1. Etiology		1.ARF **2.no** 3. CRI 4. CKF 5.Uremia +H/D 6. Nephrotic syndrom 7. Nephitis 8. GM **9. Nil** 10 renal cysts 11 polycystic kidney 12. Renal abscess 13 perinral abscess 14 APN 15 UTI 16 renal stone 17 uretral stone 18 U-GB stone 19. Staghrone stone 20 hydronephosis 21 BPH 22 Rhadomyolysis 23 acute epididimyitis 24 prostitis
9.Neoplasm		1.Y 2.N 9 nil	**14.Pul dz**		1. yes 2. no 9. nil
1.Hematology		1.AML 3.ALL 4.CML 5 CLL 6.Hodgkin's lymphoma 7. Non-Hodgkin's lymphoma 8.MM 10. MDS **99.nil** 11..Preleukemia 12. Others **2.No.**	**1. etiology**		1. COPD **2.no** 3. Ashma 4. Community pneumona 5.nosocomial pnemonia 6. Aspiration pneumonia 7. Necrotizing pneumonia 8. Lung abscess **9 nil** 10 viral pnemonitis 11 pcp 12 fungal pneumonia 13 active pul TB 14 old pul TB s/p treatment 15. Empyema 16.pleural effusion 17pneumothroax 18 hemothroax 19acure tonsilitis 20.URI 21 ILD 22.resp failure 23.ARDS 24 acute pul edema 25 embolic lung 26 URI
2.Solid tumor .		1.Brain 3.esophageal 4. Gastric 5. Intestine 6 colon 7 HCC 8. GB 10. CBD **99 nil** 11. holangiocarcinoma 12. Ut Ca 13. Ovarian Ca 14 Cx Ca 15. Skin 16 Lung Ca 17 Cardiac tumor 18 parotid tumor. **2.No. 19. Du Ca 20 carcinomatosis, abd 20 lung metastasis 21 liver metastasis 22 renal tumor 23 tumor of unknown 24 pancreate Ca 25. Prostate Ca 26. NPC 27. Laryngeal Ca**			

附件三

Legionella Identification Review Chart

Examine primary media plates at 3-7 days with dissecting microscope.

Suspicious colonies are present.

Characteristics can be:

-round, 1 – 2 mm, glistening colony at 3 days;
-cut-glass appearance, or with appearance of white pigment;
-green, or pink-purple, iridescence in young colonies, becoming opaque with longer incubation.

Pneumophila-like, not BW species

Test for autofluorescence under UV light.

Positive:

BCYE/BAP

BAP⊖ BCYE⊕ Orange or red

Perform DFA:
L.rubrilucens
L.erythra
Remel IFA

If positive by one:
Confirm ID as *Legionella* spp., *rubriucens*, *erythra*, or *Legionella* spp., not *pneumophila*

If negative by all three:
Not *Legionella*

BAP⊖ BCYE⊕ colonial blue-white

Perform DFA:
L.dumoffii, *L.gormanii*
L.bozemanii, and *L.anisa*

If positive by one:
confirm ID as that specific species.

If equivocal or cross-reacting:
Confirm as blue-white fluorescing *Legionella* spp., not *pneumophila*

If negative:
Perform Remel IFA

(+) = *Legionella* Spp., not *pneumo*
(-) = not *Legionella*

BAP⊕ BCYE⊕ Not Legionella

Negative:

Perform SAT

Positive:
Perform DFA:
Legionella pneumophila, serogroup 1 only (Lp1)

If positive:
Confirm ID as Lp1.

If negative:

Proceed with DFA by Lp 1-6 as directed in next column.

BCYE⊕ BAP⊕ → Not Legionella.

BCYE⊕ BAP⊖

Negative:
Perform DFA
Legionella pneumophila, serogroups 1 – 6 (Lp 1- 6)

If positive with one:
Confirm ID as the specific serogroup of *L.pneumophila*

If negative:

Perform Genetics DFA & Remel IFA.

(+) Both reagents = *L. pneumo.*, not 1 - 6
(+) Remel only = *Leg.* Spp., not *pneumo.*
(-) Both reagents = not *Legionella*

No suspicious colonies present: **culture negative.**

附件四.

LEGIONELLA IMMUNOFLUORESCENCE

Reagents and Materials (all assays)

(1) Special Pathogens routinely purchases the following fluorescein-labeled (FA) diagnostic reagents (conjugates).

(a) A monoclonal DFA conjugate kit for all serogroups of *L. pneumophila* (Genetic Systems, Seattle, Washington).

(b) Six individual polyclonal conjugates for each *L. pneumophila* serogroup (SciMedx, Denville, New Jersey and m-Tech Monoclonal Technologies, Inc., Norcross, GA).

(c) A polyvalent polyclonal IFA conjugate kit for 33 *Legionella* strains, including *L. pneumophila* serogroups 1 to 10 (Meridian Diagnostics, Columbus, Ohio). Remel

Note: The monoclonal DFA test from Genetic Systems is the primary assay used by Special Pathogens. Ordering details for all these reagents are found in the order card file and the Inventory, Order, and Receipt file.

Definitions

(1) Monoclonal antibody – very specific antibody that reacts with only the outer membrane of *L. pneumophila*.

(2) Polyclonal antibody – less specific antibodies that react with multiple sites on *L. pneumophila* or other *Legionella* species.

3. List of 33 *Legionella* serogroup (22 species) Antibodies Contained in the REMEL *LEGIONELLA* Test Kit.

Serogroup	Strain	Serogroup	Strain
1	Bellingham	L. gormanii 1	LS-13
1	Knoxville	L. micdadei 1	TATLOCK
1	Philadelphia	L. wadsworthii 1	81-716
2	Togus 1	L. feeleii 1	WO-44C-C3
3	Bloomington 2	L. stainthelensi	Mt. St. Helens 4
4	Los Angeles	L. jordanis 1	BL-540
5	Dallas 1	L. anisa 1	WA-316-C3
6	Chicago 2	L. spiritensis 1	Mt. St. Helens 9
7	Chicago 8	L. hackeliae 1	Lansing 2
8	Concord 3	L. maceachernii	PX-1-G2-E2
9	IN-23-G1-C2	L. jamestowniensis	JA-26-G1-E2
10	Leiden	L. cherrii 1	ORW
		L. steigerwaltii 1	SC-18-C9
		L. parisiensis	PF-209C-C2
		L. rubrilucens 1	WA-270A-C2
		L. erythra 1	SE-32A-C8
		L. dumoffii 2	NY-23
		L. longbeachae 1	Long Beach 4
		L. longbeachae 2	Tucker 1
		L. bozemanii 1	WIGA

LEGIONELLA IMMUNOFLUORESCENCE

2. *Legionella* Polyclonal Direct Immunofluorescence (DFA) Test Procedure

Reagents

Test reagents are purchased from SciMedx and m-Tech separately for this assay and not as a kit. The conjugates are immune rabbit sera against *L. pneumophila* or other *Legionella* species listed below.

(1) *L. pneumophila* individual (monovalent) conjugates:

L. pneumophila	serogroup 1	Philadelphia 1, Knoxville 1, Bellingham strains, OLDA (m-Tech only)
L. pneumophila	serogroup 2	Togus 1 strain
L. pneumophila	serogroup 3	Bloomington 2 strain
L. pneumophila	serogroup 4	Los Angeles strain
L. pneumophila	serogroup 5	Dallas 1E strain
L. pneumophila	serogroup 6	Chicago 2 strain

(2) SciMedx polyvalent I conjugate that includes all six above serogroups in one reagent.

(3) SciMedx polyvalent II conjugate that includes serogroups 1-7 plus 6 *Legionella* species.

(4) *Legionella* species monovalent conjugates for:
L. bozemanii
L. dumoffii
L. gormanii
L. longbeachae 1 & 2 SciMedx only)
L. jordani (SciMedx only)
L. anisa (m-Tech only)

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SCIMEDX CORPORATION

MATERIALS AVAILABLE:

Legionella pneumophila FITC-labeled Rabbit Globulins, polyvalent and serogrouping.

The conjugates are prepared from immune rabbit serums produced against L. pneumophila serogroups 1 through 6 and the legionella species listed below. There are individual conjugates for each serogroup, and a polyvalent conjugate for each serogroup. The conjugates have been diluted to their optimal working dilutions with 0.25% Evans Blue diluent (which serves as a counterstain) and are distributed as Lyophilized components. The products are available as follows:

Cat. No. 1504 L.pneumophila serogroup 1	0.5 ml/2.0 ml	Cat. No. 150b L.bozemanii	0.5 ml/2.0 ml
Cat. No. 1505 L.pneumophila serogroup 2	0.5 ml/2.0 ml	Cat. No. 150d L.dumoffii	0.5 ml/2.0 ml
Cat. No. 1506 L.pneumophila serogroup 3	0.5 ml/2.0 ml	Cat. No. 150g L.gormanii	0.5 ml/2.0 ml
Cat. No. 1507 L.pneumophila serogroup 4	0.5 ml/2.0 ml	Cat. No. 150lb L.longbeachae	0.5 ml/2.0 ml
Cat. No. 1508 L.pneumophila serogroup 5	0.5 ml/2.0 ml	Cat. No. 150m L.micdadei	0.5 ml/2.0 ml
Cat. No. 1509 L.pneumophila serogroup 6	0.5 ml/2.0 ml	Cat. No. 150j L.jordanis	0.5 ml/2.0 ml
Cat. No. 1520 L.pneumophila serogroups 1-6, poly	0.5 ml		
Cat. No. 1521 L.pneumophila serogroups 1-6, poly	2.0 ml		
Cat. No. 1530 L.pneu.1-7 & b,d,g,l,m,j poly	2.0 ml		

NOTE: 0.5 ml vials provide 15 tests; 2.0 ml vials, 80 tests. Upon receipt, store the conjugates at 4-8°C. The reconstituted conjugates are stable at this temperature until expired. Check labels for specific expiration dates. Once reconstituted, the conjugates may be stored at 4-8°C for up to 90 days. Thimerosal is added as a preservative at 0.01%. The conjugates provided are at their optimum working potency for both sensitivity and specificity.

Fig. 1 An Approach To Disinfection

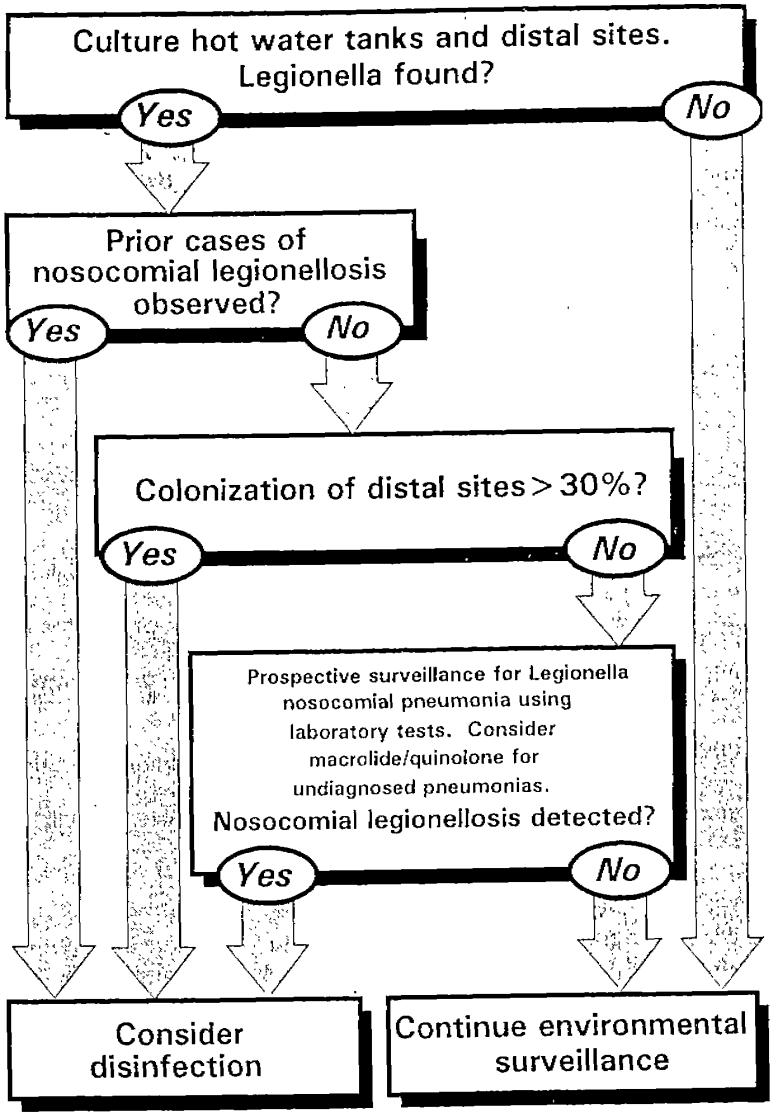

Fig. 2 Approach To Laboratory Capability For Hospitals.